Contributions and Discussion presented at the 5th International Symposium on Atopic Dermatitis

(May 22-25, 1994, Lillehammer, Norway)

Guest Editor
Georg Rajka

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Preface

The working method of this Symposium differed from that of our usual meetings: instead of regular lectures, I tried to create round-table discussions among invited leading experts on atopic dermatitis.

My idea was that since these experts know each others' work well, it might be sufficient to talk for only about 5 minutes - showing a few slides on the latest advances in the field and so giving more time to discuss in a relaxed atmosphere the 'state of the art' and allowing free questions.

In fact there was much discussion, but it was excellently organized and led by the discussion leaders, and it is essential for our understanding of the actual problems in atopic dermatitis.

Judging from several lively discussions and from the opinions of the participants, it was indeed a successful meeting.

Nevertheless, when listening to the tapes of the meeting, some problems have arisen. Several people talked freely, often without looking at their manuscript and I have tried to record in this volume exactly what was in fact said, apart from minor corrections by the authors as well as some shortening of the text and a certain adaption to written language. Originally, I intented to include tables too, which can give a better visual understanding, but for various reasons this was not possible; furthermore, only a few references are included.

I am aware that these aspects are open to criticism, but I hope that the high standard and spontaneity of the contributions will compensate for these problems.

Oslo, May 1995

Georg Rajka
Chairman of the Symposium

Invited speakers at the Fifth International Symposium on Atopic Dermatitis
Lillehammer, Norway, 22 - 25 May, 1994

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Subject: Epidemiology
Discussion leader: **F. Schultz Larsen** (Denmark)
Panelists: **Å. Svensson** (Sweden), **Th. Diepgen** (Germany), **F. Schultz Larsen** (Denmark), **T. Schäfer** (Germany), **K. Thestrup-Pedersen** (Denmark), **E. Falk** (Norway)

Schultz Larsen:
I think we should attempt to make it a productive morning. We have planned for a round table discussion, a new way of doing things, and we hope that the audience will in some way or other participate in this format.

Svensson, Å.:
At the meeting in Bergen in 1991, F. Schultz Larsen, Thomas Diepgen and I decided to perform a collaborate study about AD (abbreviation used in following for atopic dermatitis). The aim of our investigation was to study the occurrence of AD in 7-year-old children in our respective countries and to evaluate the questionnaire model for epidemiologic studies on AD.

The questions were as follows: Did your child ever have itchy rashes, infantile eczema in the elbow or knee folds, at the wrists or ankles, over the face or neck, on the hands, arms or legs or trunk? Did your child ever have any of the following: unusually dry skin, irritation of the skin from textiles (especially wool), itching of the skin while sweating, seasonal variation in severity exacerbated by psychological tension or stress? Has your child had asthma and/or hay fever or any nasal conditions? At what age was the first occurrence of the skin problems in your child? For how long did your child have itchy rashes and/or infantile eczema? Did you, your husband or the child's siblings have, or have they had, infantile eczema and/or asthma and/or hay fever?

Our first step was to translate the questionnaire into our different languages. In the spring of 1992, the pilot study was performed in Sweden, with 92 7-year old children included. The parents had to fill in the questionnaire a few days before the children went through a clinical examination. In addition, the medical records from the general practitioners were also read. Since it was our impression that most of the parents understood the questions, we continued our study. The cross-sectional study was carried out in October and November of 1992. The reply rate was high; about 90% in all three countries. The numbers of investigated children were in Denmark, 437; in Germany, 1,164; and Sweden, 1,054, in total 2,655 children.

In order to validate the score system, we sent the questionnaire to two different groups of outpatients who had visited the dermatology clinic in 1993. In one group, the patients had attended because of AD and in the other group the patients had no known history of AD. The outpatients were between 6 and 12 years old. The reply rate among patients with known AD was 100% and in the group with patients without known AD it was 90%. In this part of the study 105 patients with AD and 100 patients without known AD were included.

Diepgen, T. L.:
Dr Svensson has introduced the aims of this collaborative Danish, German, and Swedish study of AD in 7-year-old school-children and he has presented the design of the study. Before we come to the results I would like to speak about the methodological aspects of this study. The first important step is to have some conception about the quality of the diagnostic instrument used. Especially in questionnaire studies it is necessary to be able to estimate the sensitivity and specificity of the method used, according to the diagnostic procedure.

Until today there is no objective marker of AD and we do not know what is the golden

standard of the disease. We therefore validated the questionnaire by comparing outpatients with known AD and control subjects.

As we have heard from Dr Svensson, we used two different control groups; the first control was the randomly collected sample of 7-year-old school-children and it was separated from those whom we knew had AD (they had onset before they had involvement of the skin lesions and onset before 2 years of age, and the duration of the disease was more than 3 years); this was 27.5% or 198 subjects and the second control group comprised outpatients without known AD.

Evaluation of the different questions in our study was made in the following way: If the child had itchy rashes in different locations we gave between 5 and 15 points, in the elbow - and kneefolds 15 points and at the wrist or ankles also 15 points. For these atopic features we gave 5 points for unusually dry skin, irritation of the skin from textiles (especially wool), itching of the skin when sweating, seasonal variations in severity exacerbated by psychological tension or stress. Other features included a personal history of asthma, 10 points; a personal history of hayfever, also 10 points; a family history of infantile eczema, or asthma or hayfever - 10 points. For the last two features, we asked the question: at what age did the skin problem first appear in your child? In the cases where it was quite clear it was before the age of 2 years, we gave the child 20 points. Following the same reasoning, we asked: how long did your child have itchy rashes? Based on the responses, we gave between 0 and 20 points.

The most complicated question was to define the cut-off point, specifically: how many points must be achieved for a subject to belong to the group of AD patients? We all know that this is a question of sensitivity and specificity. In the light of this, the percentages of sensitivity and specificity were evaluated for all possible cut-off points. By considering all possible cut-off points one can show how the sensitivity decreases and the specificity increases. For example, if you assume that a subject with more than 20 points belongs to the AD group, then you have high sensitivity or the number of correct positive tests results is 100%. That means you will be able to recognize all AD patients as those of AD. But the specificity of the number of correct negative test results is poor because, according to a low cut-off point of 1 to 20 points, a relatively large number of controlled subjects are believed to belong to the AD group and you will have a comparably high rate of false positives. It is quite obvious that if one were to choose a high cut-off point the sensitivity would decrease and the specificity would increase. Thus, the right cut-off point depends on the sensitivity and specificity you would like to achieve with your diagnostic tests.

We thought it would be good to have both high sensitivity and high specificity and we therefore chose a crossing point of about 50 points. There should be minimal overlapping and good separation between the AD group and the control group. The result of the evaluation of the questionnaire based on our statistical analysis classifies patients within 50 points or more as belonging to the AD group and with less than 50 points as belonging to the non-AD group. With this instrument we have sensitivity and specificity of about 90%. As I have said, it is a slightly higher with a 95% confidence interval in these ranges.

There are some major points I wish to focus on regarding the results of this study. First of all, we found close agreement according to the frequencies with our questionnaire in our different countries. This is very important as we need a validated questionnaire and this works very well in Denmark, Germany and Sweden and I think also in other countries. The validation shows high sensitivity and specificity; we found a cumulative incidence of AD of 15.6% with a 95% confidence interval between 14% and 17%.

Discussion

(Remark) In these studies, all children are associated with a public school system without selection, which may not be the case in some places around the world.

(Q) What about validation from 7 to 12 years of age, with age limits and relevance for 3-year-olds and older?

(A) Many studies are based on 7-year-olds. In other age groups, there may be existing problems of information, i.e. children vs. parents, teenagers, etc. It may be unclear whether children above age 12 or their parents fill out the questionnaire.

(Q) What about language differences?

(A) There are several examples where language differences should be taken into account. For example; there is no good translation for the word 'wheezing', and the word 'trunk' may be interpreted differently.

(Q) Is there a risk of overestimation, with 10% non-responders who may be different from the responders? In Italy, pediatricians may help to fill out and complete the records, which would increase the validity.

(A) The validity was checked because the parents could not distinguish between skin diseases. As always with such high values of sensitivity and specificity (about 90%), there may be an overestimation.

Thestrup-Pedersen, K.:

Now we have just learned that the incidence of AD or frequency of AD in Germany, Sweden and Denmark is around 15.6% and we have also heard that in Italy it is around 9%, so the facts about AD seem to be that there is an increase - either two-fold, four-fold or maybe five-fold - in AD. One could discuss this at length. Our discussion leader, Schultz Larsen, has shown in his twin studies that AD seems to be a genetically determined disease. Now if this is correct it is very hard to explain how you can have a two-fold or five-fold increase. We therefore discussed if this development of eczema could somehow be linked to what I would call ectodermal dismaturation. What factors could affect the expression of AD? Just to remind you that we know for example that the incidence of a completely different disorder, such as Down's syndrome, is closely related to the age of the mother and therefore we asked: could it be due to the fact that mothers have become elder? In Denmark, the age of a primipara in 1960 was 23 years for the whole population. In 1992 it had risen to 26.8 years, so within a time period of 32 years the age of a first-time mother has increased by 4 years.

My own favourite hypothesis is that an older mother is a high-risk mother if she gives her child the genes for AD. Now suppose that you inherit the disposition for AD in an even way, and the age of the mother is important, then child 2 should have a higher risk of developing AD than child 1. We found in 2-children families where only one child had AD, that child 2 runs a statistically significant risk of developing AD.

Schäfer, T.:

The first question in the study of atopic eczema is: what type of instruments do you use? Grossly we have two possibilities. Questionnaires and dermatological examination. Both instruments need to be standardized and we have heard that already from Dr Diepgen, referring to validity, including sensitivity, specificity and precision, which means the reproducibility of your instrument. A critical factor is the characteristic of the study population, where most differences occur. This means not only the general characteristics of age, gender and size, but also selection. There is a difference as to whether you investigate the record of 18-year-old healthy male recruits or an out-patient. You should be aware of errors: random errors, which are hard to avoid, and systematic errors will shift your data in a certain direction.

Examples of systematic errors are the misclassification and the selection bias of the study population. If you find a statistical association you should ask "Does this association imply validity?" and you may answer "Not necessarily" or, on the other hand, "why not?" There are certain criteria that can prove your association, whether it is biologically plausible and whether that tends to be a more causal effect or merely a chance statistical artifact.

In our study we did the first East-West German comparison using an actual dermatological examination regarding AD in pre-school children (5–6-year-olds). We had one region in the East, Halle, where air pollution was up to 10 times higher, especially SO_2 and dust fall, and four regions in the West. The prevalence of atopic eczema in Germany was, in general, 13% with variations between the different study areas. The highest prevalence was in the East, in Halle, with 17.5%.But not significantly higher than the control area.

Regarding risk factors, genetic predisposition accounts for a high odds ratio of about 5. Interestingly, the influence of the father is regularly suppressed by that of the mother. So the maternal influence is always higher than the paternal influence. This has been confirmed by other studies as well.

The next factor, which has been shown by other studies, too, is that even for this age group of young children more girls than boys suffer from atopic eczema.The next part refers to allergen and air pollutant exposure and we found that animals, whether dead or alive, increase the risk of atopic eczema. Air pollution also has something to do with atopic eczema, maternal smoking during pregnancy and lactation leads to an odds ratio of 2.03. Use of gas without a hood shows an odds ratio of 1.68, and we have hints of outdoor exposure: if the children's home was within 50 metres of a road with heavy traffic we have more atopic eczema than in the control group. This is just an example of some of the risk factors we found in our studies.

Falk, E.:

Schultz Larsen told me to bring just one slide with me, so I will go directly to the results. This is a part of the study which I made among a Norwegian Lapp population of 3000 individuals (it has been published in Acta Dermatologica as supplement 182). I will just deal with AD. This study will start by reviewing medical records at the local health centres. I reviewed 3000 children and we found a frequency of 30.7% among children and young adults. This is not unusual, and is very much in line with other studies. We will not concern ourselves further with this study on the Lapps as they are not genetically very different from Caucasians. They have only slightly different HLA patterns.

I will go on to the next study, by Dotterud, who has investigated children 7 to 12 years old in the northern part of Norway (Kirkenes or Sørvang community) which is close to the Russian border where the pollution is very high. We had long felt that AD in particular, and atopic diseases in general was very common there, so we started a questionnaire study. Of 575 children aged 7

to 12 years, 96% answered the questionnaire and we found the frequency of AD to be 36.8%, which is clearly very high, and maybe too high (it may be the case that both urticaria and contact dermatitis are included in these figures). Asthma and allergic rhinoconjuctivitis were not particularly compared with AD.We are now talking about a cumulative incidence. I hear that people are talking about mixing cumulative prevalence and we should distinguish between these terms. This is a cumulative incidence and I believe the point prevalence is an important factor. So, if you look at mothers and fathers of these children, you will see that they have less than 50% atopic diseases compared with their children. We investigated 424 of these 500 children which is about 80%. We found that 99 children had AD and clinical signs of AD at the time of the investigation. If we demanded a positive prick test in those with mucous membrane atopy (which includes asthma and allergic rhinitis), we found that 18.1% had the disease. Patients with a history of atopy, but with no positive prick test, were called suspected cases; there were also cases we called latent atopic: patients with no history of atopy but with positive prick tests. So, altogether, we found atopic disease in 36.4% of all these children. This AD in 23.6% is very reliable, as we examined all these children and, it is slightly higher than previously shown but not much, and - as you may know, atopic disease - and AD in particular, increases the more northerly the latitude.

There are three points which could explain this. First, smoking in families is very common in northern Norway. The high pollution from the Russian melting pounds close to the border may be another point and the keeping of pets is very common in this part of the world. This is part of a continuing study which will be supplemented with indoor climate studies.

Discussion

(Q) Have you looked at the educational levels, as shown by Halwell Williams?

(A) Yes, more atopic people were found in higher social classes.

(Q) What about the influence of paternal vs. maternal atopy?

(A) This is being investigated.

(Q) Are there differences in the weather in Germany?

(A) There are no statistical significant differences between East and West Germany; otherwise varied.

(Q) What about a potential weaning difference between East and West?

(A) We have found no differences between dietary outcome of AD in previous studies.

(Remark) It is important to keep in mind that there are national guidelines in many countries, which are followed by many paediatricians.

(Remark) The differences in prevalence may also be influenced by geographical region and the potential for pollution, which should be looked at.

(Q) Is there a pollution difference between Halle and Duisburg?

(A) Yes, the SO_2 dust is higher in Halle. With regard to motor vehicle pollution, exhausts produced mostly No^x and benzene, which is higher in Halle. The high SO_2 level is due to the use of coal. It is worth mentioning that we found less asthma and hay fever in East-Germany, contrary to the hypothesis, whereas data from North Norway may support the hypothesis. It will be of interest to study highly polluted areas of North Russia versus the supposedly lower polluted areas of North Norway.

Schultz Larsen, F.:

We all know that the diagnostic guidelines from Hanifin & Rajka were a major step forward, but we are also well aware that in some circumstances they were too complicated and were of little use to physicians in family care. Due to the work of Diepgen, Svensson and myself, we have considered for some years how to arrive at a simpler proposal for diagnostic features of diagnostic guidelines for actual clinical AD.

We call them the Lillehammer criteria in honour of this olympic city, so everybody is welcome to submit suggestions. It is first and foremost simple: We have the three age groups and different clinical pictures. We are looking at the common eczematous regions. Then there are anamnestic data, laboratory data and a certain duration and it is the same in all three age groups (see Appendix).

In the future we really hope that what you have heard here today will help us to make collaborative studies, standardized procedures and I hope we can agree on common diagnostic features. In this way I am sure we can get to know more about the geographical evaluation which we have talked about here.

Subject: Infantile Eczema
Discussion Leader: E. Bonifazi (Italy)

Panelists: **T. Aoki** (Japan), **L. Businco** (Italy), **T. J. David** (England), **J. Harper** (England), **K. Yamamoto** (Japan)

Bonifazi, E.:
In our experience, the well-known diagnostic criteria of Hanifin and Rajka are not reliable in infants with atopic dermatitis aged less than 4 months. Such criteria could be modified for AD in infants aged less than 4 months. 1) Eczematous lesions located on the face and skalp with minor or no involvement of the diaper area. Criterion no. 1) should always be present and associated with criterion no. 2) or no. 3). 2) Sleeplessness or restlessness not due to other causes. 3) Families with atopic disease, asthma, rhinitis, AD in parents or siblings. When applying these criteria we can readily make a differential diagnosis from other disorders which are present in the first 3 months of life. The main problem is the differentiation of AD from seborrheic dermatitis. Seborrheic dermatitis is a label that covers numerous disorders, ranging from very common and mild ones such as cradle cap, to severe disorders such as napkin psoriasis and Leiner's erythroderma. This is why I dislike the term "seborrheic". However, by applying these criteria we can easily differentiate AD from cradle cap.

When there are lesions on the face this is not a separate dermatitis but obviously a minor form of AD.

The most difficult differential diagnosis at this age is between the so-called napkin psoriasis and AD. According to the above-mentioned criteria, napkin psoriasis can be differentiated, because the involvement of the diaper area is prominent in this case. Even in diffuse AD the involvement of the diaper area is uncommon as Yamamoto underlined 16 years ago. Furthermore, at this age even when all the skin surfaces are affected, the involvement of the face is prominent.

The so-called acute erythrodermia is not, in my opinion, Leiner's disease, but a most severe form of napkin psoriasis as the spontaneous irritation of this form demonstrates. This form is different from erythrodermic AD, because in this form there is no prominent involvement of the face, whereas in AD, even in erythrodermic type, at this age the involvement of the face is prominent.

AD starts in early infancy in most cases, onset most certainly being influenced by genetic factors.

John Harper, who works the Hospital for Sick Children in London will give us an introductory review of genetic factors.

Harper, J.:
Many aspects of AD are controversial but I think there is one thing we could all agree on and that is, that there is a genetic predisposition to the development of AD.We know that about 70% of individuals with AD have a family history of atopic disorders. We know that there is a high concordance rate of AD in monozygotic twins compared with dizygotics.Cockson et al. in Oxford first published their work correlating atopy to chromosome C11 which was subsequently located to 11Q13. Their work concerned predominantly respiratory diseases. Therefore it was logical to look at this locus in relation to AD. What we did was to look at atopy in patients recruited on the basis of diagnostically indisputable AD.

From 95 nuclear families, 407 cases were collected on the basis of at least two first relatives

having active AD. Children below one year of age were excluded because of controversies with seborrheic eczema. On dividing the children into above/below 16 years, there were as expected more diseases in the latter group. Then we had to define what we meant by atopy and that is the first major hurdle. Do you define it on clinical grounds alone or on IgE responsiveness? It seems plausible for us at times to define it as IgE responsiveness so that we can compare our data directly with Cockson's regarding those with asthma. We use the same criteria: a raised total IgE, one or more specific serum IgE - or more than one in terms of RAST tests and one more prick tests. Using three markers at about 11 to 13, we were about to exclude linkage between atopy and the low level Q13. When we took the whole group of atopic patients, we made our study. At that time, it was in agreement with several other centres around the world, which contradicted the work of Cockson and his group. But when we looked at segregation in terms of parental phenotype, we found some very interesting data.

We found a weakly positive low score of 0.8 in the 19 available families in which the father and both parents were unaffected. Other combinations were significantly negative. These data show that we cannot exclude a potential maternal influence on the genetics in atopic disease at that locus. As you are aware, Cockson has reanalysed his data in terms of parental phenotype and found that it was related to maternal inheritance at that site. Other combinations had negative low scores, in keeping with our own data. It is hard to explain the reasoning of the genetics with maternal influence, but it is exciting that the beta subunit of high affine IgE receptors is located at 11Q13. Mutations at that site will now be investigated.

To sum up: it may well be that 11Q13 is important for atopy, but this generates numerous questions. Should we really be looking at IgE responsiveness in relation to AD, and in the future also at other candidate gene sites?

Discussion

(Mudde) In Cockson's asthmatic patients the beta subunit of the IgE receptor is involved with mast cells, whereas the Langerhans cells, monocytes in AD do not use these units, which may help to explain the difference in data.

(A) It is unclear whether the beta subunit has any effect on IgE control.

(Ring) Above 100 U, IgE may often occur in non-atopics.

(A) It depends on the reference rate of the laboratory, being less in children, but it is correct that a high IgE level is not specific for AD.

Yamamoto, K.:

As some of you may already know I come from the National Children's Hospital in Tokyo, Japan, and every day I see a tremendous number of patients with infantile eczema. Typically the patients are found not in the countryside but in the bigger towns. For young mothers the most problematic thing is to determine whether or not their children are suffering from AD. This has now become a nationwide problem. Recently Hanifin joined our annual congress and the so-called open seminars, showing just how serious the situation is in Japan. I always consider the reasons why the parents, particularly younger mothers, worry so much about AD and I always reach the same conclusions. The first is that younger mothers are reminded of AD or think about it, because their children may have had a mosquito bite and they started to think this was the beginning of AD.

Secondly young parents, particularly the young mothers always get too much advice from TV and magazines or the "How-to" books on how to raise their children. Sometimes when they are sitting with their children just in front of me they start to ask "Why do not you give my son periodic examinations or psychosomatic analysis to make sure there is nothing wrong?"

This is not a medical problem, but if you consider paediatric dermatology, a patient with infantile eczema may get the same sort of answer as other patients. As you know, eczema in infants and children is very frequent in infancy and AD often starts at the age of 6 months, but this does vary somewhat. A mother may say that her baby has irritation all over the face and buttocks, but nothing around the nose. This is very important, the answer to why in the infantile stage the patient may develop skin changes more frequently than the adults or in the elderly. Even in the very severe phase, there are no skin lesions around the nose, which are seborrheic areas. All over Europe it has been noticed that this is occurring. In severe, extensive cases of infantile eczema one may fear they will develop skin lesions all over the body, yet a clear zone exists around the napkin area, particularly if good napkins, disposable napkins, are used at this age. In the napkin area of the extensive severe infantile phase, for instance, we give much attention to the outermost surfaces of the str. corneum and we can detect the impaired skin by using scanning electronmicrospic technique.

Bonifazi, E.:
Aoki will now talk about the clinical course of infantile AD, including seasonal variations.

Aoki, T.:
I brought a small selection of clinical data today and this slide shows the number of patients we have seen in our hospital. Under the age of one year, the largest number of patients visiting were aged 4 months and in our hospital the younger children come in very large numbers. I am going to talk about the clinical course of these patients: they are checked at least every 2 months and are clinically examined and scored for IgE and RAST. We examined 47 patients whom we could follow for up to one year of age and split them into 3 groups. Before I talk about skin conditions in younger children we have to say that in our country we are very reluctant to corticosteroids. Most mothers do not want to use corticosteroids on their children. We only give antihistamines and sometimes local antibacterial agents, and that is all we are doing now. Therefore, the following is a natural course of AD: about 1/2 of the patients have the peak in their skin condition at 4 months, about 1/4 at the age of 6 months, and the last 1/4 of the patients at 8 or 10 months, or they have an indifferent change in clinical condition. So, this is one conclusion I would like to present today.

Because many mothers are so upset and keen to know the cause of this change when the skin condition of their children gets worse, it is important for dermatologists to be aware that there are certain patients who have some increase of their skin condition after 4 months of age.

The slide shows that IgE value and RAST score for egg white move in parallel with these skin conditions. As you know, some patients have a positive RAST score after 4 months, especially in positive milk and soybean RAST, so set out to examine if the positive RAST to these 2 allergens can give any indication about their prognosis. The results are: at the age of 6 months if the patient has cow's milk allergy and soybean allergy, they can have less worse prognosis at the age of 8 months but at the age of one year there was no difference at all. So these tests cannot give any information about the prognosis at the age of one year. This is a second conclusion.

The next two slides show a small detail which might reflect a part of the natural course of infantile AD. We studied the months of first visits for AD seen for 5 years. The age at the first patient visits was least between July and August. This tendency can be observed up to the age of one year, but not after 2 years of age. Both child and adults patients come rather frequently in the summer. In another study of the Osaka Medical Association, the subject number was very large, and about 1/2 of the AD school children has amelioration in the summer season. I do not know what kind of information this may give, but this change may be important to note in the natural course of childhood AD.

Discussion

(Hanifin): With regard to the change in the natural course with or without steroids; could their use influence the prevalence and the number of incidents?

(A) There are no differences; the other question cannot be answered.

(Q) Comparing Japanese and German conditions, there seems to be a larger number of incidents in Japan during the summer; is it due to the hot weather and the resultant sweating?

(A) It is very likely; and during the winter, dry skin plays an important role.

(Schultz Larsen) What about the frequency and course of seborrheic dermatitis developing into AD?

(A) According to the Japanese concept, there is no relationship. Oozing may be a characteristic of AD.

(Q) Do you know anything about the natural course; you implied that none of these patients had received corticosteroid therapy, or was it a mixture of those, and is there any change in the natural course if you do not use steroids?

(A) These patients are treated without steroids or with very limited quantities of steroid. If a steroid is given we cannot evaluate the skin condition, so they are excluded from the studies. About the final course, I do not think there are any differences between the steroid group and the other group.

Bonifazi, E.:

Most infants and children with AD are not affected by growth disorders, however, the most severe cases sometimes present such problems. Professor David, could you tell us which children with AD were at risk for these problems and which are the responsible factors?

David, T. J.:

First of all, I am a paediatrician not a dermatologist. We are interested in poor growth in relation to AD. This poor growth has received very little study, whereas poor growth in asthmatic children is well documented. In fact studies on children with AD that is severe enough to require regular attendance at hospital show that about one in 5 of such cases have short stature as defined by a corrected height centile less than 10th centile when corrected for mid-parental heights. However,

there is poor understanding of the cause of short stature in AD. One possible explanation for this short stature is coexisting asthma and it may be that the asthma. Another possibility is systematic absorption of topical steroids. A few children, who are very severely affected by atopic eczema are not treated with any forms of steroids (e.g. because parents refuse to use them), and clearly in that small group any short stature cannot be due to steroid therapy. Malabsorption is known to be associated with eczema in a few atypical cases, but in general this is unlike to be the cause of short stature. However, undernutrition as a result of an elimination diet is another possible cause of poor growth in some cases. Diets that are not supervised by a dietitian are a special risk factor. Loss of sleep, near universal in children with troublesome AD, is a further possible cause of poor growth. In addition, increased energy expenditure has been shown in children with AD who scratch a lot at night, and a mismatch between intake and increased caloric requirement may contribute to poor growth. It is possible that poor growth is a non-specific effect of chronic illness; any severe chronic illness can interfere with growth. Finally, constitutional growth delay is a disorder of childhood in which the child grows slowly until puberty, enters puberty late, and then catches up and reaches the expected height. This appears to explain much of the poor growth seen in children with asthma, and it may explain some cases of poor growth and AD. There is clearly a lot of research to be done and there is no doubt that poor growth is a problem in children with severe atopic eczema.

Discussion

(Remark) It was found that melatonin (and prolactin) is depressed in adults with AD, being connected with circadian rhythm, which can be disturbed by sleeplessness.

(Q) By measuring the oxygen intake it was found that scratching in sleep increases resting energy expenditure. Does transepidermal water loss influence growth due to the expended energy?

(A) We have no data on that issue.

(Thestrup-Pedersen) I have a comment on your factor on topical steroids. We have studied a group of 13 children with severe universal atopic eczema and applied steroids for 14 days, twice daily. What we observed was that there was no significant reduction of growth during the therapy period but during the subsequent 14 days they have to catch up growth, i. e., when the eczema abated, they started to grow more significantly. There are data showing that the catch-up growth occurs when the eczema improves. The data show growth loss in protracted AD. Water loss in babies may be calculated on the basis of size and we can then calculate the quantity of kilo-calories.

Businco, E.:

The early detection of prediction of the onset of AD has captured investigator's imagination over recent decades and a number of markers have been suggested for high risk newborns for the development of atopic diseases. Some of these have been used only for research purposes, but some of them, such as the detection of total IgE in cord blood, have been widely used even in clinical practice. We could demonstrate that babies who develop AD later in life had a significantly higher level of total IgE in cord blood. However, I would emphasize that there are several

drawbacks with the detection of total IgE in cord blood, which should be known in order to avoid unreliable results.

One of the most important points is the technique of the cord blood sampling. You know that cord blood can be obtained by aspiration or by gravitation, or the blood can be collected during the first 4 or 5 days of life by capillary collection. It has been shown that there are significant differences in total IgE levels according to the methods of blood sampling. One of the main reasons for these differences is contamination with the mother's blood, detected with IgE antibodies as we know that IgE antibodies are not normally present in cord blood. When they are detected, they usually come from the maternal blood and you can see that IgE antibodies were significantly higher when the cord blood was collected by gravity, in comparison with aspiration or when the blood was taken later on during the first days of life. This is just one example of how the technique of blood sampling can significantly interfere with the results.

The clinical efficiency of high cord blood IgE is related to the family history. If the baby has elevated cord blood IgE, I mean total IgE more than 0.8, you need to correlate this with the positive family history, so we can get the best predicted value.

According to recent studies, I have summarized the sensitivity and specificity and clinical efficiency of both positive family history and elevated cord blood IgE. Both elevated cord blood IgE and family history have a quite good specificity, whereas sensitivity is rather low. My feeling is that at present the detection of total IgE in cord blood cannot be used in clinical practice for the early prediction of the onset of AD. At present, a careful clinical anamnesis is the best way to predict the definition of classification of high-risk babies. Perhaps in the future we may have more reliable tests and we are doing a lot of work on the detection of certain cytokines in cord blood, which are involved in the IgE production. We were able to demonstrate that babies that later develop AD had a significantly less pronounced production of interferon-gamma in cord blood. In conclusion, I stress at the present time that a careful family history is the best way to predict the onset of AD.

Discussion

(Ring): What do you do practically when the parents ask you to assess the risk; do you measure IgE?

(A) No, we only do IgE for research purposes. I am still convinced that a positive family history - especially when both parents are affected or when one of the parents is affected by AD - is still the best way.

Subject: Clinics
Discussion Leader: G. Rajka (Norway)
Panelist: **E. Bonifazi** (Italy), **M. K. Kägi** (Switzerland), **H. Sugiura** (Japan), **C. F. Wahlgren** (Sweden), **G. Rajka** (Norway), **N. Uehara** (Japan), **B. Przybilla** (Germany)

Rajka, G.:
Is AD influenced by dominance of maternal heredity as Harper, Cockson and others have quoted, or are there quite separate aspects involved? Please elaborate on your findings, Professor Uehara.

Uehara, M.:
I have treated AD for more than 30 years, my oldest patients now have many children and some of them have atopic eczema. So I've decided to study the descendant family history of atopic eczema. The first slide shows the published data from last year. When my old patients married normal persons or AD persons or respiratory atopic persons, the prevalence of AD in their children was 56%, 81% and 59% respectively, so we concluded from this that the mode of inheritance of AD is autosomal dominant. Secondly, from this data we concluded that AD is genetically different from respiratory atopics. We studied the influence of paternal and maternal dermatitis and the prevalence of AD children. When the father alone had AD, or when the mother alone had AD, the prevalence of AD in their children was 53% and 60%. This difference was not significant, so my conclusion is that paternal AD and maternal AD have the same influence on the indication of AD children.

Discussion
(Rajka) From your data it is absolutely obvious but there are many authors in the literature who point out that the maternal side also has some influence; how can you explain this?

(A) Most previous studies performed are *ascendent* studies. My study focused on a descendent study.

(Rajka) Dr Kägi, the differentiation between exogenous and endogenous types of AD is only on an immunological basis but immune events may appear or fade away.In other words, is this a reliable classification?

Kägi, M. K.:
We have some new data that I would like to go through. The first thing I would like to emphasize is that patients with AD can be classified according to their total and specific IgE levels, their reactivity in immediate type - skin tests and the patient's history for allergic diseases. As you all know, the scheme drawn from Prof. Wüthrich has divided AD patients into two groups: group one with respiratory allergies and group two without. From these two groups we can further differentiate between a mixed type and an extrinsic type, the so-called pure type of AD, and an intrinsic type which lacks IgE antibodies. But if one thinks about this whole classification there is a big drawback with terminology: it is imprecise. For example, extrinsic irritants, as in asthma, may trigger both forms, but skin reaction due to irritants is by definition not IgE-mediated. So even in asthma there is no sense in using the terms extrinsic or intrinsic. That is why we were thinking about new terms and we came up with non-allergic versus allergic, but this has a major disadvantage, too, because so far there is no evidence that the allergic or extrinsic form of AD is caused by allergic type-I reactions. So we thought about atopic and non-atopic: patients with the

atopic form displayed the features of atopy that is clear; patients with the non-atopic form do not display the features of atopy, but still fulfil the diagnostic criteria for AD, according to the classification of Hanifin and Rajka. Clinically, these two types cannot be distinguished; generally there is no difference in the clinical course. The atopic form may be more sensitive to seasonal deterioration than non-atopic patients. Clinically they can be differentiated by their family history for atopy, patient's history of allergies, allergic conjunctivitis, asthma, food allergies and skin tests.

Here you have the positive tests in the atopic group, while in the non-atopic group these tests are negative; they can be differentiated also in the laboratory findings. In the first group you have levels above 200 KU of total IgE, positive specific IgE's, 23CD positivity, yet non-AD patients also have high levels of eosinophils, of ECP, of activated T cells, of soluble IL-2 receptors and high levels of IL-5 in peripheral blood, and also in skin biopsies as we showed in a recent publication. The prevalence of these two types is difficult to establish: to my knowledge, there are no really good epidemiological data on these two types. But we still looked at two groups, a group of 37 adult AD patients, and there were 24% non-atopics, while in a group of 40 children with AD there were 30% non-atopics. You can't differentiate these two types very clearly when you look at cytokines in biopsies taken from atopic or non-atopic patients. There is a significant difference in IL-4 levels. On an immunological basis, one can now draw a scheme and differentiate these two types very well into the atopic and non-atopic form with a central inflammatory cell the ersinophil, causing the dermatitis. In the atopic form the allergen has a relevant role, defined through the T helper cell and cytokines such as IL-3, IL-5 and GM-CSF. On the other hand, in the non-atopic form, we still do not know what is the role of the antigen here and how the antigen is presented.

Discussion

(Q) I think it is very interesting and it is an important step but as I understood it, it is perhaps more important for theoretical investigation purposes because, in practice, if you see a patient with AD it is perhaps not so important to say that it is exogenous or not exogenous or mixed?

(A) From a clinical point of view, it is impossible to differentiate these two types, at the moment, except if one performs skin tests and measures IgE: these are two relatively easily performed tests.

(Q) In order to end the confusion, how do you classify according to your data? Patients without IgE antibodies to common allergens and positive patch tests to mite?

(A) That is still an open question. This is a subgroup that needs to be looked at further. I think nobody has performed IL-4 measurements in skin biopsies in these patients, so it would be very nice to look at their cytokine profiles in patch test reactions. I assume that they will not have high levels of IL-4 there.

(Giannetti) It has actually been done and there is no difference between high levels of IL-4 and biopsy of both groups.

(Q) Did you do make IL-4 measurements in supernatants of a skin biopsy?

(A) Yes, you will hear about this tomorrow.

(Harper) Just very briefly, from a clinical perspective, I am very worried that, as a group of

people who are interested in AD, we do not segregate AD very clearly compartmentalized from atopic respiratory disease, because if you take individuals with eczema, who are non- symptomatic for asthma and you lung function tests and histamine provocation tests, then there are several reports that at least 70% of asymptomatic eczema patients have compromised lung function and there is clearly this broad overlap of these two diseases.

(Ring) I have one linguistic or philosophical objection as the disease is not quite dermatitis but AD. An a is missing and would you really ask us to call it non-atopic; I think we are used to extrinsic and intrinsic or allergic or non-allergic, but non-atopic is rather difficult for me.

(Q) For me it is a little surprising that you have a relatively high percentage of patients with a non-atopic form. Do these patients have IgE under 200 after 1/2 year, or a change in their behaviour; do we have any prospective data on that?

(A) Actually at the moment we do not have any prospective data on that, but we are now trying hard to keep these patients at our clinic and to follow them in their coming years.

Rajka, G.:
Prof. Bonifazi - I heard that you made an investigation into the role of aeroallergens in the course of AD in different subgroups.

Bonifazi, E.:
I believe that aeroallergens are important only in a few cases of AD, but not in most cases. On this topic I performed two investigations. In the first, I compared the levels of antibodies against dermatophagoides and grass pollens in three different groups, in different populations with AD. The first population improved in summer and is the most frequent population, of course. The second population worsened in summer and this population was represented by patients with photosensitivity or with dyshidrotic eczema. And the third population, the most severely affected population, was AD without changes in the summertime. We can see the numbers of subjects 23, 23 and 42, and the average age of the three populations. The level of IgE in the three populations is of course at its highest and you can see that there are no significant differences in the level of antibodies against dermatophagoides or grass pollen in these populations, so different clinically.

In the second experiment, I studied the influence of vaccination on AD. I studied only subjects with pure AD with no asthma but with associated antibodies against dermatophagoides. Seven subjects were vaccinated for 3 years; 4 subjects were not vaccinated. We followed for 3 years these two groups from clinical and biological points of view. You can see the average of age of the two groups, their incidence of asthma (no cases of asthma in either group), the mean IgE levels and mean IgE of dermatophagoides before vaccination. After 3 years in the vaccinated group there were no significant differences between the two groups related to the frequency or the clinical course of AD; AD was present at the same rate in both groups. Asthma was more frequent in the non-vaccinated group, IgE levels were higher in this group and even specific IgE antibodies against dermatophagoides. But I would like to underline that the clinical course of AD was not influenced by vaccination against dermatophagoides. This is why I do not believe that in most cases with AD, aeroallergens play a significant role.

Discussion

(Rajka) That is an interesting opinion, though perhaps not everybody agrees with it, but the data are very convincing. You have worked with patients 7-8 years of age and Kägi had perhaps some older patients, so that means that you are of the same opinion; that subgroups and course is not closely related.

You have a lot of material on children and you know that we have here the problem of juvenile plantar dermatosis which was also among the diagnostic classification criteria. What is the situation in the South of Italy as regards this form?

(A) Cases of juvenile AD are not frequent in my country or in my region. I very rarely diagnose this disease, maybe due to a warmer climate.

(Rajka) Perhaps it is not by chance that it was described by Zachariae and specifically in Scotland. Perhaps it means that the dryness of the skin is very important, as you do not see it in the South of Europe and we see it very much in Norway.

(Businco) Did you see any side effects related to the immune therapy, because our experience with immune therapy is that it is not so well tolerated by children with AD? Frequently children with AD experience a severe itch and worsening of the skin lesions during immune therapy.

(A) I was able to observe all these side effects of immune therapy in my cases.

(Ring) Talking about vaccinations, what is your attitude towards vaccination? I think this is an issue all over the world, paediatricians and dermatologists and others all have their own opinions. Do you recommend this vaccination in AD patients?

(A) I think it is not important in most cases. Usually my decision is not influenced by the AD but by other factors such as frequent infections and high levels of antibodies against dermatophagoides.

(Hanifin) Going back to your comment on dermatophagoides or aeroallergens playing a role, do you have high mite levels in your homes there and have you done any patch testing? As one who has long maintained that the IgE-related exposures may not be as important to AD as are cellular reactions, I would resolve this by looking at cellular responses.

(A) There were investigations but I did not perform a measurement of mites, nor did I perform patch tests with mites in those cases.

Rajka, G.:

I want to discuss itching, and first I will present very briefly my own views.

I want to make a distinction between itchy skin and spontaneous itch and their relation to therapeutics. Perhaps it is most interesting to discuss in relation to antihistamines, which is always a highly debated topic and with very differing results. As you know, each stimulus affects itch receptors and then they are led by C fibers and there are many mast cells, as Sugiura has shown, and even neuropeptides are liberated. Then the stimulus is led through the medulla going up to the cortex, where the perception of itch is raised and then the efferent reflex is scratching. If we have

the classical antihistamines they have a good competition against histamine, the chief itch elicitor and they can also dampen the cortex which can be quite useful in AD. The modern antihistamines offer even better, almost perfect, competition against histamine, but they do not have the other effect. They also show an effect on the late phase reaction and the late phase reaction is characterized by an eosinophilic infiltration.But in the real, eczematous inflammation, we have many other cells and it is the whole time itching because histamine and IL-2 are liberated. Here we have the so-called itchy skin which means that the itch threshold is very low and very weak physical or chemical stimuli can affect it. But against itchy skin (*Editor's remark:* a more appropriate term is skin hyperexcitability), antihistamine has no effect and what can reduce this eczematous inflammation, this itchy skin, with substances which reduce inflammation such as topical or systemic steroids, cyclosporine or even UV. From a therapeutic point of view one has to differentiate between spontaneous itch and itchy skin.

Wahlgren, C. F.:

The pathophysiology of itch in AD is still not understood, but three factors may be of importance: (a) peripheral pruritogenic inflammatory mediator(s), (b) "itch susceptibility", and, "itchy skin".

Peripheral pruritogenic inflammatory mediator(s) The most important cause of AD itch is probably one or more pruritogenic inflammatory mediators present in the skin. They are assumed to act on the free nerve endings of C-fibres in the dermoepidermal junction zone. In urticaria, the major pruritogen is histamine released from mast cells. In AD, the peripheral pruritogen(s) and its (their) cellular origin remain to be identified. The role of histamine as pruritogen in AD has long been debated. Arguments supporting histamine as a major pruritogen in AD are the increased histamine levels in skin and plasma, the increased numbers of mast cells in lichenified plaques and the increased basophil histamine releasability in patients with AD. On the other hand, there are several arguments against histamine as pruritogen in this disease. When it is injected intradermally, a visible wheal-and flare response occurs together with itch. The dose required to provoke whealing is lower than that needed to induce itch and, in addition, whealing is not a feature of AD. Furthermore, repeated intradermal injections of histamine induce tachyphylaxis, i.e. subsequent histamine injections will not induce any vascular response or itch. Therefore the individual lesions in urticaria are transient, whereas this is not the case in AD. Several controlled studies reveal that the antipruritic effect of antihistamines is not better than that of placebo, although recent studies suggest some antipruritic effect of certain new antihistamines. However, this effect may not be related to peripheral H1-receptor blocking but to some additional effect, such as "mast cell stabilisation" or inhibition of eosinophil recruitment. In conclusion, avaible data support the view that histamine is not the major pruritogen in AD.

"Itch susceptibility" Experimental studies suggest an increased "itch susceptibility" to certain pruritogens in AD patients. For example, a lowered itch threshold for trypsin and a prolonged itch duration for mucuna pruriens (cowhage) occur in lesional skin of AD patients compared with skin of healthy controls. Moreover, the itch duration for trypsin is significantly more often prolonged in involved and uninvolved skin of AD patients than in subjects with various other eczematous lesions or psoriasis. However, the "trypsin itch duration test" seems to be of no help in the differential diagnosis of AD.

"Itchy skin" The term "Itchy skin" was introduced by Bickford, who noticed that when he touched the skin peripheral to the site of an intradermal histamine injection, he perceived itch and not only touch. The phenomenon where mild mechanical stimulation of the skin provokes itch is called "itchy skin" or "alloknesis". Following an intradermal histamine injection, "itchy skin" appears within one minute, reaches its maximum at 10 minutes and then declines within the next

10 to 20 minutes. Bickford observed "itchy skin" in patients with eczema, infestations and insect bites. Whether it occurs (e.g. around eczema lesions) in AD patients remains to be thoroughly investigated, but if so, it may in part explain their intolerance to wool fibres. The mechanisms underlaying the "itchy skin" phenomenon are not understood, but it is assumed that both peripheral and central nervous system mechanism are involved.

Discussion

(Rajka) As I said, I do not think that antihistamine should protect AD essentially - only to a degree.

(Hanifin) Do you know anything about the alloknesis dose, what concentration is necessary? I assume it is less than the concentration of histamine necessary for induced itch.

(A) You have to produce itch, you have to have itch centrally, otherwise you can't produce the state of itchy skin and you need C fiber activity. You have to release a pure itch so you have to use a histamine dose large enough to induce C fiber activity.

(Hornstein) There are several controversial results about the threshold of the histamine of the induction of itch and most studies have been done by injecting histamine. If you use iontophoresis, then you come to reverse results. I think the method is also implemented in this way as if you did it intradermally then you reach deeper fibers as you do it. We always give the same dose during the same time, so it is an intra-inter-individual factor if you give them intradermally.

(A) Yes, I know that. In the study that Prof. Hornstein published in JID the itch response was even less in patients with AD. But in a study I've done we couldn't find any difference between healthy controls and AD patients.

(Aoki) When you say there are many factors that cause itching are you thinking of substances that cause itching directly, or substances that cause the skin to pick up itch more easily?

(A) We do not know anything about that. One can speculate about the presence of some substance sensitizing the C fibers to be more itch-susceptible.

(Ring) It is a clinical fact to all of us that patients with AD, when out in the cold, have cold skin and less itch. Based on this, we made a very simple experiment: we put an ice cube on the skin of normal persons. We left it on for 30 minutes, removed it, and waited 15 minutes for the skin temperature to normalize completely and then we injected histamine. It was surprising that you had the wheal-and-flare reaction but not any itchy feeling at all during the next 1½ hours. This indicates that perception of itch needs to be a continuous metabolic process.

(A) It is a very interesting phenomenon. I do not think anyone can explain why you do not perceive itch for such a long time after removing the ice cube.

(Ring) I will try to explain, as we have been doing some preliminary studies and they tell us that it is the C fiber that is responsible for the itch and also the pain, but the stimulus more suitable to abolish itch is cold.

(A) It is well known that cold temperature relieves itching but I thought it was due to a lower C fiber activity.

(Hanifin) I have an acquaintance who is a neurophysiologist with severe AD and he has made a number of studies in both cold and hot weather. Our patients discovered that the scalding hot baths gave the most relief. There is something about temperature that does affect those fibers.

(A) I believe that if you take a hot bath, you induce pain. If it is hot enough to induce pain, then the pain relieves itching.

Rajka, G.:
Dr Sugiura, you have a report on facial irritation in a Japanese dermatological journal. Perhaps not everybody read it, so maybe you can tell us your findings?

Sugiura, H.:
We have found adult AD with facial localization, totally covered by erythema, whereas other parts of the body showed only mild lesions. The patients have applied mild steroids to the face for many years. The itchiness is variable. Biopsies from 21 patients showed eczematous changes; in others, rosaceous changes were seen, while some patients had signs of both. Thus the facial erythematous changes are not uniform, but show a mixture of two mechanisms.

(Thestrup-Pedersen) Have you performed patch tests with steroid compounds to see if the patients developed contact allergy?

(A) Yes, in about 17% of patients we had a contact allergy to topical corticosteroid. Some of the patients had photocontact allergy to a corticosteroid or a non-corticosteroid agent.

(Færgemann) I think that some of the patients may have what we are to discuss this afternoon: head and neck dermatitis. Have you looked at prick tests and so on in these patients? Has it been positive or negative against pityrosporon?

(A) The results are not uniform and vary widely from patient to patient.

(Hornstein) In many patients who have long been treated with corticosteroid, there is increased sensitivity to the sun and I think you should discriminate between photoallergy and non-allergic sun sensitivity.

Przybilla, B.:
We have compared three groups: one with atopic eczema, and the other group with only atopic respiratory allergy, i. e. asthma and hayfever, particularly, while controls had no indication of atopic disease or an atopic state. We looked for Morgan sign, facial pallor, low hair line, hyperlinearity of the palms and soles, dry skin. The main finding was that stigmata were present in both groups of atopic disorders with a slight tendency for white dermographism and dry skin to be more prevalent in patients with atopic eczema. This would mean that the atopic state is the basis for both manifestations in the skin and in the respiratory tract.

Discussion

(Rajka) Are there consequences of this study by the Diepgen-Svensson project that are perhaps not specific for AD and not specific enough of the differential diagnostic point of view?

(A) I think it is very important to look for the rate of expression of his stigmata. For a number of these signs, we found in the control group a very high prevalence, especially when we looked only for low-grade expression. In the patients with disease high expression was more prevalent.

(Diepgen) I think one problem is the subjectivity of those criteria; you have to define them exactly. Then I think it can help if you are well trained to detect there signs. We have tried this with different persons and I think you can also make a good diagnosis without these criteria but they are helpful in ambiguous forms. For experienced dermatologists I think it is absolutely necessary to know these signs and to look for them, but it is impossible for general practitioners.

(A) I think we have to differentiate between the very simple things and the sophisticated and we should be proud to be dermatologist and know a bit more than others. I think too little research has been done and this is just the beginning and we should quantify everything.

Ring, J.:

One last word to conclude this morning. This is a big adventure for all of us. There is not really an audience here; we are all experts in this forum. The common denominator for our being here is that we are friends of Georg Rajka and there is no other reason for beiing present here. This time he really gave us a challenge and especially for the discussion leaders to really bring 5 or 6 different people and to show only 3 or 5 slides and only talk 5 minutes and then allow a long discussion. I think this worked out pretty well. Because we know each other and we know our papers, we do not have to give long lectures. Everybody knows what the other one has done. We should discuss what is out in the open.

We have clarified the genetic and environmental influences and we know a little bit more than 5 or 6 years ago, but we still do not know enough and there is much research to be done. In the clinical sessions, you see that some entities can be worked out. The problem of itch is as fascinating as ever and when you talk about the cold for instance, this is the basis of our whole therapy - to produce cold. I think with the new Kägi classification, there will be a lot of discussion.

Subject: The Role of Food

Discussion Leader: **A. P. Oranje** (The Netherlands)

Panelists: **T. Langeland** (Norway), **A. P. Oranje, B. Przybilla** (Germany), **T. J. David** (England), **L. Businco** (Italy)

Oranje, A. P.:

The role of food in AD is not an easy issue. The main question for us is: does food play a role in the etiology of AD and to what extent.

One should remember that AD is a multifactorial skin disease in which genetic factors are very important. There are several factors that influence the course of AD and several factors can induce or aggravate the condition. Food is one. The therapy consists of a diet. Food allergy is especially prevalent in young children with AD, according to my own data about 25% of all small children. In older children, this proportion is less and in adults it is even smaller. The reverse is true of food intolerance. It depends on the part of the organism that is involved. When the skin is involved, then the main symptom is urticaria. Then how do we explain that AD develops from urticaria to dermatitis? One explanation is shown here and it is by scratching (that is an old theory), then dermatitis will develop.I should also mention the other symptoms of skin-related symptoms of food allergy which are angioedema, contact urticaria, food refusal, oral allergy syndrome, diarrhaea, and vomiting. They are the most common skin-related symptoms. The allergens involved in food allergy are above all cow's milk, egg and peanuts. More than 80% of all the food allergic reactions are caused by these products. I will give you an explanation of how dermatitis develops from urticaria, by scratching, but there is also another explanation and it is shown here.

We think that the food allergen can penetrate from the blood vessels, from the gut to the blood vessels of the dermis and can also penetrate the skin from outside, there is a possibility to react with the mast cell. The mast cell will explode, releasing histamine, leading to an urticarial reaction. The mast cell will also release other mediators that will attract the eosinophil that will be destroyed in the dermis and released as ECP and MBP. Thereafter, a cellular reaction occurs, with predominantly TH2 cells interacting with the Langerhans cells and so we can explain how AD develops.

As regards our children, the 25% of the young children with AD, we give them a diet that has an effect in about 40% of these children. So half of these children will react, their AD will be improved by regulating their diet. I should also mention the contact urticaria syndrome in AD. This is under-reported but, in my opinion, is common in AD children aged 0-4 years with food allergy. We have just completed a study and found that about half of the children with food allergies have immune-mediated contact urticaria. Many of them also have very irritant reactions, prevalent in young children in around 10% of the cases, direct skin reactions being potentially dangerous. We have developed a test system we call the SAFT. It is an imitation of the symptoms and can always be performed if the skin is the target organ, as in AD patients. It is our hypothesis that this skin provocation test works better than a prick test or scratch. However, a final proof will be obtained by oral provocation

Langeland, T.:

I would like to state that a prick test is not required for the diagnosis of food allergy, but it may be useful in some cases. It should be remembered that the test demonstates an immunological phenomenon (indirectly), and because the clinical reaction depends upon a number of other factors

also, a complete correlation between the test result and the clinical reaction cannot be expected. Of course, this will also be true for other tests, such as RASTs and histamine release.

To use the prick tests in a proper way we should know how this test functions. Studies dealing with this indicate that even for the best allergen (hen's egg white) the correlation between a positive test and a clinical reaction by provocation will not be better than 60%. However, a negative test will give a good correlation with a negative provocation test.

Subsequently, to do a test when the history shows that the patient is tolerant is meaningless. Testing with allergens which the patient obviously is allergy to should also be avoided. The testing should be reserved for those cases in whom there is doubt about allergy.

Another important point is that the allergen preparation is of adequate quality, which means that it contains the relevant allergenic proteins and not irritants which will interfere with the test result. The commercial preparations used for testing of food allergy have inadequate documentation, and testing should therefore be limited to a few allergens that we know at least from our own experience. At least we should have experienced that patients with known allergy to the allergen are positive in the test, and that most patients tolerating the allergen are negative. If this is not true, it is meaningless to use the allergen preparation at all.

The golden standard in the diagnosis of food allergy is double blind placebo controlled provocation. However, the method has some problems: It is time-consuming and expensive. Another important problem is if it is possible to give the patient sufficient amounts of allergen by this method. In weak allergies, which are not detectable after one single provocation with minute amounts of allergen, this method may fail. In clinical practice, open provocations carried out repeatedly may therefore be a good alternative.

Discussion

(Hanifin) For about 10 years, based on the good scientific approach that Sampson used, I've tried to look at these patient and keep an open mind. Last year I was asked by paediatric dermatologists to give a talk at the American Academy of Dermatology and I was struck by how rare it is that dermatologists, even paediatric dermatologists, are able to find AD patients in the USA who are clearly food allergic. The conclusion I drew was that it is probably the parents who discover this food allergy, but dermatologists hardly ever see it because the patients won't bother to come in for something they already know they have. Is that the experience you have here?

(A) We seldom find a new allergy in a patient and tell him to eliminate the food. Quite the contrary; we try to convince the patient that he is not allergic to those food items being eliminated, so that he can reintroduce some of them. I am convinced that food plays a role in patients with AD, though we seldom discover it.

Przybilla, B.:

I would prefer the term food ingredients of low molecular weight. We are testing particularly food colorings and preservatives such as sulfides, benzoid acid, etc. in patients with atopic eczema not responsive to the usual anti-inflammatory treatments. In 46 patients with moderate to severe diseases there was at least one challenge reaction to food ingredients of low molecular weight in about 40%. Also, 50% reacted to challenges with a series of genuine food, and only about 30% had no reaction to these tests. Mostly, these reactions are flare-up of pre-existing eczema, occurrence of new lesions, or generalized itching. These reactions seem to be of some relevance to the disease.

How do we perform these tests? First the history is taken, then skin prick tests are performed with food and food ingredients of low molecular weight; with the latter, also patch testing is done. Also, specific IgE antibodies to food are determined. Before an oral challenge, the patients must avoid the substances to be tested for at least one week. Testing is performed in the hospital according to a protocol, giving one or two substances each day. We do testing for hypersensitivity to food ingredients of low molecular weight in a single-blind fashion. The questions is whether reactions are reproducible and relevant. I believe, mostly they are. For example, there was a patient who reacted to a mixture of food colorings, and did so also when re-tested later on. This patient had a positive patch test reaction to sunset yellow, one of the ingredients of the mixture of food colorings. Upon challenge with sunset yellow, she again had a reaction. What is the mechanism of these reactions? In this patient I would suppose it is a haematogeneous contact dermatitis and not a true atopic eczema, although she had presented with a definite picture of atopic eczema. In other patients reacting to food ingredients of low molecular weight, the pathomechanism has to be considered as yet unknown; allergic or pseudo-allergic mechanisms may be discussed.

Another question is if a patient who reacts to *one* food ingredient of low molecular weight must avoid *all* of them. In most patients there are reactions to only one or few of the substances, and the patients need to avoid only these. However, if the patient reacts to many of these compounds it would be better to avoid all of them as far as possible.

Discussion

(Giannetti) You suggested that you prick test your patients with the additives. Could you give more technical details? The second question concerns the amount of additive that you administered orally.

(A) Skin tests with additives are mostly negative, but in some cases - you have seen an example with sunset yellow - reactions occur. Concentrations for the patch tests can be found in the literature. For prick tests we use rather high concentrations and have found a few reactions, particularly to benzoic acid. We have also seen late reactions, developing not immediately but after about 6 to 8 hours after prick testing. The amounts of food ingredients of low molecular weight given orally are in the range that we also use for testing in urticaria, and they do not exceed the maximum acceptable daily intake doses. Such exposure, especially in children, is near to that what will occur in daily life.

(Thestrup-Pedersen) Have you in some of these patients used a systemic provocation orally?

(A) Yes, this was all provocation testing.

(Thestrup-Pedersen) Have you re-tested some of those who had severe AD during a period when they had very little eczema?

(A) We have done this just recently and it is very interesting to see that only some of them reacted again. The initial test was usually done when the patient had severe eczema and had been treated in the hospital. It may be that these patients or many of these patients only react to these additives immediately after a severe disease. I think this is very interesting but after one year we could find also some of these reactions again.

(Comment) There is an interesting study from Finland (Kalimo), where they performed both skin and intestine biopsy and followed the mast cells. If the patient had severe eczema, they had a decreasing number of mast cells in the intestine. This could mean that if you have a severe disease, you have a changed membrane physiology in your mast cells.

(Q) In how many patients with food intolerance and atopic eczema do you see a benefit of dieting?

(A) We have followed only a few patients, but in those taking an elimination diet free of additives, we found in all provocation tests that about 50 to 60% seem to benefit. But one has to be cautious as many patients learn in the hospital how to treat their disease more effectively and this may also contradict the results.

David, T. J.:
As a result of recent reports in British newspapers of fatal food-induced anaphylaxis, mainly cases of peanuts allergy, there is now greatly enhanced general anxiety amongst the parents of children with food allergy. Thanks to the campaigning of some "adrenaline missionaries", one consequence has been the increasing supply of preloaded adrenaline syringes. The result is that in England there is considerable anxiety about anaphylaxis and many children are being prescribed syringes preloaded with adrenaline.

I will briefly list the arguments against the over-liberal use of adrenaline syringes doing this. Either one supplies adrenaline syringes to every single child who has had any history of an adverse reaction or one is selective. In Manchester, we would recommend that one is selective for the following reasons. Firstly, there is no proof that adrenaline saves lives, and some evidence to the contrary. Whilst it is clear that adrenaline is effective in nonlife threatening reactions, there is real doubt about its value in the more severe cases. Studies of anaphylaxis under controlled conditions failed to demonstrate efficacy of adrenaline. Secondly, adrenaline syringes are potentially invasive and restrictive, the child being unable to go anywhere to play without taking the syringe and the parents ensuring that there is a fully trained adult at hand who can both judge the need for injection and have the courage to plunge a needle into someone else's child. Thirdly, some parents are reassured by having adrenaline available, but for many the possession of such equipment is associated with enhanced rather than reduced anxiety. For some, fear of the possibility of having to give an injection may be greater than fear of anaphylaxis itself. Having a pre-loaded syringe is regarded by some parents as a substitute for taking care to avoid specific foods, and this may result in an increased overall danger to the child. There is a theoretical hazard of inadvertent intravenous injection, with the risk of arrhythmia and death. Also the need to eject surplus adrenaline before administration means that there is a risk of overdosage, with the possibility of arrythmia and death. In addition, the use of adrenaline (e.g. searching for syringe) may delay calling an ambulance and obtaining life-saving medical treatment.

There is a concern that in some some children who get anaphylaxis, in fact their major feature is bronchial constriction, in which case adrenaline is not the best drug to give. The final worry that we have which may be just a British worry, is that supply of pre-loaded syringes with adrenaline has been associated with exclusion from school in some cases. Some schools in the UK have said that if the threat to life is so great then they are not prepared to take the responsibility for the child and exclusion from school has resulted. In some bizarre cases, the parents requested home adrenaline for the treatment of mild allergic reactions with the specific intention of having the child excluded from school. The parents were members of "Education Otherwise", an organisation to support families who wish to practise homebased education as an alternative to schooling. In

short, to summarize, it is clear that preloaded adrenaline syringes are appropriate for children who have had a life-threatening reaction to food but there are a number of drawbacks that would make one hesitate giving preloaded adrenaline syringes to children who have had milder reactions.

Discussion

(Ring) I totally agree with you and you may know that in America there is a doctor called Frasier, who is a missionary walking around and wants to supply the whole USA with adrenalin self-injector and he has sent letters to all of the Allergy Journals and now he is obviously in Great Britain. Our philosophy is that we do this only in cases of life-threatening allergy. Of course the parents then ask what can you do. We will prescribe some emergency treatment, this normally contains an aerosol-inhaler, and even more rarely, we prescribe an injectable.

(Harper) How is it to give an aerosol of adrenalin in a life-threatening situation? The difficulty is that it is not practical in that situation.

(A) There are problems. There are inhalers that have adrenalin in them and one can use them. There are a number of worries. One is the point that Harper made, which is that children certainly are not very good at using inhalers, in fact the medihaler that is available can be attached to a volumatic inhalation device which makes it a little bit easier. There are worries about inhaled adrenalin being metabolized in the lung and you certainly need to give very large doses, something like 20 puffs or more to be of any benefit. The general recommendation is that they are of doubtful value, they may also give somebody false confidence.

(Ring) What do you do in these instances?

(A) We do not use adrenalin inhalers. We either decide to give preloaded adrenalin syringes for a child who has had a life-threatening reaction, or we do not supply those which applies to most cases and we do give or recommend that parents have a quick-acting, non-sedating antihistamine if they want. But we warn them that if they are worried about a serious reaction, they must get medical help.

(Q) We would like to know your opinion about the food challenges in children and adults with AD. So far we have been doing such challenges in the last 5 years and we have seen some reactions - even systemic reaction - with respiratory symptoms. But in most cases we have not been obliged to use adrenalin just salbutamol, sometimes for asthma and sometimes steroids.

(A) The answer briefly is that until you have had your own patients dying this way, there is an element of disbelief that it is a problem. Once you have lost a few children, you concentrate the mind wonderfully, I can tell you. We would advise extreme caution and certainly we would be very worried about a challenge in which there was a history of a severe reaction. Secondly, when we advise people to reintroduce food items, we suggest that they use a very small amount the first time about the size of a speck of dust. In Britain it is recommended that these are done in hospital because it is well known that there is a real risk of occurring in a baby who had not previously had a severe reaction.

(Q) In setting up AD without overt previous reactions to food, which is most of all the patients

we see, the parents do not detect any reaction to foods. I think this is one of the reasons why most dermatologists believe it does not exist.

(A) The risk as far as atopic eczema is concerned seems to come from withdrawing the food and then reintroducting it to a child.

Langeland, T.:
The management of food allergy is elimination. It is important that the child is given adequate elimination. This is especially important if milk is eliminated. Cooperation with a dietician is important. In questions about weak allergies, I think it is useful to differentiate between food eaten every day (cow's milk) and food eaten more infrequently, for instance once a week. If a weak reaction is suspected to food eaten once a week, I don't think it is nescessary to elimate this food. On the other hand, a weak reaction to a food item eaten every day may probably add to a clinical significant reaction. In such cases we have to go further to clarify the diagnosis by doing provocations and eliminations alternatively during periods of one or two week.

Discussion
(Færgemann) I think that in AD, patients experience allergic reactions to food, but does food really aggravate AD? I think this a completely different point. As long as we do not have any good double blind study, it is difficult to accommodate this.

(A) I have not done that double blind study, but I do think that food can aggravate AD. We have these patch tests with egg white and it is not only urticarial; if you give egg white to an egg sensitive person it may cause eczema after some time, e.g. 48 hours. I do think that this is not necessarily through urticaria, though it may contribute to the dermatitis.

(Q) The study by Ferguson concerned the course of atopic eczema. In the future it will depend on the number of solid foods featured in the first 6 months. Are there comparable studies or experiences here in the audience?

(A) They have a higher odds ratio if they give the children more than four different solid food item in the first 6 months depending on the number of foods they are fed - as eggs, cereals, etc.

Businco
Several recent studies have been performed in high-risk babies who have been followed up over the first 5 years of their life. These studies clearly show that the selected *weaning* during their life of high-risk babies has a preventive effect on the development of AD. There is no doubt that the type of feeding and mainly the type of weaning, the variety of the diet that the baby receives in life, are risk factors for AD. That has been shown by the Ferguson study, but it has also been confirmed by several well known studies on high-risk babies. I think there is no doubt that the introduction of solid food item is a risk factor for eczema, at least in the high-risk population.

We have recently done a study on Somali children. One of my colleagues, who is from Somalia, went there and did a study on the epidemiology of AD in children who were born and live in Somalia. Practically speaking, AD was unknown in such a population. However, the same study was done in Rome in Somalian children, born of Somalian immigrants. The prevalence of AD in this population was the same as in Italian children. The type of feeding of such immigrant children in Italy was similar to that in the Italian children studied. Somalian children living in

Somalia exclusively received breast milk during the first year of life, and eat no solids before the age of one year. That may be another point in favour of the importance of the type of weaning for the onset of the disease.

Discussion

(Færgemann) By moving from Somalia to Italy, you change not only the diet but also change the climate and a lot of other things that may be very important for eczema.

(Businco) It was really surprising to see that no Somalian children living in Somalia received any food other than breast milk before the age of one year. On the other hand it was astonishing to see that the majority of Somalian children born in Italy received a large variety of solids before the age of 4 months.

(Hanifin) One of the better perspective studies is (by Sager) concerns restricting milk, eggs and peanuts during the first 6 months of life. But then those differences, which were clearly significant at 12 months, disappeared in 24 months. I wonder if you have a comment on that? I'd also like to ask my earlier question: why are there such extremes? For example, at one of your meetings in Italy with paediatric dermatologists, paediatric allergists and paediatricians, they seemed to see a very close association between food allergy and AD, yet, I think you said that 25% of AD children have food allergies. Why do dermatologists never see it?

(Businco) Of course I know very well the study started by Sager and the recently published, follow-up at the fourth year of this group of children. There was a significantly less prevalent food allergy at the last follow-up for skin diseases and other manifestations of food allergy, but there was not any difference in respiratory allergy, which was the main point. Why is there such a discrepancy between paediatric allergists and dermatologists on the prevalence of food allergy? My impression is that paediatric allergists see a different population of children with AD because at least in my country, when a baby has AD and is under one year of age, usually paediatricians send the baby to a clinic of paediatric allergy. In this particular population, food allergy is more common and usually paediatricians and family doctors refer the patient to the dermatologist when the child is older.

(Oranje) I agree on the point about the population. I think my population is a biased selected group, too, but there are some figures, about 5 to 10% in a general population of young children with AD. Most children in The Netherlands, for example, are seen by paediatricians but not by dermatologists.

(Harper) I agree with that. I think that, certainly in Britain large numbers of children with moderately severe eczema would see their primary care physician or paediatrician who is perhaps a little more sympathetic to the possibility of food allergy than dermatologists. As well as the issue of recognition, one of the things that I have found quite interesting in recent years is that by the time they are brought me, most of these babies with moderately severe eczema are already on a diet and the majority have been put on soy milk. What is interesting in Britain is the increasingly recognized allergy to soy milk.

(Ring) One different factor may be the age. For very young babies, who are increasingly seen by the paediatrician, food probably plays a greater role that later. This explains why after one or two

years all the differences disappear. As Oranje has shown us, even at the end of the year or so, the prognosis was similar and this is quite natural, but it does not mean that allergy does not play a role, as the other allergies manifest then themselves. One should consider not only food, and food allergy, but many other things in the environment which we have to take a look at.

(Mudde) I would like to make a comment about the possible reason why food allergens early in life cause AD. I think it is a well-known secret among immunologists that if you elicit a primary immune response then you get IgE antibodies for a very short while, whereas if you choose a primary immune response in newborn animals, you will get an IgE response which persists for a very long period. So this is an indication in newborn infants who have not developed their immune system; and you give them food they are not suppose to eat, such as solid food as our Italian colleague demonstrated with the Somalia example. Otherwise what you get is IgE production for a very long time. I think that the fact that induces an IgE response very early in life is the cause of all the problems. Maybe I am exaggerating a little because I am biased, but I think that is where the crux lies.

Harper, J.:
We have to bring this session to a close. Not unexpectedly, this session has perhaps provoked more questions than have been answered. I would like to thank the speakers for their contributions.

Subject: The Role of Mites and Pityrosporum
Discussion Leader: **J. Færgemann** (Sweden)
Panelists: **A. Broberg** (Sweden), **L. Businco** (Italy), **Y. Tanaka** (Japan), **K. Thestrup-Pedersen** (Denmark)

Færgemann, J.:
This last afternoon section is logically divided into two parts. What I have a special interest in and what I've been working with is the Pityrosporon yeasts. We will also be talking about dust mites. So it will logically be divided into 2 parts and first we will start with the pityrosporon. I have distributed some handouts where you have a review of the program. We start with Dr Broberg's lecture and have time for discussion.

First I will try to start with the *Pityrosporum* story, and what is the role of *Pityrosporum* in AD. First, what is pityrosporon? It is a lipophil yeast, a member of the normal human cutaneous flora, at least in adults. It is especially found in the scalp, face and the upper trunk. It is not only a saprophyte but it is also associated with several diseases. What we will discuss today is its role in some forms of AD that are localized to the head and neck. The patients are typically adults. They have a lot of eczema, a lot of itch localized on the face, neck, upper trunk and even the scalp. The majority are not men, they are women - we do not know why. When you prick test these patients and compare them with patients with a more ordinary AD, you find a difference in the test results. This prick test study, which we did together with Copenhagen, showed that patients with AD localized to the head-neck-scalp were prick-test positive in 78% compared with 44% in patients with a more ordinary distribution of AD. Patients with seborrhoeic dermatitis were negative and only one healthy control was positive. Back in 1958 there was a paper indicating that one may be allergic to human dander or to scalp dandruff. Otherwise it was not until the early 1980s that this issue was brought up again. The question was "why are some patients with a lot of eczema in the scalp, head and neck areas so difficult to treat?" It was pointed out by Hjorth that they might be allergic to the *Pityrosporum*. So he prick-tested them. He cultured the *Pityrosporum* and in a double-blind fashion he treated them orally. He gave 14 patients 200 mg for 4 weeks, and 10 the of 14 improved. This was a double-blind crossover study and in fact, until today, it has been the only well-conducted double-blind study on this issue. Therefore, I would like to start now by asking Broberg if she believes in this study and if she has undertaken any other studies?

Broberg, A.:
In order for us to evaluate the effect of topical antimycotic treatment we studied patients with AD located mainly to the head and neck area. Most of the patients also had eczema on other parts of the body. During spring 1993 and until March 1994 we included 60 consecutive patients from the outpatient clinic at the Department of Dermatology in Göteborg. The study was double-blind, randomized, controlled. In addition to oral antibiotic treatment, patients in group A were given miconazole-hydrocortisone cream and ketoconazole shampoo, wheras patients in group B were given hydrocortisone cream and placebo shampoo. The study lasted 6 weeks, with return visits after 4 and 6 weeks. At the start of the study, culture for *Pityrosporum ovale* was performed. Cultures were repeated after 4 and 6 weeks. We also performed a skin prick test with a water-soluble extract of *P. ovale*. For assessment of eczema we used the SCORAD index. The assessment was performed by the same investigator each time. SCORAD index for extent was modified due to the design of the study, as we only studied the head and neck area. Instead of sleep loss we had a VAS scale called "overall condition".

Of the included 60 patients we had to exclude 7 for different reasons in the final evaluation.

In group A, we evaluated 26 patients and in group B 27. There was no difference between groups according to: sex, other atopic manifestations, result of *P. ovale* culture or SPT. At the start of the study *P. ovale* cultures were positive in 83% of all patients. After 4 weeks of treatment, there was a significant decrease in colonisation in group A but not in group B. The eczema score did not differ between the groups from study start and the decrease in eczema score did not differ between the two groups after 4 and 6 weeks. I want to conclude that in our study we found that although *P. ovale* colonisation decreased significantly in the group given antimycotic treatment no difference in decrease of eczema score between the two groups was found.

Discussion

(Hanifin) Do you ever see increasing numbers of *Pityrosporum* in steroid-treated patients? It looked as through after 4 weeks you had fewer patients with *P. ovale* in the steroid-treated group. I do not know if it was significant or not. If that were the case, would that not contradict the role of *Pityrosporum*?

(A) The significant difference between the culture was that group A had decreased cultures. So the increasing quantity was not significant. This study does not support the role of *Pityrosporum*. You can always speculate that if the *Pityrosporum* crossed-reacted with *Candida*, you cannot reach the focus of *Candida* in the gastrointestinal tract without systemic treatment. There is another aspect with the seborrheic dermatitis which Færgemann studied. You can speculate that you can have a prophylactic effect if you keep the *Pityrosporum* number low, but in the design of this study we could not show any effect.

(Færgemann) In fact we have wanted to do that if enough patients had been free of lesions after 4 weeks. If you stop treatment what happens to the patients treated with a combination of anti-*Pityrosporum* treatment, compared with the one treated with hydrocortisone? Unfortunately, we were not able to do that. There may be other answers to the question: do we reach all *Pityrosporum* with topical treatment, do we really reach *Pityrosporum* deep down in the follicle? I am not so certain about that. So if oral antifungal kills even the *Pityrosporon* present down there, I do not think we can achieve that. The culture is just a contact plate taking surface *Pityrosporon*. In fact, there was a difference between the scores, it was over 30 in group A, but was lower in Group B, about 22 (23 in the first group), not a statistically significant difference, but it might have been.

(Øhman): Did you only look at those with a positive skin prick test?

(A) Yes we did.

(Q) Did you see any difference?

(A) No, we also looked at those patients in Group A with the active treatment whose *Pityrosporum* culture turned negative, but they didn't do better than the other patients. When you do such treatment of course you decrease the number of patients, so the group will be smaller.

(Friedmann) You answered half my question in your comments about having hydrocortisone in both groups, but I was also rather surprised and sort of disappointed to see that there wasn't such an effect. One hoped that there would be an effect and I just wondered if you discussed all the

variables. You do not know whether it is the number of organisms that is important, or whether you are getting rid of them. Is there any way that you could narrow down on the clinical groups further? Is there any other estimation of who might be allergic to these organisms and who might not as everybody has the organism, as you've shown, and certainly the treatment will reduce the numbers. It is obviously something else.

(Færgemann) That is true. Maybe the treatment should have been for a longer time. As Broberg pointed out, maybe the patient will be free of the disease for a longer time compared to patients treated with only hydrocortisone. That poses a second issue, in fact, of what we are interested in. We will hopefully continue to look at that because one thing is to treat the patient but another point, especially in AD, is to keep them free of the disease for a longer time.

(A) In the study by Hjorth the patients were selected differently. All these patients were positive in SPT to *P. ovale*. As I have understood, if their patients had only eczema involving the head and neck area. Our patients had eczema on other parts of the body. This difference may mean that we have selected different subgroups of AD.

(Hornstein) I was also disappointed but I will try to give an explanation. In my opinion *Pityrosporum* is not a causative agent, it is an indicator of disturbed immunity. You have the same problem with the HIV person, they very often have seborrheic eczema. So, like *Candida*, *Pityrosporon* may be an indicator of the disturbed immunity. Your cream helps against microorganisms but it does not help against the disturbed immunity.

(A) Like in food allergies, we have a lot of positive skin prick tests and RAST positivity without any clinical significance, so this might also be the case.

(Q) It could be that this positive test is just a manifestation of atopy. They have skin with an impaired barrier and an antigen, that is the reason why they are positive. It is disappointing and a little unexpected that the allergen being applied to the skin is not causing any reaction. My question is, have you tried to do a patch test not with the extract but with the fungus itself?

(A) We have done a study published in JAAD but we were not successful. Tagami and coworkers from Japan succeeded in doing positive patch tests when they did the scratch patch test; where they scratch the skin first, they have a higher number of positive patch tests.

(Comment) I want to comment on the question whether *Pityrosporum* is an epiphenomenon or maybe an allergen. We made *in vitro* experiments with *Pityrosporum* extracts and we found only in patients having specific IgE to *Pityrosporum* which was measured in RAST test, an increase of IgE *in vitro* and only these patients showed increased proliferation and an increase of IL-2 receptor. These data indicate that there is a specific response to *Pityrosporum*, maybe an allergen from this yeast that was not found in patients with seborrheic dermatitis.

Færgemann, J.:
I think it is right that in immuno-compromised patients *Pityrosporum* may play a role. Of course it is not the etiologic agent of AD but in some patients I think it may be important. I know one study by Beck that has not been published, but I've heard about it: in an open study patients were

treated with ketokonazol capsules. He looked at specific IgE and the patients were getting better and the IgE was going down below during the treatment studied. What I would like to do in the future is to do a well-conducted study with systemic drugs, again with topical comparative studies. I do not think we have the final answer to these important questions yet.

I would like to close the *Pityrosporum* section and move on to the mites. I would like to introduce Prof. Businco from Rome who will talk about the impact of house dust mites on AD in children.

Businco, L.:

The aim of our study was to investigate the patch test response to an extract of therapy, that is the main allergen in *house dust mite* in children with AD and their parents. This study has mainly been done by one of my co-workers. As you know, such studies have already been done by other investigators, however, as paediatricians, we were interested in examining even the parents. According to our knowledge, that was never done before. We enrolled 79 children in the study who were divided into Group 1 (31 children with AD at the moment of enrollment) and 23 children who had previous AD but they didn't show any skin lesions at the enrollment as well as 25 children with a respiratory allergy with a skin test-positive RAST to house dust mite, 149 parents and 20 healthy children as a control group for the children and 11 healthy adults for a control group. All the subjects were prick skin-tested with Der. P. total IgE and specific allergy to the house dust mite measured. The patch test was done with the faecal extract, which contains 60 biological units for Der. P. The patch test was done with and without previous scratching. A score of more than 6 was considered positive.

These are the results of the study: The proportion of the children with positive patch tests to the faecal extract of Der. P: there was a significantly high difference in the prevalence of positive patch tests among the first two groups, the children with AD and the children with respiratory problems. No child in the control group had a positive patch test. I would like to stress that 30% of the children with positive patch tests had negative skin prick tests to the same allergen. The proportion of the parents of the children of the first two groups, who had a positive patch test response: there was no difference between the two groups of the parents with children with AD, but there was a significant difference in the prevalence of positive patch test between the parents of the children with AD and the parents of the children with respiratory allergy. Again no subject in the control group had a positive patch test. Our conclusion is that as it was previously demonstrated a significant proportion of the children with AD does have a positive patch test. Quite a significant proportion of these children do not have any skin test or positive RAST for the same allergen. Finally, a significant proportion of parents of the children of AD had a positive patch test response to Der. P. I would like to stress that in this population of parents, in the prevalence of previous AD there was no significant difference. Our suggestion is that parents with children with AD may have particularly prone skin, which is a particular feature of the skin which makes it more prone to the house dust mite.

Thestrup-Pedersen, K.:

We are almost finishing a study where we have looked into how much house-dust mite antigen we find in the *beds* of patients with AD. The study is using a newly developed ELISA technique. What we have done is to take 10 controls without atopy, 10 psoriasis patients to have a scaling disorder as control, then we are collecting data from AD patients. I am going to show the preliminary results. We measured the house dust mite allergen in the beds which is measured by nanogram per gram of dust. The control group had the lowest value of 61, the highest was 53,000.

In psoriasis it goes up to 58,000. If you have AD, we found some very high values. If you take the median value you get the impression that there seems to be more house dust mite antigen in the beds of AD patients. WHO in Geneva has put a limit on 10,000 nanogram per gram of dust as a limit above which you would have an increased risk of having an asthma attack. Here I have given you the percentage of patients who had actually this amount of house-dust mite i.e. ~ 33% of AD patient.

To give you the same data in a different setting; control persons except for one young man who had a very large of amount of house dust mite in his bed, were mostly below the limit of 10,000 nanogram. In the psoriasis patients you can see 3 patients above this limit.

Again, these are preliminary data. Then you would like to know what is the allergy status of these patients? These results we do not have at the moment. Now to another group. We tested 36 consecutive patients with AD, irrespective of type-1 allergies. We had the following test series: The Der pI 250 times the concentration you use a histamine prick test. The Der pII had 50 times the same. Because of the fact that house dust mite antigen does carry proteolytic activity, we also included 2 proteolytic enzymes in the test series; trypsin and papain. Two of these patients had Der pI or 2 specific tests, that is, they only reacted to the antigen but not to the antigens. Another 4 patients did have Der pI reactions, but they also showed enzyme reactivity. There is a little bit of infiltration towards one or two of the enzymes. Therefore, we consider these irritant reactions. We also found, that by using PCR by scraping the skin, one patient had a very clear upregulation of IL-8 comparable to what you find in a positive patch test.

So we are not able to confirm the results of Bruijnzeel-Koomen who found that up to 50% of patients with AD do express type-4 reactivity to house dust mite. On the other hand, she selected patients with high IgE and specific prick tests. Our group is a non selected group, where you test the patient irrespective of type I allergy and irrespective of total serum IgE. Something that should be discussed is that Businco had her antigen in petrolatum, whereas we had it in the aqueous extract that you use for the skin prick test. I would like to leave you with the uncertainty that when we do these patch tests you always run the risk of not knowing how much irritation of atopic skin contributes to our results of the patch test.

Discussion

(Mudde) I would like to reply to the remark that was just made. About 5 years ago I think we demonstrated that there was a very strong correlation between patch test reactions and IgE cells. Meantime there have been several publications that show that the patch test is not as selective as we thought it was. We have redone the study with a large panel of patients to check whether our protocol is really as good as we thought it was. To make a long story short, it is not a very sensitive method the way we do it but it is a very selected method. There is an absolute correlation between IgE, specific IgE and IgE on Langerhans cells. High IgE is not enough, specific IgE alone is not enough. The IgE has to also be present on the Langerhans cell and then you have this extremely good correlation. In our case it does not matter, whether you do the test stripping, or not, so we are not interfering with the barrier or inducing cytokines by scratching or things like that. I do not know what is going on if you scratch but I definitely think you will force the allergen deeper into the skin reaching the mast cells. I think in that case you will get away from selectivity of the patch test and also positive results in asthma patients for instance. I think the interesting thing about patch tests is that you can differentiate between asthma patients and AD patients although with low sensitivity.

(Hanifin) We biopsy it on the patch test site before. You can also biopsy on another side to see if IgE is present.

You are also saying that you cannot have a positive patch test in a patient with low IgE, because it has been shown very clearly that you have to have 3 to 4,000 kilo units per liter of IgE before you can discover the increase on Langerhans cells.

(Mudde) It is not an absolute but a very weak correlation. There is a correlation that we also find in the study that the most positive patch reactions are in those patients with the highest level of IgE, especially specific IgE, but that is not always true. Sometimes you also find patients with very high IgE levels which do not have IgE on Langerhans cells and it seems normal. Everybody has a receptor but it is a big question mark why there is no IgE on Langerhans cells, but there is a correlation.

(Q) I would like to hear your comment on the proteolytic activity of some of these antigens.

(A) They could definitely play a role in penetration so they change functions.

(Giannetti) There are many questions on the standardization of patch test mite allergen. For instance, look at the results of Thestrup-Pedersen. Did you not suggest to use your recombinant antigen in order to check if the mite antigen cells are relevant or not for AD? You find only 6 positive patch tests in 36 patients, so, that means that 6% compared with the 50% or 80%. There is a lot to do with standardization first of all. Second point, perhaps the data from Businco are right, but the situation seems to be rather complicated. In fact working on this material, we published data on the standardization by using different kinds of antigens, scratch and no scratch which gave us a different dilution of the antigen. In effect 50% of the patients in our studies, children even under 2 years of age, reacted to patch test without IgE antibodies against the house dust mite antigen. From my results it can be deduced that children under 2 years of age have patch tests that are positive.

(Færgemann) I think the standardization is very important, the antigen you use and of course, the technique. Just as for pityrosporon, scratch patch tests or regular patch tests and so forth. I like your paper a lot, especially where you had a high number of positive patch tests with your antigen. What is interesting is, that even in patients without active eczema, if I understand you correctly, you have a high number of positive tests.

(Businco) There was no difference in the prevalence of positive patch tests between the group of children who had AD at the enrollment, and the group of children who had previous AD. Our hypothesis is that children with AD, regardless of the presence of skin lesions, have something different in the skin which may be continued for life and which makes the skin more prone to the attack of the mite. As I concluded in my presentation, the same characteristics may affect the parents. It may be this defect that is inherited by the children, because it was really surprising for us to see, that there was no difference between the two groups. Our expectation was that there could be a difference, and, of course, we were very surprised to see the same high prevalence in the parents. I would like to stress that the atopic children with respiratory allergy and with positive RAST and skin tests to house dust mite didn't have such high prevalence of the patch tests, thus suggesting that atopic children without AD who are allergic to house dust mite have a different skin.

(Vieluf) We will present our data for the standardization of atopic patch tests on Wednesday morning. It is very necessary to standardize the method, because I do not believe that the data are comparable between the different groups. We have never seen with our method, only in one patient, a positive patch test without atopic eczema. It is very difficult to read the patch test in the right way, therefore it is very difficult to standardize this method.

(Comment) Just a short comment on the study of Thestrup-Pedersen. We did a similar study on the mite concentrations on patients affected with AD children and a control group and we were not able to detect any statistical significant differences in the allergen load of the homes of those children, neither in the median nor in the number of patients exposed to those very high levels (over 10,000). In the control group there were more children exposed to higher levels. Interestingly, if you looked at the AD group and made a difference between children having or not a positive RAST test to dermatophagoides, those who had a positive RAST were exposed to significantly higher levels of allergen in their homes.

Tanaka, Y.:
We can see a great number of *FceR1-positive cells* from the epidermis to the dermis in the lesions of AD. It is well-known that some of them are Langerhans cells, and the others are mast cells. Recently, it has been reported that eosinophils express FceR1.In this study, we investigated FceR1, CD23 expression and IgE binding on eosinophils in the lesions and mite patch- tested sites in AD. Ten patients with AD, and, as a control, five Bullous pemphigoid patients, were selected. Double-immunostaining with either anti-FceR1 monoclonal antibody, anti-CD23 monoclonal antibody or anti-IgE antibody and anti-ECP polyclonal antibody were done. Many FceR1-positive cells were observed in the lesions of AD, part of them are eosinophils. In addition, CD23-positive and IgE-positive eosinopils were seen in the lesions of AD. These results suggested that eosinophils may bind IgE through FceR1 and CD23 in the lesions of AD. We can see FceR1-positive eosinophils, but intensities of FceR1 staining are weak, also CD23 and IgE staining of eosinophils tended to be weak in Bullous pemphigoid. The results of mean percentages of FceR1, CD23 and IgE-positive eosinophils in the lesions of patients tested are shown: there are no significant differences in the percentages of FceR1, CD23 and IgE-positive eosinophils between the two groups. In the AD group, significant differences in FceR1, CD23 and IgE-positive eosinophils are not observed between moderate and severe patients. The severity was determined by the methods of Costa, and mild patients were not contained in this group. There were no significant correlation besides between percentages of CD23-positive eosinophils and log IgE-RIST in blood.

Next, patch tests with crude mite antigen were performed in two patients with AD by the same method reported at the 3rd International Conference on AD. We biopsied at 20 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours and 48 hours after patch testing, and checked penetration of mite antigen by staining with anti-Der pI monoclonal antibody. In one patient, two peaks of eosinophil infiltration were observed at 6 hours and 48 hours. At mite-patch tested sites, many FceR1-positive eosinophils were observed. We evaluated percentages of FceR1, CD23 and IgE-positive eosinophils at two peaks. A percentage of FceR1-positive eosinophils infiltrated at 48 hours was higher than at 6 hours. In conclusion, eosinophils express not only FceR1, but CD23 and bind IgE in the lesions of AD. These results suggested that eosinophils may trap penetrated antigen such as mite antigen by IgE on their surface, and may enhance their activity.

Discussion

(Giannetti) Did you study patients with positive patch test with dust mite antigens. Were these patients with IgE antibodies to dust mite or not?

(A) IgE score increased in patients on whom we performed patch tests.

(Giannetti) So they were positive. I wonder, if someone in this audience has seen if eosinophil infiltration in patch test is positive in patients without IgE antibodies, because in these positive patch tests there is no eosinophil infiltration.

(A) Patients without IgE antibodies do not have a positive patch test in our hands, but we are going to biopsy negative patch tests sites to see whether there is an influx or not.

(Ring) I cannot answer the histology questions either. The other effect is clear: we have positive allergy patch tests without prick or RAST. I think this is very important, otherwise we wouldn't have to do the tests.

(A) Is it correlated to the eosinophils or is it more correlated to the T-lymphocytes particular for house dust mite? That is something we do not talk about, but we found patch tests matched specific T cells in AD lesions.

Hanifin, J. M.:

I think we have learned a lot in this hour. Some of these points will be continued tomorrow. I think one of the audience members asked about the standardization of the tests. I think this is a very important thing and I do hope that tomorrow we will learn more about standardization of the tests, patch test and intracutaneous tests reading.

Scientific Program – Tuesday, May 24, 1994
Round Table Panels
Subject: The Role of T Cells

Discussion leader: **J. D. Bos** (The Netherlands)
Panelists: **C. Neumann** (Germany), **J. Ring** (Germany), **K. Thestrup-Pedersen** (Denmark), **C. Bruijnzeel-Koomen** (The Netherlands), **Jon M. Hanifin** (USA)

Good morning, we are going to begin the Tuesday morning session. I am John Hanifin, and we are going to, for the first 90 minutes, talk about the role of T cells in AD. I think you will see that there is going to be a great saturation of considerations this morning. Following this there will be cytokines and later in the day other cells. I think it covers the system quite broadly because I think in all of our dealings with the immunologic aspects of AD we have to continually consider other cells, other pathways, biochemical controls of immunologic responses and then to consider possible sites of genetic abnormality and influences of gene expression of cytokines and key enzymes in these various pathways. I turn the responsibilities over to Dr Bos and we'll proceed.

Bos, J. D.:
We are going to have this first session today on the T cells and their possible role in the pathogenesis of AD. We will also talk a little bit about the possible ways of interfering with this disease by adjusting some new knowledge that has emerged on T cells in the past few years. We have to realize that T and B cells were distinguished from each other only 20 years ago. Before that time, we only talked about lymphocytes and the existence of B and T cells was not yet known. That was what happened in the 70's. In the 80's we have seen by immunophenotyping that T cells form a major component of the inflammatory infiltrate in atopic eczema. From that time on, people are focusing more and more on the possible role of T cells in the pathogenesis of the disease. One has to look at two different aspects when we talk about these things in relation to atopy. In the first place we are talking about the possible role of T cells in the development and maintenance of the skin lesions themselves and completely different than that, but related of course is a systemic abnormality at T cells level in atopy in general, especially in relation to the fact that there has been a concept of TH0, TH1 and TH2 cells.

I would like to start with the first question of this session to be answered by Prof. Christine Neumann. What is this concept of TH1 and TH2 type lymphocytes in relation to atopy and perhaps in relation to AD.

Neumann, C.:
The concept of TH1 and TH2 cells was put forward in 1989 by Mossman and his group. It is based on the observation that CD4+ T helper cells differ with respect to their cytokine secretion. Mossman's group proposed that there are at least 3 different types of CD4+ T helper cells with respect to cytokine secretion. One he called TH0. This cell is able to produce a large range of cytokines. Depending on the signals which hit these cells, which may be either cytokines or antigen, these TH0 cells may develop into different directions. Either becoming a TH1 cell or a TH2 cell. TH1 cells are characterized by their ability to secrete interferon gamma and IL-2 but not 4 and 5 which is restricted to the TH2 subset. These subsets with respect to their cytokine secretion pattern are mutually exclusive. This concept has been confirmed in the mouse with several infectious diseases, for example, in leishmaniasis or schistosoma infection. Depending on

the type of antigen presentation and depending on the genetic background of the mouse, they may either develop TH1 or TH2 response. This has quite a substantial implication for these animals, because when they develop a TH1 response they will come up with their infection and they will survive the infection because interferon gamma obviously is a very strong activator of a delayed response, activator of macrophage. When they develop a TH2 response they will produce high levels of serum IgE and they will not come up with their infection and they die.

So, what does this concept mean to AD? In the last 3 to 4 years, several groups have shown that the atopic individual when exposed to allergens will show an early TH2-dominated response. This has been shown by cytohybridization or bronchial lavage cells after inhalation of allergen, and by cytohybridization after injection of the allergen into the skin of the individual. It has been shown in patch test lesions and spontaneously developed atopic lesions by cloning TH2 cells out of the skin. More recently, it has been shown by polymerase chain reaction done on spontaneous lesions and on allergen exposed skin lesions of atopic individuals. So this can be taken for rather safe experimental data that early in the allergen exposure time, TH2 balance dominates. I have to stress this because surely not all of the allergen specific cells are TH2. What does this mean in functional terms? When we look at the cytokines, the TH2 cytokines, which are IL-4 or IL-5, it becomes clear that these cytokines have to do with atopy because IL-4 is one of the major cytokines to promote IgE secretion. It enhances the expression of IgE receptors; it enhances IL-4 receptors leading to autocrine stimulation of the producing T cells. More recently, there is evidence that it promotes the antigen-presenting capacity of dendritic cells. Looking at IL-5, it is very well known for years that IL-5 is a promotor of eosinophil activation and expansion. IL-5 also stimulates the mast cell system. You all know that eosinophils and mast cells are activated in atopic individuals and we know that in atopic skin we find signs of eosinophil and mast cell activation. As to IL-10 there is only little data on atopic skin but if you look at the function of IL-10, which is suppression of cytokines, especially strong suppression of interferon gamma and IL-2, we all know from data of the last years that this is a typical feature of atopy at least of AD. AD individuals have low interferon gamma production of the peripheral T cells and obviously of their skin T cells as well. It may very well be that IL-10 is operating in one or the other way in early allergen exposure. Finally, I would like to make a comment on Mossman's data. It looks as if there is a group of people who have shown this, that more than 48 hours after allergen exposure, we find an upward regulation of interferon gamma in the skin of allergen-exposed atopic individuals. This can be downregulated by UV-light and there is also improvement of the skin disease. I want to leave you with a hypothesis, that allergen exposure in atopic individuals apparently leads to an early TH2 specific response followed by a TH1 response which in itself may be responsible for the continued dermatitis.

Discussion

(Giannetti) First of all, Romagnani, the spokesman of this theory of TH1 and TH2, suggests that during the evolution, because of the contact of the human body with mycobacterium, that could be the basis of this hypothesis. What do you think about it? The second question, what about TH1 prevalence or influence of a later response? I mean if you perform a close study on stabilized lesions of AD, do you find a prevalence of the TH1 closer to the infiltration?

(A) I do not think I am able to speculate on the Romagnani hypothesis, why atopy is increasing and why it is a TH2 response. The second thing I think that fits very well with our ideas, are chronic lesions. With the TH2 domination, we only find this in the very early lesions either after

allergy exposure, putting allergen on top of the skin or selecting for really early lesions. We ourselves usually work with early lesions, and we know from others who looked at other regions, that TH1 is quite dominant.

(Bos) The background in atopy is a systemic TH2 type response leaving for example allergen-specific IgE, and in the skin lesion itself we can find this TH2 type of cells. I would like to ask Prof. Ring to tell us something about what we can learn from cloning the T cells from these lesions which has been mentioned several times.

Ring, J.:
The disclosure of TH1 and TH2 cells was a major breakthrough in the history of atopy. We, as well as many other groups, have looked for this cytokine expression-pattern in the skin of atopic eczema. You can do this by several methods: you can do it by immunochemistry or by establishing clones or you can do it by *in situ* hybridization or semiquantitative PCR. The data I present are from Kilgus who joined the Hamburg group two years ago coming from Vienna. Interferon-gamma expression is definitely present in some patients with atopic eczema, but mostly in those patients who were classified as non-allergic or non-atopic (and these were contact allergy in two cases, and one with nummular eczema). I think this is very interesting: nummular eczema behaved like contact allergy and not so much like atopic eczema. IL-4 is also expressed in allergic contact dermatitis not only in AD. Interferon gamma is definetely higher in the non-atopic group.

Now I would like to speculate a little bit and follow the thoughts of Neumann. What makes those T cells go into this direction. This may be an antigen either by quantity or by quality. You see here that T cell clones, when stimulated with different antigens, all produced the classical TH1 type. Whereas with dust mite, pollens and the parasite toxicara they go to TH2's. We could say that the allergen makes it but, on the other hand, nobody knows so far what makes an allergen an allergen.

The second thing would be that maybe it is not the antigen but something in the individual. Then it has to be the recognizing structure, so we should look at the T cell-receptor. Now I want to speculate even more: Maybe besides genetic disposition some other factors drive the cell into a TH2 type. Why do some people become allergic and others have a defence against parasites? Some epidemiological studies show that if you get parasites, you may be less allergic or you get allergies. This is very naively expressed but we found similar data in our East/West German comparison. I would like to speculate on another sub-population among the TH2 cell. There may be a TH2 alpha promoting parasite defence, TH2 beta being relevant for atopy.

Discussion

(Hanifin) Were those normal clones or atopic clones and is there a difference?

(A) What I showed was no clones, it was PCR out of skin biopsies.

(Hanifin) Where you stimulated with various antigens?

(A) No, this again was not a stimulation it was a PCR out of the biopsies.

(Hanifin) Those were from biopsies of normals?

(A) Our own data was from biopsies of atopic eczema and of normal volunteers or contact dermatitis.

(Hanifin) The antigen responses were the same in both?

(A) We did not check the antigen, we just measured the cytokine expression. I cannot say anything about the specificity.

(Bos) If you do a PCR of a skin lesion, you pick up an IL-4 signal, but you probably also pick up an IL-4 signal from other cells.

(A) This is a big problem one has to bear in mind. It could be from mast cells, although usually there are not so many mast cells around the T cells.

(Q) You showed us a decrease of interferon gamma in your studies and that most studies confirm it. But there are some differences.

(A) This is a fascinating concept and, of course, some people find that elevated interferon gamma also in the atopic eczema lesion. I think it is more complicated, than we thought in the beginning. Many of us have done studies with different gamma.

Bos, J. D.:
We will now go on to Prof. Thestrup-Pedersen who will cover the same question from a different angle.

Thestrup-Pedersen, K.:
Let me present some preliminary data we have obtained in the last year. Yesterday we learned that AD may be allergic or extrinsic, but you also have patients where there is no increased IgE type I or type IV allergies, i.e. intrinsic AD. The ratio between these two types could vary from 1/3 to the intrinsic and 2/3 to the extrinsic.

If you establish an antigen-specific clone, you usually use mite as your stimulatory allergen. You perform early cloning, so you only have single cells in your culture. You use foetal cell layers, and continuous antigens stimulation.

The system we have used is simpler. We have seeded cells used directly from skin biopsies and not performed any cloning and we have not included foetal cells. We have added IL-2 and IL-4. The remarkable thing is that even though we do not make any kind of antigen stimulation, we get lymphocytes to grow fast. In antigen-specific clones, there is no continuous growth and the lymphocytes mostly represent an IL-4 cytokine profile. The cells are antigen specific because you select for antigen-specificity. The antigen-independent clone shows continuous growth. That means cell growth beyond the limit of 40 to 60 cell divisions before a T cell will stop. What we see, and we can't explain it completely, is the development of genomic instability changes in the DNA. After 150 to 200 cell doubling we see a downregulation of the T cell receptor in some cells. At the moment, we are studying the cytokine profile, which seems to be the TH0 type. We speculate that these cells are some form of immature T cells. You cannot do this from the same patients taking blood cells.

Discussion

(Mudde) I think you are aware of our cloning work that we published a few years ago in which we used IL-2 and IL-4. Practically getting the same data as you present here. We also did not stimulate these cells with antigen when we induced the patch test. That was the induction of the lesion and then we grew the cells out of that without any further stimulation. I think there is one difference, and I think it is a major difference between the findings that you present here and what we have done: we have not selected for best growth, and that was important, because our cells did not proliferate on the antigen; they needed exogenous IL-2. I think that is a major difference between our data and cloning data in general from other groups who always select for proliferation as a sign of specificity and therefore automatically for their cells which produce their own autocrine growth factors. In your case, maybe your antigen-independent clones are also depending on exogenous IL-2 from the outside and they do not produce their own growth factor.

(A) They need both IL-2 and IL-4.

(Mudde) Without IL-4 we got very few clones, but it was really nothing compared with what you got with IL-2 and IL-4. We also did not do cloning straight from the beginning, we got lines first and from that moment on we could look at the culture and we could study TCR beta expression and cytokine profiles and compare with those of the clones.

(Neumann) I would like to ask the people who did cloning from either cultures or from the whole skin lesions whether they were working with clones, because when you expand either with or without IL-2 and IL-4, you may really expand just a few cells and afterwards you are dealing with clones.

(A) We look at AD either as allergic dermatitis (the extrinsic type) or intrinsic AD not being accompanied by allergies. I do not know the ratio between these two types.

When you study AD most people tend to study the extrinsic type by establishing antigen specific clones mostly by use of house dust mite antigen. The cloning produce means you select for antigen specific T cells only. The system we have done is simpler. We have seeded cells directly from skin biopsis.

Bos, J. D.:
Professor Bruijnzeel-Koomen, what is the role of allergen specific T cells in AD?

Bruijnzeel-Koomen, C.:
The model we used in the recent years is the atopy patch test and, as I have heard from my Dutch colleagues yesterday, you already discussed the clinic of the atopy patch test. The test as it is, is well known to you. We try to standardize the clinical aspects of the atopy patch test and we succeeded in that and the work was published in the Journal of Allergy. During this standardization procedure we found that the specificity of the test is extremely high and sensitivity is rather low. There is a positive correlation with the level of antigen specific IgE. What was very peculiar in this study was that the reproducibility of the test was extremely high. In ten patients who were patch tested one year later with the same allergen, we could find again a clinical reaction pattern that was the same as the year before. For that reason we asked ourselves several questions we

tried to answer with our T cell clones. We, as others, could establish allergen-specific T cell clones from biopsies taken from atopy patch tests. As soon as 12 hours after performing the patch test, allergen specificity is already present and lasts up to 72 hours. The clones we could establish from these patch tests' reaction at several time points showed a cytokine pattern similar to that of the TH2 type. By means of this method we could prove that the atopy patch test is allergen-specific.

The next question was if we repeat the patch test in the same patient, can we then find the same allergen specific T cell clone? For that reason we did a patch test in one patient in February 1990, we repeated it in May 1991 and again in December 1993. In this patient we established T cell clones out of biopsis from patch test, we performed in February 90 and May 91. These T cell clones we used for further characterization of the T cell-receptor, meaning that we could sequence the highly variable region of the T cell receptor, the region where the antigen recognition takes place. Making primers of this region, we could use these in the PCR and with this technique we could trace them in T cell lines established from later patch test time points. For example, this is an allergen specific T cell clone which was established in February 90 out of a 24 hour patch test and we can see that this T cell clone was also present in the 48 hour patch test; the T cell clone which was established in May 91 was also present in February 90 and was also present in the 24 hour patch test reaction in December 93, all in the same AD patient.

The other point I wanted to address was the relationship between the atopy patch test and lesional skin. Using the same technique, we could demonstrate that the allergen specific T cell clones that were present in the atopy patch test were also present in the lesional skin of the same patient.

The conclusions so far are that the atopy patch test reaction is allergen-specific, but also reproducible allergen specific T cell clones have been found in several reaction times; the atopy patch test is a relevant model for AD, since allergen specific T cell clones from atopy patch test reactions have been detected in lesional skin cultures from the same AD patient.

Discussion

(Friedmann) Could you tell us whether in dermatitis there are predominantly TH1 cells in that cytokine profile?

(A) You can find the TH2 as well.

(Friedmann) My question is then, these specific clones that you found kept coming back into the lesions each time you challenged the patient and they are there in the lesional skin as well. Did you analyze those to see whether the T cell-receptor pattern that identified the clones was also found on cells with a TH1 cytokine profile? In other words, had there been any changing of the cytokine phenotype of a clone in relation to what it is that makes them decide to change today to be a TH1 cell rather than a TH2 cell?

(A) To be honest we haven't checked that, it is in fact not possible. You cannot find the same hypervariable pattern back in the TH1 clone, it is very special for T cells clone.

(Bos) There is a concept that TH1 and TH2 cells can shift to each other and we would assume that the T cells will not change during this.

(A) In fact nobody checked that. I haven't showed all the details but there were clones which did not have the same beta check.

(Mudde) There is a wide variety of TCR beta chains used in patch test biopsies. Of 24 possible beta chain families, 12 are used, but antigen-specificity is only selected by a few of these. In this technique, using the cloned type of the T cell-receptor, you do not really look for the beta chain but for that little part identifying one T cell by itself. There is no reason to assume that they change beta chain usage if they have cloned the same. What might happen is, that they have a different T cell cytokine pattern. But that is something we would like to look for, but it is very difficult because you use this technique on a bulk culture. You have DNA from a clone, and look with this typical DNA into a culture to see if you can find that message again. So you cannot really say which cytokines belong to which cell.

(Thestrup-Pedersen) I think that is an interesting observation. You have established three different T cell lines where you have a specificity to the antigen, but it seems that you have a different expression of a T cell receptor. We have from one of these patients in whom we didn't perform cloning work just looked for growth. We have established three clones, and two occasions they used the same, but the third they used a different one, meaning that actually from one patient you can establish different clones.

(Bos) What are the possible pathways of TH1/TH2 differentiation Prof. Neumann?

Neumann, C.:
From other immulogical systems we have some knowledge about what principally is able to regulate this differentiation either to TH1 or to TH2 cells. One important pathway is the allergen epitope. There are several systems in which it can be clearly shown in the mouse model that depending on the epitope of the antigen piece which is recognized by the animal, this will regulate the outcome of the cell response. Now the question is, what does this do to the genetic background. Obviously this is a very crucial part. I wanted to ask how sure are we that dermatophagoides always induce a TH1 response if the individual is not atopic. There is some data from the Dutch group showing that non-atopics promote TH1 response to dermatophagoides. So this may be a key that atopic individuals respond to a different epitope of the house dust mite, than non-atopic; this regulates the TH1/TH2 pathway. From other experimental systems we know that allergen load, or rather, antigen load, is crucial in directing. Low antigen gave a TH1 response in this experimental system and high antigen load gave a TH2 response. It is conceivable that our patients with AD, who have an impaired skin barrier, have a much higher allergen load in their skin than a non-atopic individual. This may also contribute to a more TH2 directed response early after allergen exposure. The third thing to consider is the antigen presentation itself. Ring already referred to this. There are systems where the antigen presentation has been manipulated either by UV-light for example, or by treatment with cytokines. In these systems, manipulation by UV-light abrogated the TH1 response but left the TH2 response. Disbalance may be explained by a difference or by special features of antigen presenting cells (APC) in atopics. IL-10 is a very interesting cytokine in this respect. Treatment with IL-10 produced the same results: TH1 responses are abrogated and TH2 response is surviving. Another more recent aspect is that the cytotoxic subgroup of CD8+T cells may regulate so that it leaves TH2 cells dominating and abrogates the TH1 response.

Finally one has to consider that the cytokines, which are resident in the skin itself when the

allergens hit the skin, direct the pathway. For example, mast cells which contain IL-4 may provide that IL-4 which is already present in the skin do enhance the TH2 pathway. We know that IL-4 itself enhances the development of TH2 cells, and interferon gamma when it is present, suppresses the TH2 pathway. These are the possible pathways and probably there are many more, but as related to AD I do not think we can answer this question at the moment.

Discussion

(Horsmanheimo) We have studied the last possibility that you have presented and we have found that in the development of AD lesions, the mast cells really are switched to play a more permanent role in IL-4 synthesis. There are significant differences between the lesional skin and non-lesional skin in AD compared with what happens in normal skin or in some eczemas.

(A) The question remains whether this is the hen or the egg.

(Comment) We also looked at IL-4 in skin. I can assure you that there is no IL-4 in mast cells, patch tests, non-lesional skin, or lesional skin.

Hanifin, J. M.:
About 15 years ago, having noted multiple inconsistencies in AD, I sidestepped that concern and began looking at the more consistent abnormality that was present in various types of leukocytes in AD; i.e., the cyclic AMP pathway (which has been somewhat lost in trends of G proteins, inositol phospholipid and PKC signaling). Cyclic AMP is a very potent and ubiquitous regulatory mechanism in cells. In general, with increased cAMP, inflammatory cell responses are modulated and multiple cells including monocytes, T cells, basophils, B cells and eosinophils have been shown to be affected by increased phosphodiesterase (PDE) activity in AD.

We have been particularly interested in monocytes because of their modulating effects on TH1 and Th2 responses and because they are the site of the most active atopic PDE variant. This is a very specific, highly active Type IV PDE isoenzyme, distinct from isoforms present in lymphocytes and highly sensitive to PDE inhibitors. The abnormal cAMP hydrolysis in monocytes translates to the defective regulation and immunologic dysfunction seen in AD.

In addition to the monocyte as the predominant source of PDE activity among atopic leukocyte populations, recent observations suggest that the monocyte is an important component of immune regulation. We had previously confirmed in our reports that IFN-γ production was reduced among mononuclear leukocytes from patients with AD. However, when monocytes were removed from these preparations and purified T cell production of IFN-γ was measured, atopics had higher levels. This indicated that AD monocytes had an inhibitory effect on TH1 cells. We next demonstrated high spontaneous PGE_2 production by AD monocytes in culture, along with evidence that the monocyte-derived PGE_2 inhibits production of IFN-γ by atopic T cells. We also demonstrated the PGE_2 effect by blocking with indomethacin and showing increased IFN-γ production in MNL. These findings were consonant with observations from murine and human T cell clones, showing PGE_2 regulation of TH1 responses.

A second observation comes from a recent study in collaboration with Modlin and colleagues, showing increased expression of IL-10 mRNA in PCR-generated cytokine profiles from AD skin biopsies. These findings led us to quantitate spontaneous IL-10 production by blood monocytes and we found that AD monocytes had significantly greater IL- 10 production. IL-10 is known to

suppress TH1 production of IFN-γ.Thus, atopic monocytes produce at least two suppressive factors that can to account for the reduced TH1 function and impaired cellular immunity in AD. We speculate that this abnormality may also apply to Langerhans cells and other antigen-presenting cells.

These suppressors are under control of the cAMP system, as evidenced by the reduction in spontaneous PGE_2 and IL-10 production in cells treated with PDE inhibitors. Thus, we have hypothesized that the cyclic nucleotide system is controlling not only monocytes but, indirectly, the TH1/TH2 balance in AD, shifting to a TH2-dominant system. While this regulation may be through the suppressive effects of PGE_2 and IL-10, we speculate that TH2 function might also be directly influenced by these mediators to stimulate IL-4 production. This is based on our previous studies which demonstrated increased spontaneous IL-6 production by atopic T cells. Regardless of mechanism, IL-4 production can be inhibited by phosphodiesterase inhibitors much the same way as they inhibit PGE_2 and IL-10 production by monocytes.

In summary, it is possible the central abnormality in AD resides in cells of the monocyte lineage which, because of increased PDE hydrolysis of cAMP and consequent PGE_2 and IL-10 over-production confers the elevated TH2 influence characteristic of atopic disease.

Discussion

(Q) From which patients are the biopsies taken?

(A) They were from very severe chronic AD cases.

(Reinhold) By using a PDE-inhibitor *in vitro,* is it possible to manipulate a primary monocyte response to a TH2 reaction?

(A) We can in this way decrease IL-4 production and shifting back to a TH1 control system. IFNγ acts as a PDE-inhibitor against the atopic isozyme of PDE.

(Bruijnzeel-Koomen) Has GM-CSF any influence on this activity, because according to Dr Leon, this substance delays the apoptosis of monocytes, keeping them in an activate state.

(A) Like basophil, eosinophil, the monocyte can be inhibited *in vitro* by PDE inhibitors, it is an activable state all the time. It seems that these cells are relatively quiescent until provoked and then it sets off the reaction and can't shut itself off properly.

(Bruijnzeel-Koomen) How many days are monocytes activated in culture, are they down-regulated after some days?

(A) The PDE abnormality reduces fairly quickly so we do everything within 24 hours; after that time it is very difficult to measure.

(Q) The word monocyte means something in blood, those in culture are not.

(A) You can call them macrophages by the time we get through adhering them, there may be some activation that occurs through that.

(Ring) If PGE2 plays such a role, what happens if you add cyclooxygenase inhibitors like aspirin, is it a cure?

(A) You cannot get the levels which are necessary. Like with steroids you have to impinge on multiple pathways; not only cyclooxygenase, but with its inhibitor we can reverse the PGE_2 inhibition and raise IFNg production. But they have no effect as therapeutic agents. In contrast, PDE inhibitor affects all the pathways and have a direct anti-inflammatory effect at least in atopic disease.

Bos, J. D.:
Professor Thestrup-Pedersen will present his data on the possible shift between TH1/TH2.

Thestrup-Pedersen, K.:
By switching back to TH1, we should be able to reduce total serum IgE. I will now consider the clinical point of view, by applying INFα and even some with IFNγ in AD. French authors earlier described a girl where the total serum IgE level could be reduced from 16,000 to 8,000 kU and the AD disappeared by IFNα. We worked with IFNα with highly or moderately increased IgE, giving 3 million U 3× weekly for 6 weeks without any effect; so the dose was increased to daily IFNα for 3 weeks in another 9 cases. Like other studies there was no effect on IgE levels, but histamine released from basophils dropped during treatment. We looked at neutralizing antibodies against IFN and could observe that some patients produced these. Furthermore, we have seen warts and mollusca occour during this treatment.

IFNα and β use different receptors from IFNγ but all antagonize, at least *in vitro*, the IL-4 driven IgE synthesis. Since with IFNα you can observe neutralizing antibody, one has to be careful using it.

Discussion

(Mudde) I am impressed you got these neutralizing antibodies. I will make a comment on IFNγ antagonizing IL-4 mediated IgE induction: if you have interaction between TH2 and B cells, by using superantigens, you can not inhibit IgE production. In an *in vivo* situation, IFNγ must be produced at the same time and site as IL-4, otherwise it won't work.

(A) You indicate that we are coming too late when we already have the disease.

(Hanifin) Our experience fits with this because in 80 patients we saw an increase in nonspecific IgE production with IFNγ. We did the study in the summer months, perhaps therefore IgE levels went up.

(A) In a patient with constant 6-8000 kU IgE for several years it was no help, but we found neutralizing antibodies and this might have switched off something and therefore you have an increase in IgE.

(Hanifin) We had it without antibodies, we have not got these.

(Neumann) Can you predict which patients may have infectious episodes?

(A) There must be some cross reactivity between neutralizing antibodies to interferons. A downregulation of natural resistance may happen toward viral infections.

(Neumann) Was it only patients with antibodies?

(A) Yes.

(Hanifin) In severe cases they may spontaneously develop mollusca etc, during their lifetime.

(A) With IFNγ you see neutralizing antibodies in hepatitis C or leukaemia too, but the AD person is more prone to develop this IgG2 antibodies.

(Neuber) We experienced that many patients worsen during IFNγ therapy.

(A) We saw that, too.

Bos, J. D.:
Summarizing, the emerging concept now is that in the skin early lesions involve preferentially TH2 cells and in late lesions there are extra of TH1 cells. Systematically, the TH2 preference in atopy is explained by several pathways focusing on monocytes but it is unclear why this allergen-specific preference exists only to some subsets of possible environmental allergens.

Subject: The role of Cytokines
Discussion Leader: **K. Thestrup-Pedersen** (Denmark)
Panelists: **C. Brunijnzeel-Koomen** (The Netherlands), **L. Thepen** (The Netherlands), **J. Krutmann** (Germany), **Ch. Neumann** (Germany). **K. Neuber** (Germany)

Thestrup-Pedersen, K.:
I would like to briefly discuss where the cytokines come from and how they can influence immune deviations in atopic dermatitis. Indicated from T cell studies we will perhaps end up with 50 or 60 different cytokines. Their importance is mainly as growth factors, especially within the haematopoietic system and in the clinic, especially in oncology. Their importance in dermatology is in sustaining or creating an inflammation, particularly if released in skin from for instance T cells affecting endothelial cells. So the latter regulate adhesion and other molecules, and allow cells from the circulation to go out into the skin and create the inflammation. In AD we find CD4+T cells and eosinophils due to a highly regulated mechanism. Within immunology the concept today seems to be that the TH1/TH2 theory is too simplified, it is probably more complicated. IL-10 seems to be important for directing the T cell system toward a TH1 like response, whereas IL-4 and maybe also IL-13, in relation to TH2-like response. The pressure on the T cell system stems probably from macrophage. Depending on what these cells are dealing with, we get a TH1 response to mycobacteria or to helminths, to allergens we get a TH2 response. I would now like one of the panelist to talk about evidence accepting TH2 type cytokines related to AD.

Thepen, L.:
Talking about cytokine production in AD the location of these are important for their effect. You have to know which cells produce the cytokines, then we can influence them e.g. for therapy. Technically we stain single and double with monoclonal antibodies to detect cytokines at the protein level. By this method we determine TH0, TH1 and TH2 cells staining with different markers as IL-4 and IFNγ, so by our definition a TH cell is a CD3+CD4 cell.

Whereas TH2 produces both IFNalfa and gamma, TH1 produces only alfa and TH2 shows IL-4 but no IFNγ staining. We use the atopy patch test as a model to study inflammation. Instead of dividing lesions too early and late, we have to know where the lesion starts and follow it in time taking punch biopsies at intervals. We compare lesional and nonlesional skin of the same patients and non-atopic skin. In the nonlesional skin there is always an infiltration of T cells, macrophage and a small number of mast cells, whereas in the lesional skin we see a massive influx of CD3 T cells, macrophages and of mast cells (at the same level as in nonlesional skin). The time course of patch test shows an increase in all cell types. At 24 hours one can find more IL-4 than IFNγ, thereafter IFNγ is increasing and the IL-4 slightly drops. Eosinophils are seen at 24 hours but their number decreased during the patch test. Concerning macrophages (monocytes) there is already a peak at 24 hours and they stay there. All IL-4 producing cells visualized by double staining are T cells. If you use peptides by double staining you cannot find eosinophils or mast cells staining with antibody. I wish to stress that we have to be careful with antibody staining, because of in total 45 different antibodies only 10 give some sort of staining and only three are specific. Looking at IFNγ producing cells 80% are T cells. IFNγ produced by macrophage is always localized in the lesion itself. One main interest is to find why TH2 switch to TH1 after 24 hours. If you have

inadequate antigen production and presentation by Langerhans cells you induce allergen-specific TH0 phenotype looking and acting like TH2 cells, i.e. producing IL-4 but they do not produce IL-2. The other possibility is that by macrophage produced IFNγ we get TH1 cells from TH0. A third possibility will be mentioned by Krutmann.

Krutmann, J.:
We looked at 5 patients in whom the atopy patch test was performed and subsequently biopsies were investigated for cytokine pattern by mRNA employing differential PCR. At 24 hours the IFNγ levels are increased and comparing with shore of IL-4, it is almost as in control skin, even a bit decreased. After 48 hours the picture is dramatically changed; the IFNγ is upregulated, whereas IL-4 is downregulated. Taking these and Thepen's studies together, our hypothesis published in The Lancet (January '94) is best described in a two-phase model. The initiation phase of AD is mediated by TH2 cytokines, particularly IL-4, this response is then switched to IFNγ mediated TH1 response. In recent, so far unpublished studies, we tried to learn more about the mechanism of this switch. The obvious candidate for it is the IL-12, capable of inducing a TH1 profile in T cells. At 24 hours there was a dramatic mRNA upregulation specific for a subunit of IL-12, which is the functional component of this heterodimer. This upregulation clearly precedes the IFNγ upregulation observed at 24 hours in the same biopsies. Thus IL-12 may induce the switch.

Discussion

(Neumann) Has it really been shown that after 72 or 96 hours the TH1 response is specific? It could easily be that bystander cells are coming in and there are not antigen specific T cells as in the early response. This may be valid of other types of dermatitis, too, not only of AD.

(A) The chronic phase of AD does in my opinion, not differ from e.g. allergic contact dermatitis. That may be the reason that steroids and radiation are therapeutically less effective, because they deregulate the TH1 cells response in an unspecific manner.

(Neumann) What is the experimental evidence for this late TH1 nonspecific response, because it should be stressed that also specific TH1 cells are present. Cloning from early lesions gives not only TH2 cells.

(Thestrup-Pedersen) How many cells of a patch test in AD are considered to be antigen specific, 0 or 1%?

(Neumann) By limiting illusion analysis between 0.5 and 3%, which is in a minor population. Within this population in the early response there are many TH2 cells but also some specific TH1 cells. Is the shift related or not to specific T cells, and atopy-specific, or occurring in other types of skin inflammation?

(A) We have no tools and evidence for definite answers, but my guess is that it is nonspecific for AD. We happen to be the first to demonstrate it during inflammatory disease and in this case it was AD.

(Kapp) As sources for IL-4 not only T cells but a lot of free cells as mast cells may be considered e.g. in atopic asthma and rhinitis. By *in situ* hybridization you will find IL-4.

(Thepen) Then again IL-4 measure does not necessarily mean protein production. In bronchial biopsies we can stain mast cells but not in skin as here is a difference between mucosal and skin mast cells.

(Thestrup-Pedersen) Could it be that IL-4 is gone and has left mast cells?

(Thepen) At 24 hours it looks as if there are less mast cells, they are degranulated but they will regranulate again so in lesional skin there are degranulated and granulated such cells.

(Reinhold) If there is so much IFNγ in the skin how do you explain the low expression of HLADR-Class II of keratinocytes in skin lesions, compared with other inflammatory diseases?

(Thepen) We will work on it.

(Kägi) How do you explain the very high percentage, 25%, of the TH2 cells in healthy skin?

(Thepen) We think there are antigen-specific T cells sitting in uninvolved skin.

(Thestrup-Pedersen) Have you looked at tuberculin patch test because there you have the TH1 response?

(Thepen) It is very time consuming.

(Thestrup-Pedersen) You imply that macrophage and dendritic cells make IΦNγ.

(Thepen) Yes.

(Kägi) Did the patients have associated atopy?

(A) Yes.

(Ring) Looking at staining, one has the feeling that the cells cluster more or less. Is this an artifact?

(Thepen) TH0, TH1 and TH2 are actually close together in the infiltrates.

(Hanifin) When continuing these beautiful studies you have to keep in mind to include irritant controls and allergic contact dermatitis cases for specificity.

Thestrup-Pedersen, K.:
I will now ask Professor Neumann the question whether there is a difference in cytokine expression between blood and skin in AD.

Neumann, C.:
In the literature there are data on blood derived T cells in addition to what we heard of skin lesions. We have to compare blood and skin of the same patient. We applied the antigen twice: 0 and 24 hours and biopsied at 48 hours. It is an early but not one time exposure lesion. The result is that especially in patch, but also in spontaneous lesions, there is less IFNγ produced by T cell clones than in the blood. In respect to IL-4 secretion, this is not so clear-cut, showing only a significant difference between patch test and blood, the former being higher. In spontaneous lesions the difference in IL-4 secretion was not significant. Thus in early patch tests, secretion is suppressed with respect to IFNγ and is higher for IL-4 when compared with the blood. Others also found suppression of IFNγ in atopics. Studying 12-15 clones from different individuals with AD we compared skin and blood also with respect to IL-2 and IL-10 secretions. The result was suppression of IFNγ, a little upregulation of IL-4 and strong upregulation of IL-10 in the skin clones.

Furthermore, IL-5 was strongly upregulated and IL-2 downregulated, i.e. the TH1 cytokines are downregulated and TH2 upregulated depending on whether these clones were antigen-specific or not. Comparing skin and blood derived clones, the extreme upregulation of TH2 cytokines is impressive, especially of specific clones. It means that the specific response is a TH2 response and the bystanders in the skin have only a little bit more TH2 than in the blood. I want to stress that more than 50% of the skin and blood derived clones are TH0 cells. Working with about 25-30 clones from patch tests, spontaneous lesions, and blood (if it is allowed to make a percentage from such low numbers) show that 34% in early patch were TH2 but some TH1 clones were also present, the rest was TH0. On the contrary, in blood we found very few specific TH2 cells, thus it is not a frequent event in blood, more in the allergen exposed skin. In the nonspecific compartment, TH2 was underrepresented, giving another argument for early specific response in the skin.

Discussion

(Ring) I know you work with food allergies, and predominance of TH2 in skin may be a matter of the milieu. Do you find more TH2 clones in such cases provoked orally?

(A) I have only preliminary data. In blood we found a lot of TH1 cells to dermatophagoides.

(Mudde) It is very interesting that allergen specific T cells in skin differ from blood T cells. That would point to the fact that it is not T cell immigration or that T cells immigrate into skin and there change the phenotype due to the local situation. I suggest that TH0 is moving into the skin, gets contact with immature Langerhans cells which miss their markers and are matured after 72 hours. The immature phase induces energy and shuts down IFNγ production; the product may be still there, but the mRNA is gone. When mature and specific interactions will not induce TH2 phenotype any more, you will find TH1 and nonspecific cells. Could you comment on it?

(A) I would be quite content if it were so.

(Harper) Were the patients atopic?

(A) Yes, they all had AD.

(Harper) Was it age dependent with differences between children and adults?

(A) We have not done such comparisons. In our group Jung looked at the intercellular expression of cytokines in peripheral blood lymphocytes of AD patients in different age groups. There was an IFNγ suppression in all age groups.

Thestrup-Pedersen, K.:
The last presenter is Dr Neuber.

Neuber, K.:
It is well known that about 90% of patients with AD have *S. aureus* on the skin with clinical and therapeutical consequences. In the last years it has been shown that over 50% of isolated *S. aureus* strains from AD skin produced *S. enterotoxins*. These are known as superantigens by their ability to stimulate T cells to strong proliferation. In about 60% of AD patients, specific IgE antibodies can be detected. Leung (Denver) showed that *S. aureus* enterotoxins induce IgE-mediated histamine release from basophils and our group observed that enterotoxins, such as SEB and TSST-1, induce enhanced *in vitro* IgE synthesis and IL-4 production. It is quite different from mechanisms by which usual allergens stimulate because toxins are not processed by antigen presenting cells. They bind to the MHC complex II outside the binding cleft and bind on the side of the T cell to the β–chain receptor. It induces IL-4,IFNγ production and a strong proliferation, impressive in cases of toxic shock syndrome. Here you find 70% β2+ T cells in the blood. The T cell activation is dependent and restricted to the β-chain, i.e. enterotoxin B is specialized in humans on the β 3 and β 12. We found that in normals there were low populations of β 3, 8 and 12. After stimulation with SEB, the β 3, 12 and 8 increased in AD patients. Additionally, we looked at memory and naive T cells as well as peripheral blood CD8+T cells in blood and skin. A very strong expression on memory cells and in skin of β 3 was observed in AD, but not in controls. Another important finding was that in normal donors β 5 was the dominant T cell receptor chain. In the latter, SEB induces a significant expression of cytotoxic CD8+T cells in normals according to reports, showing a cytotoxic response by superantigen-activated T cells and that T cell can kill superantigen-specific T cells by direct interaction. Furthermore, in AD, the response of cytotoxic cells was significantly reduced and the CD4+ cells exceeded.

Discussion

(Bruijnzeel-Koomen) Was there a correlation between preferential expression of β 3, 8 and 12 and with the contamination of *S. aureus* or sensitivity for *S. aureus* infection?

(A) There was a correlation. Using only SEB which restricted to B β 3 and 12 in humans, we also have this high expression of β 8 that is very strong in memory T cells. It has been shown that in mice, β 8+ T cells induce IgE synthesis, whereas β 2+ T cells do not. Maybe there is also a polyclonal activation by these strong activates of T cells which also induce T cell clones with a B

β chain-like 8, 3 or 12, which is due to a certain function of these cells and which is currently being investigated.

(Chan, Portland, Oregon) The protaglandin synthesis in antigen-presenting cells is elevated by exposure to *S. aureus* toxin. We have also shown in atopic T cells that there is an increased IL-6 production. Was there any evidence that the T cell activation also induced IL-6 production of atopic T cells?

(A) We have not measured IL-6 until now. A paper showed that superantigen may play a role in psoriasis where IL-6 plays an important role, but this is unknown for AD.

Subject: The Role of Langerhans, Mast and Eosinophil Cells
Discussion leader: **C. Bruijnzeel-Koomen** (The Netherlands)
Panelists: **A. Wollenberg** (Germany), **G. Mudde** (Austria), **A. Kapp** (Germany) **I. Schneider** (Hungary), **M. Uehara** (Japan), **M. Horsmanheimo** (Finland), **M. K. Kägi** (Switzerland)

Bruijnzeel-Koomen, C.:
I want to discuss Langerhans cells, eosinophil and mast cells. If you put the cells in a scheme there seems to be a complex interaction between the Langerhans cells, the eosinophil, mast cells and the T cells.

Wollenberg, A.:
IgE and IgF-receptors have been extensively studied in the human system. On epidermal Langerhans cells - as defined by their specific CDla surface molecule - three different IgE-binding structures have been identified so far. The IgE-receptor that gained most interest in the last few months, the high affinity IgE-receptor or FceRI, has not been described in terms of regulation. Several observations suggest regulatory mechanisms to be active in lesional atopic eczema skin as compared to other skin diseases or normal skin. During the last months, we have tried to find out how FceRl and other structures are regulated on Langerhans cells *in vivo* in lesions of chronic inflammatory skin diseases. Therefore, we took punch biopsies, isolated the epidermal cells and quantitatively analysed the Langerhans cell surface molecules by flow cytometry.

In normal skin, a single homogeneous Langerhans cell population is present with a variable FceRI expression from almost negative to moderate amounts, depending on the individual. On this LC from normal skin, the IgG-receptor CD32 is constitutively expressed, while CD36 and CD1b are negative. In lesional skin of atopic eczema there is a dramatic upregulation of FceRI in all biopsies. The constitutive CD32 expression is unaltered, while CD1b becomes weakly and CD36 strongly positive.

When we do a quantitative analysis and compare FeeRI to the serum IgE, a clear correlation becomes obvious. This correlation becomes even more significant, if you restrict the analysis to the diagnosis of atopic eczema, suggesting an at least partly common pathway of regulation for these two structurres *in vivo*.

As can be seen in the contour blot, clinically uninvolved skin of atopic eczema patients differs from normal human skin only by the higher FceRI-expression but shows a single CD1a positive cell population. In contrast, chronic lesions of atopic eczema consists of two distinct sub-populations of CD1a positive cells, as can be seen in contour blots of CD1a versus FceRI from different inflammatory skin diseases. Again you see the dramatic upregulation of FceRI, whereas in ACE the FceRI remains low. The differences are so strong that you can distinguish between classical atopic and allergic contact eczema. The second population, with less CD1a but much more FceRI expression, may be seen in atopic and allergic contact eczema, *Psoriasis vulgaris, Mycosis fungoides* and every other inflammatory skin disease, but not in clinically normal, uninvolved skin.

The first population corresponds, in terms of its FACS-profile, to normal LC, whereas the second, hithero undescribed cell population might have invaded the epidermis *de novo*. The relative percentage of this second population varies from 20% to 90%.

Since the differences in the receptor profiles of the Langerhans cells are characteristic for some skin diseases, they may be used as a helpful tool in differential diagnosis of eczematous skin reactions. Differences in the Langerhans cell-phenotype allow diagnostic access to skin diseases on a single lesion level, whereas classical skin tests (Prick, ECT) test the entire human individuum. This might become of special interest for the differential diagnosis of atopic eczema from allergic contact eczema in environmental medicine.

In conclusion, atopic eczema has a specific immunophenotype with two distinct CD1a positive cell populations, and the second, new population shows a dramatically upregulated high affinity IgE-receptor.

Discussion

(Thestrup-Pedersen) If you keep these cells in culture for 1 or 2 days and you perform your analysis again, does it change?

(A) Yes, there is downregulation of all the receptors and a moderate upregulation of the unspecific findings so everything goes down.

(Hanifin) Did you do any correlations between serum IgE and clinical activity in that second population. Can you make any conclusions from that?

(A) Most of the typical chronic lesions had a second population of approx. 60–80% and those which had less activity tended to have a lower percentage, but this is not significant at all and that is just a feeling.

Mudde G.:

The question that I had to answer today was: "Is there an active role for Langerhans cells in AD?". My answer is yes... but I am not going to talk about this, because I only got the message that I should talk about this topic, yesterday.

I would like to present data on the consequences for IgE mediated antigen presentation in allergy and specifically in AD. Langerhans cells are involved in that. Very recently Sallusto and Lanzavecchia did a contest between antigen presenting cells to see which is the most efficient, most specialized APC and it turned out that the dendritic cells in combination with specific IgG antibodies present during the complete culture period were the winners of the game. They were able to present as little as 10^{-12} M antigen. Sallusto and Lanzavecchia claim that this is the lowest concentration of antigen ever published; however, in spite of their usual high standard of work, they apparently missed our publication in Human Immunology (1993, 37; 23–30). The non specific B cells, that we used in the presence of monomeric, specific IgE were at least as good as the dendritic cells published by Sallusto and Lanzavecchia a year later. However, we preincubated the B cells, and therefore they had very little contact with the antigen. Only 1 hour and then it was over. Sallusto and Lanzavecchia had antigen and IgG present during the whole stimulation period.

In the study that I present here, we used precomplexed antigen. We mixed B cells with different concentrations of IgE and different concentrations of allergen, and the read out was allergen specific T cell proliferation. There was a straight correlation between allergen concentration and not with the IgE concentration. This means that the size of the complex is not important for antigen uptake. On the right side of the graph there is excess of allergen indicating monomeric complexes and on the left side there is antibody excess which would implicate "large"

complexes. The absence of the need for cross linking is probably different for systems where Langerhans cells are used, since they express FcER1 (high affinity receptor for IgE), which, as the data from mast cells and basophils show, needs to be cross-linked before internalization takes place. Due to expression of FcER1 in AD, monomeric IgE may be bound to monocytes, Langerhans cells, basophils and mast cells. The obvious question that always comes when I present these data, is: "Why IgE and IgG?" Monomeric IgG, and even small complexes of IgG (Bheekha Escura et al: Immunology 1995, in press), will not bind to the low affinity receptor for IgG (CD32) on B cells or dendritic cells, at least not under the conditions that we use. CD23, on the other hand, is also a low affinity receptor (for IgE), but here single chains of the receptor coil around each other forming dimers or even trimers, therefore increasing the avidity of IgE for its receptor and allowing monomeric binding. Consequently the large panel of non specific B cells, expressing CD23 become potential antigen presenting cells in the presence of IgE, but not in the presence of IgG.

We conclude from this that, IgG antibodies direct the immune response to monocytes and away from B cells, whereas IgE antibodies preferably stimulate the immune response through B cells. In allergy, patients with relatively high IgE titers will suffer from a constant stimulation of the immune system due to the very low concentrations of allergen needed. However, what is new in my presentation is that due to IgE mediated antigen presentation by B cells a patient can start with a single allergy and end up with multiple allergies. This perhaps slightly complex mechanism has recently been published in Immunol Today (1995; 16, 380–383). In short, a normal B cell, responsive to, let's say, antigen X, will under normal conditions pick up this antigen through its surface Ig receptor and present it to normal (=TH0) T cells. In the case of allergy, a normal non-switched B cells may in addition also pick up allergen Y through specific IgE against Y. So, in this case the normal B cell presents both X to a normal TH0 cell and Y to a TH2 cell (since this is an allergic donor with high IgE titers, most likely the responding T cell for Y will be of TH2 type). As a result of this double interaction, the B cell may switch to IgE due to the IL-4 produced by the TH2 cell, and the patient will now become allergic to X as a result of this. In addition, the normal Th0 cell (specific for X) may switch to a TH2 phenotype, under influence of the IL-4 produced by the TH2 cell specific for Y. So, we have here a potential time bomb, which is especially dangerous for newborn children, where e.g. allergy to food allergens such as cow milk or egg protein, may lead to allergy to aeroallergens such as pollen and house dust mites. I would therefore highly recommend that therapy and prevention of allergy be concentrated especially on this high risk patient group.

Discussion

(Thestrup-Pedersen) It is a nice model but how do you get out of it. I am talking like a clinician, I know of a study where for one year they followed newborn children and they found that 3% of these children developed milk casein allergy. They restudied the children after 3 years and found that by using skin prick tests the cow's milk allergy was gone. You must have a way to get out of this.

(A) If you come up with a way things expire, then you know it does not expand forever, somehow it stops. One of the possibilities could be that the IgE gets too diverse and that it does not work anymore. If you have too many specificities in your total IgE, then it does not focus anymore in the way it should.

(Thestrup-Pedersen) Could you speculate if you get some let's say nonspecific IgE, which would actually, so to say, blind the B cells.

(A) I think it would work. But of course I am puzzled by how things are downregulated. I know that the body has so many defense mechanisms to avoid continuous production of IgE that one of those might find a function. We are talking about immature immune responses here and later on maybe some of those cells might downregulate this particular IgE. The damage is already done and certain cells are already gone and switched to TH2.

(Bruijnzeel-Koomen) If you would like to regulate, where do you have a chance to be successful?

(A) I think if you block here, your problem is basically gone. Of course if you avoid IgE production in general, your problem is also gone. If you avoid the TH2 cells, you do not get the IgE.

(Bruijnzeel-Koomen) But then you have to do it very early?

(A) As early as possible. I would say when the patient is susceptible to become allergic from his genetic background. You should be very alert and at the first signs of allergy, you should immediately try to prevent exposure.

(Ring) Why does a B cell carrying an IgE necessarily have to activate TH2?

(A) That is a consequence of the fact that when you have high concentrations of IgE you also have TH2 cells. This correlation is present.

(Ring) Why?

(A) I do not know why, you just find it.

(Ring) What does the IgE on the B cell have to do with the activation of TH2?

(A) The presence of IgE is correlated with the presence of IL-4. The IL-4 is produced by a TH2 cell. So somewhere before this happens there is already antigen produced in another setting. In that setting this TH2 cell was activated by probably a B cell specific for that same antigen, the normal situation. But there are TH2 cells when you have IgE.

(Neuber) Do you think that superantigens fit into this concept?

(A) Of course.

Bruijnzeel-Koomen, C.:
What determines the participation of eosinophil in the inflammation in the skin of AD patient, Prof. Kapp?

Kapp, A.:

I'll try, but I do not think I will be able to argue that question because, if you look for the evolution of the different concepts of AD, there is indeed increased interest in effector cells in the last years. One of these cells are eosinophil. You have to look for the eosinophil and the use of eosinophil is a ready source in atopic diseases but they are characterized by effector cell functions. They are indeed highly proinflammatory cells, not "good" cells, because the eosinophil are able to mediate various toxic effects: they are capable of releasing oxygen radicals and extremely toxic proteins. These toxic proteins are usually suggested to be necessary for killing of parasites but these toxic proteins are also involved in atopic asthma and AD. There are some findings of eosinophil derived proteins detected in skin and it is also possible to detect the active participation in patch tests.

We also did some studies showing the activation of eosinophil by the IL-5 and fundamental changes in eosinophils. It is very interesting that the toxic proteins are released at pseudopodes which interact with the tissue. The oxygen radicals are only produced in that area, so we can speculate that the inflammation is very distinctive, located at the site of inflammation. Activation of eosinophils is determined by the release of toxic proteins and this is reflected by eosinophil cationic protein (ECP) which is significantly increased in patients with AD. The ECP being measured in serum is not the real content of ECP. It is the measurement of ECP represented in *in vitro* cellular assay because ECP is released during clotting. It depends on the time of clotting and the temperature and therefore it is difficult to evaluate the different results of ECP. But nevertheless, it was very interesting to see patients with the highest release of ECP had the most severe AD.

That was, of course, why we performed a second study only looking at the concentrations of ECP but also looking for the correlation with the sensitivity. We looked at patients with AD during inpatient therapy for the concentration of several relevant parameters. Usually we compared the laboratory parameters with the clinical examination using a scoring system. We determined that different parameter structures such as IgE or soluble IL-2 receptor because the receptor correlates very well with the body surface. We also looked for IL-2 receptor levels in these patients and determined the ECP. It was very interesting to see that there are different findings for the different parameters to show that eosinophil was effective. We had a significant decrease of the clinical score in these patients. We found that there was a significant decrease of the ECP in the patient on admission and on discharge. It is very interesting to see that even at discharge where the skin lesions were cleared there were also increased levels of the ECP. When we looked for the correlations between ECP levels as a measure of *in vivo* activation of the eosinophil there was a significant correlation of the ECP and the score measuring the disease activity in these patients. It was the only parameter changed during therapy: within 3 weeks of treatment we didn't get any reduction of the soluble IL-2 receptor and no reduction in the IgE levels, indicating that inflammation in the skin might be correlated with eosinophil activation. Taking these results we suggest that eosinophils are indeed effective for inflammatory cells offered in AD and we suggest that, based on the results from Bruijnzeel-Koomen and Yoshida, eosinophils take place in the initial phase of AD.

However, what we measure as eosinophil activation in chronic AD might be reflected by the release of eosinophil activating cytokines by TH2 cells, particularly IL-5. That is a major problem of this ECP determination, it represents eosinophil preactivation and that might indeed be induced by TH2 cytokines since we measure the artificial release of ECP during clotting. I would suggest that eosinophils could be a very good target for pharmaceutical therapy and interesting new drugs directly effecting the eosinophil activation.

Discussion

(Hanifin) Did your blood eosinophil levels go down or stay the same while your ECP levels were going down? Is there any correlation there?

(A) The eosinophil blood counts decreased, but we didn't find any significant correlation between eosinophil blood count and ECP levels. We would suggest that ECP would be a better candidate for evaluating the disease activity in these patients.

(Hanifin) I would agree. I am following a patient on cyclosporine right now whose disease is well controlled but his eosinophil count in the blood just keeps rising. Do you have that experience?

(A) It might be induced by other cytokines. In cases like this we recommend to perform evaluation of MPO levels. You can get a ratio between MPO and ECP to determine the influence of eosinophil specific cytokines.

(Businco) Did you see any influence by infection on ECP serum levels? Our experience with such tests is not so good because we showed that infections such as measles which are so common in children, did have a significant influence with the tests. My question is, did you see that infections may influence the results? The second question is, when did you do the second test?

(A) We performed the second test 3 weeks after the beginning of treatment. There is indeed an increase of ECP in infection. This is also seen in psoriasis: you can get an increase of ECP. That is, of course, why we recommend the additional determination of MPO.

Schneider, I.:
I will show you microscopic pictures: there are four kinds of granules, these are the so-called specific granules. Here we are summarizing 21 patients with AD and we examined the absolute number of circulating eosinophil cells and the cells with positive reaction to monoclonal antibodies after separation with dextrine. After separation we made smears and reacted with monoclonal antibodies to EG1 and EG2. EG1 activate catonic proteins in the eosinophils and EG2 detects the activated form of ECP and neurotoxin as well. Then we examined the serum ECP levels with radioimmune essay and the serum IgE. At first, we evaluated the actual standard of the patient with AD; then we investigated the eosinophils in skin in these smears with EG1 and EG2. These eosinophil cells reacted with EG1 and normally 20% are EG+ reactive, EG2 are 37.6%: here in AD patients, we have 3.5% EG1 positive eosinophils. Next we investigated the EG2 reactivity, and in AD we found on average 36.4% EG2 reactivity.

Discussion

(Bruijnzeel-Koomen) Have you experienced granules in slide smears with EG1/EG2 staining of cells?

(Kapp) We have some problems with the EG1 antibody because of unspecific staining of other

cells with EG1. It is very interesting that we get a different pattern of EPO and ECP translocation in the cells in patients with AD, which depends on the stimulus that is activating the eosinophil. Based on the pattern of the distribution of ECP and EPO we can distinguish which cytokine was involved in the activation.

Bruijnzeel-Koomen: Do eosinophils play a role in the skin lesions in AD?

Uehara: Eosinophils very often increase in blood but the problem is increase in skin lesion, which is not yet clear. We studied eosinophil distribution in lesions of AD: In 44 patients with severe AD, a biopsy was taken from the newly scratched site and lesion. In scratched lesions, eosinophils were very rare. In old lesions the histology was almost the same. When the skin lesions were scratched some patients showed great increase of eosinophils in EG staining and EG2 staining, but other patients showed no increase of eosinophils. A big increase of tissue eosinophils occur only in about 1/2 of the scratched lesions. My conclusion is that eosinophil *in situ* is not a primary feature of the induction of AD lesions.

Discussion

(Hanifin) Does that individual always have eosinophils and, if so, can you reproduce that artificial scratch or artificial injury and due a time course?

(A) We have only done this once.

(Fartasch) I think this rises another question; we know from barrier studies that the keratinocytes in the very early stage, the first 6 hours by PCR studies, expressed IL-8, IL-10, interferon gamma. Do you think this may influence your findings that the keratinocytes which modulate the inflammatory reaction and the barrier distribution, which certainly is present in the epidermis in atopics, might have an influence?

(Ring) I think this is a very interesting observation. How deep do we have to scratch to attract the eosinophils? There are different ways of scratching, superficially or until it bleeds. Obviously if you test when it bleeds, it would run onto the platelet. Have you tried different ways of scratching?

(A) We have not explored this.

Horsmanheimo, M. (Horsmanheimo, D. L., Harvima, I. T. et al):
T-lymphocytes infiltrating the lesions of AD belong predominantly to the TH2-subtype which produces IL-4, instead of IFN-γ. IL-4 is thought to be an important mediator of allergic response. However, TH-cells themselves require an exogenous pulse of IL-4 before they can differentiate into IL-4 producing TH2-cells. Since mast cells have recently been shown to produce various cytokines, including IL-4, they are a tempting candidate for this role.

Skin biopsies were taken from lesional and non-lesional skin of 20 AD-patients. Ten patients with nummular eczema (NE) without any atopic features or background and 5 healthy persons served as the control groups. 5 μm cryosections were cut for sequential double staining procedure.

The skin sections were first stained by an enzyme-histochemical method to visualize mast cell specific tryptase and photographed. The dye was removed and the same sections were stained immunohistochemically with a polyclonal anti-IL-4 antibody and photographed again. The percentage of mast cells positive for IL-4 was counted.

The percentage of mast cells exhibiting IL-4 immunoreactivity in the upper dermis in lesional vs. nonlesional skin was 66 ± 18% vs. 37 ± 18% in AD ($p<0.0001$, paired t-test), but only 46 ±19% vs. 31 ± 22% in NE ($p<0.09$, paired t-test, no statistical difference). Also, a statistically significant difference was found between lesional skin of AD vs. NE patients in the percentage of IL-4 containing mast cells (66 ± 18% vs. 46 ± 19%, $p<0.008$, unpaired t-test). In the skin of healthy controls, only 23 ± 25% of the mast cells were positive for IL-4. Furthermore, the total number of mast cells was slightly increased in the lesional skin of both AD and NE (10-17%).

The results show that the percentage of IL-4 containing mast cells is significantly higher in lesional skin compared to nonlesional skin of AD, whereas in NE no such increase could be seen. This suggests that increased production of IL-4 by mast cells is a characteristic feature of AD and could initiate the recruitment and differentiation of IL-4 producing TH2-lymphocytes leading to AD.

Discussion

(Kapp) I would be eager to get more information about the discrepancy between the results this morning and your results.

(A) I think that one reason might be that it was very difficult to find antibodies which do not stain all cells. Of course they looked for TH2 cells which also contain IL-4.

(Thepen) Do you see any T cells making IL-4; if not, why not?

(A) There were some TH2 cells stained. We had to develop for many years this staining so that it does not stain everything. So using these antibodies and these concentrations we are now able to do IL-4 also in mast cells. I also did interferon gamma in both these diseases.

(Mudde) I think the recent data about mast cells containing IL- 4 and their possible involvement in IgE induction has been quite remarkable and have opened speculation for pathology of diseases like this. Recently, there was a very interesting paper (J Exp Med 1994, 179, 1349–1353) where they knocked out the IL-4 gene and then reconstituted these mice which either the non-T cells fraction (including mast cells) or the T cell fraction. If you add the T cells back to these animals so IL-4 only produced T cells, you do get normal *in vivo* IgE responses in the absence of mast cells to prime them for TH2. If you restore them with the mast cells only, and no T cells, there is no IgE production whatsoever. It brings me to the conclusion that maybe IL- 4 is important as a chemokine in the skin on other levels but definitely not in the sense that it primes the T cells to become TH2 cells. They can do that without mast cells. Secondly, the option of IgE induction outside lymph nodes is not a valid one.

Kägi, M. K.:
There is increasing evidence that the activation of a selected T helper cell population producing a TH2 related cytokine pattern with IL-4 and IL-5 but no IL-2 and IFN-γ may be involved in the

pathogenesis of IgE-mediated atopic diseases and in particular of AD. Therefore, we determined cytokine profiles in lesional and non-lesional skin in patients with AD. The existence of a 'non-atopic' (intrinsic) form of AD (NAD) with normal serum IgE levels, negative RAST tests, negative immediate type skin reactions towards environmental allergens and a negative patients and family history for IgE mediated allergies raised the question whether this form may be explained by a different T cell activation and cytokine pattern. In our study we had 19 patients with the extrinsic form of AD, 14 patients with the intrinsic form of AD, 6 patients with psoriasis and 13 normal controls. The punch biopsies of the skin were mechanically disrupted, centifuged and cytokine determination was done in the supernatants immediately after disruption and after a 24 hour incubation period in freshly added medium. In the AD group there were significantly elevated levels of IL-4, whereas IL-5 was significantly elevated in all patients. The levels of IL-2 and IFN-γ were elevated but did not significantly differ between the different patient groups.The same picture was present in the 24 hours supernatants. This means that once activated the cells continue to produce the same cytokine profile. In contrast to the supernatant immediately after disruption (cut medium) the 24 hour supernatant in psoriasis patients lacked the elevated levels of IL-5, whereas IL-5 was significantly elevated in both supernatants of the AD patients. When comparing lesional to non-lesional skin, significantly lower levels of IL-5 were observed in the non-lesional skin biopsies for all patient groups. To better illustrate the different cytokine patterns one can calculate the IL-4/IFN-γ ratio. A signiflcantly increased IL4/IFN-γ ratio was found in the AD patients when compared to NAD, psoriasis and normal controls. There was basically no difference between the cut medium and the 24-hour supernatants. The close relationship between the cytokine pattern in the cut medium and the 24-hour supernatant could also be demonstrated for the individual patient by calculating the correlation coefficients for each cytokine between these two cytokine sources. Close correlations were found for IL-4, IL-5 and IL-2. No significant correlation was found for IFN-γ. In conclusion one can say that our study confirms the presence of a TH2-like cytokine profile in lesional and to a certain milder degree also in non-lesional skin in patients with AD. In my last slide I would like to speculate about the cell interaction mechanims in the skin of patients with AD and NAD. Allergens may penetrate the skin in the 'extrinsic' form (AD) and may be presented by antigen presenting cells to T cells. The T cells become activated and secrete a cytokine profile that belongs to the TH2 type with high amounts of IL-4 and IL-5. On the other hand, in the 'intrinsic' or non-allergic form (NAD) the antigen which is still not known may also be presented to T cells and again lead to the production of cytokines such as IL-5. Since IL-5 is a strong eosinophil activator, eosinophils migrate into the skin and contribute with their toxic granule products to the skin damage. (*Kägi, M. K.*, Wüthrich, B., Montano, E., et al. Int. Arch Allergy Immunol 1994; 103–340).

Discussion

(Hanifin) I am a little surprised that in your non-allergic AD you are still looking for an extrinsic factor. Most of us as we see patients clinically, see the disease going up and down at a furious rate and it has nothing to do with some environmental factors; at least we have all been trying for the last 80 years to find something. Do you have any other ideas on why that happens?

(A) I think we should definitely continue to look for antigen in this subgroup of patients because they show very similar patterns of T cell activation in blood as the atopic group does. It is really necessary to continue to look for antigens, which may also be responsible for T cell activation.

Subject: The Role of Mediators
Discussion leader: **A. Giannetti** (Italy)
Panelists: **J. Ring** (Germany), **K. Ikai** (Japan), **M. Ficcardi** (Italy), **A. Giannetti** (Italy)

Giannetti, A.:
First of all I am responsible for itch and I am afraid we have no answer to it. Could mediators justify some symptoms of the disease related to inflammation, lichenification or xerosis. What does it mean for dermo-pathology, do they influence inflammatory cells? There is a scheme in which mediators are obviously in the center of the old part of physiology of atopic eczema. They tried to explain a lot of things and you see now which is the role of histamine on AD.

Ring, J.:
As you mentioned there is not much news about *histamine*. There may be increased skin reactivity to histamine and yet you have to differentiate between wheal and flare reactions which can be quite contrary to each other. When we talk about mediators, they are not only contracting muscles and dilating vessels but they also influence immune regulation; this is often forgotten by immunologists who only concentrate on markers and cytokines. Histamine and PGE2 influence T cells. Furthermore you might use histamine metabolite measurement in the urine as a more objective marker of a positive test in food allergy. The double-blind placebo controlled challenge is wonderful but there are a lot of false negatives. This is not the golden standard. I definitely have patients who have negative double-blind placebo control challenge and yet are food allergic. Fortunately, there are a lot of mechanisms in the body preventing the outbreak of symptoms. You can measure increased release of mediators, or increased plasma-histamine levels even without objective symptoms.

Now we switch the topic from inflammatory effects to the immuno-regulatory. There is the working hypothesis of an increased *prostaglandin* production which may be involved in a depressed T cell function, or mononuclear function in atopic eczema. One parameter might be the monocyte IL-1 beta production. Cycloxygenase inhibitors can reverse this effect. We were interested in IL-1 beta major product of monocytes, macrophages.

First we sticked monocyte PGE2 production and did not find a difference here between controls and patients, although others have found this. Then, we studied the IL-1 beta production and found a significantly reduced secretion and of atopic eczema patients. There is some controversy in literature. The mechanism of this depressed T cell function is still unknown.

Discussion

(Chan, Portland, Oregon) We have data showing that spontaneous production of prostaglandin in monocyte cultures increase. One of the problems with data is the detection limit. The antibody we used (commercially available) detects other prostaglandin products and the sensitivity is about 10 fold higher than the antibody we used from Dr Bill Anderson. Secondly, when you stimulate monocytes the response of stimulations is very specific. LPS for instance, will stimulate non-specific type of prostaglandin production. We also have data showing increased IL-1 production in atopic T cells. In the same experiment we found no difference in IL-1 production, this might have to do with the condition of the T cell activation or the number of cells that are present are

producing monocytes. The third thing is, that we have a lot of data on this subject and we are trying to sort it out. The starting point is very critical because most of the data published today have detected about 10 fold higher values than what we've seen.

(A) I think it is quite clear that there are different culture conditions and I was disappointed and wanted to get the same results as you. Of course PGE2 can suppress a lot of things. I have no data and you have many of them. I believe that eicosanoids play a role. Maybe one could speculate that they are activated in this irritant type of inflammation when we talk about atopic eczema. They then influence the immune system and suddenly it shifts to the TH2, but nobody has explained how this works. I think there is a lot of interesting information still hidden in this study.

(Q) Did anybody ever look for keratinocyte PGE2 production in these patients or how it looks in lesional atopic skin?

(A) We did skin blister studies, but we did not do keratinocyte cultures from those patient. In blisters we found increased values of LTB4, PGE2, etc.

Ikai, K:

Eicosanoids are known to play a central role in the regulation and modulation of important cellular functions. Arachidonic acid derivatives such as leukotrienes (LT) and prostaglandins (PG) are potent mediators of inflammation. In a wide sense, lipid mediators include eicosanoids, such as LT, PG and platelet activating factor. These lipid mediators are active in very small amounts, very unstable *in vivo* and effective only in adjacent cells. Therefore, these compounds function as autacoids. Now I will mainly talk about leukotrienes, especially LTB4 in atopic dermatitis (AD).

Eicosanoids such as LT and PG work through their specific receptors which are coupled with GTP-binding protein localized in cell membranes. LT and PG have a significant role in the pathophysiology of AD, although they may only act as modifying, regulatory or exacerbating factors in this disease. The contribution of eicosanoids to the pathogenesis of AD is still unclear. Their role in allergy of immune functions is very complicated since most eicosanoids possess dual actions, both pro- and antiinflammatory and act synergetically with other mediators and cytokines. Recently Hanifin's group reported that PGE_2 reduces IFN-γ production and this in turn may relate to IL-4 upregulation of IgE synthesis and impaired hypersensitivity in atopy.

LTA_4 is formed from 5-HPETE, a 5-lipoxygenase product of arachidonic acid. Conversion of LTA_4 is catalyzed by LTA_4 hydrolase, which is the rate-limiting enzyme for LTB_4 biosynthesis. Biosynthesis of LTC_4 is catalyzed by LTC_4 synthase, which catalyzes the conjugation of LTA_4 with glutathione to form LTC_4. LTE_4, the final metabolite of LTs, increases in urine from patients with AD and also psoriasis. Its significance remains unknown.

We examined the enzymatic activity of LTA_4 hydrolase in peripheral leukocytes of patients with AD, since such study ought to provide new insights into the pathophysiologic role of LTA_4 hydrolase in AD and may identify new strategies for treatment. We found that the LTA_4 hydrolase activity was significantly higher in supernatant fraction of peripheral blood polymorphonuclear leukocytes (PMN) from AD cases than those from normal controls and psoriatic patients. Peripheral blood mononuclear cells from AD patients showed higher LTA_4 hydrolase activty compared to controls and psoriatics. The changes of this activity in PMN reflected the extent of the involved area during clinical course in 14 patients with AD. Many investigators reported that amount, release or synthesis of LTB_4 is enhanced in plasma, lesional skin or peripheral leukocytes in patients with AD. LTA_4 hydrolase was strongly inhibited by bestatin and captopril, suggesting

that these drugs are useful for the treatment of AD by inhibiting the synthetic activity of LTB_4 *in vivo*. However, Thestrup-Pedersen et al. already reported that bestatin therapy could not cause changes in patients with severe AD. The biosynthesis of LTB_4 is complex, it is not only regulated by LTA_4 hydrolase but also by degradation of LTB_4, the concentration of arachidonic acid or LTA_4 itself and the enzyme activities of phospholipase A_2 and 5-lipoxygenase. FLAP(5-lipoxygenase-activating protein) is also involved in the pathogenesis of AD. Furthermore, the transfer of LTA_4, a substrate of LTA_4 hydrolase, to active cells, such as keratinocytes or lymphocytes, neutrophils showing active 5-lipoxygensse activity, as well as FLAP are of importance in this relation.

LTB_4 and LTC_4 play a significant role in differentiation, movement and function of human melanocytes which can also synthetize these eicosanoids acting as autocrine regulators and mediators for postinflammatory pigmentation in AD. We detected LTA_4 hydrolase and LTC_4 synthetase activity in melanocytes but according to preliminary results, no 5-lipoxygenase activity. These results suggest that melanocytes generate LTB_4 and LTC_4 from LTA_4, although the origin of LTA_4 in these cells remains unknown. In conclusion, leukotriene and prostaglandins function as autacoids only in the adjacent areas of eicosanoid-producing cells. These lipid mediators play an important role in the pathophysiology of AD, although they are only modifying the regulation of exacerbating factors, e.g. in inflammation, pigmentation and hyperproliferation.

Discussion

(Thestrup-Pedersen) What about LTA_4 hydrolase activity in keratinocytes?

(A) We looked at it but found no activity, perhaps due to low sensitivity of the assay.

(Ring) Since description of leukotrienes (18 years ago) we have no inhibitor drug available yet.

(A) I tried a LTB_4 inhibitor which, however, was without effect in AD.

(Thestrup-Pedersen) Working with a 5-lipoxygenase inhibitor (ETH615) there was no effect either, but it is difficult to get such compounds into the skin, i.e, we also have here a pharmacological problem.

(Hanifin) We have tested local lipoxygenase inhibitors which could be used in many other fields too, and there was an effect. Thus it is worthwhile looking at such products.

Giannetti, A.:
Why study neuropeptides? Because perhaps you have only the biochemical techniques in order them. First of all the main biological reason for linking brain and immune system is that lymphocytes secrete neuropeptides. Contained in nerves and degradate a few seconds from the surrounding area to the target cells. Why study neuropeptides in AD? If you look at the physiology of neuropeptides you can find a lot of similarities. In the human body skin there is a closeup position between nerves and mast cells. Looking at skin with a lichenified lesion, mast cells can penetrate suggesting the interrelation between these two cells. Just to suggest, when you should think neuropeptides as a family of molecules. Sometimes there is an opposite effect even in several immune functions, it is important for what is concerned in the human skin diseases. The classical

approach is just to study skin reactivity to neuropeptides, to look at the immunochemistry. If you inject neuropeptides, which can induce wheal and flare, then you can measure exactly the amount of flare and wheal. In the reaction of atopic skin to injection to different amounts of neuropeptides, there is no difference between atopic respiratory patients and normal controls. In AD patients the flare and wheal are reduced in a clear cut way. Looking at time course of injection, the difference between normal and AD patients runs parallel but for a lower degree for atopic patients; even the dose-curve response showed the same response but the atopic showed a lower degree. These data can be summarized showing that the situation with the neuropeptides is more or less the same as observed with histamine injection.

If you look at immunohistochemistry, there is a increase of nerve fibers in the skin of atopic patients and a disturbance of representation of several neuropeptides. There is a substance P-like activity in AD, whereas somastatin is only present in controls. NPY was present between epidermal cells in patients with AD. This suggests once more that there is an imbalance of the presence of the neuropeptide fibers in atopic skin. One conclusion can be drawn from this finding. As you know from experimental data, VIP is really one modulator of inflammation as a depressant. If you look at the general skin of AD, itch is the main symptom. Itch can be induced by several factors including stress. For these reasons, people speculate that stress acts on skin through neuropeptides. Is this true? It is too early to say, we only have a few data about it and no data about the peptides in the brain or in the spinal cord. You know from experimental data that if you induce an irritant, direct or allergic contact dermatitis on the animal, looking at the neuropeptide content in the spinal cord, there is a difference compared with the previous situation. We know now that there is other influence apart from these two neuropeptides (VIP and substance P) on the cells but even on keratinocytes. If we go to the skin, neuropeptides are present here in a certain position, for instance, that some neuropeptides such as substance P can influence the IgE synthesis by mononuclear cells. Some of the neuropeptides can influence the level of interferon gamma and IgE as well as IgE regulation.

Discussion

(Thestrup-Pedersen) If you see heroin addicts, when they take the heroin intravenously they scratch. Have you any experience using antiopoids like naloxone in a low dose continuously in AD.

(A) Unfortunately not.

(Giannetti) The last speaker is Dr Ficcardi (O. De Pita, M. Ficcardi, P. Puddu) who will speak about IL-2, IFNγ and CD23 as biological markers in pediatric AD.

Ficcardi, M.:
It is well know that IL-2 is a cytokine able to modulate several immune functions, such as the growth and the differentiation of T-, B-, and LAK cells. Furthermore, IL-2 can induce *y*-IFN production by T lymphocytes, and a disregulation of IL-2 system seems to be involved in several immune diseases; in this light we performed a study in order to evaluate serum levels of sIL-2R and *y*-IFN in children with AD.

We have also considered the expression of low affinity receptor for IgE (FceRII) which plays a central role in the pathogenesis of allergic diseases, especially in AD. This receptor, identified

by a specific monoclonal antobody, CD23, has been quantified both on total lymphocytes and in soluble form. We enrolled 71 children aged 1-14 years with diagnosis of AD according to Hanifin and Rajka's criteria, evaluated by the Costa and Saurat method adapted to children. None of the patients suffered from asthma and/or rhinitis. Twenty healthy donors were used as controls.

Serum levels of sIL-2R, γ-IFN and sCD23 were tested in ELISA; we preferred to assay sIL-2R rather than IL-2 for the higher sensitivity of dosage.

Our results have shown that sIL-2R levels were significantly higher in patients than in controls (1790±1582 U/ml vs 573±346; $p< 0.05$), with a positive correlation between sIL-2R and the severity of disease.

Increased levels of γ-IFN(0.39 ± 0.51 UI/ml vs 0.2 ± 0.05; $p<0.05$) and sCD23 (4.86 ± 4.56 µg/l vs 5.5 ± 1.7) were observed in all patients with respect to normal donors, but no correlation with the degree of disease were found, suggesting that, in spite of sIL-2R, γ-IFN and sCD23 cannot be considered useful markers of clinical course in pediatric AD.

The expression of CD23 on lymphocytes was significantly higher in patients when compared to controls, both in percentage (3.7 ± 3.1 vs 1.3 ± 0.2; $p<0.01$) and in absolute number (92.2 ± 53.1 vs 37.1 ± 31.6; $p<0.05$); a positive correlation with the severity of the disease was observed only for the absolute number of CD23+ cells.

To better analyze our data, when we subdivided the children in patients with high and low IgE, we observed an inverse correlation between sCD23 and IgE levels in both groups of patients. This data could be due to a linking of IgE molecule to its counter receptor FceRII.

Discussion

(Thestrup-Pedersen) You say that we should have the IFN gamma very early before you switch toward the TH2, and your data tend to show that in your group of children they do have a lot of interferon gamma.

(Bos) They do have gamma interferon in peripheral blood, but perhaps not in the organs where the B cell switch occurs.

(Ring) Would VIP or other neuropeptides be a candidate to explain the phenomenon of alloknesis, which you referred to - the itchy skin? Or when you inject those neuropeptides, have you tried to study alloknesis?

(A) No, we didn't study it.

(Wahlgren) No one knows anything about that, I would say. So the phenomenon of itchy skin has not been investigated following injection of neuropeptides.

Subject: The impaired skin
Discussion leader: **O. Hornstein** (Germany)
Panelists: **R. Kiistala** (Finland), **B. Song** (Belgium), **M. Fartasch** (Germany), **O. P. Hornstein** (Germany).

Hornstein, O.:
We have heard much about cytokines, mediators and so on. We all are players in this field, but we have not talked about the piano where the player is playing, and this piano can be distuned, a cord can be disrupted, or the pedal can be broken. I think the physiology of the skin is like the piano that the cytokines are played on. So, the best way to start is to give some information about the stratum corneum, the horny layer, and then switch to the deeper layers, for instance the adnexa, for instance sweat glands, and to finish the round, I will give some information about the physiology and pathophysiology of itch. First speaker will be Mrs. Song from Brussels, Belgium, who will be give some information about what dry skin is.

Song, B.:
What about epidermal lipids? I will give today a short summary. The composition of epidermal lipids changes with differentiation of the epidermis. So in keratinocytes we find phospholipids and glucosyl ceramides. In the stratum corneum we find ceramides, cholesterol and fatty acids. The lipids in the keratinocytes have a lamellar disposition in the lamellar bodies. These bodies are present in the stratum granulosum, and their contents are extruded by exocytosis into the intercellular spaces between the granular layer and the stratum corneum. In his process - this exocytosis between these intercellular spaces - in these lamellar bodies, there are lipids but also hydrolytic enzyme. This enzyme is very important because of its role in cohesion and desquamation of the cells and its role in the desmosomes. So, in the stratum corneum we find a mixture of sphingolipid, ceramides and fatty acids, but also cholesterol, and these lipids, disposed in the layers here between the corneocyte, but with the classical tetrosmium staining we see only spaces between the corneocyte cells. In the stratum corneum, one important ceramide is the acyl ceramide which is derived from acyl glucocyl ceramide, and is bound with linoleic acid. So this acyl change between the adjacent envelope is one factor providing cohesion between corneocytes. In atopic patients, several abnomalities have been described: first, disturbed extruding mechanisms of lamellar bodies in intercellular spaces, defective maturation of lipids and fatty acids, reduction of acyl ceramides in stratum corneum with a defective structure of the intercellular multiple lipid layers, and, finally, an increased evaporative water loss and decreased barrier function. So, several questions should be posed. Firstly, is the barrier defect always "poisoned" or is it associated with dry and scaling skin? Second, is correction of scaling abnormality different from correction of the barrier defect? I hope so, the answer will be provided by M. Fartasch.

Fartasch, M.:
The keratinocytes actively participate in the inflammatory reaction of the skin. Especially in a disease like atopic eczema, which has a defective or impaired barrier, the keratinocytes seem to modulate the inflammatory reaction. In a recent publication, Nickoloff (J Am Acad 1994) has shown that irritation, e.g. disruption of the barrier by tape stripping, induces an expression of m-RNA of TNF-alpha, TGF-ß, also IL-8 and IL-10, and interferon gamma. The expression is first seen in the epidermis.

Since it has been shown that in chronic stages of atopic eczema epidermal interferon is also

expressed (like after barrier perturbation); to some degree the inflammatory process in atopic eczema might be influenced by the impaired function of the epidermal barrier.

The water permeability barrier is formed by lamellar arrangement of epidermal lipids. They consist of ceramides, cholesterol, and free fatty acids. The structural arrangement of these epidermal lipids is important for the function of the stratum corneum. With new ultrastructural techniques it is possible to depict the lamellar bodies which extrude their polar lipids (glycolipids and phospholipids) into the intercellular spaces at the stratum granulosum/stratum corneum interface, were they form long sheets. The simultaneously extruded enzymes then process the polar lipids into lamellar arranged barrier lipids. The transformation process is performed in the intercellular spaces of the lower parts of the horny layer (the first two or three layers of the stratum corneum), resulting in different composition of lipids in the lower parts of the stratum corneum (more phospholipids and glycolipids), than in the upper regions where *more unpolar* lipids such as ceramides, cholesterol and free fatty acids are found.

The uninvolved atopic skin has a disturbed barrier function. It is wellknown that atopics show a higher incidence of hand eczema in wet works. We investigated a sample of 1,004 subjects of the healthy population aged between 15 and 45 years. Their skin was investigated clinically and with various bioengineering methods. In this group of subjects there were about 81 atopics, either with a history of atopic eczema (AE-history) or with present clinical signs of flexural eczema (AE-present). In the non-AE group (n=905) 289 showed a clinically dry skin, but the TEWL values were the same as in those with normal looking skin. The subjects with a history of AE (n=47), with and without dry skin, also showed comparable TEWL values, their mean TEWL being no higher than in the non-AE group. Only in the group with present AE (n=34), a higher average TEWL was found. The elevated TEWL values seen in this group were verified not only in the subjects with "dry skin" but also in those with normal looking skin.

The outer appearance of the dry skin does not imply in all cases that the barrier function is disturbed. Studies on X-recessive ichthyosis patients by a Danish group have shown that in spite of their dry and scaly skin, these patients had normal TEWL values. Also, on the ultrastructural level patients with X-recessive ichthyosis revealed normal barrier structures.

Measuring the baseline TEWL is not sufficient to identify subjects at risk of developing hand eczema in occupations with wet work (vocational guidance). An impaired barrier function might instead be detected by functional tests. For this purpose we have irritated controls and atopics with SDS (24h, 48h occlusion). The atopics did not show higher TEWL values after irritation compared to controls. Differences were seen in the repair phase of the barrier, verified by TEWL monitoring. The atopics needed more time for the repair of the barrier. This could also be seen when a repetitive irritation was performed; the atopics showed higher TEWL values and needed more days to regain their baseline TEWL values.

We can summarize that measurement of baseline TEWL does not discriminate between atopics and non-atopics. Moreover, the comparison of peak TEWL values after irritation does not differentiate between the two groups. Instead, normalization of TEWL was shown to be prolonged in atopics. These findings corroborate our earlier ultrastructural morphometric studies, showing that the kinetics of the lamellar body secretion system in atopics was altered. Since the lamellar bodies provide the str. corneum with lipids, disturbances in lipid secretion might therefore induce changes in the regeneration phase of atopics.

Discussion

(Bruijnzeel-Koomen) Concerning the SDS study, were those AD patients or were they atopics in general?

(A:) No, these were people who had only a history of eczematous reaction. In people who have allergic contact eczema, if you subject them to irritation, they will certainly react differently, so if you do a kind of patch test or irritation test on people with active, atopic, or contact eczema, you will elicit changes.

(Giannetti) I know that you attended the meeting in Zürich on irritant contact dermatitis, and know about the experiments Sedinari did on irritation with sodium lauryl sulphate, and with ultrasound 20 MHz. It is possible to discriminate by using these techniques between atopics and non-atopics, allergic dermatitis patients, normal scaling atopics and involved skin atopics. You can predict by these techniques wherher the patient will go on to develop allergic dermatitis easier, because when you apply sodium lauryl sulphate to the skin, you then try to induce a patch test with a concentration of nickel sulphate, which can't induce allergic contact dermatitis in a normal skin. By this means, you can really distinguish between two patients.

(A) Yes, I attended the Zürich meeting where I was the keynote speaker, but I had some problems. I think I was not the only one who had problems in this field, so perhaps we can discuss this later.

(Hanifin) When you made your first reading, was it at 24 hours when you could not detect the difference between the AD and normals?

(A) It is the standardized method to do 24-hour patch testing. There are some people who say we should apply only a mild irritation, and we should use a lower concentration of SDS. This is our task to develop a new functional test to reveal differences.

(Hanifin) I totally agree with what you said. We have a study in press in the Archives of Dermatology, and I think 0.5% is excellent. Since we are also doing contact allergy testing, we happen to read those irritant SLS responses at 48 hours, i.e. in between your 24 and 72 hours, and we were able to distinguish increased irritancy, not only in AD on involved skin, but also in patients with only allergic respiratory disease and no history of AD. So we think there is again this increased inflammatory potential in the inflammatory cells that predisposes these people.

(A) Yes, then you are coming to the repair phase of the irritative reactive reactions.

(Harper) In practical terms, looking after children with atopic eczema, how do you relate the impaired barrier to the regular bathing and cleansing of the skin, and has anyone measured transepidermal water loss before and after these baths with e.g. oily additives?

(A) I was waiting for this question, actually, because the important question is how local therapy works, and we know that the atopic eczema patients will improve by using creams, oils, so this is not just because they do not have dry skin afterwards; the dry skin is only the rough skin showing the first layers only. Probably it has something to do with the transepidermal water loss,

with the loss of electrolytes, which improves when you use ointments. If you have no disturbed or *lower* disturbed barrier function, the whole inflammatory reaction elicited by the reaction of the epidermis will be that the threshold declines and the inflammation is ameliorated.

(Q) Which side of the body was selected?

(A) For irritation, always the forearm. Because we know that the transepidermal water loss is different in different parts of the body, you have to use standardized methods, and there are standardized methods in the irritant contact dermatitis group, and measurements of transepidermal water loss there.

(Q) I have a question concerning ichthyosis. You mentioned X-linked recessive ichthyosis in a control group. What is your experience with patients with autosomal dominant ichthyosis? Secondly, there is certainly something wrong with lipids in the epidermis, but in ichthyosis vulgaris the problem is probably more related to enriched protein and the pathway. No one has mentioned anything concerning the role of protein and water-binding molecules in dry skin, so what is your opinion on this?

(A) Yes, this is a question we all face. We know that the water permeability barrier is formed by the lipids which are in between, as the water passes the keratinocytes; they are polar. So the only thing that will hinder the water from passing or something is penetrated, are the lipids in between. And of course the hydration of the corneocyte is another issue. Regarding the water permeability barrier, this is a function of lipids. We did studies - there is the study of Johansen et al. on 13 patients with X-recessive ichthyosis, and there is a defect of the cholesterol sulphate, but this obviously has an influence only on the desmosome on the cohesion. We did the same studies on ichthyosis congenita, and they had all a barrier impairment, and one could see this altered structure there too. As to the last group, viz. autosomal dominant ichthyosis, I have not yet seen my electronmicroscopical slides of these parts, but I think that the barrier is impaired there, though I cannot give you any data on this group yet.

(Rajka) To the clinician it is very easy to understand what dry skin is, but scientifically it is a slightly more delicate question. As Song has shown, many criteria are needed to fulfil the criteria for dry skin, and Professor Uehara has shown that in dry skin there is always also some cellular infiltration. I do not know how you can say that this is dry skin and the other is not, and, secondly, perhaps xerosis is the most frequent combination with AD. Perhaps it would be worthwhile to look at this, too.

(A) The point was, if we see dry skin, the barrier function is undisturbed, which you can see in x-recessive ichthyosis, but if you see the scaly dry skin of other forms of ichthyosis, psoriasis for example, there might be a barrier impairment. Of course there is always a clinical question in atopics, whether this is already a dry skin or not. They are questions you can ask the patient: e.g. after you take a bath, do you need an ointment or not? And of course, there is your clinical way of seeing the dry skin.

Kiistala, R.:
The history of sweating in AD surpasses that of other common skin diseases and derives from

investigations of miliaria in AD. In the 1950's Sulzberger et al. were the first to suggest heat induced hypo- or anhidrosis in AD. They suggested sweat duct occlusion in atopic skin and a similarity with the sweating disturbance in miliaria. Although this hypothesis received support in the beginning, it was gradually discarded. In the 1970's studies concentrated on cholinergic local sweating stimulation. Contrary to Sulzberger's findings these studies suggested an increased sweating response in AD. Our own studies of this decade again suggest a depressed cholinergic response, especially in areas with dry-looking skin. At present, as the circle of studies on sweating is closing, I can show you results similar to those of Sulzberger in the 1950's.

My topic is thermal, i.e. heat-induced, sweating in AD. Studies on thermal sweating in AD are very scant and thus I refer to our own two studies. In Study 1 we tested the sweating response in AD to moderate thermal stress in 22 non-atopics and 26 patients with AD. In Study 2 with 26 non-atopics and 17 patients with AD, the sweating response was studied in the Finnish sauna. All subjects were male conscripts aged 18 to 29 years.

In Study 1 the patients with AD were divided into two groups: patients with normal looking skin and patients with dry-looking skin. A further subdivision of these patients was based on their sporting habits. The test-area was non-eczematous back skin. The tests were performed during winter time. The temperature of the test-room was 33°C and the relative humidity 23%. Sweating is known to start when the environmental temperature exceeds 30°C.

In Study 2 the mean temperature in the sauna was high, 79°C, and the relative humidity 12%. Both the non-atopics and the AD-patients remained in the sauna for 20 min.

A gravimetric method was used to measure local sweat secretion. The absorbent gravimetric pads were encased in transparent colostomy bags. In Study 1 the collectings pads were removed at 40, 60 or 80 min and in Study 2 at 5, 10, 15 or 20 min. After sauna, sweat collection was continued, and the pads were removed at 10, 40 or 70 min.

As it is not possible to present all results, only the cumulative sweat loss values for all collecting times in Study 1 will be shown. Median levels of the whole AD-group were significantly lower than in the control group ($p<0.02$). There were poor and profuse sweaters in both groups. However, sweat loss levels were lower in the AD-subjects at all intervals. The first 40 min sweat loss levels were about one fourth - and the later levels about one half - of the control levels, indicating a retarded onset of sweating. In AD the lowest levels were found in the groups with dry-looking skin.

Active participation in sports was related to a tendency of slightly elevated sweating response. However, a statistically significant difference could not be found between the sporting and non-sporting groups.

In the sauna, Study 2, sweating seemed to start more slowly in the atopics than the controls. In the controls, the initial 5 min and the 20 min sweat levels were about two-fold higher than in the atopics. During the recovery period after sauna, no further increment was observed in the sweat accumulation in the non-atopic controls. But in the AD-patients, the median sweat amounts further increased from the sauna level by about 40% at the post-sauna 10 min interval. The greatest increases in sweating in the recovery period were observed in the subjects with the lowest values in the sauna.

In conclusion, in these studies, the sweating response in patients with AD to moderate and to intense thermal stress was depressed and particularly the onset of sweating seemed to be delayed. Atopics and non-atopics involved in active sports tended to exhibit higher sweat outputs than non-exercising subjects.

Discussion

(Hanifin) Sulzberger hypothesized that there was a blockage of the sweat duct , and yet as you pointed out, cholinergic sweating seems to be increased in some studies. Do you believe there is a blockage, or do you think the reduced sweating is a physiological event?

(A) Already in the histologic studies, they could not show any blockage in the sweat duct orifice, but I regard it as a swelling of the stratum corneum.

(Bruijnzeel-Koomen) Were there any patients with AD who produced normal amounts of sweat?

(A) Yes.

(Bruijnzeel-Koomen) Was there a correlation with itch in those patients?

(A) We did not investigate that.

(Hornstein) I think it is a good example of what we call bioengineering techniques. Some people are trained to learn sweating, and the itching and sweating improve. You told us about military persons who did sports, and they improved better than the others.

(A) Persons who do a lot of physical training have more active sweat glands than those who are not sporting, and their sweat output is larger.

Hornstein, O. P.:

As we have heard, there is a problem about histamine response in the skin. Nobody has considered the fact that the amount of histamine is increased. There are many studies - several important ones in the 1980's - about this, and I think it is adequately confirmed, but what has remained obscure until recent years is how the nerves react, the itch receptors, and not the receptors to histamine. And perhaps you know that Gisela Heyer and others in my team work on this histamine induced iontophoresis, and that in comparison with controls, they found a decreased sensitivity to itching. It was highly statistically significant, and they did the same with substance P, and they found the same, and they had several studies I will not repeat, as I think they are known to you. So I think the method of applying histamine is important. Iontophoresis is another method as intradermal injection. This is the first point.

The other point is that rating of itching is important. How do you measure what the subject feels?

The second question is, what is itching, what are the sensations that the patients feel? There are different sensations. Some people report "sticking" (stinging) and even burning sensations, so we should concentrate not only on "yes" or "no", but what kind of sensation is experienced. I will devote the next 10 minutes to this, and will show some slides.

We have made another study by G. Heyer. We studied non-atopic eczema patients with eczema, and also controls, and a skin subject with atopic score prone to atopy and some with a history of atopic eczema following a flexural atopic eczema or repeated flexural atopic eczemas. There are quite significant differences in the itch rating between the controls; they had a better

rating than those with a history. So for us it was important to make this study not only during atopic eczema, but also after, and also on those prone to this eczema.

How do we do this rating? We train the persons for itching and burning. There is a scale, and they can, after a fixed interval indicate the point where they feel itch, no itch, maximum itch, and it is then removed. After the next iontophoresis application every rate is taken away, so we can exclude the possibility of self-induction. One should assess the quality of itching; here you see it is the same. As regards itching and burning, after one minute those with atopic eczema history reported both itching and burning; after 5 min the different groups are alike. During the first 4 min there is a significant difference between the rating for these different sensations.

We made another study where we asked: is histamine the chief pruritogenic mediator, or could it be some other? We took acetylcholine. It was given by injection, indeed, as a double injection. At 1, 5, 10 min, and also at the end of the study, itching was prominent, but at the beginning, the controls had only a sensation of burning, but no feeling of itching, while those of atopies had both. After 10 min controls gave no information and the atopics had only itch, so we have some indications that it is not histamine; it is another substance, probably acetylcholine. Another substance would be bradykinin - that would explain some late reactions in some of these patients. If you give bradykinin, and then make the same histamine iontophoresis, it increases the itch. So there are some conditioning mediators, or neuropeptides that influence the extent and in the quality of the sensation.

Now witht our hypothesis about this diminished histamine reactivity in atopic eczema, there may be a downregulation of histamine receptors on the C-fibres, there may be a hyposensitization of the C-fibres for histamine, there may be an increased turnover (as you know, histamine is increased in the skin), but there may be histaminase hyperactivity, and there are also some thoughts about a central nervous mechanism (for instance, placebo effects can only be explained by a central nervous mechanism) and there are other items to confirm the last possibility. So these are the four possibilities open for discussion at present.

As we heard from Dr Wahlgren yesterday, the term alloknesis was coined by an English scientist before the Second World War, but recreated by La Motte in 1988. *Allos* means other, and *knesis* is a Greek word for itch. Alloknesis means that slight stimuli, e.g. a slight touch, elicit pruritus. And this slight touch elicits another fibre, as does histamine. It is a mechanoreceptor unit, it is excited, and the central nervous system may be involved. Now, just to explain a few terms: C-fibres or nociceptor fibres are unmyelated, slow-conducting peptidergic afferent neurons, they are responsible for pain and itch. A-delta-fibres, mechanoreceptors, have ten times faster velocity of conducting the sensations, they are myelinated, fast-conducting, are responsible for touch, pressure and Meissner bodies and so on. They are terminal receptors for these A-delta fibres. If we cause alloknesis, then we induce these mechanoceptors with A-beta fibres. This should not be confused - there is a different population and subpopulations of fibres in the skin, which are handling this. After the application of iontophoretically applied histamine, there is in normal skin an area out of the flare, and there is this feeling of itching. If you do the same in the atopics, they have diminished alloknesis: about half of our 20 subjects had no alloknesis. Twenty patients and 20 controls were studied: the histamine iontophoresis was always done with 20 mg. It was studied on the forearm and the scapular area at the same time, and induction of alloknesis was done after 2 and 3 min intervals. Rating was done at one min intervals. Then, after 8 min, a cold stimulus of 10 sec was given,which rapidly alleviated the sensation of itch, as is known, but before this, there is also a decreased sensation, and decreased alloknesis.

So, what is the message? Significantly smaller alloknesis areas in 9 patients, no alloknesis in 11, also histamine-induced itch, and before the cold stimulus, significantly diminished at back. The

area of the flares was, of course, also reduced. So it was the only way to show what we should do in the future about itching. Itching is not the same, as it depends on the method, it depends on the rating, and it depends on the quality of the itch. There is also a mixture of what you feel, as atopics often report: the report on itch, and on burning. I must confess, before I did not ask. And when I did ask, I was astonished to find how often they report another feeling of itching.

Discussion

(Rajka) I am impressed by your results; I only have one additional explanation. If you give histamine repeatedly as you have done, you may perhaps even expect some tachyphylaxis.

(A) Yes, we did it twice, and there wasa greater response reduction. Tachyphylaxis would occur if you gave it repeatedly, very often. We did not do this. We did it together with bradykinin, together with acetylcholine, and my message is: acetylcholine would be a major candidate for itch - but not histamine.

(Wahlgren) During the 1950's, it was stressed that if you injected histamine too deeply intradermally, burning was a rather common sensation, rather than itching. Now, if you deliver histamine by iontophoresis, how deep do you get the histamine delivered into the skin? If you deliver it very deeply, can that explain the diminished itch response?

(A) Maybe the reason why we switched to iontophoresis instead of intracutaneous injection was the inequality and intrapersonal variability of the technique, and perhaps, even if we try to give it intra-epidermally, there may be a reason for this other feeling. It is known that if you give histamine subcutaneously you do not feel itch, but pain, so it is surely an effect of the different nociceptors and the different A-beta and C-fibers. It is surely both an anatomical and a physiological response.

(Wahlgren) That was what I meant. If you have a defective barrier on the skin and apply histamine iontophoresis into a patient with AD, then maybe histamine penetrates better, and then you perceive burning and not itching, and you get a less pronounced response.

(A) That was why we repeated our study after the eczema. In clinically normal skin, in atopics, of course, there was a reason to exclude this hypothesis. It could only be a factor of faster penetration.

(Aoki) I will just tell about our data, which are not published yet. Following histamine injection, we monitored the skin temperature with a thermograph, but this reflects rather deeper skin, and the result was that the temperature increase occurred at places around the injection site. It appears like a spot, and then it becomes confluent, but the maximum temperature was just the same in atopic patients and in control subjects.

(Hanifin) What technique did you use?

(Aoki) Infrared.

(A) Yes, we did the same, and we found a diminished area, and only a small increase in temperature in comparison with the controls. The controls had an increase of about 0.5%, and the atopics had an increase of between 0.5 and 0.1%. They had an increase, but only a very small one. I think all these studies need a control group.

(Ring) These are all very important works, but one question: how long does itch last with the iontophoresis.

(A) It lasts about 10-20 min.

(A) I am sure that a mixture or a cocktail of cells and of cytokines is involved. What I said initially is, we are playing with many, many cytokines, but we also have to look at the piano, is it mistuned? I think eosinophils and granules, neurotoxic granules, they all have an influence. But I think this is not the only modulator, there are many involved. I think we have to study this problem from two sides, from the cytokine/mediator side, and the receptor side where the neurofibers are.

Subject: Diagnosis and severity
Discussion leader: **F. Ring (Germany)**
Panelists: **T. Langeland** (Norway), **J. F. Stalder** (France), **A. Taïeb** (France), **D. Vieluf** (Germany)

Bonifazi, E.:
After receiving a lot of theoretical information yesterday, we now come back to practical, clinical problems. Today the session is devoted to diagnosis, severity, prophylaxis, and therapy of AD. The diagnosis of AD is usually very easy. Some problems, arise, however, when trying to assess or establish the severity of the disease.

Ring, F.:
The session called *Diagnosis and Severity* will be split into two parts. One part is, let us say, the results of "The European Task Force on AD", devising the common scoring system you know. Everybody may have read about SCORAD; it will be presented by the leader of this group, A. Taïeb from Bordeaux. *Special Aspects* will be covered by Jean Francois Stalder, then Tor Langeland will talk to us a while about the problem of the influence of duration and course of the disease when estimating the severity. Then we shall leave scoring and devote the rest of the time to the procedure we call atopy patch tests, which Dr Vieluf will tell us something about. Now I would like to ask Dr Taïeb to start.

Taïeb, A.:
On behalf of the "European Task Force on Atopic Dermatitis" I will present the method we use to assess the severity of AD. Our group began to work in 1990 in Bordeaux on the occasion of the 3rd Congress of the European Society for Pediatric Dermatology. It comprises a majority of pediatric dermatologists but also dermatologists interested in adult disease.

Our consensus paper (Dermatology 1993; 186: 23–31) was the result of several work sessions to agree on definitions and basic methods for assessment of extent, intensity and subjective symptoms. To help investigators, we have included an atlas featuring five out of the six items chosen for grading intensity, namely erythema, papulation/edema, oozing/crusts, excotiations, and lichenifications. A 0–3 scale is used for all intensity items. Dryness was not possible to photograph adequately and is assessed on non-inflamed skin. Other intensity items are graded independently on "representative areas" corresponding to the mean intensity øf the item on three different sites.

For extent we have explored two methods prospectively: one based on distribution of each intensity item and one corresponding to the rule of 9. The rule of 9 was found more accurate because it scored extent independently of intensity and especially because the product intensity × distribution led to a majoration of scoring errors. Extent is scored after drawing the inflamed lesions on a front-back figure found on the evaluation sheet.

For subjective symptoms we have chosen two analogue scales corresponding respectively to daytime pruritus and sleep loss for the three last day/nights. Children above 7 years can answer directly but in infants and younger children the method is less reliable since parents usually upgrade symptoms.

The second question addressed by the ETFAD was about the creation of a cumulative index for AD which could summarize the current disease status of the patient in one number like a snapshot. This index was actually developed after a mathematical manipulation by Michel Poncet at Sophia Antipolis, and named SCORAD for Scoring Atopic Dermatitis (Arnold Oranje's

suggestion). To create this index, data from 88 patients gathered at 5 centres were pooled and analysed using the principal component method. The first component, which represents the linear combination of the parameters which has the largest variance among the 88 patients is a severity component. SCORAD was defined using the best regression of the 1st component versus extent, intensity and subjektive symptoms [SCORAD (0–103) = Extent/5 (0–20) +3.5 × intensity (0–63) + subjektive symptoms (0–20)]. The distribution of SCORAD was normal in the original series of patients and this has been verified in larger series.

Principal-component analysis also allowed us to extract a second component unrelated to the first one, corresponding to a clinical profile. It showed that for a group of patients with similar global severity, one subset has high subjective symptoms associated with erythema/edema but no/low dryness or lichenification, while another subset mostly has dryness and lichenification and low subjective symptoms.

Finally, I would like to emphasise that SCORAD is quite easy to use after a period of training. It takes around 5 minutes to fill out the SCORAD sheet. We are currently assessing between observer validations at scoring sessions and JF Stalder will give you an account of our findings.

Stalder, J. F.:
After publishing the SCORAD index, we needed to test it *in vivo*, so we organized to workshops. We organized the first one in Hamburg, organized by Johannes Ring, with children and adults, and the second one in Bordeaux with six children. I would like to give you briefly the first preliminary results.

The data from Hamburg first. You can follow on these curves the three items extension, intensity, and the SCORAD index. As you can see, there are some high scores that are fairly pessimistic, some optimistic scores with low index, and on these curves you can see it is probably more difficult to assess extension than to assess intensity. I would like to give you two examples from Bordeaux.

This 3 year old boy presented a high score in intensity as you can see here - the median extension rate was 34 with a large range from 21 to 60, a very high variance index at about 50%. The median intensity rate was 11.8 with a range from 10 to 14, and a variance index of ± 14%. Finally, the medium SCORAD was 57, and the variance index was 12%.

Second girl: Here you can see the extent. It is not easy to evaluate the surfaces involved. Here are the results: medium extension 24 with range between 12 and 44. Medium intensity 6.7 from 3 to 10, and medium SCORAD index of 41 < 10% variance index.

These are only two examples, and to summarize, here you have the six patients from Bordeaux, here you can see a global good distribution between 10 and 60 for the SCORAD index, and the variance index is globally not so bad; about 20%.

I would like to give you some brief comments. I think it is difficult to organize *in vivo* training. It is more difficult than to do assessment on slides. As you can see, there are high variations concerning extent and intensity. And the most reliable items for extent are erythema and edema. But finally, the global SCORAD index has a good value index < 20%. During the meeting yesterday, the Task Force suggested to organize training sessions and certainly to create educational material - brochures, booklets, or CD-ROM, on computers, it is probably better with a lot of slides, and finally to produce a spacial software for direct calculation.

Discussion

(Bruijnzeel-Koomen) Can you use it in adults too, and does it reflect the variations of the clinical course?

(A) Yes, it has been used in adults. The first session we had on seven patients in Hamburg was in adults - in fact you have seen the first sheet - it is about the same in variation as we have found in children. But we still have problems: Probably the point to improve is better education of scorers, there are still high scorers and low scorers. Your second question: there is some consistency in high scorers and low scorers. We had nine physicians attending the Hamburg and Bordeaux meetings, and there were consistently high and low scorers. So we thought, when the same physician is scoring the same patient, you have some consistency, but you may also reflect on the shift in the evaluation of the patient. We have now data on file on more than 200 patients, and the picture of SCORAD fits quite nicely with the evolution of the disease, but there is no way to take into account the respiratory symptoms.

(Ring) To make it clear, this has nothing to do with judging the overall severity of an atopical individual. This is a momentary picture of the skin lesions solely. That is the purpose of the score, otherwise you cannot follow the severity. We will come to the problem of overall assessment with Tor Langeland´s comment.

(Hanifin) I certainly applaud the effort to try to make a standardized scoring system. I think it would be wonderful, because we all do mostly pharmaceutical studies to try to find new treatments. The thing I like about it is the idea of having standardized photographs. I am impressed that erythema and edema are coming out consistently, those are the key things. With the other things, I have the same problems as Carla Bruijnzeel-Koomen had. It seems that it is awfully weighted toward the pediatric side, oozing as something which we never see in adults, and crust, but relatively less often edema and pruritus. The other comment I make is someone from the international side, and I think our Japanese colleagues will second this, then you would need a scale of about 12, because in Africans-Americans, Africans, Asians, the degree of lichenification is much more than your 3, so I think it does take an international group to really have the full spectrum.

(Ring) Well, we concentrated on caucasians. The ethnic problem is totally left out. It is a very good point.

(Gieler) I think you have made a very important work because it is a one-sheet paper. But I have two critical points that I would like to point out. I feel that firstly the extent has very great variance. And so I feel the 9% system is not very good, because we have made a very similar severity index as well, and we have made a whole figure system, so, for instance if you have an erythrodermia, it is very difficult to have this scoring index.

The second point is the subject of impairment. I think the two questions you have in your scoring index are not enough to look at the life quality or to look at the subject of impairment. I feel it is very necessary to include one or two additional questions for the subject of impairment, because coping with the diseases is as important as the severity. Perhaps Dr Taïeb can make a comment on this.

(A) I think your points are good. We admit that grading extension is quite difficult, and that should be improved. In this index, extent accounts for only 20% of the total score. It was 30% in the previous SCORAD, and the one that was proposed at the workshop here by Dr Hanifin. So this mathematical model gives 20%, and it is good, because it will reduce these great shifts in differences. But I agree, we have to improve in assessing this item. For the subjective symptoms we already made this proposal of about ten questions to assess the quality of life of our patients. For routine use, it is very difficult. We first use this overall analogue scale, but in fact it was highly related to pruritus, and this was a reason to discard it from the final SCORAD, and this was based on routine examination goals, and on mathematical considerations.

(Schäfer) We used the SCORAD in our study in the East-West German comparison, and we faced at least two uncertainties. One was, again, the visual analogue scale where the mother has to assess the status of the child, and this brings in a second subject of component. It was sometimes difficult for the mother to fill out the visual analogue scale.

The second was, the minor forms of atopic eczema where you very well knew the child had atopic eczema, but is in a very good state now and only has e.g. a little bit of lichenification which was pretty hard to assess, too.

(Ring) It is a fact. We used it already in epidemiology, this is again an important starting point. We count cases of atopic eczema. It was good, but now we give them means to differentiate the different cases. Europe is a continent of great variety, and it was not so easy, as you can imagine, to come up with a common score. There was a lot of disagreement, but we are all friends now!

(Langeland) It is very important to practice, because the nice results that we sometimes come up with are, of course, due to the fact that we are practicing. I think we should emphasize the fact that when this is fully developed, it will be useful when you get a lot of practice.

(Ring) This is very important. You should not just use it the first time and be very disappointed with the result. What we have in mind is national training centers in each country, otherwise it is no use, it will spoil the whole method. We have to invite people first to train with the slides, but slides and real life are two different things.

We could come now to the second issue which was raised by Dr Bruijnzeel-Koomen: How can we assess the overall severity, including the duration and the clinical course. Tor Langeland is going to tell us something about this.

Langeland, T.:
When entering patients into a study, we have to specify what we mean by mild, moderate and severe. For this purpose Rajka and I published a simple system for grading patients with AD some years ago. This system was designed so that, on the basis of a single consultation, we were able to make a rough descrimination between patients with AD, according to the severity of the disease. We used a sort of average, taking into account the course during the last year, and the intensity, by means of the influence of itch on the night sleep during the last month. In addition we used the rule of nine in order to estimate the extent of the dermatitis at the examination.

I would like to emphazise that this method is useful for a rough discrimination between patients with AD. If the need is to record changes in the activity of the dermatitis for instance during a clinical study, this method cannot be used. Then a more sensitive method should be applied. SCORAD was designed for this purpose.

Discussion

(Bruijnzeel-Koomen) I still have the feeling that if you use SCORAD patients with the same score show a totally different clinical picture. What kind of patient did you use?

(A) I think that when defining the clinical material, you have to use something more than these scores. Of course, you have to say whether they have respiratory allergies and something about their immunological status. Then you will have, in the group of severe AD, a great variety of patients. So I agree that you have to use more than just a simple score.

(Ring) I think if you want to bring it together, and you add your parameters of duration, you could come up with some additional system for the SCORAD, because I think the nice thing about the SCORAD, is not lost, you got all the information, and you can do pattern analysis as Alain Taïeb told us, you can put out only the lichenified lesions, only the dry, or the oozing ones, you can do all you want, you have all the information there, but you have a score in the end, and the essence of a score is that you count "oranges and apples" - it is never logical.

(Thestrup-Pedersen) I understand that this is a very difficult issue, and I like your point that you can have exactly the same score - number of points - but have a completely different clinical picture. And we all know the erythrodermic, the head-and-neck dermatitis, the papular forms of AD, and others. We even have the irritant hand eczema of the AD. Have you discussed if you want to bring a kind of clinical subdiagnosis into the SCORAD, or would you say, this is a head-and-neck, this is an erythema, this is a papular, this is a hand eczema, and add that to your SCORAD index?

(A) We had such considerations in the first consensus paper and, in fact, we decided to address the common presentation of the disease and to exclude black skin, prurigo lesions, and so on. It is now possible to have variance of the SCORAD index, Ann Broberg for instance has presented her paper about head-and-neck dermatitis, and she makes just small adjustments for extent, in order to break down the extent only to the upper extremity, and it works quite well. So it is possible to have variance for special clinical purposes, but overall, the system was designed for common presentation of AD, and I think, when you are a clinical investigator, you can make the effort to include similar patients.

(A) We cannot test with different SCORAD indexes at different visits. This patient is followed during four different visits, and we can add the different SCORAD in order to have a good evaluation of the severity of this patient. I think it is probably not so different from yours, because with this follow-up you can evaluate the global severity of the patient.

(Ring) So you see, we are only at the beginning. Creativity is called for, and dermatological knowledge to go on. We will leave SCORAD now and come to another aspect of diagnosis.

The diagnosis of this disease has two levels of wisdom. The first one is to diagnose the atopic eczema. Here we have the criteria of Hanifin and Rajka, and I think no one doubts that you can diagnose this disease. But this is only half of the story. The more important part of the diagnosis is to find out the provoking, or eliciting factors which influence this disease in the individual. This has been forgotten by many of our colleagues the last ten or twenty years. They just said, atopic eczema, steroids and emollients, that is it. The real part of diagnosis is finding out the underlying

causes, and one instrument in order to evaluate the role of aeroallergens or allergens is the so-called atopy patch-test, and Dr Vieluf from Hamburg will tell us something about this.

Vieluf, D.:
Studies with patch tests using aeroallergens in patients with atopic eczema have varied widely in the methods used. In the last years we tried to standardize the Atopy-Patch-Test (APT).

After withdrawal of antihistamines, systemic and topical (test area) steroids for at least seven days the test substances (1,000 and 10,000 PNU/g of house dust mite, cat dander and grass pollen in petrolatum or hydrogel) were applied for 48 hours in large Finn chambers on clinically uninvolved not treated back skin. The evaluation of test areas was performed after 48 and 72 hours. Grading of positive atopy patch test reactions was principally similar to the criteria used in conventional contact allergy patch testing.

Some patients showed questionable positive reactions with erythema without infiltration. In clear-cut positive reactions we saw mostly follicle-bound papules on an erythema with infiltration. Therefore we tested in all patients as a control not only the vehicles, but also sodium lauryl sulphate to control irritable skin.

The analysis of the results of the atopy patch test with two different vehicles (petrolatum and methylcellulosegel) in different allergen concentrations (1,000 vs. 10,000 PNU/g allergen) showed that the number and the intensity of positive reactions in the APT were higher with petrolatum as vehicle than with the hydrogel. Our results showed that the allergen concentration needed to elicit positive APT-reactions is more than 1,000 PNU/g allergen because we saw more often and more intense positive reactions to 10,000 PNU/g allergen than to 1,000 PNU.

We performed the APT in 53 patients, 37 women and 16 men, 3–69 years old, with moderate to severe atopic eczema and 14 controls, 8 non-atopic individuals and 6 patients with allergic rhinoconjunctivitis without atopic eczema. In addition we performed skin prick tests and determination of specific IgE.

We obtained positive APT-reactions in 23/53 patients (44%) with atopic eczema and in one patient with only allergic rhinoconjunctivitis, none in non-atopic controls. The most frequent positive reactions could be detected to house dust mite (20/23), in 14 patients we saw reactions to cat dander and in 8 to grass pollen. There were different reaction patterns in these patients.

The analysis of the data included the history and the distribution of the skin lesions. Positive atopy patch test reactions occurred more often in patients with atopic eczema predominantly in air-exposed skin areas like face, neck and upper extremities/hands. This was statistically significant in comparison with patients with skin lesions predominantly in other areas. The correlation of APT and skin prick test or RAST showed allergen-specific concordance of max. 0.53 (prick-test) and 0.69 (CAP-RAST). Some patients with negative skin prick test and/or RAST showed clear-cut-positive APT-reactions.

With a standardized atopy patch test the actual clinical relevance of IgE-mediated sensitizations for the eczematus skin lesions in patients with atopic eczema might be proved. The reproducbility of this test procedure has to be evaluated in further studies with aeroallergens in different concentrations in petrolatum.

Discussion

(Thestrup-Pedersen) Thank you for this interesting study. I must say that you have done a lot to standardize your tests. I would like to give you some results that we have obtained. If it is so

that you have an AD patient, you have clinical normal-looking skin, if you perform epidermal scrapings and you for example measure the expression of interleukin 8, you will find it in most patients. So normal skin and AD may not be normal skin. Secondly, by occlusion of atopic skin you do see an increase in the interleukin 8. This is the cytokine we have looked at specifically because of its chemotactic activity. So, just by occlusion, you can increase the cytokines in the epidermis, and I wonder if the reason why you choose the large Finn chambers is that you see a lot more clear-cut positive reactions, instead of having the normal ones.

(A) Yes, we see more positive and more intensely positive reactions with the large skin chambers. We made controls with only petrolatum, with only the hydrogel, or without anything Finn chambers, and we saw only positive reactions to aero-allergens. We have one patient who reacted to all the chambers, then we excluded it. We have really specific and dose-dependent reactions in these patients. I know that atopic skin is not normal in most of these patients, because most patients have severe eczema, but we tested them in a really stable phase and without visible skin lesions on the back. They were not treated with sterioids or something like that seven days before. We had many controls, and they reacted to only one or two of the allergens, some to three, but only in special areas and mostly to the 10,000 PNU and not to the 1,000 PNU. Therefore I think it is very specific.

(Thestrup-Pedersen) Did you try to include an irritant or proteolytic nonspecific agent?

(A) Sodium lauryl sulphate 0.5%.

(Businco) Did you count allergen in p.n.u. as you say? I think that is a rather unreliable way, because the protein nitrogen unit has been quite abandoned in the last decade. I think for such test a more reliable way of standardization should be used, such as biological unit, because there is a tremendous change in the response with evaluation of the batch.

(A) That is a real problem of standardization of allergens, but we have to use this because of the pharmaceutical aspect. We are very well aware of this point, but this is rather expensive, and you could not get micrograms of the Der-P1 for this test.

(Giannetti) In many patients with IgE specific antibodies, you found your test positive?

(A) In the RAST?

(Giannetti) Negative RAST patients and positive atopy patch test.

(A) Four patients = 10%.

(Giannetti) We find that 50% of patients without IgE antibodies as deposited in our series have no irritant reaction at all, and I can confirm it by using a specific measure analysis system.

(Ring) These are the most exciting patients, and returning to what we heard yesterday, those are the ones we are looking for, with reactions in the skin and not in the blood.

(Bruijnzeel-Koomen) Concerning the specificity, we have tested at this moment I think 100

nonatopic controls, and none of them showed a positive patch test reaction, and in a group of atopics without the AD - this group consisted of 35 patients - there were only three positive reactions, two of them were urticarial, disappeared after two hours, and one persisted to 24 hours.

(A) I hope that one time we all use the same tests, because I think it is necessary to compare the data.

(Hornstein) Why did you use 48 hours instead of 24 hours - firstly, you have an occlusional effect, and secondly, you can only follow the crescendo or decrescendo for another 24 hours. During these 48 hours there are many reactions going on under the patch, e.g. the urticarial reactions you told us about, flare, and follicular reactions. I think it would be better to test open or to test after 24 hours and not 48 hours.

(A) We tried all, and looked at the tests after 20 minutes, 24 hours, 48 and 72 hours, and our best results were after 48 hours.

(Reinhold) I find your one patient with rhinitis and positive reaction very interesting. I would like to ask whether this patient ever had a skin disease before, and do you remember the age of that patient?

(A) I do not remember the age of the patient, and we think that he might not have known that he had a little eczema in childhood.

(Diepgen) You showed a very interesting slide, in which only 6 out of 36 patients showed identical reactions. I think that this is a very important point to make. Make more patch tests and comparisons with a huge number of controls, which is also important to find out if it works or not.

(A) The identical reactions were for petrolatum.

(Diepgen) Yes, but you do not have the golden standard, so it is very hard to judge, is it really allergic reactions or is it irritation?

(A) No, I think it is real allergic reactions, and the vaseline is the best vehicle.

(Ring) The problem is that if the golden standard does not exist this would be a provocation test. But tell me how we can do a provocation test with house dust mite and eczema?

Subject: Prophylaxis
Discussion leader: **A. Broberg** (Sweden)
Panelists: **L. Businco** (Italy), **U. Gieler** (Germany), **B Kunz** (Germany), **K Yamamoto** (Japan).

Broberg, A.:
We will now start the session about prophylaxis. First I would like to introduce to you the topics of prophylaxis, which we of course have touched many times during these two days. There are many ways of looking and speaking about prophylaxis, and this is just my way. Of course, it all starts and ends with the family, the patient we meet in the practice. I just want to highlight the question of prophylaxis by telling you the story of a mother. She is married to a man who had a very severe atopic disease and eczema from one year of age. Today they have a son, and he is one year. Just before I left for this conference, she said to me: today of course you know more about AD, so please can you help prevent that our child will have as many problems as the father? And I just want to highlight the amount of knowledge we have gained during the recent years. So I looked to see what I could read in the journals from when the father was one year old: that man when atopic does have peculiar antibodies present, and these reaginic antibodies are present in all tissues and in the blood. It was just before we were to name IgE. Thirty years later, when the child is one year old, this is a figure of some of the immunological events that can occur. And today we know even more. So of course we have gained a lot of knowledge during the 30 years between the one-year-old father and today. So I think the roles of the dermatologist and the pediatrician have changed a lot during the years, and of course, this is for us well known. It begins with the AD, and I think it is when we meet the patient for the first time, it is before the respiratory allergies have started. This is so well known, and we will not discuss it further. But the hand-out you all got is a reference list of all the problems that can occur in the AD patient. When we have a problem, we start to think about how to prevent the problems, of course, and these are some of the problems which perhaps we can highlight during this session.

If we would like to discuss each item, we can start with: What kind of knowledge do we want to give the patients, or our risk group patients, and how are we going to communicate to give them this knowledge? A third question is, of course, what group of patients do we want to give this information to?

I would now like to introduce Barbara Kunz, and she starts with the risk group. The criteria for the definition of the at-risk baby for AD.

Kunz, B.:
I would like to focus on the target group, as Dr Broberg told you. If we want to do primary prevention, we have to find reliable criteria for the definition of the *at-risk group*, the group of patients whom we will give the advice to do primary prevention. The classical criteria used until now have been, of course, a family history of atopy, and the cord blood or neonatal serum IgE. This seems rather simple, but as we get into more detail, it gets more complicated. I would like to start with the cord blood IgE, the studies done on the cord, the predictive value of cord blood IgE. This topic has been studied extensively for 20 years, and during the 80's there were several studies that showed a good predictive value for cord blood IgE for atopic eczema. The specificities are not listed here but were in all the studies quite high, and also a good sensitivity was found. The predictive value found in these studies was around 70%. However, later studies have found quite contradictory results. The sensitivity was as low as 9% in a Swedish high-risk group. Most of these studies are based on unselected newborns, and two of them, Hartevig and

Roos, studied high-risk infants with a double parental history of atopy. Also in these populations they found a very low sensitivity, always with a good specificity. The predictive values were as low as 26% for AD. So, in conclusion, there is a vast variability among the study populations, among the methods used and among the results achieved. But it seems that cord blood IgE cannot be used as a screening method for the evaluation of at-risk groups.

Some of the investigators here have tried to improve the predictive value by combining different markers, such as combining the cord blood IgE with family history. This, indeed, increases the quality of the predictive value, but it increases significantly the sensitivity. So, since cord blood IgE is not reliable enough, other markers have been proposed to be a predictive value, and as you see here, most of the immunological alterations present in atopic eczema have been investigated and have been found to be altered also in cord blood. But, the drawback with these studies are that most of the methods are expensive, laborious, time consuming, and not suitable for routine use.

Furthermore, only in a few of these studies has AD been specifically addressed, as for instance in the study of eosinophils. Unfortunately, the predictive value has been shown not to be superior to cord blood IgE. So, none of these methods have been proven to have good predictive value up until today, because most of these things have not been investigated for their predictive value. A promising candidate seems to be the soluble CD23. There is one study from Japan, which showed an increase. There is a working group in Hamburg who is currently studying this marker, and maybe in the future this will become available.

So as of today, we do not have any alternative biological or immunological markers for AD - no objective markers. So what we do in clinical practice is - this has been dealt with in the past days. I would like to discuss with you whether any of you use a score like that. Schellmann proposed in 1984 his family allergy score. He gives 2 points for an obvious atopic disease in first-degree relatives, and 1 point for probable atopic disease in first-degree relatives. So he arrives at a score. If the score is above 4, he proposes a prevention program. If the risk seems to be medium, he proposes to do a cord blood IgE determination. As I told you, this is a debatable point, but I would like to know what you do in your practice.

Finally, the most reliable criterion still seems to be the family history for atopic eczema and here I will show you a slide from the review by Dr Schultz Larsen, showing the empirical risk figures for atopic eczema, depending on the genetic load the infant brings. This means that double parental history, of course, shows the highest risk, and the range shows you that it is of importance for the type of atopic disease that is present in the parents. So if you have a biparental history of eczema, the risk shifts to the higher level. If you have a respiratory history, the risk shifts to the lower level for atopic eczema.

I think this is the mainstay of atopic eczema we should cling to in deciding on a definition of the at risk for atopic eczema prevention programs.

Broberg, A.:
The next speaker, Louisa Businco, will speak of the role of diet during the period of lactation and during early life. I think the diet problem is one of the main questions in the prevention of the IgE priming in the child.

Businco L.:
In my very short talk I will try to convince you that at present it is impossible to prevent the onset of AD in *high-risk prone babies*. The first question that we would like to answer is this: does maternal avoidance of allergenic foods such as cow's milk and egg during the period of lactation

prevent the development of atopy in the baby? There are several well conducted studies which clearly show that this really may occur. The Linköping group showed that babies whose mothers received a diet during the period of lactation had significant less prevalence of AD early in life, at six and nine months. At 12 months, there was no significant difference, however, the babies whose mothers had an avoidance regimen during the period of lactation, still had less AD. Now there is a follow-up of this population at the fourth year of age. It clearly showed that at four years, the prevalence of AD was significantly less in the group of babies whose mothers received a diet during the first three months of lactation. I would like to point out that the offending foods that were eliminated were cow´s milk and eggs. Of course, everybody knows that allergens can be transferred through the milk to the baby, but I would like to point out that the amount of such potential antigens is extremely low in human milk. For instance, it has been calculated that 40 ml of a cow´s milk formula contains betalactoglobulin in amounts equal to the amount provided by about 19 years of nursing one liter of human milk per day. That is an important point for pediatricians, because you know, very commonly newborn babies in the nursery receive the so-called "Idem" bottle of cow´s milk formula before the onset of lactation. So, this is tremendously low in terms of potential allergenicity in comparison with human milk.

As you know now, there is general agreement that the best thing for babies at high risk for atopy is to be exclusively breastfed for the first six months. We are working on this in a large program for the prevention of atopic diseases in children, and we are chairing a multicenter study and all the yellow points represent maternity hospitals which collaborated in this program. At the present, 2,200 high-risk babies have been enrolled in this project.

In the four year-olds study group the children received prolonged breastfeeding and selected weaning after six months of life. At the last follow-up, there was a significant difference in the prevalence of AD, and no difference in the prevalence of respiratory allergies, although asthma seems to be less in the study group.

These results are in agreement with other studies performed in other countries - especially in Sweden, and USA, California by Bob Sager. I would like to stress that I think the main preventive measure, in addition to breastfeeding, is the selected weaning and no solids before six months of age.

The problem is, when breastmilk is not available, what is the best formula for feeding such high-risk population? Of course, we know that cow's milk contains such large amounts of potential allergens. As you know, recently so-called hypoallergenic formulae have been developed with the aim of feeding such a high-risk population. Recently, The European Society of Pediatric Allergy and Immunology has delivered a position paper on hydrolyzed cow´s milk formulae. I would like to point out that there are different types of such products. Some of them are extensively, some of them are partially hydrolyzed. The partial ones contain as this position paper stressed, a large amount of non-degradate cow´s milk proteins which can be notably allergenic in an already sensitized individual, but even immunogenic in a predisposed host as a newborn at risk for atopy.

I would like to stress, as I have already mentioned, that some of these products which are delivered and marketed as hypoallergenic, are not hypoallergenic, and they can be harmful when given early in life to a predisposed baby.

What is realistic to say on the prevention of food allergies? According to recent studies, we can say that it is possible to obtain a lower prevalence of food allergies, including AD. I would like to stress early, because all the studies that have been done are now too young to conclude that it is possible to prevent for life food allergies. It is possible to have a milder severity of the disease (that the disease will develop in a milder degree), especially of AD. Finally, an important point,

it is possible to obtain an early diagnosis, and therefore an easier identification of the offending food. I would like to give some conclusion for an high-risk mother: Stop smoking during pregnancy. Eat whatever you like during pregnancy, because avoiding certain foods does not seem to be useful, avoid cow's milk during lactation, no solid foods for the first six months of life.

I would like to conclude my presentation reminding you of the fascinating legend of the two twins, Romulus and Remus. As you perhaps know, the babies were abandoned by their mother on the bank of a Roman river, the Tiber. And they were fed by a wolf. Despite this very strange way of feeding newborn babies, they grew healthy enough to be able to build the wonderful city of Rome where I was lucky enough to be born and to live, thus stressing that perhaps human beings are very strong when it comes to overcoming many difficulties. However, in modern life, the environment is so different from that of Romulus and Remus, and there is no doubt that for high-risk babies now, the type of feeding should be much more different than that of those times.

Broberg, A.:
We all know that Rome was not built in one day, not even the prevention program. The next speaker is Professor Yamamoto. We have now come to the practical point of the issue, how to manage the prophylaxis in every-day clinics.

Yamamoto, K.:
In our country, Japan, there are materials, methods, and the so-called medicines directed to *prophylactic* use, and also therapeutic use on the market. But frankly, most of them are so-called "monkey business". For instance, in February this year, I had a twelve year old girl and the parents who brought her to my clinic and I asked why the lesion had become like that, and the answer was something like this: Six months ago, in the autumn, they brought her to some association. They found this association's name in a so-called health magazine, and in that paper they declared that you can treat your child with AD without steroids. So they brought her to that association, and I asked, how they got membership, and they answered something like: First of all you have to pay 1.5 million yen to get the membership, then they send you every day some so-called secret, hot-spring water to your home, and by using this every day, after six months you may have a good result, and your child's skin condition will disappear. So they started this thing, and after six months, that was in February, they realized that it had had no effect, and also that the lesion had become much deteriorated. So they visited the association located near Tokyo, but the office had disappeared. So the lesion has not disappeared, but offices can disappear after six months. You often see this in Japan when it comes to the business of prophylactic treatment.

Today, I am going to show you some of the methods used to prevent an atopic condition. But this is not monkey business, it does work well. By using the tape stripping corneocyte investigation, by using scanning electromicroscope, there is a disturbed outermost layer of the stratum corneum in patients with AD. So you can find out easily the pathogenic substance will pass through the outermost layer of the patient.

In Japan and other parts of the world you find many mites living in homes. So you have to eliminate mites, and there are many ways to do that. But I will show you only one example. This is a so-called MicroGuard (AllerGaud) fabric; the appearance came from the electronmicroscopic findings. If you use this type of fabric, the mite and dust cannot pass through to the outside or inside. So, you can use this for the culture of the mite, because the standard mesh or fabric is easy to pass through for the dust and mite, but if you use these special types of fabric, they cannot pass through.

Many children like to play with dolls, and we can collect many mites from this type of dolls.

Many companies now make the dolls with this type of special fabric and they even use this special suture, so this way the mite cannot pass or move on. And also, for the bed materials, we can find pillows, covers or mattresses that are made out of this type of fabric. At least you can think about letting the babies or children have much better circumstances if you compare this with the slide I showed before.

I would like the AD patients to have at least a little bit better sleep during the night. This method is not 100% complete, but you can add many examples of this type to prevent AD. We can do much more with the environment, and this way the children may have a good life.

Broberg, A.:
We are now going to hear about an AD prevention program as it is used by Uwe Gieler.

Gieler, U.:
When I as a clinician read papers about the immunology reactions and receptors and mediators, I wonder about how I can pass this on to the patient, in order that the patient may not only fare better but also cope better with eczema. Our idea was to put these four steps together: dermatological treatment, allergy, nutrition as well as dermatological education, and some kind of psychological treatment for coping with the disease together with the patients. This was the start of our prevention program. I will show you the study which we have carried out for the last five years.

We tried four therapy conditions with 120 patients, and it was a randomized study, matched for age, sex and severity. We made a follow-up at one year, two years now, and we have just started the five year catamnesis these days. Design: after recruitment, four treatment groups were established and one control group with only 12 months' follow-up. The contents of the prevention program was to show what is going on in prevention, and what is the best prevention program possible for the adult patient. We made 1) a relaxation training program, 2) a dermatological education program, and 3) a psychological cognitive-behavioural training program. The fourth group was a combination of 2) and 3) - we put together the dermatological education and the psychological training. We expected this to be the best one, but you will see that it was not.

It was a prevention program for our patients, so we put together 5-8 patients once a week for 12 sessions. So we needed 3 months to bring this prevention program over to the patients. We started with these 4 groups in one month, and 3 months later with all of the groups. At first we thought it would be a problem with subjective measurements. We started with this rating index because the SCORAD index did not exist when we started. We made a questionnaire about coping with this disease, to look for the subjective impairment of life-quality. The questionnaire was standardized for five scales: helpnessness, social stigmatization, psychosomatic complaints, deficit in problem solving, as well as impact on quality of life. The quality of life is what is now discussed all the time, but the scale is unimportant. The questionnaire can be used for psychological therapy as well as atopical treatment. You can use it before and after treatment for an individual patient, you can use it for a regimen, or for large groups; this is our standardization for atopic patients. There were nearly 300 patients. If you compare it with psoriasis, for example, you see a very special clinical finding which I am sure you know already from your clinical experience: the social stigmatization is significantly higher in psoriasis patients, although the helplessness and the psychosomatic complaints are much higher in atopic eczema patients. We use this questionnaire to find out not only the severity changes, but also subjective changes, subjective impairment. This questionnaire also makes it possible to examine discrimination subgroups, and in clinical practice.

I will now present the results of our study at the 3 month and 1 year follow-ups. There have

no significant differences in any of the prevention program groups, and the only significant difference was between the four groups and the control group. So you can say, the prevention groups are better regardless of what they do; much better than the control group, or standard or intensive dermatological treatment.

We also examined the subjects after two years. It can be seen on the slide that the dermatological education fared a little bit worse, but there is no significant difference. On the severity index, we have the percentage of extent and the percentage of intensity. The questionnaire is quite similar to SCORAD, but it differs as to the extent.

My last slide will show what happens to social anxiety, only one of the subjective measurements - and it is the same result - we have very good outcome in the combination group, but it is not a significant difference compared with the other groups. I feel that relaxation training, for instance, is very effective, which was very against our hypothesis, but we have a significant difference as well compared with the control group, and there is no change in the subjective impairment or in the coping with the disease.

I cannot give you all the results, of course, of this very large study, so if you are interested, you can read some of the papers about the contents of the dermatological prevention program. (Ehlers A. Et al. Int J Behavioural Med 1:107, 1994; J. Clin Psychology 1995, etc).

Prevention of atopic eczema is much better than no prevention at all, or, only routine dermatological treatment. I think this study can show this, and I hope I will be able to show you the results of the 5-year catamnesis as well.

Discussion

(Broberg) What we have discussed at large is the research. We have communicated the research among ourselves and we are representatives of research consumers. You have to decide on the knowledge and the way of communication, and also what group is concerned.I would first like to ask Barbara Kunz: In our clinical practice, how do we find the risk groups? Regarding the cord blood, the ideal levels are perhaps not a way of doing it in everyday practice.

(Kunz) That is right. In everyday practice, we mainly rely on the family history of atopic diseases, and especially in the families of AD. A family history of two first-degree relatives, mainly the parents, is the group that is prone for primary prevention. I have to admit that we are not often in that situation, because the patients that come for primary prevention are very often seen by pediatricians, not by us dermatologists.

(Broberg) I would like to comment on that, because for example in one family, the father has been a patient for a long time, and I think that you can prevent many things before the child is born. You should discuss with the father, for example, not to have a pet. In Sweden, many investigations have shown that more than half of all Swedish families have a pet or go horsebackriding, so it is advisable to try to advice the family not to have an animal. It is easier not to buy the animal than to get rid of it. I think it is important to find the risk group before the child is born.

(Businco) Although I am a pediatrician, I am still convinced that dermatologists have a crucial role in this field. My feeling is that a dermatologist is the best person to select the future mother or father of a high-risk baby. Therefore I think that the primary selection should start before pregnancy. You see the patients, and that is very important.

(Bruijnzeel-Koomen) Which criteria do you advise in a prevention program?

(Businco) The criteria we stress when starting a prevention program for a pregnant mother are the following: We inform about some dietary measures which are: prolonged breastfeeding for the first six months of age, with total avoidance of cow's milk during the whole period of lactation; only during the period of lactation. Then: no solids before six months, then highly potentially allergenic foods such as eggs, fish should not be given before the end of the first year. In addition to this dietary advice, we inform them about some environmental measures: no smoking and thorough cleaning of the house in order to reduce the proliferation of the house dusts mite; no pets, and, in addition, if possible, we advice no dependence of day care centers.

(Broberg) A program presented at the American Academy of Allergy in 1994 is really something that you can work out from for these families.

(Businco) This has been published in several papers, e.g. in the position paper of the European Society of Pediatric Allergy and Immunology, which has been has been published in the *Pediatric Allergy and Immunology* (the first issue this year).

(Broberg) I would like to discuss prophylaxis more. We do not only have food allergy problems in the atopic patients, we also have respiratory allergies. In a recently published paper, Atherton highlighted asthma in the AD patient, and also, in the same same journal there is an article about hyperreactivity in those children. I would like to ask one of the panelists to answer this question: What is important, what can we do? We know this hyperreactivity, and we try to eliminate the mites, and we have heard Professor Yamamoto. Can we do anything else?

(Yamamoto) This is a very difficult question to answer. For instance, as you know, in our country, social medicine covers the health care costs completely. Actually, there are many systems of social medicine. First of all, you have the disease, we give the patient an examination, then we treat. The prophylactic aspect sometimes does not belong to the clinician.

(Broberg) Now we go on to the communication of the knowledge. I would like to ask Uwe Gieler about the prevention program. Is you prevention program also used for children? Would you like to comment on when it is suitable to use a group, because this is a matter of how many people can enroll in such a program, and the cost of such a program.

(Gieler) This is an important point. We have very similar studies with parents of atopic eczema children, but not in groups, only parents of one child. We have quite similar results. Of course, I think it is better, and the costs are reduced, if you gather the patients in groups and can inform them collectively, this is better than having to say the same things twenty times a day, as happens when you have out-patients. I therefore think that such prevention programs are related to cost/benefit. In addition, I think that the pediatricians and dermatologists have to go through the same steps as for asthma education or diabetes, with prevention programs for *our* patients. That is my aim.

Of course we had very high costs, because it was a scientific investigation. But I would like to stress two points. I feel it is very necessary for the outcome of the eczema of all patients to learn how to put the ointment on the skin. We know from the studies that we have compliance of about 50%. Only 50% are going through the skin, and these 50% are mostly not going in the right

way. What we can teach, and we have done this in training, is how to actually put the ointment on the skin, and which ointments are necessary. For instance, as I have explained to the patients, they need four things at home: an antiseptic, a hydrocortisone, a fatty and a non-fatty ointment.

The second point is, to reduce stress. We know that nearly half the patients are stress responders. If they are have individual stress, they will experience exacerbation of the eczema, and we have to reduce this stress. We were really astonished at the results that the relaxation training was so effective, so, I emphasize that it would be very helpful in atopic eczema as well.

(Broberg) Can we involve other disciplines in the prevention program? I am thinking about dietitians. Another thing is, in Sweden we have an allergy society, and I know in other countries, for instance Great Britain, they have a national eczema society, do you think it is useful to use the society for a prophylactic program?

(Gieler) Yes, I think so. Of course, there are difficulties, and different countries will work differently. I feel it is necessary to have a dietitian and a psychologist with me, but I think it depends on what possibilities you have in your own city - there are a lot of practical concerns. It is better to have four or five persons, but normally you have only one.

(Thestrup-Pedersen) I was very interested in the results you presented, Dr Gieler. But if you try to analyze these results, is not the most important thing to have a concerned physician?

(Gieler) Yes, you are quite right. What I have not shown are the results from the parent studies. Because the physicians who had treated the patients also had carried out the adult studies, they were so well prepared, and we had no differences between the parents of the normal treatment group and the preventive group.

(Harper) I would like to bring you back to diets, because I think it is really a fundamental issue. Are you suggesting that this is a protocol only for those who are the most at risk, for instance when both the parents are affected? Because there is a worry here, that if it was a recommendation for pregnant mothers, that is, there is a total avoidance for these things, and you start challenging these children after a long period of time, you definitely put them more at risk of severe reactions after the age of one.

(Businco) We give thus advice only to high-risk babies. According to our large experience, we have never seen a systemic anaphylaxis when babies were first fed with the solids following the first sixts month of age.

(Harper) You are only restricting cow's milk and eggs, and in the group that I have seen which have really severe eczema, where the mothers have already been put on a dairy product free diet, and this is not working. Sometimes when they stop breastfeeding completely and their babies go on to a hypoallergenic milk, this really does work, suggesting that there are many other foods and substances that these babies could be allergic to.

(Businco) Yes, this could of course occur, but it is a very rare phenomenon. If you try to see what is really happening in some of these babies during the first days of life, very frequently you find that they have received some supplementation of a cow´s milk formula. I would like to stress that, at present, we have hard scientific evidence that it is possible to prevent early-life food allergy and

food induced AD, but at present we do not have any evidence that it is possible to prevent respiratory allergy.

Subject: Therapy

Discussion leader: **J. M. Hanifin.**

Panelists: **P. S. Friedmann** (England), **J. Harper** (England), **J. Krutmann** (Germany), **B. Melnik** (Germany), **M. Morren** (Belgium), **U. Reinhold** (Germany), **E. Søyland** (Norway), **J. M. Hanifin** (USA)

Hanifin, J. M.:

We have a busy agenda. We will start with Dr Morren, who will talk about diagnosis and management of contact allergy to corticosteroids, which is an increasingly important part of management. Then John Harper will talk about traditional Chinese medicine, Peter Friedmann about treatment and withdrawal of cyclosporin A, John Krutmann will talk about UVA effects on TH-1, TH-2, Uwe Reinhold will talk about gamma interferon, and then B. Melnik will talk about essential fatty acids, as will Dr Søyland, and I will finish up with phosphodiesterase inhibitor therapy.

Morren, M.:

When a patient with apparently classical atopic eczema, does not respond to an appropriate treatment with topical corticosteroids, one has to consider the possibility of corticosteroid allergy.

Important reasons why the extent of corticosteroid contact allergy has been underestimated in the past are: that corticosteroids are naturally present in the body, what placed them beyond suspicion, and that the anti-inflammatory capacity of corticosteroids masks the allergic reaction to it. The clinical picture is almost always misleading: it is exceptional that acute reactions occur, mostly a slight aggravation of the lesions or failure to respond to a correctly performed topical treatment are the only signs. Changing to a more potent corticosteroid frequently does not result in improvement because cross reactions between different molecules are common. Poly-sensitisation, e.g. to components of the base, is another pitfall. Optimal testing conditions for the vehiculum and the concentration, have until now not been found. Because of the anti-inflammatory capacity of corticosteroids it is very important to perform late readings, as late as five to seven days after applying the patch tests. There are so many different corticosteroid molecules - more than 50 - on the market, and therefore it is mandatory to find good markers to trace the whole range of corticosteroids in a first phase of testing. Based on a statistical study and a computer-based conformational analysis, we suggest tixocortol pivalate as a marker for hydrocortisone and prednisolone allergies and budesonide - by far the most common and potent allergen - as a marker for the acetonides such as triamcinolone acetonide and amcinonide but also for the esters like hydrocortisone-17-butyrate, alclomethasone dipropionate and prednicarbate. However the most recent data suggest that hydrocortisone-17-butyrate allergy is not infrequently unrelated to budesonide allergy and should be a third marker molecule. It is also important to test all the corticosteroid preparations the patient has used, and if possible all the ingredients separately. When these markers are added to a standard series more than 90% of contact allergies can be detected. When a contact allergy is detected to one of these molecules we advise to test all the different corticosteroid molecules in a second phase, one month later, to allow correct recommandation as to which corticosteroids can still be used.

At our department corticosteroids are among the top ten of most frequent allergens. It was a complication in the therapy of generalised atopic eczema in seven patients, among them four children, of merely atopic hand eczema in another ten patients and of allergic rhinitis in three patients. In four of the patients with generalised atopic eczema corticosteroid allergy was not suspected before testing but found because we tested markers in our standard series. Considering

the frequent use of topical corticosteroid preparations in atopic eczema, this complication is however not frequent.

The management of corticosteroid allergic patients is not easy. If the suspicion arises of corticosteroid allergy betamethasone valerate, betamethasone dipropionate, flumethasone pivalate and the more recent momethasone furoate in a simple petrolatum base can be recommended, awaiting the test results. In our experience these molecules are far less allergenic. When the allergy is objectivated by the tests and the responsible allergens are found, a list with the preparations the patient cannot use any more can be handed out.

In conclusion we can say that corticosteroids are still the cornerstones in the treatment of atopic eczema. However, in therapy resistant cases and in those patients who flare up during treatment, we should not forget the possibility of corticosteroid allergy.

Hanifin, J. M.:
I think we are going to have to hold questions and have discussions in the end, but I think this problem of contact allergy is a very real one, and I think we miss a good deal of it. Of course, steroids are the most insidious of this group, and I think managing them and the suggestion of using a few of the less frequent allergens may be the most practical management approach. Next we will go to John Harper, who will update us on traditional Chinese medicine therapy for AD.

Harper, J.:
The Chinese are not only good at proverbs; they may also hold the key to a better treatment for eczema. You can imagine my surprise when patients with very severe eczema, problematic eczemas, were sent to us and many of these eczemas were suddenly dramatically better and first one, then repeatedly other parents were saying to us; we don't quite know how to tell you this, but we have actually abandoned your treatment, and we have turned towards Chinese herbs. Additionally, one tended to dismiss this along with so many other altaernative therapies, but it became more and more common, and other doctors were saying the same thing, and one had to take this seriously. At that time, there were only a handful of Chinese practitioners in London, and one here, which was the one which we have liaised with, was able to prescribe a mixture of herbs which are taken as an oral drink, an oral tea, with dramatic improvement. I wanted to see what this daily dose of treatment looked like, and I was horrified, but there are a number of projects you can attempt to do scientifically, you can attempt to look at these in a double-blind placebo controlled trial, and the other area that we will be working in the continuing of our program of research is analysis - what is actually in these herbs. It is a minefield with many different compounds pharmacologically.

These observations led to the original letter published in *The Lancet* by myself and a group of workers at The School of Pharmacy in London, including a professor of pharmacognocy. He is the only professor of pharmacognocy in England, i.e. the study of plant medicines, and he has continued a close collaboration from a phytopharmacological analytical approach.

Over the next two years, Mary Sheehan and David Atherton at the Department started with a standardized formulation of the herbs that were most commonly used in a double-blind, placebo controlled study. They treated 47 children and showed quite clearly that it had a statistically beneficial effect over against placebo, looking at both erythema and surface damage. Then the study was repeated at another hospital, the Royal Free Hospital in London, looking at adults and with similar results.

My interest in this is, what is it in it that has this most definite clinical beneficial effect? The plant materials themselves contain a potpourri of chemicals, groups of chemicals that are listed

here. I have included the word steroids, but these are not biologically active steroids in the same way as we understand cortisone effects. The patients do not become cushingoid, and when we test plasma and urine cortisol metabolism, it does not seem to have an effect in that way, but the material does contain a significant number of plant steroid chemicals, which may or may not be relevant.

The interest in it has cascaded, and there is now quite a major public interest in this type of treatment. That in itself has brought problems, because we are now aware of the potential toxicity related to hepatotoxicity which has been reported. The mechanism of this hepatotoxicity is unclear, but obviously this aspect needs to be taken into account. It is therefore not a treatment which we would routinely recommend, it is very much done on a clinical trial basis.

There is a future here. I generally believe that within these plants there are chemicals that may well have a beneficial effect on eczema. We have demonstrated pharmacologically that the mixture as a whole is antiinflammatory, it is also sedative, and it is also antimicrobial. We need to do further studies, and perhaps at the end of this there will be new treatments for eczema. But on the last note; it is very easy as Western doctors to cast this aside. It is very difficult for us to understand the Chinese philosophy of treatment, but perhaps there is something there. When we think that when we are treating eczema we are using a mixture of treatments anyway, there is a similarity here, but perhaps one needs to save this subject for our next meeting.

Hanifin, J. M.:
P. Friedmann will now talk on therapy in withdrawal of cyclosporin. I think the withdrawal aspect is something I have had several questions about at this meeting, and P. Friedmann has been working with this drug for a long time. Hopefully he can answer these questions.

Friedmann, P. S.:
In order to be brief, I will actually go through much of this very quickly, because it has recently been published in the *British Journal of Dermatology,* and you can read the details there if you want to. We set out on this study at a time when many centers were picking up on the use of cyclosporin, and it is now, I think, well enough established in the literature, with many reports, and its efficacy as a means to suppress the inflammation of AD is well-known. The problems are how to use it safely. Of particular concern is the side effect of renal damage, that may be lasting. We started a study at the same time as others, to do a randomized, double blind, cross-over study - I think to help Sandoz get licensing approval, because it seems now that this is such an effective drug that to compare it with placebo is no longer a relevant thing to do. We took 24 patients and randomly allocated them to two groups, assessed them at two visits beforehand, and then at two weekly intervals for the area, the severity, and a whole variety of signs and symptoms. We quantified the amount of topical steroids they were using. We found that there was tremendous clinical benefit in all the people on the active treatment. They underwent a cross-over period so the first 12 started on active treatment and the other 12 on placebo, and after a certain length of time, they changed over. To give you an impression, this is a young woman whose life was thoroughly miserable with bad AD. After a few weeks on cyclosporin, she had seen Nirvana. And this is one of the problems which we are going to have: when we use this drug, people will want more of it, and therefore the question of how to use it in a way that will not destroy their later life qualities is very important.

Eight weeks on the drug, you can see that the active treatment has produced rapid benefit, and most particularly, the itching is almost all gone within two weeks. Then, after withdrawal of the drug, a very rapid return of all symptoms again, itching in particular, comes back most quickly.

The other group, when they change to the active treatment, show the same rapid drop. All symptom parameters get better very quickly. There were a few people who withdrew from the study for a variety of reasons, none due to significant side effects on the drug. Two had problems, one was found in the pretreatment work-up to have an elevated uric acid.

The next question was how to withdraw the drug. So we took all the people who had gone to that stage of the study without dropping out - 19 people, rerandomized them, and said, let us withdraw the drug in two different ways to see what effect there is on the return of the eczema, as well as on the continued safety profile. One group, having started on 5 mg/kg per day, which I suppose is now regarded as the absolute upper limit of what we would want to use. Every two weeks they step down by 1 mg/kg per day. The other group continue to take 5 mg/kg, but they increase the intervals between doses, so that going from this on an every day basis, they went to every second day, and then, two weeks later, every third day, and then to every fourth day, then to every fifth day, and then finally we stopped the drug. They had two weeks on each dose first. Before they started the withdrawal, of course, we had to get everybody on to 5 mg/kg/day for two weeks to restore remission. The people who had been on placebo for the last part of the study had to be brought under control again. The people who were coming off the active period were given another two weeks. So everybody was more or less on the same scale. We were reducing by 1 mg/kg/day, or 5 mg/kg every other day, every third, every fourth. Basically, there is very little difference in clinical results. Looking at the area of eczema involvement, not until we get to the very low doses does there seem to be some separation of the two groups. The same was really very apparent with the itch. But even, for example, at this dose with an average of 3 mg/kg/day or 5 mg/kg every third day, we were still observing excellent control and no side effects. The crucial thing is that here these people are taking, in the one group, 3 mg/kg/day, but these people are taking 1.75 mg/kg/day, average. So, for an equivalent therapeutic effect, they are on a much lower dose. And essentially, that is the message. Overall, we should now be exploring how techniques using alternative days or spaced out regimes. I think that long-term maintenance with those kind of doses is going to be a much safer and much better thing to do.

Discussion

(Kägi) I have a question concerning the follow-up of these patients. Do you have follow-up over several years now of these patients, and, do you have patients that are still on cyclosporin?

(A) These were short term studies, up to three months, or not much longer. We have done other studies where we have had people on for nine months continuously, looking for optimal dosage, on a daily basis maintenance, and we have found that with AD on a daily basis, one can normally maintain people on 2½ mg/kg/day. During that length of time - we have not followed them for many years at all; when we have had people in, it has been on an intermittent treatment course, and as far as we can tell, there are no lasting consequences from that. But new studies where people get repeated courses are beginning, and in our patients whom we have followed for about a year of continuous treatment in general we found no lasting problems. We have not treated large numbers of patients; there are many others who have treated many more people than we have.

(Thestrup-Pedersen) I am going to ask this question because I am concerned: When you give cyclosporin, do you treat the normal reactive T-cells or do you treat the diseased T-cells. So the

question is, have you or anyone else seen a development of pseudolymphoma in patients with AD - during or after cyclosporin treatment, which is a complication you see in transplant patients?

(A) We have not seen it in any of our recent studies. We had a patient who appeared to have it long ago when we were on to high doses in psoriasis; it was over when we stopped the drug. But that is not answering your question whether we are treating diseased T-cells or not.

(Hanifin) We have not seen pseudolymphoma, but John Koo has had one case of T-cell lymphoma.

(Thestrup-Pedersen) I have seen one case of the real type of malignant T-cell lymphoma in a case of actinic reticulosis, which I felt was due to the five months of cyclosporin therapy (which worked beautifully in that case).

(Hanifin) What dose?

(Thestrup-Pedersen) 5 mg/kg/day.

(Hanifin) So, that is two, when we look at all the psoriasis and atopic patients treated with relatively low doses.

(Ring) We have lost one patient in Munich for kidney nephrosclerosis. So I am very reluctant.

(Harper) I think this has also been reported in London, in an adult with psoriasis.

(Thestrup-Pedersen) In Ann Arbor they have seen 3 out of 60 cases where they saw papules on histology, but that was on a high dose.

(Friedmann) And long term therapy, presumably.

(Hanifin) Presumably in Ann Arbor they have not hesitated to keep on using it. Questions for John Harper: Should we still send our patients to London for herb therapy? (I got from you the other night that hepatoxicity was becoming more worrisome and you were not necessarily using this drug).

(Harper) I think it must be made absolutely clear that we are not sending, and never have, patients directly from us to Chinese practitioners. We are looking at this very critically, as a potential source of new compounds for treatment. Certainly hepatotoxicity has been increasingly reported, that is of concern to us. One last point, we must stop using the word herbs, it is technically completely wrong, many of these plant materials are actually roots as well. Many drugs that we use in medicine are actually derived from plants, so there is absolutely no reason why there cannot be new medicines from some of these.

(Langeland) I have a woman who went to see this Chinese women in London, and she was very happy with it, but after some months she experienced that the treatment did not work any longer. Can you comment on that?

(A) Sure, it is well documented that a proportion does seem to develop tachyphylaxis; it is a suppressive treatment in the same sort of way as steroids, in the sense that it becomes slightly less effective after between six and nine months.

(David) I think there is a worry that hepatotoxicity has been underreported. There is some interesting data from Oxford, suggesting that hepatoxicity may itself be associated with improvement in eczema. The final point is that there is no evidence submerging of cardiotoxicity, which is not being systemically sought, and I think there is a real worry, not only about the short-term toxicity of this approach, but also about the long-term toxicity, and we certainly would counsel extreme caution. We would not encourage any of our patients to go for this treatment because of these worries. I think one has to be extremely careful.

(A) This mixture certainly contains large numbers of chemicals, some of which are very potent, and it is not surprising that there are going to be toxic effects. But what is important is that this is analyzed and researched in a very systematic way, taking out individual isolates and processing them in the same sort of way as we would do with toxicology studies on all new Western medicines. I would support what Dr David just said, that there is a degree of caution here about patients going to have this treatment on their own accord. It is a completely unlicensed and unregulated set-up in the UK, and that question is being addressed at the moment by the Government.

(Morren) In Belgium there was a scandal with Chinese herbs. They were used for obesity, and there were several patients who developed severe renal insufficiency.

(A) That is a different set of herbs, and that has been well documented in *The Lancet* in many papers. It was a specific toxin that should not have been there. It makes it very essential that this is looked at very critically. I have done so in the same way as with other medicines.

(Hanifin) Jean Krutmann will talk on UVA-1 in therapy, and especially its effects on TH-1 and TH-2.

Krutmann, J.:
Three years ago at the Bergen meeting, Georg Rajka gave me the opportunity to discuss with you results from a pilot study which we did in our department, in which patients with severe exacerbation of AD were treated by radiating them to high doses of ultraviolet A-1 radiation, which is UV radiation with a range of 340–400 nanometers. This pilot study showed, that high-dose UV-1 therapy was clearly superior in its therapeutic effect as compared to conventional UV therapy. During the last three years we have done a continuation of this work. We first did a multicenter trial, in which we compared high-dose UV-1 therapy to topical steroid treatment and to conventional UV therapy. The major conclusion of this multicenter trial was that it is going to confirm the therapeutic effectiveness of high-dose UV-1 therapy in the treatment of patients with AD. We also started to analyze the photoimmunological mechanisms which may account for the therapeutic effectiveness of high-dose UV-1 therapy in the treatment of patients with AD. I would like to share with you some of the results we have generated thus far, which may help us understand what is going on in the skin of our patients.

Before I do this, I would like to briefly mention a study that has recently been done in my laboratory, in which we were interested in analyzing the cytokine pattern which has been expressed

in lesional skin of patients with atopic eczema. We took biopsies from our patients, and then extracted the total RNA and analyzed the cytokine expression, in particular the expression of the TH-1 cytokine interferon gamma, and of the TH-2 cytokine IL-4 by using the differential PCR. In a total of 15 patients there were increased amounts of interferon gamma in 13 of 15 patients, whereas IL-4 expression was increased in 4 out of 15 patients. Even more interesting, if we biopsied the same patients after the skin had clinically improved, we found an increase in the interferon gamma mRNA expression but not in the IL-4 expression. The major conclusion from this study was that in expression of the TH-1 derived interferon gamma is linked to the clinical severity of the AD. How does high-dose UVA-1 radiation therapy affect the cyto-expression of interferon gamma in lesional atopic skin? We took one biopsy of this patient before and after high-dose UVA-1 therapy, the biopsy was taken from a lichenified chronic eczematous lesion on the flexural elbow of this patient, and there are increased amounts of interferon gamma mRNA expressed in lesional skin before treatment and increased interferon gamma mRNA expression is downregulated after high-dose UVA-1 therapy. This downregulatory effect appears to be relatively specific, since, in another patient we were able to detect increased amounts not only of interferon gamma mRNA but also of IL-4 before therapy. There was a clear downregulation of interferon gamma mRNA after high-dose UVA-1 therapy, whereas IL-4 mRNA expression was not downregulated. In 7 of 9 patients we were able to observe increased amounts of interferon gamma mRNA before therapy, and in none of these patients interferon gamma mRNA was increased after high-dose UVA-1 therapy. This is in contrast to IL-4 mRNA expression. In three out of 9 patients, IL-4 mRNA expression was increased before, and in none of these patients was it decreased after high-dose UVA-1 therapy.

The overall conclusion here would be that high-dose UVA-1 therapy is capable of downregulating interferon gamma mRNA expression in lesional atopic skin. How is high-dose UVA-1 capable of downregulating the expression of this TH-1 derived cytokine? There are two possibilities. One would be that high-dose UVA-1 radiation is capable of exerting direct effects on the TH-1 cells within the skin, and the second possibility is that high-dose UVA-1 radiations induce the release of soluble mediators which may be produced by, for example keratinocytes, which would then act in an indirect manner on the TH-1 cells and shut off the interferon gamma production.

We currently think that at least one of the mechanisms may be operative, and that UVA-1 radiation induced downregulation of *in situ* expression of interferon gamma mRNA may be due to an increased production of IL-10 production, and we are currently assessing whether our *in vitro* results are also relevant for the *in vivo* situation.

In conclusion, I think there are actually two sides to ultraviolet A1 radiation. One is that we have a new modality for the treatment of AD. The other one is that if we learn more about the underlying photoimmunological mechanisms which may be relevant for the therapeutic effectiveness of high-dose UVA-1 therapy, we will also obtain interesting information about the pathogenesis of AD.

Discussion

(Bos) This is very interesting work in relation to your results with UVA-1, but I would like to warn against the certainty with which you bring forward your data. For example your gamma interferon and IL-4 in biopsies have not been confirmed, and I know of several groups who have found the opposite. Also, the fact that you state that humans can produce IL-10 has not been

confirmed, and I know of several groups who absolutely cannot prove that human keratinocytes can produce IL-10.

(A) Let us start with the first point. I think the fact that at a chronic stage of the disease, you primarily see the interferon gamma, and the fact that interferon producing cells clearly outnumber alpha-producing cells at protein level was confirmed yesterday in a very elegant manner by Thepen and Bruijnzeel-Koomen. So I do not have any doubts that in the chronic stage what we are looking at is a TH-1 mediated response. With regards to the abstract by M. Tønnesen, which was presented at the last SSD meeting and to within you are referring, I know that there are several groups which have problems detecting significant amounts of IL-10 produced by human keratinocytes, but it very much depends on the primers you use. It is not that primers, which give you a clear signal in T-cells automatically also give you a clear signal in human keratinocytes. There are a lot of laser kits available on the market which are not capable of detecting IL-10, which is produced by human keratinocytes. I know about at least three other, independent studies which clearly can show that human keratinocytes are capable of producing IL-10, including Steve Katz at the NIH. I think that Thestrup-Pedersen would like to comment on that, because he told me more than a year ago that they see IL-10 production by human keratinocytes; and Thomas Luger and Thom Schwartz from Münster have had identical data.

(Mudde) I think this is a very interesting study. In relation to your data from yesterday, did you notice what happens to T-cells leaving the skin, or, if you do a patch test, for instance, when you use UV radiation, do you see cells with a TH-1 or TH-0 phenotype move in, or do you just shut down the message for IL-2 and IFN-γ?

(A) What we know thus far about the patch tests lesions is that if we preradiate the skin with UVA-1 and then do the patch-test, we can inhibit the patch test. What we do not know yet is which cytokine we shut off, whether it is an early stage or later stage; but we are working on that.

(Giannetti) Going back to your clinical results, in your original paper you describe an improvement of approx. 80–85% of the patients. Could you predict what kind of patients did not respond to such a therapy?

(A) My first guess was that it may have to do with the skin type, and that patients who belong to skin type 1, may be more difficult to treat with UV radiation than skin type 3 or 4. What we did was that we also analyzed the photobiological mechanisms which are relevant, or underlying these photoimmunomodulatory effects, and there it appears that most of the effects were mediated via the generation of singled oxygen. Different individuals show marked differences in the capacity to quench free radicals. So, I think one explanation for this may be that the capacity of the skin system to quench for instance singled oxygen or other free radicals which may be involved in this system may differ.

(Q) What about long-term effects and side effects with this treatment? You have treated patients for 2-3 years.

(A) We have never seen a rebound, but we see, of course, reexacerbation of AD. However, it is not more frequent than in the group that was treated with the steroids. From all the patients I have seen thus far we have only had two with acute side effects, and that was exacerbation of eczema.

Hanifin, J. M.:
Two observations: clinically UVA-1 has not impressed me because the patients are required to be in a box for so long. You mentioned the other day that you are hoping in the new trials to decrease it from an hour to 25 minutes, and maybe even less in the type 1 and type 2 patients. Secondly, you and I talked, because we have seen some increased gamma interferon expression in biopsies also, and then there is this paradox, and maybe Dr Reinhold will talk about it, why should we be treating with gamma interferon when it seems to be a marker for bad disease, but remember it is only one snapshot in a whole lifetime of AD. Those were the very severe chronic adult patients who were the least responsive to it. So there may be something there that may be predictive. Dr Reinhold will enlighten us about treatment with gamma interferon.

Reinhold, U.:
As we have already discussed yesterday very extensively, AD is a disease which might be associated with TH-2 activities. This became apparent in 1987 when we could show that peripheral blood T-cells show decreased interferon gamma production and increased IL-4 production. Several groups have now confirmed that TH-2 cells may accumulate also in skin lesions of AD. There is thus strong evidence for the importance of a TH-2 like phenotype cytokines secretion pattern in the pathogenesis of the disease. From these data, a new concept in the treatment of AD has been developed, modulation of TH-2 activities. This includes inhibition of the induction of TH-2-mediated inflammatory response, antagonism of TH-2 derived cytokine activities, restoration of TH-1 growth rate and normalization of the TH-1/TH-2 balance. One of the agents chosen for the modification of the TH-2 response was interferon gamma. For cancer patients, we know that interferon gamma has several immunomodulatory effects *in vivo*. This includes: increased expression of MHC class I and II receptors, a slight increase of the expression of Fc receptors on the monocytes, and decreased expression of CD 14 on monocytes. Other activities of interferon gamma have been exclusively studied *in vitro*. This includes inhibition of the ongoing IgE production, as well as of the IL-4 used IgE production and inhibition of the IL-4 receptor expression on T-cells.

We also know from *in vitro* studies that spontaneous IL-4 induced IgE production in the hyper IgE syndrome can be blocked by interferon gamma. Also, there are some studies showing clinical improvement in the hyper IgE syndrome with interferon gamma.

Then several studies came up, indicating clinical improvement in severe AD in response to interferon gamma. Professor Hanifin has presented a placebo-controlled, randomized study demonstrating clinical improvement in about 50% of patients. The response rate was different, depending on the age of the patients, a higher response rate was seen in the group with 3-20 years old patients.

We also have done an open trial recently published, an open trial with 14 patients with severe AD, and we had similar data, with a response rate in about 50%.

The question is, how does interferon gamma work *in vivo*? What we have seen, is that the serum IgE levels were not decreased, we only saw that the spontaneous IgE production *in vitro* was reduced, but the total serum IgE, and also the slightly antigen specific IgE, was not different during and after therapy.

Interesting results were obtained from a child with Omenn's syndrome, recently published in the European Journal of Immunology. Omenn's syndrome is a combined immunodeficiency syndrome. Clinically it is characterized by erythroderma, hepatosplenomegalia and repeated infections. Immunological disturbances include hypereosinophilia, increased serum IgE and dective T-cell proliferation. Interestingly, it was shown that circulating T-cells in that syndrome

spontaneously express IL-4, IL-5, IL-10 mRNA. But they do not express IL-2 and interferon gamma mRNA.

After treatment with 40 µg of intereron gamma per day, clinical improvement, decrease in eosinophil count and disappearance of spontaneous IL-5 and IL-10 mRNA were observed. On the other hand, IL-4 on RNA expression and serum IgE was unchanged during treatment. However, the study clearly indicates that interferon gamma can downregulate certain TH-2 activities *in vivo*.

In conclusion, it becomes apparent that interferon gamma might represent one therapeutic agent which may downregulate certain TH-2 activities *in vivo*. The observation indicates that interferon gamma treatment was associated with modification of a pattern of cytokines produced. Possible mechanisms include inhibitory activity of the IL-4 induced IgE production as well as inhibition of the IL-4 induced T-cell proliferation. Also, it has been shown that interferon gamma can downregulate IL-4 receptor expression on T-cells *in vivo*. Furthermore, downregulation of IL-5 and IL-10 mRNA expression in Omenn's syndrome indicates that interferon gamma can downregulate certain TH-2 activities *in vivo*, and may favor the development of TH-1 cells during primary induction of T-cell response.

Discussion

(Hanifin) I might just point out that if that list of eight possible mechanisms is frustrating to immunologists and clinicians, it is a good sign. We had the same thing with cyclosporin A, and the more mechanisms you have, the more pathways are being affected, and I think this is the reason why these drugs have an effect in diseases like this.

(Mudde) That is exactly one of the things I wanted to mention - why do you still suggest that IgE downregulation or IL-4 downregulation could be a mechanism, if you show in your study that IgE is or IL-4 is not affected? The other thing that I would like to state again here is that in an antigen specific interaction between T-cells and B-cells, interferon gamma may not be able to down-regulate IgE switching. This is another indication that it might work like that, so I think what we should concentrate on the other effects of interferon gamma.

(A) Yes, you see decrease of spontaneous IgE production *in vitro*, but - no change of serum IgE.

(Hanifin) Again, it is very inconsistent and different - so many of these things are laboratory artifacts. The point is, it works in lots of different places, and I think it is one of the reasons it is helpful.

(Ring) Peter Rieber from Munich has shown that the nuclear factor IL-4 act at the same responsive element interferon gamma. So, if you are first there, you may stop IL-4. So, maybe we should give interferon gamma, much earlier.

(Hanifin) I think that there is an enormous amount that could be learned with gamma interferon if only it were not so expensive, and did not have such low priority for a disease that is so common.

(Thestrup-Pedersen) Is there a correlation between clinical efficacy and the development of lymphopenia in the patients?

(Hanifin) No. We have not seen any such correlation. One of our adults who did not respond at all happened to be Board member of the Eczema Association, and I was terribly embarrassed, but she got severely neutropenia while on the drug we had - she was one of the few we had to drop from the study. I think age may be a factor.

Now we will have a slight diversion, talking about fatty acids in AD, and Dr Melnik will talk about essential fatty acids like eicosanoids and postnatal T cell maturation, implications for treatment and prevention.

Melnik, B.:

In my talk I want to focus on the role of omega-6 essential fatty acids (FA) in AD. There is a whole list of papers reporting a deficiency of long-chain omega-6-FAs in patients with AD and respiratory atopy. There are very interesting recent data on deficient omega-6-FAs in breast milk lipids of mothers with infants suffering from AD and deficient omega-6-FAs in cord blood and T cells of infants at risk for atopy. The cause of the imbalance of the TH-1 and TH-2 subtypes of lymphocytes in the atopic situation is not yet known. Recent data point to a maturational deficiency in CD4 T cell function of atopic T cells in early infancy. The major problem at the beginning of atopy might be an abnormal T cell maturation in the perinatal period and in early infancy.

A deficiency of essential FAs within the postnatal period of the rat results in a deficiency of omega-6-FAs in the thymus phospholipids associated with ultrastructural damage of the thymus, a decrease in thymus weight, a decreased mitogen-stimulated proliferation of thymus and spleen lymphocytes and a disturbed development of the neuroendocrine system. These postnatal deficiencies in omega-6-FAs result in life-long alterations in cell-mediated immunity.

At present, we do not know the effect of a deficiency of omega-6-FAs in the atopic situation. There is very striking information from the Italian group of Galli et al. and collaborators of the Department of Immunology of the Karolinska Institute who performed a prospective study on 57 infants with increased risk of atopic disease. Of these 57 infants, 13 developed atopic diseases within a one year follow-up period. Ten developed AD, three allergic asthma. These 13 infants exhibited a decrease of the omega-6-FAs dihomogammalinolenic acid and arachidonic acid within the first three months of life prior to the manifestation of atopic disease. Galli et al. suggest the deficiency of long-chain omega-6-FAs as a predictor of atopy.

So one may speculate about the role of essential FAs in early T cell regulation and maturation. It is known that T cells mature within the thymus medulla in close vicinity to thymic macrophages and dendritic cells. There are cellular structures, like the thymus rosettes, showing the close relationship of T cells and supporting cells. Thymus macrophages play an important role for the thymus microenvironment and are responsible for the secretion of eicosanoids. These are involved in the regulation of T cell maturation. Prostaglandin E2 of thymic macrophages is involved in apoptosis of autoreactive T cells and leukotriene B4 stimulates the generation of suppressor T cells.

Thus, I want to ask two questions: Is there anything wrong in thymic T cell maturation in atopic individuals? Is the deficiency of long-chain omega-6-FAs related to disturbed thymic Tcell maturation leading to the imbalance of the TH-1 and TH-2 subtypes of lymphocytes in the atopic immune system?

So one may speculate about the role of essential fatty acids in T cell regulation and

maturation. But from the literature we do know that T cells are mature within the thymus medulla in a very close vicinity to macrophages and dendritic cells. And there are known structures like the thymus rosettes showing this close relationship. From other papers of the literature we know that the thymus macrophages play an important role in the thymus microenvironment. They are responsible for the secretion of the eicosanoids, which are involved in the regulation of T cell maturation, so we know that PGE-2 of thymic macrophages is involved in apoptosis of autoreactive T cells and leukotrienes are believed to be involved in the generation of suppresive T cells. These processes are very much involved in the maturation of the immune system. I want to stop with the question: is there anything wrong with thymic T cell maturation in atopic patients? Are the long-chain essential fatty acids, which may be diminished also in atopic thymus, a problem of T cell maturation, and could this be a possible cause of the pathogenesis in the atopic situation?

Hanifin, J. M.:
We will have to go on now to Dr Søyland's presentation: the effect of fatty acids on T cell activation, and possible mechanisms.

Søyland, E.:
We recently conducted a clinical study. This was a multicenter study, including 145 patients, and we wanted to see how N-3 fatty acids worked on patients with AD. We compared the results with those of a control group who got corn oil.

There was no significant difference between the two groups, and we can therefore not exclude the possibility that the beneficial effect observed as compared to baseline values is due to a placebo effect.

Corn oil consists of approx. 60% lineolic acid, which is an n-6 fatty acid. However, we wanted to study the effect of this fatty acids on some immunologic parameters *in vivo* as well as *in vitro*. So, we isolated mononuclear cells from the patients before and after 4 months of treatment, and we evaluated the secretion of some immunologic parameters like TNF, IL-2, IL-6, as well as the expression of IL-2 receptor. What we observed was increased cell death among the activated T cells in the presence of these polyunsaturated fatty acids. This cell death may be caused by necrosis or apoptosis, also called programmed cell death. Preliminary results show that fatty acids seem to induce apoptosis rather than necrosis in our cell cultures. This may partly be due to the inhibitory effect of the fatty acids on the cell activation and the proliferation of T cell that we observed by these fatty acids. Recently Issemann and Green showed that fatty acids interact with a group of nuclear receptors that bind to certain regulatory regions of DNA, and thereby are able to alter transcription of regulatory genes. So one possible explanation of the effect on T cells by the fatty acids may be that the fatty acids interact with a group of nuclear receptor proteins and thereby alter the transcription of the regulatory genes responsible for the programmed cell death.

However, there are a lot of questions to be answered, as for example, do the fatty acids act on subtypes of T cells? If you look into the cell and all its pathways there are many possible ways in which the fatty acids may act, for instance, diazyl glycerol consists among other molecules of arachidonic acid or another polyunsaturated fatty acid, and has been shown to directly influence the activity of protein kinase C. Protein kinase C actives proteins by phosphorylation, and this again can activate gene transcription and induce protein synthesis cell growth and cytokine release or differentiation or proliferation.

We have seen that fatty acids are strong inhibitors of T cell activation *in vitro* and T cell

proliferation. But they do not seem to induce satisfactory clinical improvement *in vivo*. But is it possible that they can potentiate other immunosuppressive treatments? One important benefit of the fatty acids is that they do not have any known unwanted side effects in humans.

Discussion

(Bos) You have shown that this T cell proliferation can be inhibited with free fatty acids within the range of 50-60 µmol. Do you know of any location in the body where T cells are in an environment with similar levels of free fatty acids?

(A) You can obtain this concentration in serum. But in serum the fatty acids are bound to albumin, and if we are doing the same experiments with fatty acids bound to albumin, we have to go up in concentration. But the concentrations we have used where we get approx. 30-40% inhibitory effect you will find in serum when you have eaten a lot of omega-3 fatty acids.

(Ring) The results of this study reminds me of the rounds we had when we presented eicosapentaenoic acid at one of the earlier meetings here used as a control for saturated fatty acid. This was the study where placebo turned out to better than verum. No statistician has helped me so far to find out what I should do with those data. No pharmaceutical company was interested in doing a study with saturated fatty acids.

(Q) How many patients did you have in your study?

(Ring) Twenty each. It was a big study over half a year.

(Hanifin) I would just like to give some background to this whole thing. It started in the 20s when Hansen said that rats who were essentially fatty acid deficient looked like they had eczema. That got propounded, and of course the Efamol evening primrose oil cartel picked up on those things and I guess convinced even the national health services to put on the formulary. The problem I have nowadays when I see studies that show abnormal levels of one thing or another - and I know nothing about lipids, as is true for most of us - I go to a person who is a biochemist, a lipobiochemist and a nutritionist, and say, what about this, is it real? The study always seems to break down because they did not do proper diet loading, and the controls are not right, and we never seem to get to a point where the basic abnormality is defined, and what I would like to ask is whether there have been any studies that stand up to scrutiny and can be published in the Journal of Biological Chemistry about these abnormalities in AD?

(A) Yes, I think there are a lot of studies which are convincing, there are very good statistical studies showing a deficiency of dihomogammalinolenic acid and arachidonic acid in the atopic individual, especially if you are looking into T cell lipids - for instance Galli´s group has shown this in a quite big number. There is much evidence showing that there is a deficiency. The problem with the treatment, I think, is that there could be a very critical time for response to treatment, because the essential fatty acids are very important in the postnatal period. If you treat the adult atopics with already manifested disease, there could be a problem in the response. Our experience is, - and this is also an Italian experience - (Bordoni) - that if we treat young children, infants, you have a much better response in the clinical improvement. So my suggestion is that we should look into

the immediate postnatal period, and this is already being done by the Karolinska Institute now: This also coincides with the observation in the infantile seborrhoeic eczema where Tollefsen and Fritz observed deficiency of the omega-6 fatty acids cascade, and they applied topical borage oil on these infants, with considerable success.

(Hanifin) Let us just take one segment at a time. We have to be very careful of accumulative evidence, because there is a wealth of studies that did not stand up to scrutiny. So, we have to be very critical of the basic defect.

(A) I think it is very important to do a dietary history when you are measuring the fatty acids in serum. We did it on the patients with AD and compared them with patients with psoriasis in a similar multicenter study. We did not find, in patients with AD, high levels of linoleic acid as compared to arachidonic acid, and when we did the dietary history, we saw that these patients had a significantly smaller intake of polyunsaturated fatty acids, which could explain it.

(Hanifin) There is also the malabsorption problem but I do not know if this has been taken into account. So there may be some deficiencies there, but this is no clear evidence. Then we go on to the therapy phase, and you said, borage oil topically helps, evening primrose oil, I think when you look at the cumulative studies, not Horrobin's meta-analysis, but the cumulative studies where you look at the ones that are done critically, you see very little. Borage oil systemically orally has not been very effective. The problems there is when you do a study, and you do it for six weeks, twelve weeks, they say, the dose was not high enough, you should have done it with three times the dose with twenty-four weeks, so all the negative studies get shot at the side. Now you say that topical borage oil may be effective, and then you are saying corn oil or fish oil may be effective, and we are waiting for definitive trials.

(A) But the problem should be the postnatal period. The latest evidence points to a delta-6 saturatase deficiency in the postnatal period, these are also the data of the Karolinska Institute. When they applied the borage oil topically, they had to apply it for seven months and then the eczema disappeared; if they stopped the treatment after two months, it relapsed. So they say it could be a postnatal maturation problem of the enzyme.

(Hanifin) Was that a controlled study? Of course, eczema does disappear during seven months.

(A) Yes, it was.

Hanifin, J. M.:
As I mentioned yesterday, there is a number of functional conscquences of elevated cyclic AMP phosphodiesterase (PDE) activity in atopic leukocytes including increased basophil histamine release, B cell IgE secretion, T cell IL-4 production and eosinophil chemotaxis in addition to the monocyte PGE_2 and IL-4 production. Significantly, each of these defects is normalized by phosphodiesterase inhibitors. As an example, the Type IV PDE inhibitor, Ro 20-1724, resulted in very consistent reduction in the *in vitro* atopic mononuclear leukocyte IL-4 production stimulated with anti-CD3. These PDE inhibitors act by this mechanism in many cells, they affect multiple functional pathways and may lead to new therapeutic approaches for allergic diseases. In a multicenter blinded study done a number of years ago (and never published), we compared

vehicle with 2.5% and 5% Ro 20-1724 cream. At the end of one week, we demonstrated significant improvement in AD.

Recently we have studied a much more potent PDE inhibitor, which a number of companies have developed around the world for depression, cardiac inotropic effect or asthma. Unfortunately, when given systemically, all of these inhibitors cause nausea, just like theophylline does in high doses. Most companies have largely abandoned systemic treatment with these agents, but some drugs are coming to trial as topical inhibitors of inflammation. In a paired comparison trial of a new PDE inhibitor vs. placebo on two sides of the body, we showed improvement in clinical score in 16 of 19 on the side with active drug but on only 3 of 19 on the placebo-treated site. This is the only non-steroidal drug that I have seen that works topically for AD and it appears to be as effective as mid-strength corticosteroids.

Focusing next on the systemic effects of PDE inhibitors is a study of Chan, assessing the PDE activity in monocytes of normal and AD patients. Leukocyte PDE activity was elevated before therapy and was significantly reduced after three months of daily interferon-gamma (IFN-γ) injections. Thus, IFN-γ acted as a PDE-inhibitor *in vivo*, confirming previous *in vitro* findings. Clearly when you affect multiple pathways, you inhibit factors and in this study the reduction in the PDE activity was accompanied by clinical improvement, suggesting anti-inflammatory mechanism in systemic treatment of AD. There are companies in many countries that have good PDE inhibitors and we should encourage them to develop these much needed alternatives to corticosteroids.

Discussion

(Q) Possible side effects fo these topical agents?

(A) These have not been studied in long-term applications. But there has been no evidence in the preliminary toxicology studies, neither in animals or humans. We have seen no problems with them. I am informed that the Schering compound which may or may not be a PDE inhibitor - it is at least a 5-LO inhibitor and a cytokine inhibitor - that compound did cause headache, and may not be developed even though it is an excellent drug topically. We are not expecting to see much. We may get into nausea problems with patients with widespread body areas, but those are the kind of patients who need other than topical therapy anyhow. We are talking about severely ill patients, about infants, we need an alternative to corticosteroid topically.

Rajka

Thank you for this excellent session, and also for keeping the time schedule. Now this meeting has come to an end, and of course, to make an evaluation: the method was an experiment, and I am the last person who should evaluate it. But I feel, from the level of the discussions, that it worked, and I got many positive signals. So, what is the secret? Somebody who already left said to me yesterday: how could you gather the best people? That was the secret, and therefore, I am very grateful to all the people who were here, on the floor, all panelists, the chairmen, but, particularly, the discussion leaders. I also have to apologize for my schoolmasterly method, keeping within the schedule, I am aware of it, but perhaps sometimes it is needed. I am grateful to all the people who kept the schedule, like Anna Broberg, a real soft iron lady, who made it.

I am only a little worried about how we will be able to make this supplementum only from listening to the tapes.

I have nothing more to add than to remind you that the next meeting will take place on 7–9 June, 1996 in Aarhus, organized by Dr Thestrup-Pedersen and myself, and perhaps Dr Thestrup-Pedersen will have one word.

Thestrup-Pedersen
Just one word: I have been wondering how I can make you all come to Aarhus in 1996. I know that you are already going to too many meetings. I know that you need 720 days a year to do everything you want to do. So why Aarhus in 1996? For two reasons. First of all, I think we owe it to the patients to work on this disease and in the surroundings where we will use three days to discuss AD only. And secondly, being an optimist, I promised Georg Rajka that we will solve the puzzle of AD before the year 2000. So I need your help in 1996.

Rajka
Thank you for coming. The meeting is closed.

Appendix

Clinical Criteria in Diagnosing AD
The Lillehammer Criteria 1994

F. Schultz Larsen, T. Diepgen, Å. Svensson

I. The infantile phase (age up to 2 years)

A. Clinical
1. Eczema over the face or neck.
2. Eczema on the trunk.
3. Eczema on the arms or legs (extensor or flexural sites).
4. Itching or scratch effects, incl. lichenification or impetigo.

B. Anamnestic
5. A history of relapsing course or seasonal variation.
6. A history of dry skin.
7. A history of itching when sweating or wool intolerance.
8. A history of respiratory atopy or positive family history of atopy in first degree relatives.

C. Laboratory
9. Elevated serum IgE or positive skin prick tests.

D. Duration
10. Duration of more than 6 weeks.

II. The childhood phase (age 2-12 years)

A. Clinical
1. Eczema over the face or neck.
2. Eczema in the elbows or the knee folds.
3. Eczema at the wrists or ankles.
4. Eczema on the hands or feet, incl. dermatitis plantaris sicca.
5. Pityriasis alba or reversed eczema above (below) elbows/knees or toilet seat dermatitis.
6. Itching or scratch effects, incl. lichenification or impetigo.

B. Anamnestic
7. A history of relapsing course or seasonal variation.
8. A history of dry skin.
9. A history of itching when sweating or wool intolerance.
10. A history of respiratory atopy or positive family history of atopy in first degree relatives.

C. Laboratory
11. Elevated serum IgE or positive skin prick tests.

D. Duration
12. Duration of more than 3 months.

III. The adult phase (age over 12 years)

A. Clinical
1. Eczema over the face or neck.
2. Eczema in the elbows or the knee folds.
3. Eczema at the wrists or ankles.
4. Eczema on the hands or feet, incl. dermatitis plantaris sicca.
5. Pityriasis alba or nummular eczema on the arms or legs, or eczema on the upper trunk, incl. nipple eczema.
6. Itching or scratch effects, incl. lichenification or impetigo.

B. Anamnestic
7. A history of relapsing course or seasonal variation.
8. A history of dry skin.
9. A history of itching when sweating or wool intolerance.
10. A history of respiratory atopy or a positive family history of atopy in first degree relatives.

C. Laboratory
11. Elevated serum IgE or positive skin prick tests.

D. Duration
12. Duration of more than 3 months.

Diagnostic criteria
Visible eczema in at least one of the regions (A), and at least one positive of the anamnestic or laboratory criteria (B, C), and at least three of the clinical, anamnestic or laboratory criteria (A, B, C) fulfilled. In addition, as a fourth criterion, the skin disease should always have a duration of at least 6 weeks in the infantile phase or 3 months in the childhood and the adult phases.

Comments
The effort of establishing disease definitions is an assignment open to criticism. On the one hand simplicity is desirable, on the other some vague or uncharacteristic cases easily falls outside the frame of distinct outlines of any definition, and in a largely visual science as dermatology the task will always involve an inborn error of indistinctness as for example in diagnosing eczema which implies knowledge of the variability of the micro- and macroscopic appearance of the eczematous skin reaction. Thus it is essential to be familiar with the diagnosis of eczema, otherwise it is not possible to diagnose AD.

The guidelines are built up around the following theme:

(A) The distribution of eczema and common eczematous reaction patterns in the different phases, and at the time of the clinical examination eczema should be present in at least one of the described anatomical regions.

(B, C) Anamnestic and laboratory criteria that are identical for the three age groups.

(D) The eczema should have been present during a certain length of time.

This approach is based on the following ideas:

(A). The distribution of the eczema in the three phases follows the common course of the disease and the criteria concentrate upon eczema of larger characteristic areas, while often characteristic signs in small areas are avoided. For example, it is not considered to be reasonable that a clinical diagnosis of AD should rest only upon the involvement of the lips or folds of the earlobe. It is considered to be a hallmark that visible eczema is present at the time of the clinical examination in at least one characteristic region. And, in general, more or less associated features are incorporated into one criteria.

(B). For simplifying and didactic reasons the four anamnestic criteria are identical in the three phases, although we are well aware that for example seasonal variation is usually an inappropriate criterion in early infancy. We do not find it advisable to suggest certain fixed and prepared questions to be asked as the usefulness or the validity is highly dependent upon the patients or the families background for answering medical enquiries. It is considered to be more important that the physician clarifies the questions according to the definitions.

(C). The laboratory criteria about IgE and skin prick test have been placed separately. Initially we were reluctant to include these relative expensive and invasive procedures among the criteria as most cases of AD can be confidently diagnosed without laboratory tests. However, there laboratory investigations are often performed and might be helpful for accepting or rejecting the diagnosis of AD in patients with ambiguous skin diseases.

(D). Although the specialist might diagnose a newly developed case of AD we urgently feel it is necessary that the criteria comprise a certain length of time of the disease. In this way we avoid the often diagnostic confusion that might exist in relation to transient skin manifestations of childhood.

After these underlying reflections the criteria will be commented.

(1). *Eczema*. In infancy the eczema may be dry, scaly and erythematous in the milder cases or more exudative and crusting in the more severely affected patients. As in all the phases a certain degree of symmetry in the distribution is often present but can never be used as an absolute criterion. In childhood the eczema becomes modified by the pruritus towards epidermal thickening or lichenification, and in adults a chronic, dry, thickened and lichenified eczema may predominate.

(2). *Itching*. Itching or pruritus is a sensation which leads to a desire to scratch. As itching and the clinical effect of itching is highly associated these features are incorporated into one criterion.

(3). *Lichenification*. Lichenification is a cutaneous response to repeated scratching. It is characterized clinically by a thickened appearance of the skin with accentuation of the skin markings in a way that the affected area may resembles tree bark.

(4). *Impetigo*. Impetigo is a bacterial inflammation of the skin characterized by the appearance of vesicles (bullae) or pustules which later dry to form superficial yellowish crusts.

(5). *Dermatitis plantaris sicca (juvenile plantar dermatosis)*. Dermatitis plantaris sicca is a dry, fissured dermatitis usually affecting both plantar surfaces of the forefeet and especially the great toes.

(6). *Pityriasis alba.* Pityriasis alba is a non-specific dermatitis of unknown origin which characteristically produces round or oval slightly erythematous and scaly patches with indistinct margins which subside to leave areas of depigmentation. The lesions are often multiple and localized to the face, neck, shoulder and upper arms.

(7). *Reversed eczema.* Reversed eczema is irregular eczematous lesions of the extensor sides of the extremities accentuating above/below the knees and elbows.

(8). *Toilet seat dermatitis*. Toilet seat or gluteofemoral eczema has been recognized as a fairly distinctive entity and is characterized by an often lichenified and excoriated eczematous reaction localized corresponding to the toilet seat.

(9). *Nummular eczema*. Nummular eczema is characterized by circular or oval plaques of eczema with clearly demarcated edges, often localized to the extremities and the lower trunk.

(10). *Nipple eczema*. Nipple eczema is a chronic lichenfied, fissured or weeping dermatitis over one or boths nipples.

(11). *Relapsing course* means phases of outbreaks alternating with periods of remissions.

(12). *Seasonal variation* means that the ezcema varies with the seasons. Most patients with AD have improvement of their eczema in the summer and deterioration in the winter.

(13). *Dry skin*. Dry skin means the patient's subjective judgement of dryness of a substantial part of the skin.

(14). *Itching when sweating* means iching of the non-eczematous skin following any stimulus to sweating.

(15). *Wool intolerance* means that the mechanical irritation from wool fibres causes itch in the skin.

(16). *Personal respiratory atopy* is defined as current or previous history of allergic rhinoconjunctivitis or asthma.

(17). *Family history of atopy* is defined as current or previous history of AD, allergic rhinoconjunctivitis or asthma in first degree relatives.

(18). *Elevated serum IgE* is defined as increased serum total IgE level above the upper normal limit given by the reference laboratory.

(19). *Positive skin prick test* is defined as positive skin prick tests to common standardized allergens.

The criteria suggested rest upon our previous clinical and laboratory studies centered on the diagnostic features of AD. There is an array of additional symptoms and signs which might be helpful in cases difficult to diagnose, but they have been ommitted due to the intended simplicity of the proposed guidelines. Thus, we have to admit that the criteria represent a great deal of subjective judgements, which should be evaluated scientifically by appropiate clinical investigations. The specificity of the diagnostic guidelines should approach 100%, i.e. that all those who fulfill the criteria have definite AD without doubts in the mind of any dermatologist, and that the sensitivity surpasses 90%. Such validated and generally accepted criteria may have an enormous impact, not only in the clinical situation but also on the continuous analysis of the natural history of AD.