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AN IMMUNOLOGICAL STUDY OF PATIENTS WITH BULLOUS PEMPHIGOID

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Abstract. T-lymphocyte subpopulations, serum IgE levels and *in vitro* IgE synthesis were investigated in patients with bullous pemphigoid. Most of the 11 patient studied had an increase in serum levels of IgE and, compared with controls, their lymphocytes also showed augmented IgE production *in vitro*, both spontaneously and after pokeweed mitogen stimulation. A significant increase in the ratio of Leu-3 to Leu-2 positive lymphocytes was found in the peripheral blood of patients with pemphigoid, indicating a relative decrease in T-cells with suppressor function. The hypothesis that a defective regulative T-cell function may be responsible for the production of anti-basement membrane zone antibodies and the tendency to a rise in synthesis of IgE antibodies in patients with pemphigoid is discussed.

Key words: Bullous pemphigoid; IgE; T-cell subpopulations

Pemphigoid is a bullous disease which affects particularly the elderly. Its aetiology is unknown. Auto-antibodies, usually of the IgG type, against the basement membrane zone (BMZ) are found in the skin and often also in the serum of patients with pemphigoid. In support of an auto-immune aetiology for this disease are reports of an association between pemphigoid and systemic lupus erythematosus (7, 8) and rheumatoid arthritis (11). Suppressor lymphocytes, representing a subpopulation of T-lymphocytes, have been reported to be implicated in the mechanisms behind various auto-immune diseases (10). In elderly people, age-related changes occur in the immune apparatus, leading to reduction of several functions (4) and it has been presumed that this may cause increased incidence of auto-immune diseases with increasing age. Another sign of disturbed immune system in patients with pemphigoid is the finding that many of these patients have increased levels of serum IgE (2, 3, 6, 9). It is known from animal systems that IgE production is strongly influenced by suppressor and helper functions of T-cells. During recent years, there has been a rise in support for the theory that failure of T-cells with suppressor function are responsible for the tendency of excessive IgE production in patients with atopy (5, 13).

The aim of the present investigation was to study the hypothesis that patients with pemphigoid have defective immune regulation and that this disturbance might explain the production of auto-antibodies and the tendency to an increased formation of IgE. For this reason the patients were investigated with regard to disease activity, serum levels of anti-BMZ antibodies, serum levels of IgE, IgE synthesis *in vitro* and peripheral blood-T-lymphocyte subpopulations.

MATERIAL AND METHODS

Patients and controls

The patient material was obtained from the Department of Dermatology, Södersjukhuset, Stockholm. The diagnosis of bullous pemphigoid was made on the basis of clinical examination, histological investigation and standard direct and indirect immunofluorescence staining. In all patients direct immunofluorescence examination showed a linear deposition of IgG and or C3 along the BMZ. Only one of the patients (no. 4) was on oral corticosteroid medication (Prednisolone, 25 mg/d) when the tests were conducted and the patients were given no other immunosuppressive drugs.

The controls consisted of patients visiting the Department of Dermatology. Six had healed or almost healed leg ulcers, two had seborrhoeic keratosis, 2 had earlier been treated for basalioma and one for acrodermatitis chronica atrophicans. The blood samples from one patient were studied simultaneously with the samples from one control. Except for the eldest patient (no. 2), who was 98 years old, the controls were matched with regard to sex and age.

Cell culture technique

The lymphocyte culture technique was essentially the same as described previously (5). Mononuclear cells from peripheral blood were separated by the Ficoll-Isopaque method and washed four times and, 2×10^6 lymphocytic cells were cultivated in a 1 ml volume of Mishell-Dutton medium supplemented with 5% heat-inactivated calf serum. Cultures were set up without mitogens for deter-

Table I. *Results of immunological studies in patients with bullous pemphigoid and controls*
P = patient, C = control

Case no.	Sex	Age (y.)	Disease activity[a]	Anti-BMZ ab. titer	Leu-2 pos. 1 y. (%)	Leu-3 pos. 1 y. (%)	Leu-3/ Leu-2 (Ratio)	IgE/s (U/ml)	IgE synthesis in vitro (pg/ml) Spontaneous	PWM	ConA
P1	M	71	++	1/640	–	–	–	355 (304[b])	1 300	1 900	1 200
C1	M	74			–	–	–	–	<200	<200	<200
P2	F	97	+	<1/10	15	61	4.1	533 (322[b])	350	1 450	<200
C2	M	73			34	41	1.2	–	250	550	<200
P3	F	86	0	1/640	14	51	3.6	124 (253[b])	250	450	<200
C3	F	83			44	49	1.1	–	<200	<200	<200
P4	F	73	++	1/80	13	63	4.9	179 (4[b])	1 450	650	700
C4	F	77			25	43	1.7	–	1 000	1 000	500
P5	F	82	++	1/20	24	50	2.1	41 (30[b])	350	900	1 150
C5	F	77			21	62	3.0	–	750	850	<200
P6	F	74	0	1/320	16	67	4.2	535 (495[b])	1 600	1 800	1 000
C6	F	78			19	53	2.8	20	200	200	<200
P7	M	84	+	1/80	20	56	2.8	275 (246[b])	250	950	<200
C7	M	80			31	52	1.7	80	<200	250	<200
P8	F	83	+	1/20	20	44	2.2	43	<200	<200	<200
C8	F	85			19	51	2.7	–	<200	<200	<200
P9	F	86	+	1/320	11	29	2.6	800 (>1 000[b])	1 200	2 250	500
C9	F	80			28	28	1.0	–	<200	<200	<200
P10	F	74	+	1/160	19	54	2.8	730 (>1 000[b])	300	250	<200
C10	F	73			42	49	1.2	–	<200	<200	<200
P11	M	78	0	1/80	21	61	2.9	351 (121[b])	2 850	–	1 550
C11	M	79			25	62	2.5	–	650	–	250
Mean P		81			17	54	3.2	361			
Mean C		78			29	49	1.9	Normal value <120			

[a] Disease activity: ++, new bullae appearing every day; +, new bullae and/or eczematous lesions appeared the week prior to the test; 0, no new lesions appeared the week prior to the test.
[b] Test made on a different occasion.

mination of spontaneous in vitro IgE synthesis and with the following mitogens: Pokeweed mitogen (PWM) 4 μg/ml (Serva Finbiochemica, Heidelberg, GFR) and Concanavalin A (conA) 50 μg/ml (Pharmacia Fine Chemicals, Uppsala, Sweden). The tests were run in duplicate. The culture tubes were gassed with 10% CO_2 and incubated for 7 days. The supernatants were frozen and stored until IgE determinations could be performed.

IgE levels were determined on the supernatant fractions by a modified PRISTmethod (PRIST; Pharmacia, Uppsala, Sweden). In order to be able to measure low IgE values, the PRIST discs were incubated for 24 h with 0.8 ml of the supernatant. With this method, determinations were obtained for IgE levels as low as 200 pg/ml. In vitro IgE synthesis was assessed as IgE in the supernatant fraction after the culture period minus IgE in the supernatant fraction from tubes in which the cells had been killed by repeated freezing and thawing at the start of the culture period.

The conventional PRIST method (Pharmacia) was used to determine the serum levels of IgE. With this method, the normal value (geometric mean ±2 SD) of IgE/serum in non-atopic Swedish adults is < 120 U/ml.

Determination of T-lymphocyte subpopulations

Mononuclear cells from peripheral blood were separated by the Ficoll-Isopaque method. Phagocytosing cells were removed with a magnet after treatment of the cell suspension with carbonyl iron. Fluorescein-conjugated monoclonal antibodies were used to identify T-lymphocyte subpopulations. One million lymphocytic cells were incubated for 45 min at 4°C with monoclonal anti-human Leu-2a and Leu-3a antibodies (Becton Dickinson Facs Systems, 490-B Lakeside Drive, Sunnyvale, CA 94086, USA). Leu-2a was diluted 1/100 and Leu-3a 1/80 in a 50 μl volume of PBS containing 5% calf serum. Thereafter, the cells were washed twice in ice-cold PBS and kept on ice. Three hundred lymphocytic cells were counted and the percentage of fluorescent cells was determined in a Leitz fluorescence microscope.

Statistics

Student's *t*-test for paired observations was used in the statistical analysis.

RESULTS

The results of the investigation are shown in Table 1. All but 2 patients had increased serum levels of IgE (>120 U/ml). In all tests but one the lymphocytes from the patients with increased serum levels of IgE also produced more IgE *in vitro* than the corresponding control, both spontaneously and after PWM stimulation. There was a tendency to decreased in vitro IgE production in the lymphocyte cultures after ConA stimulation (decrease in 9 of 11 cultures from the patients and in 5 of 11 control cultures).

Compared with their respective controls, the patients had a statistically significant increase in the Leu-3/Leu-2 ratio; mean value in patients was 3.2 and in controls 1.9 (*t*-test for paired observations shows $p<0.02$). The 2 patients with normal serum IgE levels had the lowest Leu-3/Leu-2 ratios of all patients and both also had low anti-BMZ titres. The material was too small to draw any conclusions as to whether there were any correlations between disease activity and parameters such as titres of anti-BMZ antibodies, IgE/s levels, IgE synthesis in vitro or Leu-3/Leu-2 ratios.

DISCUSSION

The present study confirms the reports of earlier investigations (2, 3, 6, 9) that patients with pemphigoid often have increased serum IgE levels. In most cases the lymphocytes from the patients also produced more IgE *in vitro* than lymphocytes from the controls, and this was valid for both spontaneous IgE production and after PWM stimulation. ConA is a T-cell mitogen which preferentially stimulates suppressor T-cell functions (12). In most cases conA stimulation of the lymphocytes from the patients resulted in decreased *in vitro* IgE synthesis. This may indicate that stimulation of suppressor T-cell functions may decrease (normalize) IgE production in patients with pemphigoid.

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Ahmed et al. (1) have found normal percentages of T-cells, but diminished *in vitro* lymphocyte rephigoid. Khan et al. (6) have reported a low T-lymphocyte count, impaired lymphocyte transformation response to PHA and high serum level of IgE in one patient with pemphigoid; these tests normalized after transfer factor treatment. The results of the present investigation also support the hypothesis that patients with pemphigoid have T-cell disturbances. Leu-3 positive lymphocytes are associated with helper/inducer functions and Leu-2 positive cells are associated with suppressor/cytotoxic functions. The patients had an increased Leu-3/Leu-2 ratio which may indicate a relative decrease in T-cells with suppressor/cytotoxic function. In this age group most individuals have symptoms of age-related diseases, for example arteriosclerosis. As there could have been differences between patients and controls regarding their general state of health, it cannot be excluded that this might have influenced the results. Nor can the possibility be ruled out that the T-cell disturbance is secondary to the disease. However, the findings can also signify disturbed regulative functions of the T-cells. A defective suppressor function of the T-cells or a hyperfunction of helper T-cells may thus be responsible for the synthesis of anti-BMZ antibodies and the tendency to increased IgE production in patients with bullous pemphigoid.

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