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MECHANISMS OF DECREASED ANTIBODY-DEPENDENT CYTOTOXICITY MEDIATED BY MONOCYTES AND NEUTROPHILS IN ATOPIC DERMATITIS

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Abstract. By using IgG-sensitized erythrocytes as target cells, we found the antibody-dependent cytotoxicity mediated by monocytes and neutrophils to be depressed in atopic dermatitis. This change was accompanied by a reduction of phagocytosis of target cells by the effector cells, and also by a decreased lysozyme liberation from, and hexose monophosphate shunt activation in, the effector cells during the cytotoxic reaction. However, the binding of target cells to effector cells remained normal. In individual patients, cytotoxicity and phagocytosis were equally depressed. No relation was found between cytotoxicity and lysozyme liberation in atopic dermatitis, but a relation between cytotoxicity and hexose monophosphate shunt activity was highly significant. These results suggest that the decreased cytotoxicity mediated by monocytes and neutrophils in atopic dermatitis is caused by a diminished production of oxygen radicals during the respiratory burst. This reduced respiratory burst was not due to any defect in the enzymes responsible for this process, since the superoxide production of monocytes and neutrophils stimulated with phorbol myristate acetate was found normal in atopic dermatitis. It therefore seems that although binding of target cells to effector cells was normal, the signals created by the binding process were defective in activating monocytes and neutrophils in atopic dermatitis.

Key words: Antibody-dependent cell-mediated cytotoxicity; Atopic dermatitis; Hexose monophosphate shunt; Lysozyme liberation; Monocytes; Neutrophils; Phagocytosis; Superoxide production

In atopic dermatitis (AD) the in vitro function of monocytes determined as antibody-dependent cell-mediated cytotoxicity (ADCC) has been found to be depressed (12, 15). This defective ADCC in AD is caused by neither humoral factors (15) nor lymphocytes (16). The cyclic AMP response elicited in monocytes has been found diminished in AD, but ADCC did not correlate with the cyclic AMP response (16).

The aim of this study was, therefore, to investigate whether any of the processes involved in ADCC, viz. binding of target cells, degranulation, activation of the hexose monophosphate shunt, and phagocytosis (5, 6, 7, 10), were disturbed in AD, and to examine whether similar defects could be found in neutrophils.

MATERIALS AND METHODS

Patients

14 patients (7 females and 7 males) were studied. Their age ranged between 13 and 31 years with an average of 24 years. All had atopic dermatitis with typical cutaneous morphological characteristics and distributions of lesions. Cutaneous infections had not been clinically apparent for at least 2 months. The extent and severity of the dermatitis were graded according to Rogge & Hanifin (17). Both variables were quantified on a scale of 1 to 5. Most of our patients had moderate AD with an average score for severity of 2.5 and for extent, 1.7. Four of the 14 patients had a past history of allergic rhinitis and/or asthma. None had received systemic treatment; all were being treated topically with hydrocortisone or hydrocortisone–butyrate. Seven healthy individuals with an age and sex distribution comparable to the patient group served as controls.

Isolation of monocytes and neutrophils

Monocytes and neutrophils were isolated from heparinized venous blood as described elsewhere (4, 13). In esterase-stained preparations 89% to 97% (mean 93%) of the recovered mononuclear cells were monocytes. Occasionally 1–3% neutrophils were present. Judged by morphological criteria the isolated neutrophils were more than 96% pure.

Antibody-dependent cell-mediated cytotoxicity

The assay was performed as described previously (14). The tests were set up in duplicate in volumes of 300 μl. Cytotoxicity was measured as ^{51}chromium-release from prelabelled human type A erythrocytes sensitized with human hyperimmune anti-A serum. By subtracting the release in control tubes containing no phagocytes the specific cytotoxicity was defined. Results were expressed as the number of targets lysed per phagocyte:

Table 1. *Events during antibody-dependent cytotoxicity mediated by monocytes and neutrophils in atopic dermatitis*

	Atopic dermatitis ($n=14$)	Healthy controls ($n=7$)
Monocytes		
% rosette forming cells[a]	88±2	90±2
Phagocytosis (erythrocytes ingested per monocyte)[b]	0.46±0.03**	0.62±0.03
Cytolysis (erythrocytes lysed per monocyte)[b]	0.58±0.03**	0.78±0.02
% lysozyme liberation[b]	18.7±1.5*	22.0±2.0
cpm $^{14}C\bullet_2$ from [1-^{14}C]glucose[b]	$2.29\times10^3\pm0.16$**	$3.19\times10^3\times0.07$
Neutrophils		
Phagocytosis (erythrocytes ingested per neutrophil)[b]	0.53±0.03**	0.74±0.03
Cytolysis (erythrocytes lysed per neutrophil)[b]	0.72±0.05**	0.98±0.03
% lysozyme liberation[b]	20.0±1.5*	24.1±1.7
cpm $^{14}CO_2$ from [1-^{14}C]glucose[b]	$3.58\times10^3\pm0.25$**	$5.11\times10^3\pm0.17$

[a] 1×10^5 monocytes were incubated with 1 % (v/v) erythrocytes sensitized with IgG for 15 min at 20°C.
[b] 1×10^5 phagocytes were incubated with 4×10^5 erythrocytes sensitized with IgG for 30 min at 37°C.
* $p<0.05$. ** $p<0.01$.

$$\frac{\text{number of targets} \times \text{specific cytotoxicity}}{\text{number of phagocytes}}$$

Phagocytosis

For determination of phagocytosis the method of complement-mediated lysis described by Holm (8) was adopted. After incubation at 37°C under the same conditions as for ADCC, the tubes were chilled in ice water, and 1 mM iodacetamide was added in order to stop further phagocytosis and lysis. Non-phagocytosed erythrocytes were then lysed by reincubation at 37°C with a 10-fold dilution of fresh human serum.

After 30 min with frequent agitation, half of the supernatant was withdrawn. This supernatant (P) and the residue (R) were counted in a gamma counter. Controls were included as for the ADCC assay. Phagocytosis was calculated as the fraction of targets protected from complement-mediated lysis:

$$\text{Phagocytosis} = \frac{(R \text{ cpm} - P \text{ cpm})}{R \text{ cpm} + P \text{ cpm}}$$

In control tubes without anti-A serum, phagocytosis was almost nil. Results were expressed as the number of target cells phagocytosed per phagocyte:

$$\frac{\text{number of targets} \times \text{phagocytosis}}{\text{number of phagocytes}}$$

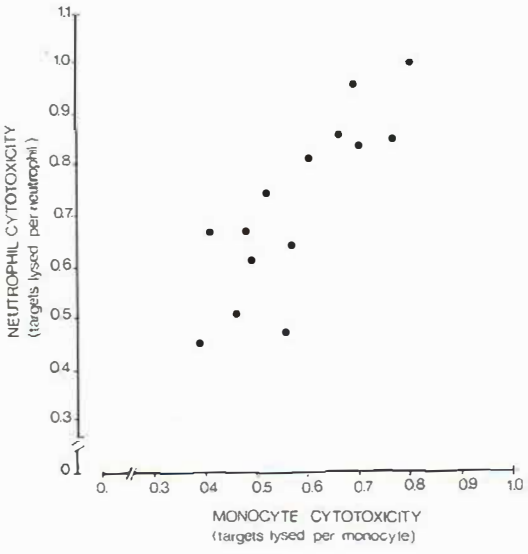

Fig. 1. Relation between monocyte cytotoxicity and neutrophil cytotoxicity in 14 patients with atopic dermatitis ($R=0.84$, $p<0.01$). 1×10^5 phagocytes were incubated with 4×10^5 erythrocytes sensitized with IgG for 30 min at 37°C.

Hexose monophosphate shunt

$^{14}C\bullet_2$ liberation from [1-^{14}C]glucose during the ADCC reaction was measured by incubating the cells in the presence of 1 mM labelled glucose, specific activity 2.2 mCi per mmole. After 30 min the reaction was terminated by injecting 100 µl 1 N NaOH through the caps into the reaction mixture. After 1 hour the content of each tube was withdrawn and transferred to a conical flask containing 800 µl 1 N HCl. The flask was immediately sealed with a rubber cap and the killed reaction mixture and the 1 N HCl were mixed to drive out the CO_2 dissolved. A centre well, 1 cm in diameter, contained 400 µl 1 NaOH for the absorption of CO_2. After standing overnight in a shaking bath, the content of the centre well was withdrawn and counted in 19 ml Instagel. We have previously demonstrated that $^{14}CO_2$ liberation from [6-^{14}C]glucose is unchanged during ADCC. Thus, the stimulation of $^{14}CO_2$ liberation from [1-^{14}C]glucose reflects activation of HMPS. (5)

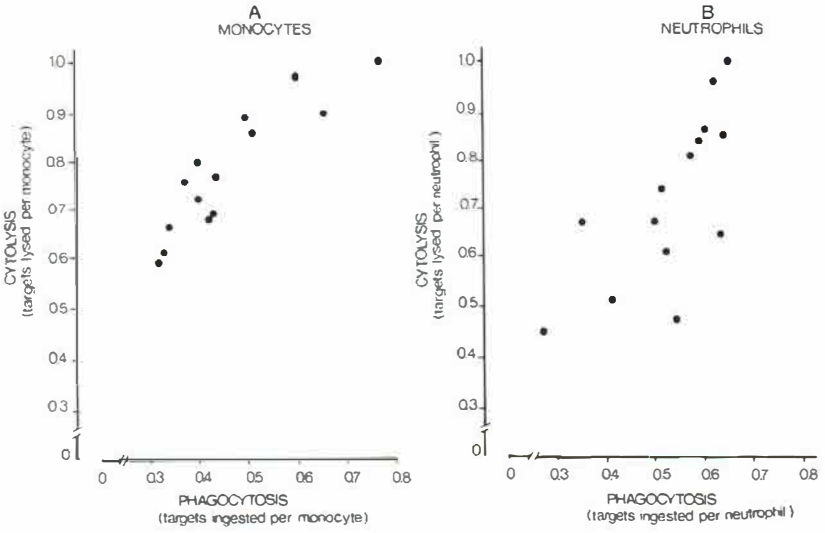

Fig. 2. Relation between cytotoxicity and phagocytosis in 14 patients with atopic dermatitis. (A) Monocytes, $R=0.90$, $p<0.001$. (B) Neutrophils, $R=0.75$, $p<0.01$. 1×10^5 phagocytes were incubated with 4×10^5 erythrocytes sensitized with IgG for 30 min at 37°C.

Degranulation

Degranulation was determined as the liberation of lysozyme from monocytes and neutrophils during ADCC. Assay mixtures were incubated as for the cytotoxicity assay. After 30 min 200 μl of the supernatant was withdrawn for assay of lysozyme and lactate dehydrogenase (LDH). Total lysozyme and LDH were measured after ultrasonic disruption of unstimulated cells in 0.2 % Triton-X-100. Lysozyme was measured turbidometrically by the lysis of *Micrococcus lysodeikticus* (11). LDH was measured as described by Bergmeyer (1). Liberation was expressed as a percentage of the total amount of enzyme in unstimulated cells.

Superoxide production

Phorbol myristate acetate (PMA) stimulation of superoxide production was measured as described elsewhere (3) by monitoring the increase in absorbance at 550 nm of a cell suspension containing 0.2 mM cytochrome *c*.

Binding of IgG-sensitized erythrocytes

The ability of monocytes to form rosettes with erythrocytes sensitized with IgG was determined as described previously (9). After incubation at 20°C for 15 min, acridine orange was added and, under the fluorescent microscope, monocytes with at least 3 erythrocytes attached were counted as rosettes.

Statistical analysis

Statistical significance was assessed by using Wilcoxon's test for two samples. A p-value below 0.05 was considered significant. Spearman's coefficient of rank was applied in the correlation studies.

RESULTS

In Table I the processes involved in ADCC are shown. Both monocytes and neutrophils exhibited a slightly but significantly depressed ADCC in AD. In individual patients, monocytes and neutrophils were almost equally depressed in their cytotoxic activity (Fig. 1). The cytotoxic level was higher in neutrophils than in monocytes, but in principle nearly identical results were obtained for the two types of phagocyte in AD (Table I). The depressed ADCC was accompanied by depressed phagocytosis (Table I) and, in individual patients, ADCC and phagocytosis were closely related (Fig. 2).

During the ADCC reaction both lysozyme liberation from, and hexose monophosphate shunt activity in, monocytes and neutrophils were depressed in AD (Table I). The ability to form rosettes with IgG-sensitized erythrocytes was found normal in monocytes (Table I), but it was not determined in neutrophils from patients with AD.

We were not able to show any relation between lysozyme liberation and ADCC in AD ($R=0.31$, $p>0.10$ for monocytes; $R=0.39$, $p>0.10$ for neutrophils). However, a highly significant relation was demonstrated between ADCC and hexose monophosphate shunt activity (Fig. 3).

The superoxide production (mean ± S.E.M.) of PMA-stimulated phagocytes was the same in AD (2.7 ± 0.1, $n=14$ for monocytes; 13.2 ± 0.1, $n=14$ for neutrophils) as in controls (2.6 ± 0.1, $n=7$ for monocytes; 13.4 ± 0.1, $n=7$ for neutrophils).

DISCUSSION

Antibody-dependent cell-mediated cytotoxicity with monocytes or neutrophils as effector cells is

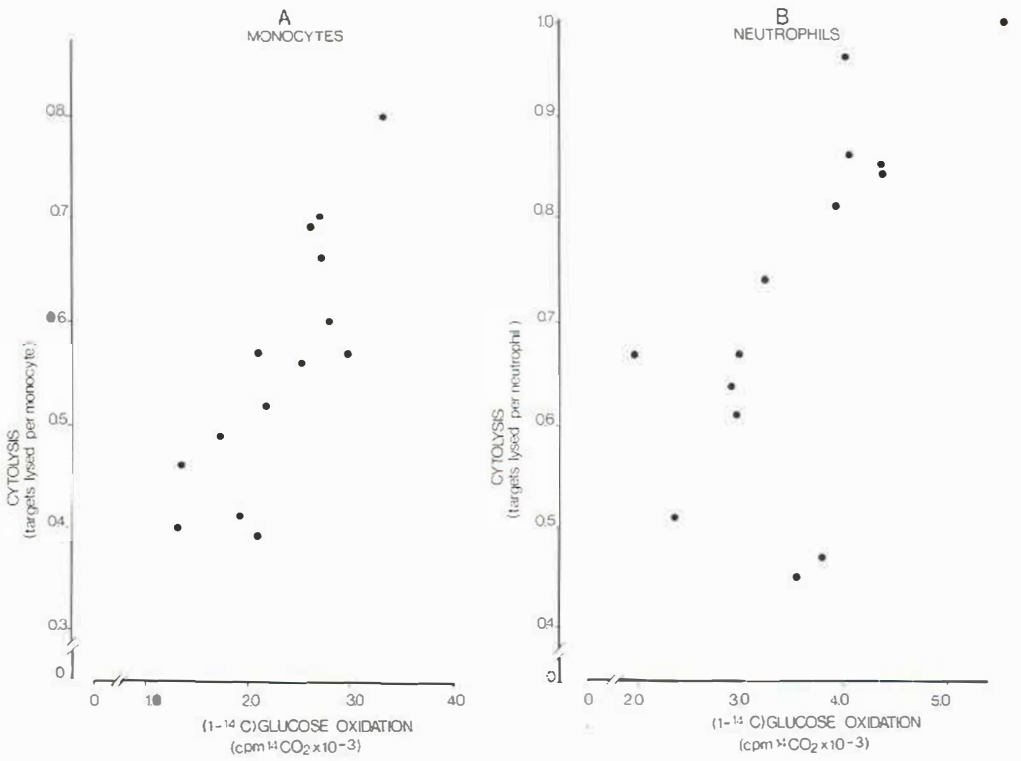

Fig. 3. Relation between cytotoxicity and hexose monophosphate shunt activity in 14 patients with atopic dermatitis. (A) Monocytes, $R=0.84$, $p<0.001$. (B) Neutrophils, $R=0.72$, $p<0.01$. 1×10^5 phagocytes were incubated with 4×10^5 erythrocytes sensitized with IgG for 30 min at 37°C.

a complex reaction involving binding of the target cells to effector cells and subsequent phagocytosis and lysis of the target cells. During this reaction the effector cells are stimulated as observed by both the degranulation and the activation of the hexose monophosphate shunt which closely accompany the ADCC reaction (5).

Both lysosomal enzymes liberated during degranulation (7), and oxygen radicals formed during the respiratory burst (5, 6, 10) have been ascribed a role as effector molecules in ADCC mediated by monocytes and neutrophils. The generation of the oxygen radicals is attributed to a superoxide forming system, which through the interaction of $NAD(P)^+/NAD(P)H$ is connected to the hexose monophosphate shunt as the donor of electrons for the reduction of oxygen (2). We have previously shown that there is a close relationship between ADCC, activation of the hexose monophosphate shunt, and superoxide production in cells which are defective in their ability to generate superoxide (5). Our findings that the activation of the hexose monophosphate shunt during ADCC was depressed in AD, therefore indicates that the generation of reduced oxygen radicals during ADCC was defective. However, this did not result from abnormalities in the enzymes responsible for the generation of the respiratory burst, since superoxide production induced by the soluble phagocytosis stimulator PMA was normal in AD.

Although lysozyme liberation was depressed in AD it did not correlate with the depression of ADCC. Lysozyme liberation was taken as a parameter of the liberation of lysosomal enzymes. Hence it cannot be the defective degranulation which is responsible for the depression of ADCC in AD.

The strong correlation between ADCC and hexose monophosphate shunt activity indicates that it is the defective generation of reduced oxygen radicals which is responsible for the depression found in AD. However, the observation that the respiratory burst, degranulation, and phagocytosis all are depressed in AD, despite normal binding of the

target cells to effector cells, points to a defective mounting of the membrane signals which transduce the Fc receptor stimulus into activation of the effector cell, as being the primary defect in AD. The same defect, in or near the cell membrane, might be of importance in the activation of other cell functions.

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REFERENCES

1. Babior, B. M., Curnutte, J. T. & McMurrich, B. J.: The particulate superoxide-forming system from human neutrophils. J Clin Invest *58:* 989, 1976.
2. Bergmeyer, H. V.: Methods of Enzymatic Analysis. Verlag Chemie, 574 pp. Academic Press, New York and London, 1974.
3. Borregaard, N., Johansen, K. S. & Esmann, V.: Quantitation of superoxide production from normals and 3 types of chronic granulomatous disease. Biochem Biophys Res Commun *90:* 214, 1979.
4. Borregaard, N., Johansen, K. S., Taudorf, E. & Wandall, J. H.: Cytochrome *b* is present in neutrophils from patients with chronic granulomatous disease. Lancet *i:* 949, 1979.
5. Borregaard, N. & Kragballe, K.: Role of oxygen in antibody-dependent cytotoxicity mediated by monocytes and neutrophils. J Clin Invest *66:* 676, 1980.
6. Clark, R. A. & Klebanoff, S. J.: Studies on the mechanism of antibody-dependent polymorphonuclear leukocyte-mediated cytotoxicity. J Immunol *119:* 1413, 1977.
7. Fleer, A., van Schaik, M. L. J., von dem Borne, A. E. G. Kr. & Engelfriet, C. P.: Destruction of sensitized erythrocytes by human monocytes in vitro. Effects of cytochalasin B, hydrocortisone and colchicine. Scand J Immunol *8:* 515, 1978.
8. Holm, G.: Mechanisms of antibody-induced hemolytic activity of human blood monocytes. *In* Wagner, W. H. & Holm, H. (eds.): Activation of Macrophages, p. 63. Excerpta Medica, Amsterdam, Elsevier, New York, 1974.
9. Jensen, J. R., Cramers, M. & Thestrup-Pedersen, K.: Subpopulations of T lymphocytes in atopic dermatitis. Submitted for publication.
10. Katz, P., Simone, C. B., Henkart, P. A. & Fauci, A. S.: Mechanisms of antibody-dependent cellular cytotoxicity. J Clin Invest *65:* 55, 1980.
11. Klass, H. J., Hopkins, J., Neale, G. & Peters, T. J.: The estimation of serum lysozyme: A comparison of four assay methods. Biochem Med *18:* 52, 1977.
12. Kragballe, K.: Antibody-dependent monocyte-mediated cytotoxicity in severe atopic dermatitis. Allergy *34:* 35, 1979.
13. Kragballe, K., Ellegaard, J. & Herlin, T.: Antibody-dependent monocyte-mediated cytotoxicity. Isolation and identification by esterase staining of monocytes detached from monolayers by lowering the temperature. Scand J Haematol *24:* 399, 1980.
14. — Antibody-dependent monocyte-mediated cytotoxicity. The effects of adherence and removal of mononuclear phagocytic cells, and of in vitro incubation. Scand J Haematol *24:* 405, 1980.
15. Kragballe, K. & Herlin, T.: Impaired monocyte mediated cytotoxicity in atopic dermatitis. Arch Dermatol Res *269:* 21, 1980.
16. — Antibody-dependent monocyte-mediated cytotoxicity in atopic dermatitis. Relation to clinical state and cylic AMP response. Allergy, in press.
17. Rogge, J. L. & Hanifin, J. M.: Immunodeficiencies in severe atopic dermatitis. Arch Dermatol *112:* 1391, 1976.

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