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Impaired Monocyte-mediated Cytotoxicity in Atopic Dermatitis

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Summary. Twenty-three adult patients with atopic dermatitis of different severity and extent all without present cutaneous infection were investigated for antibody-dependent cytotoxicity mediated by purified monocytes. Compared to a healthy control group the monocyte cytotoxicity was significantly decreased for patients with more widespread dermatitis. Eight patients with acute contact dermatitis and 13 patients with extrinsic asthma or allergic rhinitis showed normal cytotoxicity. Decreased monocyte cytotoxicity in atopic dermatitis was not related to the serum IgE level. In vitro cultivation of defective monocytes from atopics did not increase cytotoxicity, nor did normal monocytes preincubated with patient serum show abnormal function. In atopic dermatitis the total number of Fc receptor bearing monocytes was normal. However, the affinity of this receptor was lower than in normals.

Serial studies are needed to establish whether reduced monocyte function is a basic pathophysiologic defect in atopic dermatitis.

Key words: Monocytes – Antibody-dependent cell-mediated cytotoxicity – Atopic dermatitis – Contact dermatitis – Extrinsic asthma – Allergic rhinitis

Zusammenfassung. Dreiundzwanzig erwachsene Patienten mit atopischer Dermatitis verschiedenen Ausmaßes und Ausprägung ohne Zeichen einer Hautinfektion wurden auf antikörperabhängige Cytotoxicität von gereinigten Monocyten untersucht. Verglichen mit einer gesunden Kontrollgruppe war die Toxicität der Monocyten bei ausgedehnter atopischer Dermatitis signifikant vermindert. Acht Patienten mit akuter Kontaktdermatitis und 13 Patienten mit „extrinsic asthma" oder allergischer Rhinitis zeigten normale Cytotoxicität. Die verminderte monocytäre Toxicität bei atopischer Dermatitis zeigte keine Beziehung zum Serum-IgE-Spiegel. Die Kultur von Monocyten atopischer Patienten zeigte keine vermehrte Cytotoxicität, auch zeigten normale Monocyten, die mit Serum von Atopiker inkubiert waren, keine Besonderheiten. Bei atopischer Dermatitis war die Gesamtzahl der Fc-Receptor-tragenden Monocyten normal, jedoch war die Affinität dieser Receptoren schwächer ausgeprägt als im Kontrollkollektiv.

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Weitere Studien sind notwendig, um festzustellen, ob die reduzierte Monocytenfunktion ein pathophysiologisches Grundphänomen bei atopischer Dermatitis ist.

Schlüsselwörter: Monocyten – Antikörper-abhängige, zellübertragende Cytotoxicität – atopische Dermatitis – Kontaktdermatitis – „Extrinsic Asthma" – allergische Rhinitis

Within recent years attention has been paid to the immunological aspects of atopic dermatitis (AD). An increased susceptibility to infections is well-known and depressed in vivo delayed reactions and increased levels of IgE have been recognized. Defects in in vitro T-lymphocyte function have also been proposed, but the results are controversial [6, 7, 12, 23, 24]. Recently, monocyte and neutrophil chemotaxis has been studied [5, 20, 21]. In these studies the defective chemotaxis was more common in monocytes than in neutrophils.

The monocyte-macrophage system plays a prominent role in the defence against a variety of infectious agents [2, 8, 11] and has a cooperative and effector function in immune response [3, 13, 19]. These functions may be of importance in AD characterized by infection, anergy, and faulty immunoglobulin synthesis. We have therefore studied the in vitro function of purified blood monocytes from patients with AD of different severity and extent. To avoid the influence of infections only patients without clinical cutaneous infections were selected for the study. Monocyte function was expressed as antibody-dependent cell-mediated cytotoxicity (ADCC).

Material and Methods

Patients and Controls

Twenty-three patients (11 females and 12 males) were included in the study. Their age ranged between 16 and 40 years with an average of 28 years. All had atopic dermatitis with typical cutaneous morphological characteristics and distributions of lesions. In all but 4 cases the disease appeared in the childhood. The extent and severity of the disease were graded according to Rogge and Hanifin [20]. The extent of disease was quantitated on a scale of 1 to 5 as follows: Grade 1: less than 10% of the body area involved; grade 2: 10–25%; grade 3: 25–50%; grade 4: 50–75%, grade 5: 75–100%. Severity was likewise graded on a scale of 1 to 5, on the basis of pruritus, lichenification, excoriation, and erythema. Hence, grade 1 severity indicated mild lichenification without pruritus, while grade 5 referred to extreme excoriated erythroderma with intractable pruritus. Most of our patients had moderate atopic dermatitis with average score for severity: 1.83 and average score for extent: 2.83. Thirteen had a past history of allergic rhinitis and/or asthma. Ten had AD alone. In case of antihistaminic therapy this drug was withdrawn 2 days before the investigation. All were treated topically with hydrocortisone or hydrocortisone-butyrate. They had been folowed closely in our out-patient clinic during several months. Five were in remission at the time of investigation, while 3 were in a progressive state. Cutaneous infections had not been clinically apparent within the last 2 months.

A group of healthy individuals with an age and sex distribution comparable to the patient group served as controls and were investigated concomitantly. Eight patients with acute allergic contact dermatitis, of an extent comparable to AD, 6 patients with extrinsic asthma, and 7 with allergic rhinitis, all with active disease, were included as patient controls. Patients with contact dermatitis had been treated with topical steroids for up to 1 week. Treatment with systemic methylxanthines and/or beta-receptor stimulating agents were withdrawn from asthma patients 2 days before the investigation. None of the investigated persons had been treated with corticosteroids systemically within the last year.

Preparation of Monocytes

Heparinized venous blood (20 IE preservative free heparin/ml) was centrifuged over Ficoll-Isopaque as described by Böyum [1]. The resultant interface layer, which contained the mononuclear cells, was

washed twice in Hanks balanced salt solution (HBSS) with 2.5% (v/v) heat-inactivated fetal calf serum (FCS), (Gibco, Grand Island,NY). The washed cells were resuspended in medium RPMI 1640 (Gibco, Grand Island, NY) supplemented with 25% (v/v) FCS and dispended to tissue culture flasks (Nunc, Roskilde, Denmark). After 1 h at 37° C in a humidified atmosphere containing 5% CO_2 the nonadherent cells were decanted and the flasks rinsed with three changes of HBSS/2.5% FCS at 37° C, leaving behind the adherent monocytes. After introducing HBSS with 2.5% FCS into the flasks they were placed on ice bath for 30 min. Detached monocytes were then decanted and the flasks rinsed three times with HBSS/2.5% FCS.

Identification of Monocytes

a) Esterase Activity. Using alpha-naphtyl acetate as substrate cytocentrifuged preparations were stained to detect activity for nonspecific esterase [10]. The median percentage of monocytes showing diffuse cytoplasmic activity was 91, ranging from 82 to 96. Occasionally, 1 – 3% granulocytes were found. The other contaminating cells were lymphocytes.

Phagocytosis of Immunoglobulin-coated Latex Particles. The latex particles (Difco, 0.81 μm diameter) were incubated in human immunoglobulin 100 mg/ml (Statens Seruminstitut, Copenhagen) at pH 8.5 for 60 min at 37° C. After three washings in HBSS the latex particles were resuspended in HBSS at 1×10^{10} particles/ml. To 500 μl cell suspension (1×10^6/ml FCS) 10 μl latex particles were added. After an initial centrifugation the mixture was incubated at 37° C for 1 h and finally centrifuged twice over FCS to remove extracellular latex. The percentage of phagocytosing cells was estimated by light microscope regarding cells with four or more particles ingested as positive.

Monocyte Cytotoxicity

Target Cells. For all experiments fresh type B Rh-negative human erythrocytes from the same donor were used. Equal volumes of washed erythrocytes (200×10^6/ml) and sodium-^{51}chromate (Amersham, England, 1 mCi/ml, 2 – 10 μg Cr/ml) were mixed and incubated for 60 min at 37° C. After labelling, the erythrocytes were washed twice by centrifugation.

Antibody. Human hyperimmune antiserum to type B human erythrocytes was obtained from the Blood Bank and Blood Grouping Laboratory, Aarhus Kommunehospital. Throughout all experiments the same lot was used. Serum was heat-inactivated and stored at −20° C. A dilution inducing maximal target cell lysis in the ADCC assay, was used.

Assay Procedure. 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 μg/ml streptomycin and 5% FCS, finally adjusted to pH 7.4. To 100 μl monocytes (1.0×10^6/ml) 100 μl ^{51}Cr-labelled erythrocytes (1.0×10^6/ml and 8.0×10^6/ml) and 100 μl antiserum dilution were added. Controls in which the monocytes were replaced by unlabelled erythrocytes (1.0×10^6/ml) and anti-B serum replaced with human AB serum were included. The mixtures were centrifuged at 150 *g* for 1 min and incubated in a humidified atmosphere containing 5% CO_2 at 37° C. After 1 h and 18 h the tubes were centrifuged at 300 *g* for 10 min. On each occasion half of the supernatant was withdrawn. Both this supernatant (S) and residues (R) were counted separately in a well-type gammacounter. Chromium release was calculated according to the following formula:

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

For each reaction, the specific release was obtained by substracting the release in control tubes from the release in tubes containing monocytes as well as anti-B serum. Results were expressed as the number of target cells lysed per monocyte:

$$\frac{\text{Number of targets} \times \text{Specific release}}{\text{Number of monocytes}}.$$

In Vitro Cultivation of Monocytes

Adherent monocytes from AD patients and healthy controls were incubated in medium RPMI 1640 containing 10% pooled AB serum for 24 h at 37° C in a humidified atmosphere containing 5% CO_2. After cultivation monocytes were detached and the cytotoxicity assay performed as usual.

Preincubation of Monocytes with Patient Serum

To test the influence of serum upon monocyte cytotoxicity, normal monocytes and monocytes from patients with depressed function were resuspended in medium RPMI 1640 containing 50% normal AB serum or serum from patients with depressed function (fresh or frozen at −80° C). Incubation at 37° C in a humidified atmosphere containing 5% CO_2 was stopped after 0.5 h or after 24 h followed by three washings by centrifugation at 4° C. Finally, the monocytes were resuspended in assay medium and the cytotoxicity assay performed as usual.

Erythrocyte-antibody (EA) Rosetting Test

One volume of a 2% suspension of ox erythrocytes (E) was mixed with an equal volume of heat-inactivated rabbit anti-ox serum (A) at increasing subagglutinating dilutions (1:256, 1;64, 1:16, and 1:4). After 1 h at 20° C the cells were washed three times and resuspended in HBSS with 10% FCS to the original volume. One hundred microliters of EA and 100 µl monocytes (1.0×10^6/ml) was mixed and centrifuged at 45 *g* for 5 min. After 15 min at 20° C, acridine orange was added and under fluorescent microscope monocytes with at least three EA attached were counted as rosettes.

Serum IgE

Levels of IgE were determined by a radioimmunoassay technique (Medicinsk Laboratorium, Copenhagen). Values higher than 150 U/ml were considered increased.

Statistical Analysis

For statistical evaluation a Wilcoxon test for two samples or for pair differences was used. A *P*-value below 0.05 was considered significant.

Results

In Table 1 the monocyte ADCC in patients with AD is compared to the control group. The rate of lysis (defined as the amount of lysis after 1 h) and the capacity for lysis (defined as the amount of lysis after 18 h) are shown. At low target to effector cell ratio (1:1) the cytotoxicity in AD was normal at both times tested. However, in the region of target cell excess (8:1) the rate as well as the capacity were significantly reduced ($P < 0.01$). The rate of lysis was not more sensitive than the capacity for lysis to disclose impaired cytotoxicity. Patients with allergic contact dermatitis and patients with other atopic manifestations expressed normal cytotoxicity. In patients with AD a coexisting asthma or rhinitis could not be shown to influence the results.

A close correlation between the extent of the dermatitis and the monocyte ADCC could not be demonstrated. However, patients having an extent of more than 25% showed significantly decreased ADCC compared to controls ($P < 0.005$) (Table 2). Since the patients were rather uniform with respect to severity of lesions a correlation study for this parameter was not performed.

To evaluate if a high proportion of immature blood monocytes contributed to the decreased function in AD cytotoxicity was measured again after in vitro incubation of monocytes for 24 h at 37° C. Following incubation of defective monocytes their ADCC was unchanged (Table 3). In contrast, the proportion of monocytes phagocytozing opsonized latex particles increased for patients as well as for controls.

Table 1. Antibody-dependent monocyte-mediated cytotoxicity in atopic dermatitis

Target to effector ratio	1:1		8:1	
Incubation time (h)	1	18	1	18
Atopic dermatitis ($n=23$)	0.18 (0.11–0.19)	0.48 (0.43–0.60)	0.36* (0.22–0.51)	1.28* (0.73–1.33)
Contact dermatitis ($n=8$)	0.20 (0.13–0.24)	0.52 (0.37–0.61)	0.49 (0.40–0.63)	1.50 (1.39–1.70)
Asthma or allergic rhinitis ($n=13$)	0.17 (0.13–0.22)	0.50 (0.36–0.63)	0.46 (0.40–0.61)	1.48 (1.36–1.70)
Healthy controls ($n=36$)	0.18 (0.14–0.20)	0.52 (0.39–0.60)	0.48 (0.42–0.60)	1.47 (1.34–1.69)

Values are expressed as median number of target cells lysed per monocyte. In brackets, the 95% confidence limits are indicated. For statistical evaluation a Wilcoxon test for two samples was used
* $P<0.01$

Table 2. Monocyte cytotoxicity in atopic dermatitis of different extension

	Cytotoxicity
Atopic dermatitis	
< 25% of skin involved ($n=12$)	1.29 (1.15–1.57)
> 25% of skin involved ($n=11$)	0.89 (0.51–1.35)*
Healthy controls ($n=36$)	1.47 (1.34–1.69)

Cytotoxicity is expressed as the median number of target cells lysed per monocyte. Target to effector ratio 8:1. Incubation time 18 h. In brackets, the 95% confidence limits are indicated. For statistical evaluation a Wilcoxon test for two samples was used
* $P<0.005$

Table 3. Monocyte cytotoxicity after in vitro cultivation for 24 h in pooled AB serum

	% esterase pos.	% ingesting latex	cytotoxicity
Atopic dermatitis			
Day 0	89 (86–94)	80 (74–83)	1.03 (0.84–1.23)
Day 1	91 (88–95)	88 (81–88)*	1.06 (0.80–1.30)
Healthy controls			
Day 0	91 (88–94)	81 (75–86)	1.49 (1.30–1.66)
Day 1	92 (87–96)	87 (82–89)*	1.48 (1.28–1.64)

Values are expressed as medians. In brackets, the range for six experiments are indicated. Cytotoxicity is expressed as the number of targets lysed per monocyte. Target to effector ratio 8:1. Incubation time 18 h. For statistical evaluation a Wilcoxon test for pair differences was used
* $P<0.05$

We examined the possibility that the presence of inhibitory, or the absence of stimulatory serum factors, might be responsible for depressed cytotoxicity. This was done by preincubating monocytes from normal individuals with fresh serum from AD patients with reduced ADCC and vice versa. Neither after 0.5 h nor after

Table 4. Cytotoxicity after preincubation of normal monocytes with 50% patient serum or vice versa

	Preincubation for 0.5 h		Preincubation for 24 h	
	Normal AB serum	Patient serum	Normal serum	Patient serum
Normal monocytes	1.51 (1.43–1.58)	1.48 (1.40–1.56)	1.48 (1.44–1.52)	1.47 (1.42–1.51)
Monocytes from a patient with atopic dermatitis	1.03 (0.97–1.07)	1.04 (0.96–1.09)	1.02 (0.95–1.06)	1.05 (0.96–1.09)

Cytotoxicity was expressed as the number of targets lysed per monocyte. Target to effector ratio 8:1. Incubation time 18 h. Values are medians. In brackets, the range for experiments with six different sera is indicated. For monocytes from another healthy control or from another patient nearly identical results were obtained

Table 5. The percentage of EA-rosette forming monocytes as a function of the degree of erythrocyte sensitization

	Serum dilutions used to make EA			
	4	16	64	256
Atopic dermatitis ($n=14$)	65 (58–78)	40 (33–50)	20 (16–29)	8* (5–10)
Healthy controls ($n=14$)	69 (58–89)	43 (34–56)	23 (18–39)	14 (8–18)

Values are expressed as medians. In brackets, the 95% confidence limits are indicated. For statistical evaluation a Wilcoxon test for two samples was used
* $P<0.05$

24 h preincubation was any influence of AB serum or patient serum observed (Table 4).

Since binding of the antibody-coated target cell preceeds cytotoxicity the ability to form EA-rosettes was tested in 14 atopics (median ADCC 0.99) and in 14 normals (median ADCC 1.42). In Table 5 it is shown that the percentage of rosette-forming cells was a function of the degree of erythrocyte sensitization. At all four dilutions the median value was lower for the patients with AD, but only significantly lower for the highest dilution ($P<0.05$).

The serum IgE was elevated in all of 23 patients studied. Association between IgE levels and monocyte ADCC could not be demonstrated.

Discussion

In an earlier study we demonstrated that monocyte ADCC was substantially depressed in severe AD during flares of their disease [9]. In the present investigation of patients with mild disease activity and without infection these results are extended. For the whole group of patients a mild to moderate decrease in cytotoxicity was observed. In other atopic diseases monocyte cytotoxicity was normal, and the inflammatory skin involvement in contact dermatitis was not

followed by an altered function. Monocyte cytotoxicity was not closely related to the extent of the dermatitis, but the group of patients with an extent of the dermatitis of less than 25 % of body area showed normal values. An influence of the applied corticosteroids on monocyte function has not been ruled out. However, since systemic administration of prednison in doses up to 80 mg every other day does not impair monocyte in vitro function [16] an effect of topical steroids of low potency is unlikely.

Defects of monocyte chemotaxis have been looked for and found in three studies of AD patients [5, 20, 21]. Chemotactic defects were in these studies more common for monocytes than for neutrophils. Since monocytes constituted only 20 % of the mononuclear cells studied, it cannot be excluded that depressed function was due to disturbed monocyte-lymphocyte interaction. Thus, it has been demonstrated that depressed monocyte chemotaxis in mycosis fungoides is due to lack of lymphocytes and not to an intrinsic monocyte defect [15]. In contrast to the cytotoxicity data, reduced monocyte chemotaxis is not restricted to atopic patients with dermatitis but also occurs in those with respiratory allergies [5].

Monocytopoiesis may be stimulated in dermatological diseases, including atopic dermatitis [14]. If patients with AD have an increased proportion of immature monocytes in their circulation, such cells may show low cytotoxicity. In vitro cultivation for 24 h, however, did not increase cytotoxicity of dysfunctional monocytes.

The existence of an immunosuppressive factor in serum from patients with AD which could modulate the function of leukocytes as well as other cell series has been suggested [7, 20], but has only been demonstrated in test systems where patient serum is present during the assay procedure [7, 20, 21, 24]. For monocytes a cell-directed chemotactic inhibitor was found in patient serum in one study [21] but not in another [5]. Under experimental conditions used in the present study an inhibitory or stimulatory factor would be required to be absorbed to the cell surface or to have a long term effect on the cell. However, our results provided no evidence for serum factors affecting monocyte ADCC.

Since binding of IgG antibody-coated target cells to monocytes is required for the occurrence of lysis [4] a decreased number or affinity of Fc receptors might impair the cytotoxic process. Recently, the existence of a subgroup of human monocytes without Fc receptors has been pointed out [17]. In AD we found that the proportion of Fc receptor negative monocytes was not increased. However, functional expression of monocyte Fc receptors was decreased for low degrees of sensitization of the EA test system, indicating a reduced receptor activity. The significance of this finding is doubtful, because the cytotoxicity assay was performed at optimal antibody concentration where we found normal rosette-forming capacity.

An abnormality in the system of cyclic nucleotides seems to be common to the atopic diseases [18, 22, 25] and may in some respects explain abnormal in vitro findings in AD. Defective monocyte chemotaxis has been demonstrated in AD as well as in asthma [5], whereas decreased monocyte-mediated cytotoxicity is restricted to AD. The cyclic nucleotides was not investigated in this study; therefore, it is still unknown whether the impaired regulation of cyclic nucleotides may be responsible for the immunological defects in atopic dermatitis.

Since monocytes and macrophages not only destroy microbial agents but also are essential cells in the regulation of immune response, a defective monocyte function may be a basic pathophysiologic defect in a disease as AD, where infection, anergy, and faulty regulation of immunoglobulin synthesis is common. The observed abnormality, however, could also be a secondary phenomenon, since decreased function was not found in all patients. Therefore, controlled prospective studies on the same patient during different stages of disease are needed.

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