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Detection of Circulating Immune Complexes in Patients with Atopic Dermatitis and Psoriasis

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Sera from 32 patients with atopic dermatitis and 22 patients with psoriasis were examined for the presence of circulating immune complexes (CIC) in comparison to 51 healthy controls using a PEG-precipitation laser nephelometer technique. Different patterns of the precipitated proteins were found in both diseases. In atopic dermatitis C3 and IgG were significantly elevated in CIC. Furthermore, significantly increased amounts of IgE were found in the precipitates. Groups with high and low serum IgE levels showed no significant differences in the quantity of precipitated proteins. Skin involvement did not correlate with CIC. In psoriasis patients, a different pattern with significantly increased IgA, IgG, IgM and C3 was found in the precipitates. IgE was also significantly increased in comparison to the controls. No difference was found between patients with psoriasis vulgaris and psoriasis guttata. CIC in psoriasis and in atopic dermatitis thus showed a characteristic composition. However, a detection of CIC was not directly related to the cutaneous manifestation of the disease. *Key words: PEG precipitation technique; IgE.* (Received July 3, 1985.)

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Atopic dermatitis (AD) and Psoriasis are well-known inflammatory skin diseases. Several reports describe abnormalities of the immune system in both diseases (1, 2, 3, 4, 5). Although, there is little information about the role of the complement system and complement activating factors in these disorders, activation of the complement system in AD be considered since decreased total haemolytic complement activity has been described (6, 7). Complement split products of C3 have been found in the serum of AD patients (6). Detection of C5a in the scales of psoriatic patients (8) and detection of the complement split product C3a in the plasma (9) of the patients are additional signs of the activation of the complement cascade. This activation is possibly triggered by immune complexes. Circulating immune complexes have been described by several authors in both diseases (10, 11, 12, 13, 14, 15, 16). Our interest was to investigate the presence and possible differences in the composition of CIC in both diseases using a PEG-precipitation laser nephelometer technique. Furthermore, the presence of IgE in the precipitates was to be determined.

MATERIALS AND METHODS

Thirty-two patients suffering from AD (as classified by Hanifin & Rajka (17)) were examined. Only patients with mild to intermediate disease were tested. The psoriasis group consisted of 22 patients. Fourteen patients exhibited symptoms of psoriasis vulgaris, whereas 9 patients showed the guttata type of psoriasis. In parallel to AD only patients with mild to intermediate disease were tested. None of the patients had received systemic or local steroid therapy or therapy with UV light 6 weeks prior to blood collection.

Table I. *Levels of CIC in the sera of patients with atopic dermatitis*

	2.75% PEG-precipitates		
	Atopic dermatitis	Controls	
IgA ($g/l \times 10^{-3}$)	22.8±2.8[b] (32)[a]	19.1±1.3 (48)	NS[c]
IgG ($g/l \times 10^{-3}$)	123.3±11.2 (32)	73.3±6.0 (51)	$p<5\times10^{-5}$
IgM ($g/l \times 10^{-3}$)	15.6±4.6 (30)	27.4±2.4 (51)	$p<0.013$
C3 ($g/l \times 10^{-3}$)	13.3±1.1 (32)	9.2±0.6 (48)	$p<5.2\times10^{-4}$
C4 ($g/l \times 10^{-3}$)	3.8±0.5 (32)	4.3±0.4 (44)	NS
log IgE (kU/l)	0.657±0.112 (32)	0.087±0.006 (20)	$p<3.4\times10^{-4}$

[a] Number of probands.
[b] Mean±SEM.
[c] Not significant.

Controls

The control group consisted of 51 and in the case of IgE of 20 healthy non-atopic blood donors. Atopy was excluded through history and laboratory findings.

Collection of serum samples

For the measurement of CIC 10 ml blood was collected by venipuncture and kept at room temperature for 60 min and then at 4°C for another 30 min. After inmediate centrifugation the serum was frozen at −70°C.

Detection and characterization of circulating immune complexes

CIC in the sera were determined according to Krapf et al. (18). The serum samples were precipitated at a final concentration of 2.75% PEG. In the washed precipitates IgG, IgA, IgM, C3 and C4 were measured using an automated nephelometer (Hyland Laser-Nephelometer PDQ/Disc 120) with a special program for use in the Hewlett-Packard 9815S calculator. Furthermore, IgE was determined in dissolved 2.75% PEG precipitates using Enzygnost-IgE (Behringwerke, Marburg).

Statistical analysis

Statistical significance of differences between the tested groups were calculated using two-tailed Student's t-test. IgE values were transformed logarithmically before statistical analysis.

RESULTS

Atopic dermatitis

IgG and C3 were significantly elevated in the precipitates of patients with AD (Fig. 1, Table I). IgA and C4 showed no significant differences in comparison to the controls, IgM was significantly diminished. Furthermore, IgE was significantly elevated in the precipitates (Table I). After separation of the patients in the groups with high and low IgE serum levels, no significant differences in precipitated IgA, IgG, IgM, C3 and C4 were found. IgE was significantly elevated in the precipitates of patients with high serum IgE (Table II). Unspecific precipitation could be excluded, since addition of IgE to normal serum did not lead to increased IgE content in precipitates (data not shown). Furthermore, there was no significant correlation between the extent of body surface involvement and the level of precipitated proteins (Table III).

Psoriasis

Also in psoriasis IgG and C3 were significantly elevated in the precipitates (Fig. 1, Table IV). Furthermore, in contrast to AD, IgA and IgM were significantly increased in the CIC. C4 was significantly diminished. In addition IgE was found to be elevated significantly. No

Table II. *Levels of CIC in the sera of patients with atopic dermatitis—relation to serum IgE*

	2.75% PEG-precipitates		
Serum IgE (KU/ml) ...	<100	≥100	
IgA ($g/l \times 10^{-3}$)	23.3±6.1[b] (10)[a]	22.6±3.1 (22)	NS[c]
IgG ($g/l \times 10^{-3}$)	110.4±16.5 (10)	129.1±14.6 (22)	NS
IgM ($g/l \times 10^{-3}$)	13.0±5.8 (8)	16.6±6.0 (22)	NS
C3 ($g/l \times 10^{-3}$)	12.4±2.2 (10)	13.7±1.3 (22)	NS
C4 ($g/l \times 10^{-3}$)	4.0±1.3 (10)	3.7±0.4 (22)	NS
log IgE (kU/l)	0.092±0.092 (10)	0.914± 0.123 (22)	$p<0.00016$

[a] Number of probands.
[b] Mean±SEM.
[c] Not significant.

Table III. *Levels of CIC in the sera of patients with atopic dermatitis—relation to extent of skin involvement of the disease*

	2.75% PEG-precipitates		
	Percentage of skin involved ...		
	0≤30	30≤60	60≤100
IgA ($g/l \times 10^{-3}$)	26.4±5.1[b] (14)[a]	17.4±2.9 (9)	22.8±5.2 (9)
IgG ($g/l \times 10^{-3}$)	120.6±19.2 (14)	133.4±16.2 (9)	117.2± 22.9 (9)
IgM ($g/l \times 10^{-3}$)	13.0±4.4 (12)	24.1±11.2 (9)	10.6±9.1 (9)
C3 ($g/l \times 10^{-3}$)	13.6±2.0 (14)	13.0±1.5 (9)	13.2±2.3 (9)
C4 ($g/l \times 10^{-3}$)	3.9±1.0 (14)	3.9±0.4 (9)	3.6±0.8 (9)
log IgE (kU/l)	0.472±0.154 (14)	0.748±0.200 (9)	0.855±0.246 (9)

[a] Number of probands.
[b] Mean±SEM.

significant difference was found between patients with psoriasis guttata and psoriasis vulgaris (Table V).

A comparison of the precipitate patterns (Fig. 1) between the investigated diseases showed the distinctly different composition of CIC. In AD, the relatively large amount of IgE compared to the IgG contents was noteworthy, whereas IgA and IgM were not found to be elevated. In psoriasis, all four Ig classes were found in CIC, the relative amount of IgE was less pronounced, and the total contents of Ig were found to be higher than in atopic dermatitis.

DISCUSSION

In AD we found elevated CIC containing IgG, IgE and C3. Our results support the findings of Brostoff who described complexed IgE in atopic patients' sera (11). IgG containing immune complexes were also found in patients with atopic eczema and milk protein allergy (19). Ferguson et al. (12) described IgG containing immune complexes in children with atopic eczema, but no measurement of IgE concentrations was performed. In all studies including our own, no significant correlation with the grade of severity of the

Table IV. *Levels of CIC in the sera of patients with psoriasis*

	2.75% PEG-precipitates		
	Psoriasis	Controls	
IgA ($g/l \times 10^{-3}$)	40.6±5.3[b] (22)[a]	19.1±1.3 (48)	$p<1.1\times10^{-6}$
IgG ($g/l \times 10^{-3}$)	234.2±39.9 (22)	73.3±6.0 (51)	$p<1.2\times10^{-7}$
IgM ($g/l \times 10^{-3}$)	40.9±6.7 (18)	27.4±2.4 (51)	$p<0.019$
C3 ($g/l \times 10^{-3}$)	16.2±1.1 (19)	9.2±0.6 (48)	$p<4.2\times10^{-8}$
C4 ($g/l \times 10^{-3}$)	1.3±0.4 (22)	4.3±0.4 (44)	$p<3.4\times10^{-6}$
log IgE (kU/l)	0.687±0.165 (22)	0.087±0.06 (20)	$p<0.0018$

[a] Number of probands.
[b] Mean±SEM.

Table V. *Levels of CIC in the sera of patients with psoriasis—comparison between psoriasis guttata and psoriasis vulgaris*

	2.75% PEG-precipitates	
	Psoriasis guttata	Psoriasis vulgaris
IgA ($g/l \times 10^{-3}$)	39.8±11.3[b] (8)[a]	41.1±5.8 (14)
IgG ($g/l \times 10^{-3}$)	276.9±103.6 (8)	209.9±24.8 (14)
IgM ($g/l \times 10^{-3}$)	31.8±8.2 (8)	48.2±9.9 (10)
C3 ($g/l \times 10^{-3}$)	15.6±1.8 (8)	16.6±1.4 (11)
C4 ($g/l \times 10^{-3}$)	1.5±0.6 (8)	1.1±0.6 (14)
log IgE (kU/l)	0.360±0.246 (8)	0.873±0.208 (14)

[a] Number of probands.
[b] Mean±SEM.

disease could be detected. The source of antigens leading to immune complex formation is presently unknown. The presence of food antigen has to be considered (19, 20). Brostoff reported the existence of IgE-egg albumin immune complexes of high molecular weight in the serum of the patients (20). Complement and immunoglobulin deposits have been reported in the skin of patients with AD (21), but whether there exists any correlation between immune complexes in the skin and CIC in the serum of the patients is presently unknown. Alterations of the complement system have also been described in the serum of patients with AD (6, 9). However, whether the activation of the complement system in AD is merely a consequence of immune complex solubilization remains to be determined.

In psoriasis several authors report the existence of CIC (10, 13, 14, 15, 16). In a pattern different to patients suffering from AD we found elevated levels of IgA, IgG, IgM and C3 in the precipitates. Considerable amounts of IgE were also detected. This finding is paralleled by elevation of serum IgE in psoriasis (1). Hall et al. (13) recently described elevated levels of IgA containing immune complexes in psoriasis. In contrast to their results we also found IgG and IgM to be significantly increased in the precipitates. The differences may in part be due to the different assay systems used for detecting CIC. The role of IgE containing CIC in psoriasis is unclear at present. Since CIC were found in soluble substances from psoriatic scales (22) the source of antigen may possibly be located in the dermis of the patients. Deposition of immunoglobulins and complement in the dermis (23) has led to the suggestion that immune complexes may play a role in the inflammatory process in psoriasis. Furthermore, alteration of the complement system and

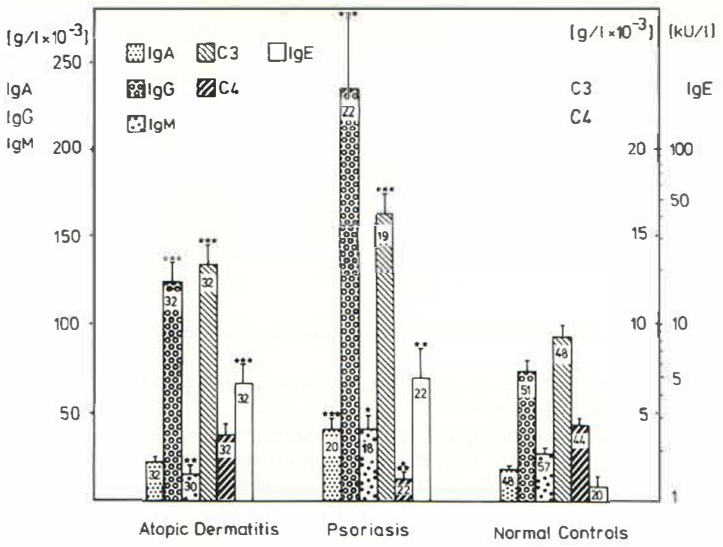

Fig. 1. Composition of CIC in the sera of patients with atopic dermatitis and psoriasis in comparison to normal controls. Values represent mean ± SEM.

elevation of complement split product C3a was described in psoriasis (9). C5a could be detected in the psoriatic scales (8). Taken together, there is suggestive evidence that the complement system is activated by immune complexes in the dermis. Since there was no corelation to skin involvement of the disease and no significant difference was found between psoriasis guttata and psoriasis vulgaris, we suggest that CIC only reflect the situation in the dermis of the patient. Conflicting results have been reported on the concentration of CIC under therapy (13, 24, 25, 26). So it is not clear if these CIC play a primary or secondary role in the pathogenesis of psoriasis.

Since we found significantly elevated concentrations of CIC both in AD and psoriasis, these may be responsible for the alteration of the complement system described in both diseases, since solubilization and removal of CIC apparently is a physiological function of the complement system. Activation of the complement cascade leads, however, to the generation of complement split products C3a and C5a which may be important for the development of inflammation in both diseases.

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