

Antibody-Dependent Monocyte-Mediated Cytotoxicity in Severe Atopic Dermatitis

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Antibody-dependent monocyte-mediated cytotoxicity was studied in nine patients with severe atopic dermatitis during flares of their disease. In all cases the cytotoxicity was depressed. The disturbed function may contribute to the severe cutaneous infections seen in these patients. It remains to be seen if these patients have an intrinsic monocyte defect or if an inhibiting factor only is operating during active disease states.

Key words: antibody-dependent cellular cytotoxicity; atopic dermatitis; monocytes.

There is evidence for disturbed cell-mediated immunity in severe atopic dermatitis. In addition to severe cutaneous infections hyporeactivity in cell-mediated immunity has been demonstrated with both *in vivo* (17,21,26) and *in vitro* (17,21,24,32) methods.

Stimulation or activation of monocyte-macrophages is a common, perhaps invariable, requirement for the development of cell-mediated immunity against both tumours and microorganisms (2,10,20). Increasing numbers of patients with malfunctioning monocyte-macrophages have been reported. In patients with atopic dermatitis, however, studies on monocyte-macrophage function have only recently been reported (29), showing impaired monocyte chemotaxis.

The aim of the present investigation was to measure the monocyte effector function in terms of antibody-dependent monocyte-mediated cytotoxicity (ADMMC) in patients with severe atopic dermatitis during flares of their disease.

MATERIAL AND METHODS

Nine patients with serum IgE levels higher than 1,500 u (normal range 30-500 u, 1 u corresponds to approximately 2 µg/ml) were included in the study. The investigation was performed during an active state of the disease, but none were erythrodermic. Their age ranged between 21 and 45 years with a median age of 27 years. Several had cutaneous infections mainly with staphylococci. Beside antihistamines, which were withdrawn 48 h before the test, none had received systemic therapy. All patients were treated with potent topical corticosteroids from time to time, but not for the last 2 weeks.

A control group of 14 healthy persons was included, their age ranged between 19 and 48 years with a median age of 26 years.

Monocytes. 40 ml venous blood was mixed with the dipotassium salt of EDTA. Following Ficoll-Isopaque separation (4) the

mononuclear cells were washed twice in Hank's balanced salt solution (HBSS) with 2.5% heat-inactivated fetal calf serum (FCS) (Microbiological Associates, Bethesda, Maryland). The washed cells were suspended in medium RPMI 1640 (Microbiological Associates, Bethesda, Maryland) with 50% FCS ($2-4 \times 10^6$ cells/ml) and dispensed in plastic petri dishes (Nunc, Roskilde, Denmark). After incubation in a humidified atmosphere containing 5% CO_2 for 90 min at 37°C the medium was aspirated, and the plates rinsed five times with HBSS with 10% FCS, prewarmed to 37°C . The dishes were then refilled with HBSS with 2.5% FCS, and the adherent cells were suspended by gentle scraping with a rubber policeman. For removal of dead cells a second Ficoll-Isopaque fractionation was carried out. The cells harvested in the interface were washed twice and finally adjusted to 0.5×10^6 cells/ml.

Target cells. Fresh type-B human erythrocytes were drawn from the same donor. Prior to use, the erythrocytes were washed four times in 0.88% saline. $100 \mu\text{l}$ erythrocytes (20×10^6) were incubated in HBSS-2.5% FCS with $100 \mu\text{l}$ sodium⁵¹ chromate (Amersham, England, 1 mCi/ml, $2-10 \mu\text{g}$ Cr/ml) for 60 min at 37°C . After labelling, the erythrocytes were washed twice.

Antiserum. Human hyperimmune antiserum to type B human erythrocytes was obtained from the Blood Bank and Blood Grouping Laboratory, Aarhus Kommunehospital. The serum was heat-inactivated and stored in aliquots at -20°C .

Cytotoxicity assay. The tests were set up in duplicates in roundbottomed plastic tubes. All dilutions were made in medium RPMI 1640 supplemented with 25 mM hepes, 2 mM glutamine, 100 IU/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin and 5% FCS, finally adjusted to pH 7.4. To $100 \mu\text{l}$ monocytes (0.5×10^5) were added $100 \mu\text{l}$ ⁵¹Cr-labelled

erythrocytes at four different concentrations (1×10^5 , 2×10^5 , 4×10^5 and 6×10^5) and $100 \mu\text{l}$ antiserum-dilution, or in serum controls heat-inactivated AB serum instead of antiserum. Controls in which the monocytes were replaced by 0.5×10^5 un-labelled erythrocytes were included. The mixtures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 . After 18 h the tubes were centrifuged at 300 g for 10 min. Half of the supernatant was withdrawn. Both this supernatant and residues were counted separately in a well-type gamma counter.

Identification of cells. The cell-viability was assessed by trypan blue exclusion. The number of monocytes present was obtained by differential counting of cytocentrifuge preparations stained to detect cells containing non-specific esterase (33). Lymphocytes forming rosettes with sheep erythrocytes (E-RFC), with antibody-coated erythrocytes (EA-RFC) and with antibody- and complement-coated erythrocytes (EAC-RFC) were identified in cytocentrifuge preparations as described before (18).

Expression of results. The results were presented in the form of per cent⁵¹Cr release as determined by the following formula

$$\%^{51}\text{Cr release} = \frac{\text{supernatant c.p.m.} \times 2}{\text{supernatant c.p.m.} + \text{residue c.p.m.}} \times 100$$

for each reaction the per cent release was corrected by subtracting the release in control tubes.

RESULTS

Isolation of monocytes

After separation 78-90% of the cells were monocytes. 0-2% polymorphonuclear neutrophils (PMNs) were present. The other contaminating cells were lymphocytes. Among lymphocytes the percentage of E-RFC, EA-RFC and EAC-RFC was unchanged

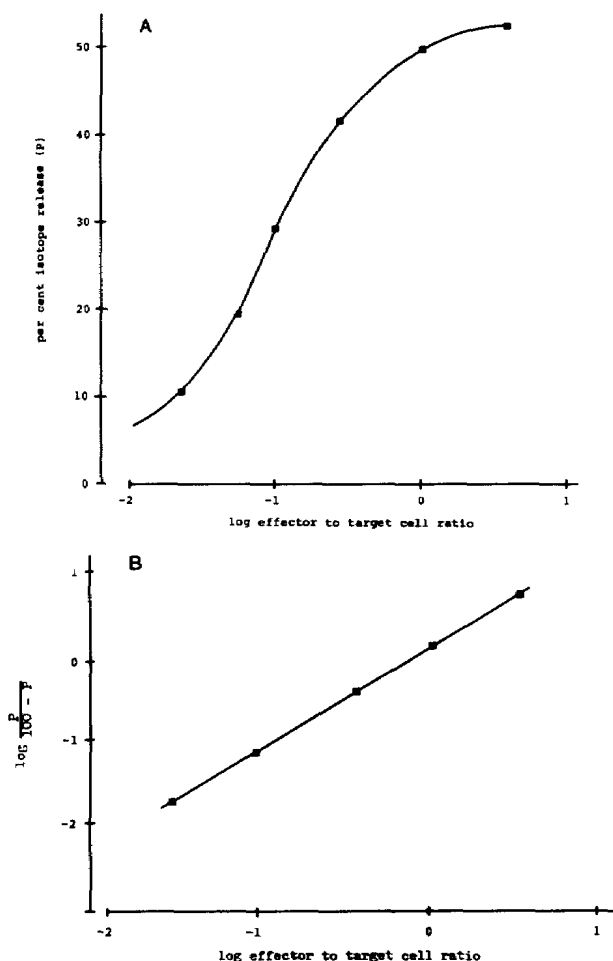


Fig. 1A. Relationship between effector to target cell ratio and per cent cytotoxicity (P). The abscissa expresses logarithmic values. The position of the curve is the mean for the control group. B. Logit transformation of the curve in A. The numbers given on the ordinate are $\log \frac{P}{100-P}$.

before and after the surface adherence. The recovery of monocytes was 52–68%, and the mean viability of cells was 95%.

Characteristics of ADMMC

At optimal antiserum concentration the relationship between the effector to target cell ratio and cytotoxicity was analysed (Fig.

1). The per cent ^{51}Cr release increased sigmoidally with log increase in effector to target cell ratio. At very high ratios the release decreased. Converting the per cent ^{51}Cr release (p) to a value Z using the formula.

$$Z = \log \frac{p}{100-p}$$

the curve was linearized (23).

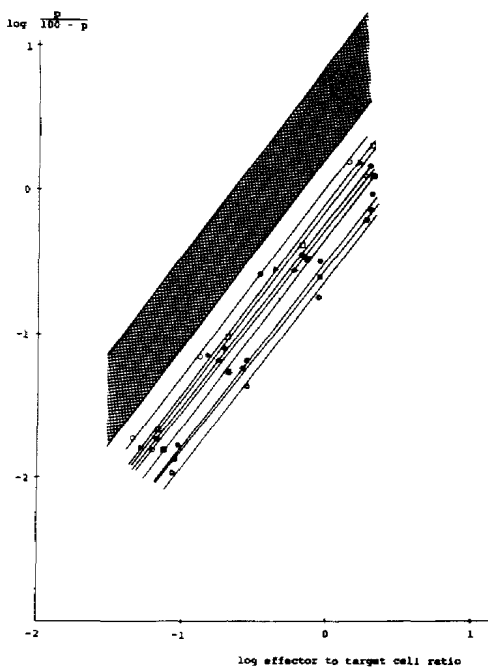


Fig. 2. Relationship between effector to target cell ratio and monocyte cytotoxicity. The results are plotted on a double logarithmic scale. The hatched interval indicates the control group. The curves represent the patients with atopic dermatitis.

Using this logit transformation the dose-response curves obtained with different cell suspensions resulted in parallel lines, and the Z value at a given effector to target cell ratio gave a direct measure of the cytotoxic potential. In subsequent experiments the ^{51}Cr release was tested at four different ratios, and the Z value at ratio 0.2 was read off the dose-response line.

In Fig. 2 the interval which includes the whole control group is shown. The limits of the interval represent the highest and lowest activity measured. Four of the 14 controls were investigated twice, and in every case nearly identical curves were obtained. The mean value and the marginal values are given in Table 1.

The results in patients with severe atopic dermatitis are presented in Fig. 2 and Table 2. At the time of investigation all patients showed impaired monocyte cytotoxicity. Among the patients the activity varied moderately, but compared to the control

group a marked depression was observed in every patient. The present data showed no relationship between the monocyte cytotoxicity and the presence of cutaneous infection.

DISCUSSION

In a selected group of patients with severe atopic dermatitis it was demonstrated that ADMMC invariably was depressed during flares of their disease. As monocytes play an important role in host defences the *in vitro* findings may be relevant to the susceptibility of these patients to severe cutaneous infections.

The full range of mononuclear phagocyte function is just becoming apparent, but there appear to be five major areas of monocyte-macrophage function 1) defence against microorganisms, 2) removal of effete cells, cell debris, and inorganic materials, 3) regulatory interactions in hematopoiesis, 4) cooperative and effector function in immune

Table 1
Monocyte cytotoxicity in normals

Experiment No.	Z
1	-1.41
2	-0.62
3	-0.70
4	-0.12
5	-0.07
6	-0.20
7	-0.33
8	-0.38
9	-0.55
10	-0.42
11	-0.18
12	-0.01
13	-0.28
14	-0.46
Mean	-0.40 (-0.07-0.70)

Cytotoxicity (P) is converted to a value Z using the formula: $Z = \log \frac{p}{100-p}$

The actual value is obtained at the effector to target cell ratio 0.2. In brackets the longest and lowest values are indicated.

response and 5) synthesis of biologically active components (7). One of the important ways in which monocyte-macrophages perform their *in vivo* functions is to act as cytotoxic effector cells (9,14).

Antibody-dependent cell-mediated cytotoxicity refers to the *in vitro* lyses by non-immune effector cells of target cells coated with an appropriate anti-target cell antibody. The reaction is due to effector cells with surface receptors for the Fc portion of IgG molecules which interact with IgG antibody bound to target cells (5). When human erythrocytes are used as target cells, monocytes as well as PMNs are involved as effector cells, whereas lymphocytes have no effect (15,16,22). In the present study the monocytes were contaminated with less than 3% PMNs.

Clinical application of an ADMMC assay has revealed defective cytotoxic activity in patients with the Wiskott-Aldrich syndrome

Table 2
Monocyte cytotoxicity in atopic dermatitis

Experiment No.	Z
1	-1.28
2	-1.41
3	-1.13
4	-1.54
5	-1.44
6	-0.99
7	-1.07
8	-1.02
9	-1.16
Mean	-1.30 (-1.54-0.99)

Cytotoxicity (P) is converted to a value Z using the formula: $Z = \log \frac{p}{100-p}$

The actual value is obtained at the effector to target cell ratio 0.2. In brackets the longest and lowest values are indicated.

(25), and there is evidence to suggest that an abnormality in monocyte function is to some extent responsible for the altered immune response observed in these patients (1). Previously there has been one study of monocyte function in atopic dermatitis (29) showing impaired monocyte chemotaxis in four out of four patients with erythrodermal atopic dermatitis. Serum IgE was significantly raised in all four patients and both the PMN chemotaxis and lymphocyte phytohemagglutinin stimulation were depressed. In addition the patients did not manifest delayed cutaneous hypersensitivity when tested with five intradermal antigens.

The mechanisms responsible for the depressed function of monocytes remain totally speculative. The presence of cutaneous infections in several of the patients in the present study may contribute to the findings, but could not account for the defective function in all patients. The administration of systemic glucocorticoids results in a wide range of effects on immunologic processes, and monocyte-macrophage function, however, normalizes

about 1 day after withdrawal of treatment. None of our patients had received systemic therapy with corticosteroids, and topically applied fluorsubstituted steroids were discontinued at least 2 weeks prior to the study.

All the patients were characterized by high levels of serum IgE. T-lymphocyte rosette formation is inhibited, when normal lymphocytes are incubated in atopic serum with high IgE concentration (12), but in the present study IgE was not present in the medium. Recently, aggregates of IgE have been detected in serum from patients with atopic dermatitis (3). By blockade of the Fc receptor sites aggregates of IgE and immune complexes inhibit ADMMC under *in vitro* conditions. Possibly aggregates of IgE could act in similar manner. In addition to IgE a high proportion of atopic dermatitis patients have elevated levels of IgG₄ in serum (30). But IgG₁ and IgG₃ are the only IgG subclasses that inhibit the cytotoxic process (19).

The existence of an inhibitory plasma factor which could modulate the function of monocytes as well as other cell series has been suggested (29). When PMNs and lymphocytes are incubated in serum from patients with atopic dermatitis the chemotaxis and mitogenic responses decrease (29). An effect of a plasma inhibitory factor, however, seems unlikely in the present study as the monocytes during isolation were washed several times. This would require the factor to be absorbed to the cell surface or to have a long-term effect on the cell.

The changed ADMMC could be a function of alterations in the system of cyclic nucleotides, a pathway common to most cell series and through which different cellular functions are modulated. Particularly, the cytotoxic activity mediated by lymphocytes has been shown to be inhibited by increases in cyclic AMP (13,31); for cyclic GMP the inverse relationship exists (31). In another study an altered lymphocyte cyclic AMP response to agonists was observed in patients with atopic dermatitis (27), suggesting a defect antecedent to activation

of adenylyl cyclase and specific for the beta-adrenergic receptor.

The question remains whether an intrinsic monocyte defect is responsible for the decrease in ADMMC in our patients, or if the suppression only is present during bouts of the dermatitis. Studies are being prepared to examine atopic dermatitis of different degrees, during both active and inactive disease states. Besides, further studies are needed in order to disclose the nature of the inhibiting mechanisms.

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