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Involvement of Complement in Atopic Dermatitis

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There is little information about the role of complement in atopic dermatitis (AD). We studied the levels of both normal complement components and activation products in peripheral blood of patients with mild to intermediate disease. 35 patients had not received systemic or topical steroid therapy 6 weeks prior to blood collection. C3, C4 and C1 INA were determined in serum by radioimmunodiffusion. C3a and C5a levels were measured in EDTA plasma by radioimmunoassay. Compared to healthy non-atopic controls C3, C4 and C1 INA were found to be increased significantly. There was a tendency towards increased C3a levels but the difference was not significant. No measurable amounts of C5a were detected. Elevations of C3a were correlated with elevated levels of C3. The results suggest that the complement system participates in the inflammatory process in AD. *Key words: Atopic dermatitis; Complement; Anaphylatoxins; C3a; C5a.*

There is little information about the role of complement in atopic dermatitis (AD). Some authors measured complement C3 levels and found them to be mostly within normal limits or even increased (7, 8, 11). Split products of C3 were detected in the peripheral blood of AD patients (8). Also decreased total serum complement hemolytic activity (CH 50) was reported (8, 12). In some patients a significantly decreased hemolytic activity of complement component C2 (C2H 50) and C3 (C3H 50) was detected (8). We could recently show that in patients with severe AD the complement split product C3a was significantly increased as compared to non-atopic controls. But it could not be excluded that this activation of complement was associated with a secondary infection of the skin in this group of patients (6).

In the present study we investigate if signs of complement activation can be detected in peripheral blood of patients with mild to moderate disease.

PATIENTS AND METHODS

Patients. 35 patients suffering from AD according to the criteria of Hanifin & Rajka (3) were examined. The group consisted of 11 male and 24 female patients between 14 and 81 years of age with a mean age of 31±3 years. Only patients with mild to moderate disease were included. None of the patients had received systemic or topical steroid therapy, or therapy with UV light 6 weeks prior to blood collection.

Controls. The control group consisted of 27 (measurements of C3a and C5a) respectively 16 (measurements of C3, C4 and C1 INA) healthy nonatopic blood donors. Atopy was excluded by history and laboratory findings (3).

Collection of serum and plasma samples. For measurements of C3a and C5a blood was collected in disodium ETDA (Vacutainer, Becton and Dickinson, No. 6453). Plasma was separated by centrifugation and frozen immediately, within 30 minutes following venipuncture, at −70°C. For measurements of C3, C4 and C1 INA 10 ml blood were collected by venipuncture and kept at room temperature for 60 minutes, then at 4°C for another 30 minutes. After immediate centrifugation the serum was frozen at −70°C.

Measurement of C3, C4 and C1 INA. Measurements were done by immunodiffusion using commercially available plates (Behring, Marburg, West Germany). All measurements were done at the same time with the same batch of immunodiffusion plates.

Measurement of $C3a_{desarg}$ and $C5a_{desarg}$. C3a and C5a levels were determined in EDTA plasma by radioimmunoassay (Upjohn Diagnostics, Kalamazoo, Michigan, USA) (5).

Statistical analysis. For statistical analysis the Student's *t*-test was used.

RESULTS

C3a, C5a, C3, C4 and C1 Inactivator (C1 INA) were measured in the blood of patients with AD. The measurements of C3a and C5a were done in EDTA-plasma to avoid in vitro activation of the complement system (5). C3, C4 and C1 INA were determined in the serum of the patients. Effects of systemic or local therapy and the possible influence of superinfection of the skin were excluded in the tested patients.

We found C3, C4 and C1 INA to be increased significantly in the patients (Table I) and there was a tendency towards increased C3a levels (Table I). No measurable amounts of C5a could be detected in the EDTA plasma of the patients. Elevations of C3a were correlated with elevated levels of C3 ($r=0.55$, $p<0.001$), C4 ($r=0.5$, $p<0.003$) and C1 INA ($r=0.61$, $p<0.001$).

DISCUSSION

The present results suggest that the complement system participates in the inflammatory process in AD.

Reduced serum complement levels may be due to complement consumption, perhaps caused by in vivo formation of antigen antibody complexes, or decreased synthesis or increased catabolism of complement (5). In contrast, the significance of elevated serum complement levels is not clear. Many diseases are reported to be associated with elevated serum complement concentrations. For example, acute rheumatic fever, ulcerative colitis, Reiter's syndrome, gout and obstructive jaundice (10). The most likely mechanism is overproduction, possibly by activated macrophages (4).

In contrast to our results other authors found levels of C3 normal or even slightly increased in AD (7, 8, 11). However, in most studies the influence of therapy is not excluded. Also the measurements of normal complement components in serum is difficult without using a standardized blood collection technique to avoid artificial in vitro activation of the complement system (9). Furthermore, measurements were done at the same time and with the same batch of media and assay kits in all patients. Non-standardized blood collection techniques may lead to an in vitro activation of the complement system, which may reduce elevated complement levels.

We could also demonstrate that levels of C3a anaphylatoxin as a sign of in vivo activation of the complement system, were elevated, but not significantly in AD. The elevated levels of C3 were correlated with C3, C4 and C1 INA. C5a could not be detected in measurable amounts. This may be due to the rapid binding of C5a to cells and its subsequent internalization and degradation (2).

The generation of split products of the complement system in AD could be the result of a local activation in the dermis of the patients, maybe triggered by antigen antibody complexes (1) or by other unknown complement activating factors. The elevation of C3a in

Table I. *Levels of C3, C4, C1 INA and C3a in atopic dermatitis (mean±SEM)*

	Atopic dermatitis	Controls	
C3 (mg/dl)	107±5 (35)[a]	71±5 (16)	$p<0.0005$
C4 (mg/dl)	50±3 (35)	30±2 (16)	$p<0.0005$
C1 INA (mg/dl)	47±2 (35)	37±2 (16)	$p<0.005$
C3a (ng/ml)	199±23 (35)	161±8 (27)	NS

[a]*Numbers of probands in parenthesis.*

AD was not significant, but it is difficult to detect C3a in significant amounts in peripheral blood, if the activation is in dermis, particularly in patients with mild to intermediate disease. Our results are in keeping with the findings of Ring et al. who found split products and decreased complement hemolytic activity in some patients with AD (8).

In conclusion, we propose that alterations of normal complement components in AD are caused by rebound production following a possibly continuous local complement consumption induced by immune complexes or other complement activating factors. The measurement of normal complement components might be useful to determine the disease activity in follow-up studies.

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