Acta Derm Venereol 2001; 81: 263–267

INVESTIGATIVE REPORT

Expression and Function of CD43 and CDw60 on T Cells from Patients with Atopic Dermatitis

NAOYUKI HIGASHI[1,2], KAIDA WU[1], CHRISTIAN GRØNHØJ LARSEN[1], METTE DELEURAN[1], SEIJI KAWANA[2], KAZUYA YAMAMOTO[3] and KRISTIAN THESTRUP-PEDERSEN[1]

Department of Dermatology, [1]Marselisborg Hospital, University of Aarhus, Aarhus, Denmark, [2]Nippon Medical School, Tokyo, Japan, and [3]Aiiku Hospital, Tokyo, Japan

Signalling via the CD43 and CDw60 epitopes has been reported as providing two novel pathways of T-lymphocyte activation. In Wiskott-Aldrich syndrome, which has atopic eczema-like skin symptoms, there is a defective expression of CD43, while CDw60 is strongly expressed on T cells from rheumatoid arthritis synovial fluid and from psoriatic skin lesions, and on blood mononuclear cells from patients with cutaneous T-cell lymphoma. We therefore studied the expression and function of these phenotypes on peripheral blood mononuclear cells and on CD4+ and CD8+ T-cell subsets from patients with atopic dermatitis. We observed a significant increase in the percentage of CD43+ cells among the blood mononuclear cells in patients with atopic dermatitis and an enhanced proliferation of CD4+ T cells following stimulation with anti-CD43 antibody. There were no changes in the CDw60 expression or function after stimulation with anti-CDw60 antibody. Thus, CD43 expression was not decreased but rather increased in blood mononuclear cells from patients with atopic dermatitis, whereas CDw60 expression did not differ from healthy controls. *Key words: azathioprine; CD4+; CD8+; lymphocyte proliferation; PMA.*

(Accepted June 21, 2001.)

Acta Derm Venereol 2001; 81: 263–267.

Naoyuki Higashi, Department of Dermatology, Nippon Medical School, 1-1-5, Sendagi, Bunkyou-ku, Tokyo, 113 8602, Japan. E-mail: nhi_ton@hotmail.com

Wiskott-Aldrich syndrome is an X chromosome-linked, recessive disease characterized by atopic dermatitis (AD)-like eczema, thrombocytopenia, impaired humoral and cellular immune function and early mortality in affected boys (1–7). A defective expression of CD43 has been found in Wiskott-Aldrich syndrome (2). CD43, which is also called sialophorin or leukosialin, consists of 385 amino acids with a mucin-like extracellular domain of 234 amino acids that are extensively *O*-glycosylated with 75 to 85 *O*-linked carbohydrate chains (4, 8). It has been proposed that this large surface molecule is an adhesion molecule capable of interacting with ICAM-1 (9), human serum albumin (10), E-selectin (11), galectin-1 (12), and MHC class I (13). However, CD43 has also been documented to have negative effects on T-cell adhesion (14).

CDw60 was originally defined by the UM4D4 monoclonal antibody (mAb), which was generated against T-cell lines and clones from synovial fluid and tissue from patients with rheumatoid arthritis (RA), where CDw60 is strongly expressed on T cells. Since then, CDw60 has been shown to be highly expressed on T-lymphocytes and keratinocytes in psoriatic skin lesions, and on peripheral blood mononuclear cells (PBMC) from patients with cutaneous T-cell lymphoma (15–18). The natural ligand for CDw60 is still unknown, but the anti-UM4D4 mAb has been found to bind to the *O*-acetylated form of ganglioside G_{D3} (19).

The aim of this study was to determine whether CD43 and CDw60 expression and function were changed on PBMC, CD4+ and CD8+ T lymphocytes taken from patients with AD.

MATERIALS AND METHODS

Patients and controls

Blood samples were obtained from 13 patients (9 men and 4 women; mean age 33 years, range 24–47 years) with AD according to the criteria of Hanifin & Rajka (20). Seventeen healthy persons (11 men and 6 women; mean age 42 years, range 25–55 years) served as controls. The patients received only topical treatment; none received systemic immunosuppressive therapy. Blood samples were also taken from a man and a woman aged 25 and 31 years, respectively, who prior to blood sampling had been treated for 1 month with azathioprine 100 mg/day for AD. Informed consent was obtained prior to inclusion in the study, which was approved by the Ethics Committee of Aarhus County.

Cell staining and flow cytometry

PBMCs were isolated by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). CD4+ and CD8+ T cells were positively purified from PBMC using magnetic microspheres in accordance with the manufacturer's instructions (Dynal, Oslo, Noweay). One million PBMCs, CD4+ or CD8+ T cells in Hanks' balanced salt solution (HBSS) with 1% fetal calf serum (FCS) were incubated with 20 µl FITC-conjugated anti-CD43 mAb (DF-T1) or anti-CDw60 mAb (M-T41) (both Becton Dickinson, Brøndby, Denmark) for 30 min at 4°C. The cells were then washed twice in HBSS with 1% FCS and fixed in 100 µl 4% paraformaldehyde-HBSS for 15 min at 4°C. Finally, cells were washed twice in HBSS with 1% FCS and resuspended for analysis in 500 µl of HBSS with 0.5% paraformaldehyde. Flow cytometry was carried out using EPICS XL-MCL (Coulter, Luton, UK) in accordance with routine procedures.

The purity of the CD4+ and CD8+ T-cell subsets was measured in 4 samples from patients with AD and controls using antibodies from DAKO, Copenhagen. The purity was similar for patients and healthy persons, and was on average 96% with the lowest value being 92.3%.

Cell stimulation with anti-CD43 or CDw60 antibody

Lymphocyte proliferation assay was performed as previously described (21). A total of 100,000 CD4+ or CD8+ T cells was incubated in 96-well flat-bottomed plates (Nunc, Roskilde, Denmark) in 200 µl of RPMI-1640 with glutamax (Life Technologies, Paisley, UK) supplemented with 2% FCS, 10 IE/ml penicillin G, 100 µg/ml streptomycin, and 25 µg/ml gentamycin. In pilot assays we studied the concentrations of antibodies and phorbol 12 myristate 13-acetate (PMA) (Sigma, St.

 ISSN 0001-5555

Louis, MO) as well as incubation time. We selected the optimal culture conditions, i.e. 5 μg/ml of anti-CD43 mAb (DF-T1, Immunotech, Marseille, France), or 2.5 μg/ml of anti-CDw60 mAb (UM4D4, Harlan Sera-Lab Ltd., Loughborough, UK), either without or in combination with 2.5 ng/ml of PMA. Culture time was 72 h at 37°C in 5% CO_2. During the final 18 h of culture, the cells were pulsed with 1 μCi of [^{3}H] thymidine, following which they were harvested according to routine methods using a semiautomated cell harvester (Skatron, Lier, Norway). Counting was done in a liquid scintillation counter (Packard Instrument, Meriden, CT) and mean counts per minute (cpm) of triplicate cultures were calculated.

Cell aggregation assay for CD43

Aggregation assays were carried out as described (22) with slight modifications. One million CD4+ or CD8+ T cells were plated in a 96-well flat-bottomed plate with the addition of 5 μg/ml of anti-CD43 mAb (DF-T1) for 24 h, following which the cells were resuspended by pipetting up and down 20 times. The cells were then stained with 0.2% trypan blue and transferred to a hemocytometer, where the number of free cells and total cells were counted. Aggregation was determined as (1 – number of free cells/total cell number) × 100.

Statistical analysis

We performed Wilcoxon's signed rank test for paired data and the Mann-Whitney U-test for unpaired data using statistical software (Statview, Abacus Concepts Inc, Berkeley, CA). Values of $p < 0.05$ were considered of significance. All results are presented as mean ± 1 SD.

RESULTS

Phenotype measurements

The results from flow cytometry analysis of PBMC, CD4+ and CD8+ T-cell subsets are given in Tables I and II. We observed a significant increase of CD43 expression on PBMC ($p < 0.03$), but not on CD4+ and CD8+ T cells from patients with AD. CD4+ and CD8+ T cells had the same percentage of CD43+ cells, with no difference between the patients with AD and the controls. Also, the mean fluorescence intensity (MFI) of CD43 expression in PBMC, CD4+ and CD8+ T cells did not differ between the patients with AD and the controls (data not shown). Interestingly, azathioprine treatment led to a reduction in CD43 expression on both the CD4+ (from 91.9% to 76.8%) and CD8+ (from 94% to 77.3%) subsets of T cells from patients with AD.

CDw60 expression did not differ between the patients with AD and the controls. Although CD43 was equally expressed on both CD4+ and CD8+ T cells, CDw60 was not. It was, rather, expressed significantly less on CD8+ T cells than on CD4+ T cells in AD (CD4/8 ratio, $p < 0.05$). The MFI of CDw60 expression in PBMC, CD4+ and CD8+ T cells also did not differ between the patients with AD and the controls (data not shown). Again, azathioprine treatment led to a substantial reduction of CDw60 expression on CD4+ (from 50.8% to 33.9%) and CD8+ (from 35.3% to 17.8%) T cells from patients with AD.

Function of CD43 and CDw60 expression

We then stimulated purified subsets of CD4+ and CD8+ T cells with either anti-CD43 or anti-CDw60 antibodies ± the addition of PMA. The use of highly purified T-cell subsets led to a low cpm count not comparable to what was seen in PMA stimulation of PBMC, where monocytes are present. The results are presented in Table III. In contrast to the normal controls, we observed that anti-CD43 mAb stimulation led to a higher proliferation of CD4+ T cells, but not of CD8+ T cells in AD. PMA stimulation significantly increased the proliferation of both T-cell subsets, but further addition of anti-CD43 mAb did not cause any further increase. However, AD T cells had significantly higher responses to PMA than did T cells from normal healthy controls ($p < 0.03$). Anti-CDw60 mAb stimulation did not induce a significant proliferation of T cells from either patients with AD or controls.

Although we analysed the correlation between the expression of CD43 (MFI, the percentage of positive cells) and the cell proliferation results, we could not observe any meaningful correlations in patients with AD and in controls. Also, in

Table I. *CD43 positive cell percentages in peripheral blood mononuclear cells (PBMC) and isolated CD4+/CD8+ T-cells (mean ± SD)*

	PBMC (%)	CD4+ (%)	CD8+ (%)
Controls	85.5 ± 4.6 ($n = 6$)	90.7 ± 5.9** ($n = 13$)	94.4 ± 3.2 ($n = 13$)
Patients with AD	92.1 ± 3.4* ($n = 6$)	91.9 ± 8.2 ($n = 12$)	94.0 ± 5.1 ($n = 12$)
AD treatment with azathioprine	–	76.8 ± 18.5 ($n = 2$)	77.3 ± 18.8 ($n = 2$)

*$p < 0.03$ vs. controls.
**$p < 0.03$ vs. CD8+ T-cell in controls.
AD = atopic dermatitis.

Table II. *CDw60 positive cell percentages in peripheral blood mononuclear cells (PBMC) and isolated CD4+/CD8+ T-cells (mean ± SD)*

	PBMC (%)	CD4+ (%)	CD8+ (%)	CD4/CD8
Controls	50.7 ± 17.3 ($n = 6$)	48.2 ± 13.2 ($n = 13$)	40.2 ± 16.3 ($n = 13$)	1.3 ± 0.3
Patients with AD	52.2 ± 13.0 ($n = 6$)	50.8 ± 8.1 ($n = 12$)	35.3 ± 13.5 ($n = 12$)	1.6 ± 0.5*
AD treatment with azathioprine	–	33.9 ± 1.8 ($n = 2$)	17.8 ± 0.9 ($n = 2$)	1.9 ± 0.2

*$p < 0.05$ vs. controls.
AD = atopic dermatitis.

Table III. *The results of lymphocyte proliferation assay*[a] *(mean ± SD)*

Stimulation	Control ($n = 6$)		Patients with AD ($n = 6$)	
	CD4+ T-cell	CD8+ T-cell	CD4+ T-cell	CD8+ T-cell
Medium	78 ± 12	79 ± 25	82 ± 17	75 ± 11
Anti-CD43 mAb	100 ± 31	113 ± 46*	245 ± 316**	263 ± 431
Anti-CDw60 mAb	87 ± 30	76 ± 22	98 ± 17	93 ± 42
PMA	860 ± 285	2754 ± 3172	2889 ± 2093†	11 803 ± 11 095#
PMA + anti-CD43 mAb	984 ± 398	3706 ± 3461	3413 ± 1474††	12 670 ± 8890#
PMA + anti-CDw60 mAb	1021 ± 461	2819 ± 3136	2583 ± 1861†††	11 555 ± 10 001#

[a] Lymphocyte proliferation assay were carried out using [^{3}H] thymidine incorporation.
*$p < 0.03$ vs. medium in CD8+ T-cell from controls.
**$p < 0.05$ vs. medium in CD4+ T-cell from patients with AD.
†$p < 0.03$ vs. CD4+ T-cells from controls.
††$p < 0.01$ vs. CD4+ T-cells from controls.
†††$p < 0.05$ vs. CD4+ T-cells from controls.
#$p < 0.03$ vs. CD8+ T-cells from controls.
AD = atopic dermatitis; PMA = phorbol 12 myristate 13-acetate.

analysing the correlation between the expression of CDw60 (MFI, the percentage of positive cells) and the cell proliferation results, there was no correlation in AD patients and in controls.

Lymphocyte aggregation after anti-CD43 mAb stimulation

Isolated CD4+ and CD8+ T cells were cultured with anti-CD43 mAb alone for 24 h, following which we estimated the degree of cell aggregation. CD4+ T cells from healthy controls showed a significantly higher degree of aggregation than CD8+ T cells ($p < 0.03$) (Table IV), but there was no difference between patients with AD and healthy controls. No obvious correlation between the degree of cell aggregation and MFI or the percentage of CD43 positive cells was seen in either patients with AD or control subjects.

DISCUSSION

This study is the first to analyse the expression of CD43 and CDw60 phenotypes on PBMC and on CD4+ versus CD8+ T cells drawn from patients with AD. We observed a significant increase in CD43 expression in PBMC from patients with AD, but not in purified T-cell subsets. This finding indicates it is likely that CD43 expression is increased among the monocytes or natural killer T cells. The stimulation of CD4+ T cells with anti-CD43 mAb led to a significantly higher proliferation of AD CD4+ T cells. In a previous report it was found that the signalling via CD43 can induce IL-2 production and up-regulation of CD25 expression in T cells, and that autocrine production of IL-2 can then induce T-cell proliferation (23). In another report it was found that CD43 enhances the antigen-specific activation of T cells (2). Thus our results, in which stimulation with anti-CD43 mAb could enhance the proliferation of CD4+ T cells drawn from patients with AD, indicate that a large population of antigen-specific CD4+ T cells can highly express CD25 molecules in patients with AD. This is in accordance with a previous report in which a high expression of CD25 was observed in circulating T cells from patients with AD (24). In the result of cell aggregation assay, CD4+ T cells from healthy controls showed a significantly higher degree of aggregation than CD8+ T cells ($p < 0.05$), while the percentage of CD43-positive CD4+ T cells from healthy control increased compared with CD8+ T cells ($p < 0.03$). There is a negative correlation between CD43 expression and cell aggregation in T cells from healthy controls, but not from AD. This is in accordance with a previous report in which CD43 has also been documented to have negative effects on T-cell adhesion (14). These findings seem to exclude the possibility that there is a faulty expression or function of CD43 on either mononuclear or T-cell subsets in patients with AD, as has been observed for Wiskott-Aldrich syndrome.

Table IV. *Percentage of cell aggregation by anti-CD43 mAb (mean ± SD)*

	CD4+ T-cell (%)	CD8+ T-cell (%)
Controls ($n = 6$)	44.4 ± 14.1*	26 ± 12.2
Patients with AD ($n = 6$)	42.6 ± 15.2	31.4 ± 15.4

*$p < 0.05$ vs. CD8+ T-cell in controls.
AD = atopic dermatitis.

Kruse et al. have recently reported that CD43 polymorphisms (R337C, L341F) in atopic subjects were not associated with altered IgE responsiveness, but concluded that the polymorphisms R337C and L341F adjacent to phosphorylation sites in the intracellular region of CD43 should be elucidated (25). Further study of monocytes, NK cells and some populations of B cells in CD43 may provide new evidence.

Meanwhile, the expression and function of CDw60 did not differ between the patients with AD and the healthy subjects. In patients with AD, CDw60 was present on more CD4+ T cells than CD8+ T cells. It was interesting to see, as a preliminary observation, how azathioprine treatment led to a substantial reduction in the expression of both CD43 and CDw60. This points to the fact that these markers are necessary for normal T-cell reactivity.

Azathioprine, which is a common immunomodulatory drug, has been available for not only various skin diseases, such as pemphigoid, pemphigus, chronic actinic dermatitis, AD and dermatomyositis, but also for RA and multiple sclerosis (26, 27). During treatment with azathioprine in patients with

RA, the percentage of CD4+ T cells increased, and the total number of lymphocytes decreased (28). Also, Salmaggi et al. have reported that both the CD4/CD8 ratio and the percentage of CD4+ CD45RA+ T cells increased in multiple sclerosis patients treated with azathioprine (29). However, so far, no relationship between CDw60 and treatment with azathioprine has been observed. Since a high expression of CDw60 was reported in T cells from RA synovial fluid, our result suggests that the clinical effect of azathioprine on RA may be partially associated with the down-regulation of CDw60 expression on activated CD4+ and CD8+ T cells. On the other hand, although we did not observe an enhanced expression of CDw60 in PBMC, CD4+ or CD8+ T cells from patients with AD, the clinical improvement of AD after treatment with azathioprine may also be partially associated with a reduced CDw60 expression in the T cells. In addition, CDw60+ CD8+ T cells have recently been characterized as secreting high levels of IL-4 and as providing B-cell helpers for IgG and IgA synthesis such as the Th2 type (30, 31). Thus azathioprine can inhibit IL-4 production from CDw60+ CD8+ T-cell in AD.

Isolated CD4+ and CD8+ T cells from patients with AD showed dramatically higher proliferative activity than those from controls when T cells were stimulated with either PMA alone or PMA in combination with anti-CD43 mAb or anti-CDw60 mAb. A depressed lymphocyte response to T-cell mitogen *in vitro* has been described in AD and has been related to the elevated monocyte's production of PGE_2, which enables inhibition of mitogen-induced lymphocyte proliferation (32, 33). In accordance with previous reports, our observations suggest that isolated CD4+ and CD8+ T cells, which were depleted of monocytes from PBMC in AD, showed a higher cell proliferative response to PMA stimulation as compared with control subjects.

Although the biological significance of our observations is not immediately evident, many phenotypes and functions are upregulated in AD, including CD43. Future studies should concentrate on new potential markers for the immunological disturbance observed in patients with AD (34).

ACKNOWLEDGEMENTS

We are grateful for the co-operation of Danish patients with atopic dermatitis. This work was supported by generous grants from the Velux Foundation, the NOVO-NORDIC foundation and the Institute of Clinical and Experimental Research, University of Aarhus. We thank LEO Pharmaceutical Research Foundation for its generous support of Dr. Naoyuki Higashi.

REFERENCES

1. Mentzer SJ, Remold-O'Donnell E, Crimmins MA, Bierer BE, Rosen FS, Burakoff SJ. Sialophorin, a surface sialoglycoprotein defective in the Wiskott-Aldrich syndrome, is involved in human T-lymphocyte proliferation. J Exp Med 1987; 165: 1383–1392.
2. Park JK, Rosenstein YJ, Remold-O'Donnell E, Bierer BE, Rosen FS, Burakoff SJ. Enhancement of T-cell activation by the CD43 molecule whose expression is defective in Wiskott-Aldrich syndrome. Nature 1991; 350: 706–709.
3. Remold-O'Donnell E, Rosen FS. Sialophorin (CD43) and the Wiskott-Aldrich syndrome. Immunodefic Rev 1990; 2: 151–174.
4. Remold-O'Donnell E, Kenney DM, Parkman R, Cairns L, Savage B, Rosen FS. Characterization of a human lymphocyte surface sialoglycoprotein that is defective in Wiskott-Aldrich syndrome. J Exp Med 1984; 159: 1705–1723.
5. Aldrich RA, Steinberg AGI, Campbell DC. Pedigree demonstrating a sex-linked recessive condition characterized by draining ears, eczematoid dermatitis and bloody diarrhoea. Paediatrics 1954; 13: 133–138.
6. Huntley CC, Dees SC. Eczema associated with thrombocytopenic purpura and purulent otitis media: report of five fatal cases. Paediatrics 1957; 19: 351–361.
7. Saurat JH. Eczema in primary immune-deficiencies: clues to the pathogenesis of atopic dermatitis with special reference to the Wiskott-Aldrich syndrome. Acta Derm Venereol 1985; Suppl 114: 125–128.
8. Cyster JG, Shotton DM, Williams AF. The dimensions of the T-lymphocyte glycoprotein leukosialin and identification of linear protein epitopes that can be modified by glycosylation. EMBO J 1991; 10: 893–902.
9. Rosenstein Y, Park JK, Hahn WC, Rosen FS, Bierer BE, Burakoff SJ. CD43, a molecule defective in Wiskott-Aldrich syndrome, binds ICAM-1. Nature 1991; 354: 233–235.
10. Nathan C, Xie QW, Halbwachs-Mecarelli L, Jin WW. Albumin inhibits neutrophil spreading and hydrogen peroxide release by blocking the shedding of CD43 (sialophorin, leukosialin). J Cell Biol 1993; 122: 243–256.
11. Sawada R, Tsuboi S, Fukuda M. Differential E-selectin-dependent adhesion efficiency in sublines of a human colon cancer exhibiting distinct metastatic potentials. J Biol Chem 1994; 269: 1425–1431.
12. Baum LG, Pang M, Perillo NL, Wu T, Delegeane A, Uittenbogaart CH, et al. Human thymic epithelial cells express an endogenous lectin, galectin-1, which binds to core 2 O-glycans on thymocytes and T lymphoblastoid cells. J Exp Med 1995; 181: 877–887.
13. Stockl J, Majdic O, Kohl P, Pickl WF, Menzel JE, Knapp W. Leukosialin (CD43)-major histocompatibility class I molecule interactions involved in spontaneous T cell conjugate formation. J Exp Med 1996; 184: 1769–1779.
14. Manjunath N, Correa M, Ardman M, Ardman B. Negative regulation of T-cell adhesion and activation by CD43. Nature 1995; 377: 535–538.
15. Fox DA, Chan LS, Kan L, Baadsgaard O, Cooper KD. Expression and function of the UM4D4 antigen in human thymus. J Immunol 1989; 143: 2166–2175.
16. Baadsgaard O, Tong P, Elder JT, Hansen ER, Ho V, Hammerberg C, et al. UM4D4+ (CDw60) T cells are compartmentalized into psoriatic skin and release lymphokines that induce a keratinocyte phenotype expressed in psoriatic lesions. J Invest Dermatol 1990; 95: 275–282.
17. Hansen ER, Vejlsgaard GL, Cooper KD, Heidenheim M, Larsen JK, Ho VC, et al. Leukemic T cells from patients with cutaneous T-cell lymphoma demonstrate enhanced activation through CDw60, CD2, and CD28 relative to activation through the T cell antigen receptor complex. J Invest Dermatol 1993; 100: 667–673.
18. Skov L, Chan LS, Fox DA, Larsen JK, Voorhees JJ, Cooper KD, et al. Lesional psoriatic T cells contain the capacity to induce a T-cell activation molecule CDw60 on normal keratinocytes. Am J Pathol 1997; 150: 675–683.
19. Kniep B, Peter-Katalinic J, Flegel W, Northoff H, Rieber EP. CDw60 antibodies bind to acetylated forms of ganglioside GD3. Biochem Biophys Res Commun 1992; 187: 1343–1349.
20. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980; Suppl 92: 44–47.
21. Wu K, Volke A, Lund M, Bang K, Thestrup-Pedersen K. Telomerase activity is spontaneously increased in lymphocytes from patients with atopic dermatitis and correlates with cellular proliferation. J Dermatol Sci 1999; 22: 24–30.
22. Babina M, Weber S, Henz BM. CD43 (leukosialin, sialophorin) expression is differentially regulated by retinoic acids. Eur J Immunol 1997; 27: 1147–1151.
23. Axelsson B, Youseffi-Etemad R, Hammarstrom S, Perlmann P.

Induction of aggregation and enhancement of proliferation and IL-2 secretion in human T cells by antibodies to CD43. J Immunol 1988; 141: 2912–2917.
24. Piletta PA, Wirth S, Hommel L, Saurat JH, Hauser C. Circulating skin-homing T cells in atopic dermatitis. Selective up-regulation of HLA-DR, interleukin-2R, and CD30 and decrease after combined UV-A and UV-B phototherapy. Arch Dermatol 1996; 132: 1230–1232.
25. Kruse S, Kuehr J, Forster J, Deichmann KA. Two common polymorphisms in the coding part of the CD43 gene are not associated with atopy. Int Arch Allergy Immunol 1998; 117: 244–247.
26. Younger IR, Harris DWS, Colver GB. Azathioprine in dermatology. J Am Acad Dermatol 1991; 25: 281–286.
27. Dutz JP, Ho VC. Immunosuppressive agents in dermatology. An update. Dermatol Clin 1998; 16: 235–251.
28. Pedersen BK, Aabom B. The *in vivo* effect of triethlphosphine gold (auranofin), sodium aurothiomalate and azathioprine on lymphocyte subsets of patients with rheumatoid arthritis. Allergy 1988; 43: 396–398.
29. Salmaggi A, Corsini E, La Mantia L, Dufour A, Eoli M, Milanese C, et al. Immunological monitoring of azathioprine treatment in multiple sclerosis patients. J Neurol 1997; 244: 167–174.
30. Rieber EP, Rank G. CDw60: A marker for human CD8 + T helper cells. J Exp Med 1994; 179: 1385–1390.
31. Birkhofer A, Rehbock J, Fricke H. T lymphocytes from the normal human peritoneum contain high frequencies of Th2-type CD8 + T cells. Eur J Immunol 1996; 26: 957–960.
32. Schöpf E, Kapp A, Kim CW. T-cell function in atopic dermatitis controlled examination of concanavalin A dose–response relations in cultured lymphocytes. Arch Derm Res 1978; 262: 37–44.
33. Jakob T, Huspith BN, Latchman YE, Rycroft R, Brostoff J. Depressed lymphocyte transformation and the role of prostaglandins in atopic dermatitis. Clin Exp Immunol 1990; 79: 380–384.
34. Thestrup-Pedersen K, Ellingsen AR, Olesen AB, Lund M, Kaltoft K: Atopic dermatitis may be a genetically determined dysmaturation of ectodermal tissue, resulting in disturbed T-lymphocyte maturation. Acta Derm Venereol 1997; 77: 20–21.