Acta Dermatovener (Stockholm) Suppl. 92: 52–56, 1980

CHEMOTAXIS INHIBITION BY PLASMA FROM PATIENTS WITH ATOPIC DERMATITIS

Jon M. Hanifin, Janet L. Rogge and Richard H. Bauman

Department of Dermatology, University of Oregon Health Sciences Center, Portland, Oregon 97201, USA

Abstract. We previously reported depressed polymorphonuclear leukocyte and monocyte chemotaxis in patients with severe atopic dermatitis. The degree of impairment roughly correlated with the disease severity and chemotaxis was noted to improve rapidly with clinical remissions. This rapid improvement suggested the presence of a short-lived plasma inhibitor of leukocyte function. We used a radiolabeled PMN chemotaxis assay to assess plasma effect on migration. PMN's were incubated in plasmas from patients with varying degrees of atopic dermatitis then washed and assessed for migration toward endotoxin-stimulated serum attractant. Inhibitory effect varied widely and correlated directly with clinical extent and severity of dermatitis. Serial studies on individual patients showed lessening of plasma inhibitory activity during remissions. PMN's from patients with atopic dermatitis showed improved migration after incubation in normal plasma. There was no evidence for circulating chemoattractants nor chemotactic factor inactivators. There appears to be a circulating inhibitor of chemotaxis present in plasma during acute flares of atopic dermatitis; the molecular nature of the inhibitor remains to be elucidated.

Key words: Polymorphonuclear cell chemotaxis; Atopic dermatitis

Individuals with atopic dermatitis (AD) have depressed cell mediated immunity (CMI) (7, 10, 11) and increased susceptibility to viral and staphylococcal cutaneous infections (4). We have reported that polymorphonuclear leukocyte (PMN) and monocyte chemotaxis, as well as lymphocyte response to phytohemagglutinin (PHA) were simultaneously depressed in patients with atopic erythroderma (11). PMN chemotaxis was noted to improve rapidly with clinical remissions. Dysfunction of three major host defense cells during erythrodermic flares may contribute to the symptomatology and cutaneous infections of atopic dermatitis. These transient functional deficiencies of PMN's, monocytes and lymphocytes may provide basic insight into the relationship between inflammation and immunity.

Numerous abnormalities of human leukocyte chemotaxis have been described, including intrinsic cellular defects, deficient chemotactic factor production, humoral inactivators of chemotactic factors and humoral inhibitors of leukocyte migration. Snyderman et al. (13) have reported inhibition of normal monocyte chemotaxis by serum from severe AD patients. In this communication we report studies showing that plasma from patients with AD inhibited chemotaxis of normal PMN. Plasma inhibitory activity correlated with the severity of dermatitis and there was no evidence for chemotactic factor inactivators.

MATERIALS AND METHODS

The diagnosis of AD in patients was based on a constallation of typical clinical features, and extent and severity of skin involvement were graded on scales of 1 to 5 as previously outlined (11). Thus, a patient with generalized erythroderma would carry a rating of 5/5. Patient ages ranged from 3 to 62 years; most were young adults. Some of the patients were under treatment with systemic antibiotics when studied, but none were receiving corticosteroids, antihistamines or other agents. None of the patients had evident systemic disease. Control subjects were normal, healthy, young adults receiving no therapy.

Preparation of leukocytes

Heparinized venous blood was centrifuged on a Hypaque-Ficoll gradient and mononuclear cells were removed. Cells below the gradient included PMN's and erythrocytes (RBC's). The RBC's were sedimented with 3% dextransaline; residual RBC's in the PMN-rich supernate were then osmotically lysed. The remaining PMN's were suspended at 2.6×10^6/ml in Gey's solution containing 2% bovine serum albumin (GBSS) then subjected to the migration assay detailed below.

PMN chemotaxis assay

The isotope-labeled PMN chemotaxis assay of Gallin et al. was used in all studies. (Briefly, this assay measured the migration of ^{51}Cr labelled PMN's through one filter and onto a lower filter (2).) Radioactivity was quantitated using a gamma counter. All samples were run in triplicate; results were expressed as average counts per minute of three lower filters corrected for labeling variability between different donor cell populations as follows:

$$\frac{\text{Mean CPM of 3 filters} \times 10\,000.}{\text{CPM per } 10^6 \text{ PMN}}$$

This value was designated corrected counts per minute (CCPM).

Preparation of chemoattractant

Two ml normal AB or atopic serum, 1 ml sterile, *E. coli* lipopolysaccharide (Difco; 250 mcg/ml saline), and 17 ml veronal buffered saline were mixed and incubated at 37°C for 60 min followed by 56° for 30 min. The chemoattractant was then diluted with equal parts of veronal buffered saline and small aliquots were stored at −70°C until used.

Preparation of plasma and serum

Heparinized plasma was drawn into plastic syringes and immediately spun in plastic centrifuge tubes at 2 000 g for 30 min in at 4°C, then stored at −70°C until used for incubation studies.

Before addition to chemotaxis chambers, isotope-labeled PMN's were suspended in 1.0 ml plasma or serum (7.8×10^6 cells/ml) for 90 min at 37°C. The incubation tubes were periodically agitated and after 90 min the cells were washed once and resuspended in 3 ml GBSS. This cell suspension was then placed in the upper compartment of the chemotaxis chamber and migration was quantitated as described.

RESULTS

Effect of atopic plasma on normal PMN chemotaxis

In all studies the migration of normal PMN's after incubation in atopic plasma was compared with parallel migration of cells incubated in pooled, normal AB plasma. The same standard attractant was used in all cases.

Initial studies tested plasmas obtained from four patients during acute exacerbations of generalized atopic erythroderma (i.e. extent/severity ratings of 5/5). As shown in Table I, inhibition ranging from 42% to 82% was observed when the cells were incubated in plasma from these severely involved atopic dermatitis patients. Pilot studies compared chemotaxis of neutrophils incubated for 15, 20, 60 and 90 min in these plasmas and inhibition was found to be maximal between 60 and 90 min. The 90 min incubation period was used for all subsequent studies. In no study did Trypan blue or phagocytic tests show decreased viability (nor was either gross or microscopic aggregation of cells noted after incubation in atopic plasma.)

Table I. *Inhibition of normal PMN chemotaxis by plasma from patients with erythrodermic atopic dermatitis (mean CCPM ± S.E.M.)*

Normal plasma	Atopic plasma	Depression (%)
2 846 ± 309	1 675 ± 175	42
3 061 ± 28	629 ± 31	79
4 817 ± 439	829 ± 256	82
2 788 ± 525	1 184 ± 309	58

Table II. *Comparison of clinical extent and severity of atopic dermatitis with atopic plasma inhibition of normal PMN chemotaxis*

Based on extent/severity grades of 1–5

	Chemotaxis inhibition		
	≤ 20%	> 20%	*p*-value
Mean extent	2.8	4.1	< 0.001
Mean severity	2.3	3.8	< 0.001

We subsequently performed 91 studies from 28 patients with mild to severe atopic dermatitis. Cells preincubated in atopic plasma showed a mean migration of 1 845 CCPM (±S.D. 992) compared with 2 149 CCPM (±S.D. 1046) after normal control plasma. A *t*-test for correlated data showed these differences to be significant with a *p*-value of < 0.001.

Inhibitory activity of plasmas varied considerably with the clinical severity of disease. Extent and severity ratings were recorded for comparison with chemotactic inhibition by 30 atopic plasmas. Statistical comparisons of the mean extent and severity ratings of the 16 plasmas causing less than 20% vs. the 17 plasmas causing greater than 20% inhibition showed significant differences for both parameters (Table II). There clearly appears to be a correlation between clinical severity of atopic dermatitis and presence of a plasma chemotactic inhibitor.

Effect of normal plasma on atopic cells

Since incubation in atopic plasma could decrease normal PMN chemotaxis, we next investigated the effect of incubating atopic cells in normal plasma to reverse the inhibition. For these studies, chemotaxis of freshly isolated PMN's was compared to that of the same cells which had been incubated for 90 minutes in normal plasma. PMN's from 7 of 8 patients with atopic dermatitis showed increased migration after exposure to normal AB plasma (Table III). The mean increase from 1 482 to 2 239 CCPM was significant with $p < 0.001$ by paired comparison *t*-test.

Table III. *Effect of normal plasma pre-incubation on atopic PMN chemotaxis (mean CCPM ± S.E.M.)*

Exp. no.	Atopic cells Non-pre-incubated	Atopic cells after normal plasma	% Δ
1	3 035 ± 102	3 034 ± 85	0
2	1 802 ± 66	2 692 ± 336	+ 33
3	353 ± 85	1 396 ± 78	+ 75
4	1 185 ± 41	1 927 ± 70	+ 39
5	887 ± 52	1 441 ± 54	+ 38
6	1 303 ± 122	1 710 ± 34	+ 24
7	496 ± 28	1 225 ± 99	+ 60
8	2 799 ± 351	4 489 ± 280	+ 38
Mean	1 482 ± 331	2 239 ± 369	+ 38

Parallel control studies of normal cells in normal AB plasma showed no change in migration.

Chemoattractant activity of non-stimulated atopic plasma

We questioned whther the cellular inhibition observed with atopic plasma was due to random stimulation of neutrophils by chemotactically active molecules. To evaluate inherent chemotactic activity, we measured normal PMN migration using as attractants undiluted inhibitory plasmas obtained from 11 patients with severe AD. Migration varied widely but we were unable to detect significant chemoattractant activity of atopic plasmas compared with normal plasmas (Table IV). In one study normal plasma caused significantly greater migration, but this was not a consistent finding.

Effect of atopic serum on standard attractant

To detect the possible presence of circulating chemotactic factor inactivators, we mixed fresh serum from patients with AD 1:10 with standard attractant (endotoxin-stimulated normal serum). Chemoattraction of this mixture was then compared with

Table IV. *Comparison of normal and atopic plasmas as chemoattractants (mean CCPM ± S.E.M.)*

Figures in parentheses represent number of plasma tested in each experiment

Exp. no.	Normal plasma	Atopic plasma	*p*-value
1	(1) 1 270 ± 70	(5) 1 908 ± 294	< 0.1
2	(1) 1 639 ± 98	(2) 2 712 ± 327	< 0.2
3	(2) 4 335 ± 83	(3) 3 579 ± 419	< 0.3
4	(1) 2 850 ± 42	(1) 1 472 ± 25	< 0.001

Table V. *Effect of atopic serum on standard attractant*

Exp. no.	Attractant plus 10% normal serum	Attractant plus 10% atopic serum
1	2 791 ± 71	2 732 ± 73
2	2 623 ± 81	2 883 ± 68
3	3 974 ± 118	4 060 ± 51

10% normal control plasma in standard attractant. Results (Table V) showed no evidence of chemotactic factor inactivation by atopic serum.

DISCUSSION

Multiple basic defects have been identified to account for the various human leukocyte chemotaxis deficiencies. Abnormalities may be intrinsic to the cell or may be due to external influences directed at either the cell or the chemoattractant (12). In our studies of patients with AD we noted that neutrophil chemotaxis tended to be only transiently abnormal. Patients with depressed PMN migration during severe flares of dermatitis showed nearly normal chemotaxis 48 hours later, as their erythroderma subsided in the hospital (11). We hypothesized that a transient circulating factor may be associated both with leukocyte abnormalities and with cutaneous exacerbations.

This report documents inhibition of normal neutrophil chemotaxis by plasma from patients with AD. Inhibition was maximal when cells were incubated for 90 minutes prior to being washed and placed in the upper compartment of a chemotaxis chamber. As with the direct chemotaxis studies of atopic cells (11), the magnitude of plasma inhibition varied directly with the clinicial severity of disease. Plasmas obtained during remissions generally failed to cause significant inhibition.

Since cells had diminished chemotactic function after exposure to atopic plasma, we questioned whether the defect was reversible. Migration of cells from patients with AD significantly increased after incubation in normal plasma while normal control PMN chemotaxis did not increase. These findings indicate that atopic PMN's are not intrinsically defective, but are reversibly inhibited by a factor acting either on the internal metabolism of the cell or on the surface membrane. A previous study of chemotactic defects in the hyperimmunoglobulinemia-E syndrome failed to restore chemotactic responsiveness when cells were incubated in normal plasma

for 30 min (4) but in a later publication by the same authors, normalization of migration was observed when patient's blood was kept at room temperature for 12 hours before cells were isolated (5). Possibly because of differing migration assays, we noted reduced chemotaxis of both normal and atopic PMN's when we attempted to reproduce those findings.

The molecular nature of the atopic plasma inhibitor has not been characterized. We used cell and platelet-free, undiluted heparinized plasma for PMN preincubations throughout this study. Preliminary studies showed that preparation of blood with compounds such as trasylol, EACA and hexadimethrine bromide, to prevent activation of potentially chemotactic molecules in plasma did not offer any advantage. Heparinized plasma prepared in plastic equipment consistently supported greater migration than plasma plus these additives. Significantly less migration was seen with cells preincubated in serum. Incubation of cells in plasma-free media caused marked depression of chemotaxis as did manipulation of plasmas with heating or dialysis.

Decreased leukocyte chemotaxis has been associated with increased serum chemotactic factor inactivator in anergic patients with Hodgkin's disease (16), sarcoidosis (9), and cirrhosis (14). Our studies indicate that the atopic plasma inhibitor acts directly on cells rather than on chemoattractant. The cells are washed before transferring to chemotaxis chambers; it seems unlikely that enough of the factor could be transferred on cell membranes to cause inactivation of the standard attractant in the lower compartment of the chamber. We further investigated this possibility by directly combining atopic sera with our endotoxin-stimulated serum attractant and found no reduction in chemoattraction.

We also postulated that cells might be "inactivated" by circulating chemotactic factor. Chemotactically active molecules have been shown to cause random stimulation of PMN's, decreasing their directed migration (15). There was no difference in random migration between cells incubated in normal plasma and those incubated in atopic plasmas. Furthermore, we were unable to demonstrate chemoattractant activity with untreated atopic plasma.

Hill & Quie (4) have studied PMN chemotaxis in patients with hyperimmunoglobulinemia-E syndrome consisting of recurrent cutaneous infections, high levels of serum IgE and eczema. Serum from these patients did not inhibit chemotactic activity of normal PMN's. Since IgE is known to mediate histamine release, they suggested this agent might be responsible for the abnormal chemotaxis. They showed *in vitro* inhibition of PMN chemotaxis by histamine concentrations ranging from 10^{-3} to 10^{-5} M (4).

We have been particularly interested in this possibility because histamine could explain many of the abnormalities associated with atopic dermatitis, including cutaneous erythema and depressed lymphocyte responsiveness in addition to inhibition of chemotactic response. In unpublished studies, we have found elevated histamine levels in three atopic plasmas which caused inhibition of normal PMN chemotaxis; this has not, however, been a constant finding. Our observations of the cyclical flares of dermatitis in these patients suggests that histamine may be only briefly present before it is cleared or inactivated. Consistent documentation of a correlation between plasma histamine levels and chemotactic inhibition, therefore, would be difficult.

Histamine levels are elevated in the skin of patients with AD, and one might suspect that chemotaxis through involved skin might be decreased. We have used skin window preparations to demonstrate significant reductions in total cells and in the PMN to mononuclear cell ratio on the coverslips after three hours. Migration appears to be merely slowed, since differences between normals and atopics is negligible after 6, 12 and 21 hours (S. Grewe and J. M. Hanifin, unpublished data).

Thus, our *in vitro* findings appear to reflect an *in vivo* abnormality. This slowed migration through skin may well account for cutaneous anergy in patients who have normal lymphocyte transformation responses to microbial antigens (11).

Certainly, there are innumerable factors which could be responsible for the impaired chemotaxis in patients with atopic dermatitis. At this point we can only say that there is a circulating inhibitor of chemotaxis; this inhibitor is most active during acute flares of dermatitis and the exact nature of the inhibitor remains to be elucidated.

REFERENCES

1. Elliott, S. T. & Hanifin, J. M.: Delayed cutaneous hypersensitivity and lymphocyte transformation: Dissociation in atopic dermatitis. Arch Dermatol *115:* 36, 1979.
2. Gallin, J. I., Clark, R. A. & Kimball, H. R.: Granulocyte chenotaxis: an improved *in vitro* assay employing ^{51}Cr-labeled granulocytes. J Immunol *110:* 233, 1973.

3. Hanifin, J. M., Bauman, R. & Rogge, J. L.: Chemotaxis inhibition by plasma from patients with atopic dermatitis. Clin Res *24:* 198 A, 1977.
4. Hill, H. R. & Quie, P. G.: Raised serum-IgE levels and defective neutrophil chemotaxis in three children with eczema and recurrent bacterial infections. Lancet *i:* 183, 1974.
5. Hill, H. R., Quie, P. G., Pabst, H. R., Ochs, H. D., Clark, R. A., Klebanoff, S. J. & Wedgwood, R. J.: Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. Lancet *ii:* 617, 1974.
6. Keller, H. U., Hess, M. W. & Cottier, H.: Inhibitory effects of human plasma and serum on neutrophil random migration and chemotaxis. Blood *44:* 843, 1974.
7. Lobitz, W. C., Honeyman, J. F. & Winkler, N. W: Suppressed cell-mediated immunity in two adults with atopic dermatitis. Br J Dermatol *86:* 317, 1972.
8. Maderazo, E. G. Ward, P. A. & Quintiliani, R.: Defective regulation of chemotaxis in cirrhosis. J Lab Clin Med *85:* 621, 1975.
9. Maderazo, E. G., Ward, P. A., Woronick, C. L., Kubik, J. & DeGraff, A. G.: Leukotactic dysfunction in sarcoidosis. Ann Intern Med *84:* 414.
10. McGeady, S. I. & Buckley, R. H.: Depression of cell-mediating immunity in atopic eczema. JACI *56:* 393, 1975.
11. Rogge, J. L. & Hanifin, J. M.: Immunodeficiencies in severe atopic dermatitis. Arch Dermatol *112:* 1391, 1976.
12. Snyderman, R., Pike, M. C. & Altman, L. C.: Abnormalities of leukocyte chemotaxis in human disease. Ann NY Acad Sci *256:* 386, 1975.
13. Snyderman, R., Rogers, E. & Buckley, R. H.: Abnormalities of leukotaxis in atopic dermatitis. J Allergy Clin Immunol *60:* 121, 1977.
14. Van Epps, D. E. & Williams, R. C.: Serum chemotactic inhibitory activity: heat activation of chemotactic inhibition. Infect Immun *13:* 741, 1976.
15. Ward, P. A. & Becker, E. L.: The deactivation of rabbit neutrophils by chemotactic factor and the nature of the activatable esterase. J Exp Med *127:* 693, 1968.
16. Ward, P. A. & Berenberg, J. L.: Defective regulation of inflammatory mediators in Hodgkin's disease. Supernormal levels of chemotactic factor inactivator. N Engl J Med *290:* 76, 1974.