Acta Dermatovener (Stockholm) Suppl. 92: 103–105, 1980

MONOCYTE CYTOTOXICITY IN CLINICAL EXACERBATION AND REMISSION OF ATOPIC DERMATITIS

Knud Kragballe and Troels Herlin

Department of Dermatology, Aarhus University, Marselisborg Hospital, Aarhus, Denmark

Abstract. Six adult patients with severe atopic dermatitis were followed up for an average time of 9 months, through periods of clinical exacerbation and remission. Sequential studies of monocyte function measured as antibody-dependent cell-mediated cytotoxicity showed depressed function which did not normalize during clinical remission.

Key words: Antibody-dependent cell-mediated cytotoxicity; Monocytes; Atopic dermatitis; Remission; Exacerbation

Several studies have suggested immunologic dysregulation in patients with atopic dermatitis. However, only a few sequential studies have been performed. In these studies there has been a lack of agreement over the correlation of the clinical state with serum IgE levels (4, 9). For a decreased lymphocyte transformation test, no influence of the clinical state was noticed (1), while for polymorphonuclear leukocytes, chemotaxis normalized following clinical remission (7).

Earlier we showed that monocyte function measured as antibody-dependent cell-mediated cytotoxicity (ADCC) was reduced in patients with severe atopic dermatitis during exacerbation of their disease (2). The purpose of this investigation was to determine by sequential studies on individual patients whether or not monocyte ADCC fluctuated with exacerbations and remissions in skin involvement.

MATERIALS AND METHODS

Patients and controls. Six patients with recurrent widespread dermatitis were included. Their ages ranged from 21 to 33 years (median 24). Two of them had in addition asthma, though not during the observation period. None had received systemic glucocorticoids.

Patients were observed over an average period of 9 months. The first blood sample was taken during acute widespread dermatitis and repeated when there was no sign of dermatitic activity. Between these two samples the patients were treated with potent topical glucocorticoids and/or tar. After remission, blood sampling was repeated every second months over a period of 6 months and on the same occasions the extent and severity of the disease were graded according to Rogge and Hanifin (7). During this period all patients experienced some fluctuation in their disease state, but the treatment was limited to topical hydrocortisone or hydrocortisone-butyrate from time to time. Antihistamines were withheld 72 hours before every blood sample. Controls were 36 healthy volunteers, their ages ranging between 17 and 55 years (median 30).

Preparations of monocytes. Heparinized venous blood (20 I.E. preservative-free heparin/ml) were spun over Ficoll-Isopaque as described by Böyum (4). 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, N.Y., USA). The washed cells were resuspended in medium RPMI 1640 with 25 mM HEPES (Gibco) supplemented with 25 % (v/v) FCS and dispensed to tissue culture flasks (Nunc, Roskilde, Denmark). After incubation in a humidified atmosphere containing 5 % CO_2 for 1 h at 37°C the non-adherent cells were decanted and the flasks rinsed with 3 changes of HBSS with 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 an ice bath for 30 min. Detached monocytes were then decanted and washed once. Finally, the cell concentration was adjusted to 1×10^6/ml. Judged by non-specific esterase activity in cytocentrifuged preparations (33) the median percentage of monocytes was 91, ranging from 82 to 96. 0–3 % granulocytes were found. The other contaminating cells were lymphocytes. As assessed by trypan blue exclusion, the viability was always higher than 95 %.

Labelling of erythrocytes. Equal volumes of washed type B human erythrocytes (200×10^6/ml) and sodium ^{51}chromate (Radiochemical Centre, 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.

Antiserum. Human hyperimmune antiserum to type B human erythrocytes was obtained from the Blood Bank and Blood Grouping Laboratorium, Aarhus Kommunehospital. Serum was heat-inactivated and stored at −20°C. A dilution inducing maximal lysis was used.

Cytotoxicity assay. The tests were set up in duplicate in round-bottomed 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 was added 100 μl ^{51}Cr-labelled erythrocytes (1×10^6/ml and 8×10^6/ml and 100 μl antiserum dilution.

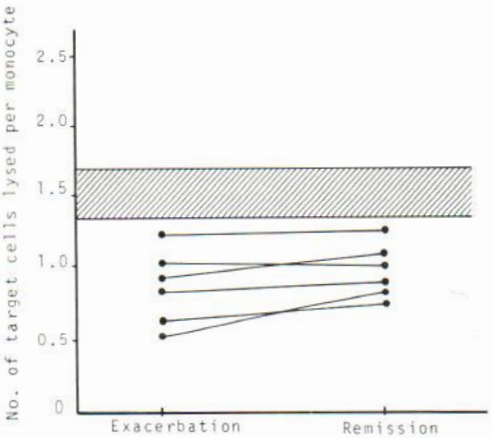

Fig. 1. Monocyte ADCC in patients with atopic dermatitis at time of clinical exacerbation and remission. The lines connect the paired results from each person. The hatched interval marks the 95% confidence limits for normal individuals. (●) denotes patients with atopic dermatitis.

Controls in which the monocytes were replaced by unlabelled erythrocytes (1×10^6/ml) were included. The mixtures were centrifuged at 160 *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. Half of the supernatant was withdrawn. Both this supernatant (S) and residues (R) were counted separately in a well-type gamma counter. Cytotoxicity was calculated as:

$$S \times 2/(R+S)$$

By subtracting the release in control tubes the specific cytotoxicity (C) was defined. Results were expressed as the number of erythrocytes (E) lysed per monocyte (M):

$$E \times C/M.$$

RESULTS

During clinical exacerbation, monocyte ADCC was invariably decreased (Fig. 1). In clinical remission, the function in 5 cases increased slightly without reaching normal values. In the last case a minor fall in cytotoxity was observed from exacerbation to remission.

Between exacerbation and remission, patients were treated with potent topical glucocorticoids. To answer the question whether this treatment had maintained impaired function even though clinical remission had occurred, 4 out of the 6 patients were observed for a further period of 6 months (Fig. 2). During this period some fluctuations of disease activity were observed, but patients were only from time to time treated with topical glucocorticoids of low potency. As illustrated, only minor fluctuations in monocyte ADCC occurred. Only for one patient did the function normalize, but on the following occasion this patient again displayed depressed function. There was no significant correlation between changes in monocyte function and the clinical state of the disease.

DISCUSSION

Because of the rapid fluctuations which may be seen in the severity of lesions in patients with atopic dermatitis, we chose to observe individual patients through exacerbations and remissions. Our observations suggest that decreased monocyte ADCC tends to remain low in quiescent phases of the disease. Even though potent topical steroids were used only during exacerbation, it could not be ruled out that concurrent therapy was responsible for the hyporeactivity. Besides, it was not possible to say from this investigation, if or when normalization would take place if the patients had been free from dermatitis for longer time.

In spite of the rapid turnover of human monocytes which under normal conditions leave the vascular space with a half-time of about 8 hours (5), it has been shown that suppression of normal monocytes following thermal injury can be prolonged. In patients with mild or moderate burns the phagocytic functions returned to normal after 3 to

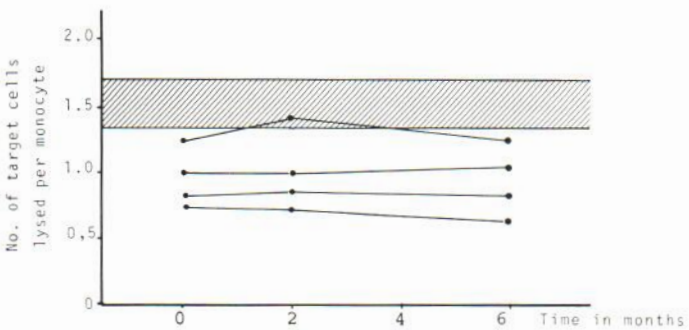

Fig. 2. Individual variations in monocyte ADCC in atopic dermatitis. The lines connect results from one person (●). The hatched interval marks the 95% confidence limits for normal individuals.

6 weeks, but in the group with severe burns, the dysfunction persisted after 7 weeks (3).

There has been some controversy over the relationship between altered immune reactivity and the clinical state of patients with atopic dermitis. Sequential estimation of total levels of serum IgE and specific IgE has shown no significant correlation between IgE levels and the severity of skin involvement (4, 9), but in another report some correlation was shown (6). For lymphocytes, a decreased number of circulating T lymphocytes and hyporeactive lymphocyte transformation test to PPD and herpes simplex antigen have been found during both exacerbations and remissions (1). In serial comparisons of PMN chemotaxis in 4 out of 4 patients with erythrodermal atopic dermatitis a dramatic parallel with clinical improvement was found (7). The same patients also expressed decreased monocyte chemotaxis during exacerbation, but monocytes were not tested again in quiescent periods. Impaired monocyte chemotaxis has also been found by another group of investigators, and they were not able to correlate this finding to the severity of the dermatitis. However, the individual patients were studied on one occasion only (8).

It is difficult to compare our results with other sequential studies, because different cell types have been tested in different assay systems. As mentioned, serum IgE also tends to remain abnormal in clinical remissions. Nevertheless, a common factor behind elevated IgE levels and low monocyte function is unlikely, as depressed monocyte ADCC is not correlated to serum IgE levels (in preparation).

The association between severe atopic dermatitis and monocyte function remains speculative. However, the fact that monocyte ADCC remains depressed despite fluctuations in the clinical state could suggest that a defective monocyte function is part of a constitutional basis for atopic dermatitis.

REFERENCES

1. Hovmark, A.: An *in vitro* study of depressed cell-mediated immunity and of T and B lymphocytes in atopic dermatitis. Acta Dermatovener (Stockholm) *57:* 237, 1977.
2. Kragballe, K.: Antibody-dependent monocyte-mediated cytotoxicity in severe atopic dermatitis. Allergy *34:* 35, 1979.
3. Lloyd, R. S. & Lerich, P. L.: Blood monocyte dysfunction following thermal injury. Burns *3:* 245, 1977.
4. Mackie, R., Cobb, S. J., Cochran, R. E. I. & Thomson, J.: Total and specific IgE levels in patients with atopic dermatitis. Clin Exp Dermatol *4:* 187, 1979.
5. Meuret, G. & Hoffman, G.: Monocyte kinetics studies in normal and disease states. Br J Haematol *24:* 275, 1973.
6. Ogawa, M., Berger, P. A., McIntyre, O. R., Clendenning, W. E. & Ishizaka, K.: IgE in atopic dermatitis. Arch Dermatol *103:* 575, 1971.
7. Rogge, J. L. & Hanifin, J. M.: Immune deficiencies in severe atopic dermatitis. Arch Dermatol *112:* 1391, 1976.
8. Snyderman, R., Rogers, E. & Buckley, R. H.: Abnormalities of leukotaxis in atopic dermatitis. J Allergy Clin Immunol *60:* 121, 1977.
9. Öhman, S. & Johansson, S. G. O.: Immune globulins in atopic dermatitis. Acta Dermatovener (Stockholm) *54:* 193, 1974.