

Antibody-Dependent Monocyte-Mediated Cytotoxicity in Atopic Dermatitis

Relation to Clinical State and Cyclic AMP Response

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Antibody-dependent monocyte-mediated cytotoxicity was investigated in adult patients with atopic dermatitis. In patients with active dermatitis monocyte cytotoxicity was reduced. Serial studies of individual patients demonstrated that a reduced cytotoxicity in clinical exacerbation did not normalize during clinical remission. The reduced monocyte cytotoxicity was not caused by an abnormal influence of lymphocytes. During the cytotoxicity reaction a rapid and transient elevation of monocyte cyclic AMP occurred. In atopic dermatitis this elevation was decreased, but no relation was found between the monocyte cytotoxicity and the cyclic AMP response in these patients.

Key words: antibody-dependent cell-mediated cytotoxicity; atopic dermatitis; cyclic AMP; lymphocytes; monocytes

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Both the cause and pathogenesis of atopic dermatitis (AD) remain obscure. However, during recent years two major proposals have been advanced – the immunological hypothesis and the beta-adrenergic blockade hypothesis of Szentivanyi (21). Studies on blood monocytes have been productive. Patients with AD have both decreased monocyte chemotaxis (5, 19) and decreased antibody-dependent cell-mediated cytotoxicity (ADCC) (12, 15). In our earlier studies (12, 15) all patients had active dermatitis, but in order to evaluate the significance of the results, it is necessary to perform serial investigations of individual patients during periods of quiescent dermatitis.

The original demonstration of a diminished cyclic AMP response to beta-adrenergic stimu-

lation (2) has recently also been shown to stimulation with prostaglandin E₁ (16) and histamine (3). This applies to monocytes (9) as well as to other leukocytes. These discoveries indicate a more general defect of membrane activation in AD. A defect that might provide a possible linkage between the immunological and pharmacological theories.

This paper reports studies concerning monocyte ADCC in relation to the clinical state of AD including investigations of the cyclic AMP response during the ADCC reaction.

MATERIAL AND METHODS

Patients

For the serial study six patients (four females and two males) aged from 21 to 33 years with widespread dermatitis typical of AD but without cutaneous infection were selected. Two had a past history of asthma, but received no treatment for asthma during the observation period. All were observed for a period of approximately 9 months. The first blood sample was obtained during acute widespread dermatitis when patients were treated with potent topical steroids. The investigation was repeated in clinical remission when steroids had been withdrawn for more than 2 weeks. After remission blood samples were repeated every second month over a period of 6 months, and the extent and severity of the disease was graded on a scale 1 to 5 according to Rogge & Hanifin (18). During this period all patients experienced some fluctuations in the clinical state of their dermatitis, but treatment was limited to intermittent use of topical hydrocortisone and hydrocortisone-butyrate.

For the study of the cyclic AMP response in monocytes, 16 patients (eight females and eight males) aged from 14 to 30 years with a slight to moderate AD (mean extent score: 2.3 and mean severity score: 1.5) were included. Six patients had a past history of asthma and/or allergic rhinitis. Treatment consisted of topical hydrocortisone or hydrocortisone-butyrate. Patients receiving antihistamines had these withdrawn for at least 48 h before the investigations. No other systemic therapy was given. A group of healthy staff members with an age distribution comparable to the patient group served as normal controls. Four female carriers of X-linked chronic granulomatous disease characterized by a depressed monocyte ADCC due to a defective respiratory burst (1) served as patient controls.

Isolation of monocytes

Monocytes were isolated from heparinized venous blood as previously described (13). The isolation procedures included an adherence

step allowing the separation of monocytes from lymphocytes. The adherent monocytes were detached by lowering the temperature to 4°C and identified by esterase staining.

In control persons the mean purity was 94% (range 90–96%) and in patients 92% (range 89–96%). The contaminating cells were lymphocytes. Occasionally up to 3% neutrophils were found. For some experiments monocytes were diluted with non-adherent lymphocytes (containing about 5% monocytes), or highly purified monocytes were obtained by subjecting the adherent monocytes to a second adherence step.

Antibody-dependent monocyte-mediated cytotoxicity

The assay was performed as previously described (14). Tests were set up in duplicates in volumes of 300 µl. After incubation of monocytes (1.0×10^5) with ^{51}Cr -labelled human type B erythrocytes ($1-8 \times 10^5$) and anti-B serum for 1 h or 18 h, half of the supernatant (S) was withdrawn and counted together with the residue (R) in a gamma counter. ^{51}Cr -release was calculated according to the following formula:

$$^{51}\text{Cr-release} = \frac{\text{S cpm} \times 2}{\text{S cpm} + \text{R cpm.}}$$

By subtracting values for control tubes the specific release was defined. Results were expressed as the number of erythrocytes lysed per monocyte:

$$\frac{\text{number of target cells} \times \text{specific release}}{\text{number of monocytes.}}$$

The standard deviation of the differences between the duplicates (s.d.) was generally smaller when ADCC was small. After incubation for 18 h at target to monocyte ratio 8:1 s.d. was 0.02 for ADCC below 1.00, 0.04 for ADCC between 1.00 and 1.50, and 0.06 for ADCC above 1.50. At mean ADCC (1.56) for 32 normal persons the coefficient of variability was 0.038. In eight normal persons the determination of ADCC was repeated twice. The mean difference between de-

terminations on 2 successive days was 0.04 ranging from 0.02 to 0.06, after 2 months 0.06 ranging from 0.04 to 0.08. Using the formula $\text{mean} \pm 2.0 \times \text{s.e.m.}$ it could be calculated that the differences were consistently within the 95% limits for the duplicates.

Cyclic AMP formation

Unlabelled type B erythrocytes and anti-B serum were treated as for the ADCC assay (14), except that all dilutions were made in Hanks' balanced salt solution with 5% (v/v) foetal calf serum. All suspensions were preheated to 37°C for about 1 min. To 54 μl monocytes ($15 \times 10^6/\text{ml}$) were added 6 μl erythrocytes ($150 \times 10^6/\text{ml}$) suspended in anti-B serum. In control tubes anti-B serum was replaced by normal AB serum. The tubes were immediately placed in a water bath at 37°C. At the time indicated the reaction was stopped with 60 μl boiling potassium phosphate buffer (pH 5.5, 4 mM theophylline 9 mM 2-mercaptoethanol). After boiling for 8 min the tubes were placed in an ice-water bath. Denatured protein was removed by centrifugation. Then supernatants of 50 μl were withdrawn in duplicates. Cyclic AMP was determined by the protein binding competition method, using a cAMP assay kit from Radiochemical Centre, Amersham, England.

Statistical analysis

Statistical significance was assessed using Wilcoxon's test for two samples or for paired differences. A *P* value below 0.05 was considered significant. In the correlation studies the Spearman's coefficient of rank was applied.

RESULTS

In the serial studies patients with decreased monocyte ADCC during the clinical exacerbation were investigated (Fig. 1). From exacerbation to remission the mean ADCC increased from 0.88 to 0.96, but this increase was insignificant. In no case were normal values obtained. To solve the question whether the treatment with potent topical steroids had maintained im-

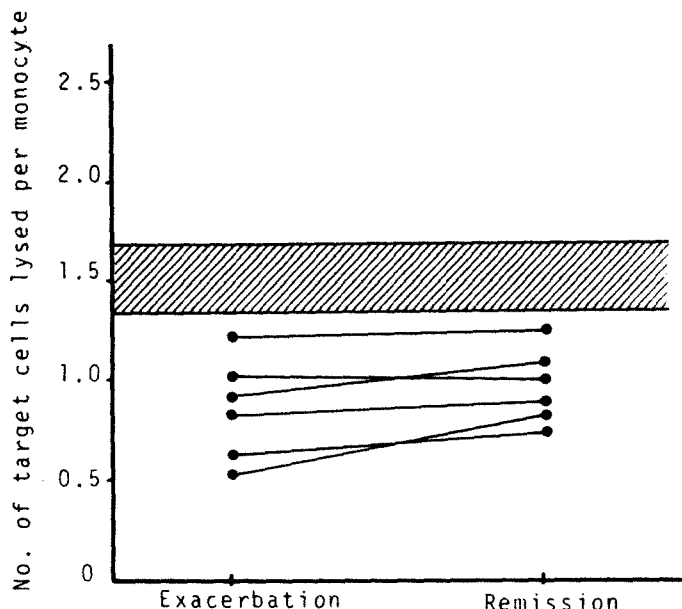


Fig. 1. Monocyte ADCC in atopic dermatitis in relation to clinical exacerbation and remission. Each line connects the pair of results from one patient. The hatched interval indicates the 95% confidence limits as determined in 32 normal controls. Erythrocyte to monocyte ratio 8:1 incubation time 18 h.

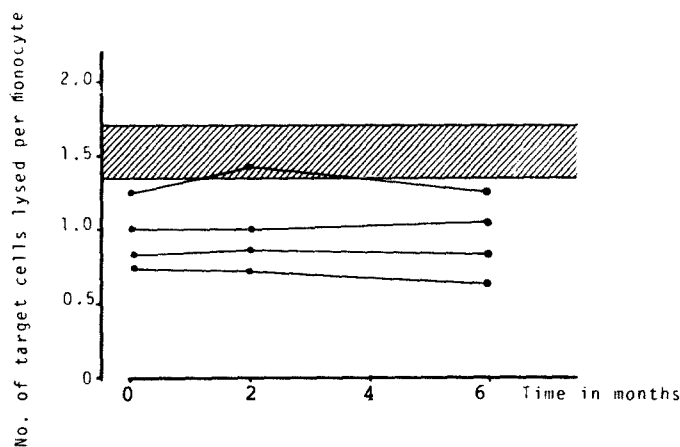


Fig. 2. Serial investigations of monocyte ADCC in atopic dermatitis. At time zero all patients were in remission. During the 6 month observation period all patients experienced variations in the activity of the dermatitis. The hatched interval indicates the 95% confidence limits as determined in 32 normal controls. Erythrocyte to monocyte ratio 8:1. Incubation time 18 h.

paired monocyte ADCC, even though clinical remission had occurred, four of the six patients were observed for a period of 6 months (Fig. 2). During this period all patients experienced some fluctuations of their disease activity, but only received intermittent therapy with topical steroid of low potency. As illustrated only insignificant alterations of monocyte ADCC occurred.

Table 1
Effect of lymphocytes on monocyte ADCC in atopic dermatitis

Cell population	% lymphocytes	No. of targets lysed per monocyte
Adherent mononuclear cells (after 1 adherence step)	8 (4–11)	1.11 (0.97–1.31)
Adherent mononuclear cells subjected to a second adherence step	1 (0–3)	1.06 (0.99–1.24)
Mixture of adherent and non- adherent mononuclear cells (1:1)	51 (49–53)	1.12 (1.01–1.33)

Target cell to leucocyte ratio 8:1. Incubation time 18 h.

Values were expressed as means. In brackets, the range for six patients is indicated.

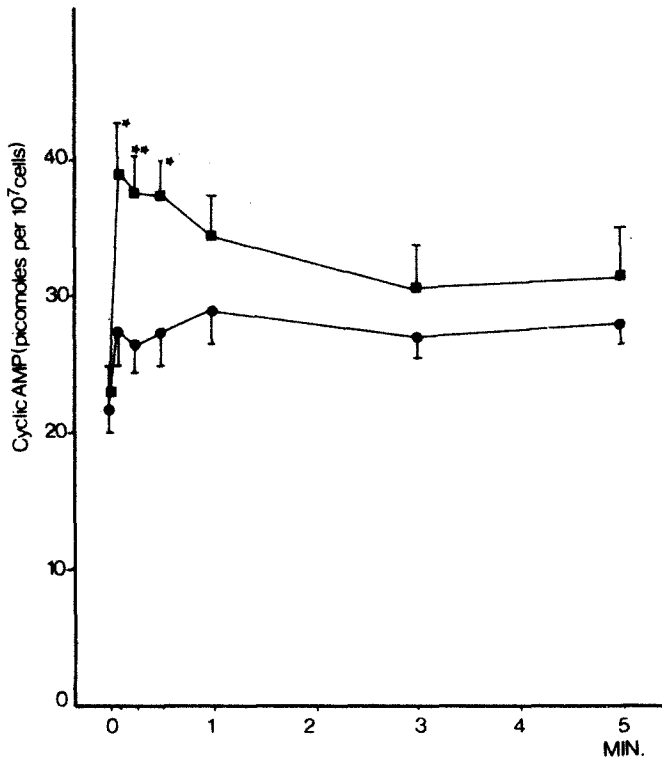


Fig. 3. Time-course for monocyte cAMP after addition of IgG-sensitized human erythrocytes in healthy controls, $n = 10$ (■) and in patients with atopic dermatitis, $n = 16$ (●). Erythrocyte to monocyte ratio 1:1. Values were expressed as mean $\pm$ s.e.m.

* $P < 0.02$, ** $P < 0.01$.

In order to evaluate a possible interference by residual lymphocytes with monocyte ADCC, cell suspensions containing lymphocytes in different proportions were used as effector cells (Table 1). The amount of ADCC mediated per monocyte was not altered by the use of either highly purified monocytes or diluted monocyte suspensions.

In patients with active AD the monocyte cAMP response during the ADCC reaction is shown in Fig. 3. At time zero monocyte cAMP in AD was comparable to that of controls. After adding type B erythrocytes and anti-B antibody to monocytes a burst in cAMP formation was observed. In AD this increase of cAMP was significantly lower than in controls. After 5 min monocyte cAMP in controls had returned to a level not statistically different from the basal level, whereas cAMP in AD was still elevated ($P < 0.01$). However, after 15 min baseline levels were obtained also in AD (data not shown).

The cAMP content of erythrocytes was negligible compared to that of monocyte ($0.24\text{--}0.30 \times 10^{-12}$ mol/ 10^7 erythrocytes, $n = 3$).

Expressed as the maximum increase of monocyte cAMP during the observation period, the increase of monocyte cAMP (mean $\pm$ s.e.m.) was higher in controls (21.7 ± 6.8 , $n = 10$) than in patients with AD (11.4 ± 2.0 , $n = 16$) ($P < 0.02$). However, no relation could be demonstrated between the ADCC and the maximum cAMP increase in AD (Fig. 4). Both patients with AD and carriers of chronic granulomatous disease exhibited decreased monocyte ADCC, but the diminished cAMP response during the ADCC reaction was only present in AD (Table 2).

Most patients with AD showed elevated serum-IgE levels, but neither the ADCC activity nor the cAMP response in monocytes were related to serum-IgE levels (data not shown).

Table 2

Monocyte ADCC and cyclic AMP response in patients with atopic dermatitis and in carriers of chronic granulomatous disease

	<i>n</i>	No. of targets lysed ^a per monocyte	Maximum cAMP response (picomoles per 10 ⁷ monocytes)
Atopic dermatitis	16	1.19 ± 0.05*	11.4 ± 2.0**
Carriers of chronic granulomatous disease	4	0.85 ± 0.08*	25.0 ± 3.6
Healthy controls	10	1.56 ± 0.04	21.7 ± 6.8

^a Target cell to monocyte ratio 8:1. Incubation time 18 h. Values were expressed as mean ± s.e.m.

* *P* < 0.01, ** *P* < 0.02.

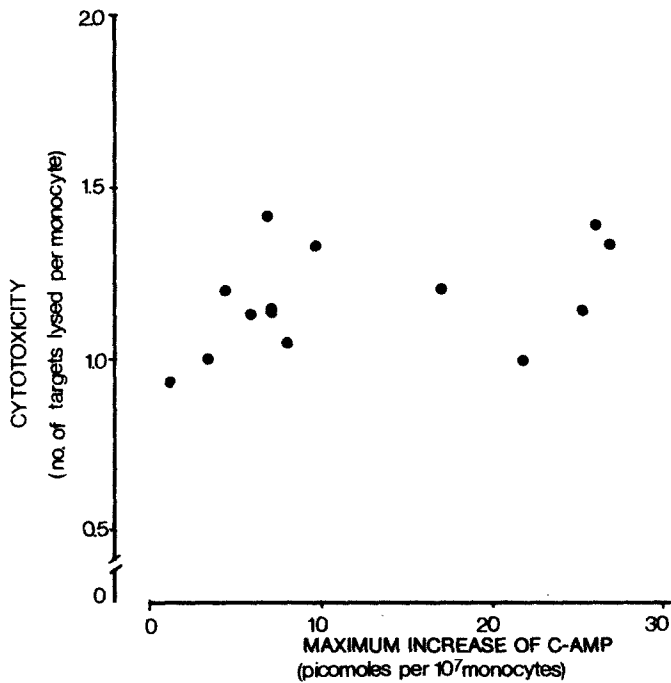


Fig. 4. Relation between monocyte ADCC and maximum monocyte cAMP increase in 16 patients with atopic dermatitis. *R* = 0.42, *P* < 0.10. The ADCC values shown were obtained at erythrocyte to monocyte ratio 8:1 after incubation for 18 h. When ADCC values obtained after incubation for 1 h were compared to the maximum monocyte cAMP increase the same relation was found.

DISCUSSION

A large number of studies have suggested immunologic dysfunction in AD (17). However, only a few sequential studies have been performed. Most investigators have failed to demonstrate significant fluctuations of serum IgE levels in spite of considerable variations in the activity of the dermatitis (4, 20, 22). No influence of the clinical state was noticed upon de-

creased lymphocyte transformation *in vitro* (10) while neutrophil chemotaxis normalized following clinical remission (18). Our results suggest that an initially decreased monocyte ADCC remains low in quiescent phases of the disease. Although potent topical steroids were only used during exacerbation it can not be completely ruled out that concurrent therapy had contributed to the hypofunction. It is not possible to say whether normalization would take place in our patients if they had been free from dermatitis for a longer time. Serum-IgE levels have been reported only to return to normal when clinical remission has persisted 1 year (11).

It has been postulated that diminished cAMP responses were characteristic for AD and other atopic diseases (2, 16, 21). Therefore, the persistence of reduced monocyte ADCC might be associated with this defect. The dualism of biological control where cAMP and cGMP mediate opposing influences on cell function (7) also applies to ADCC (6, 9). In these studies hormonal stimulation revealed that elevated cAMP levels depressed ADCC. The hypothesis of an inverse relationship between ADCC and cAMP seems, however, to be inadequate for the understanding of depressed ADCC in AD. According to this hypothesis it would be expected that reduced cAMP responses resulted in an increased rather than a decreased ADCC. In this study, however, an immediate cAMP formation was demonstrated during the initiation of the ADCC reaction. The observed elevation of cAMP was weaker than seen after hormonal stimulation (9), but a similar transient elevation of cAMP has been de-

monstrated in neutrophils phagocytosing latex particles (8). Since the cAMP elevation can be induced by inert particles (8), it is not likely that the cAMP elevation found during ADCC was generated by adenyl cyclase in erythrocytes.

The inhibition of the initial cAMP elevation observed in AD might have led to a faulty activation of the processes involved in monocyte ADCC. But the lack of correlation between ADCC and cAMP response in AD indicated that these events were not intimately related. The normal cAMP response in carriers of chronic granulomatous disease despite decreased ADCC supported the idea that the diminished cAMP response was characteristic for AD and not a phenomenon shared by diseases showing decreased ADCC.

Since the persistence of the abnormal monocyte function in AD neither reflected an influence of lymphocytes nor was related to a diminished cAMP response, it is suggested that processes directly involved in the ADCC reaction are defective in AD.

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