IgA-associated Inhibition of Polymorphonuclear Leukocyte Chemotaxis in Neutrophilic Dermatoses

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The chemotactic activity of normal human polymorphonuclear leukocytes (PMNs) confronted with heat inactivated sera from patients with psoriasis as well as various chronic proliferative diseases was determined using modified Boyden chambers. By the addition of phorbol myristate acetate (PMA) at a concentration of 1 ng/ml the chemoattractant activities of the sera were greatly potentiated. However, the chemotactic migration of normal PMNs was strongly inhibited by sera from patients with long standing and widespread psoriasis, pyoderma gangrenosum, severe acne conglobata, Sweet syndrome, and some patients with chronic arthritis following rheumatoid fever. In acute guttate psoriasis and atopic dermatitis increased migratory activities were seen. The inhibition of chemotaxis correlated with increased serum IgA levels as determined by radial immunodiffusion. Column chromatography (Sephacryl S-300) revealed serum fractions of strong inhibitory potency at a molecular weight near 200,000 Dalton. These inhibitory fractions were seen in patients with long standing neutrophil related diseases and could not be detected in normal control sera. It appears that inhibition of PMN chemotaxis is a secondary phenomenon and may play an autoregulatory role in PMN related inflammation.

One of the outstanding features in psoriasis is the migration of polymorphonuclear leukocytes (PMNs) through the epidermis toward the periphery. These cells form microabscesses along the inner lining of the stratum corneum in response to a chemotactic stimulus which is believed to be located within the upper epidermis. Several studies have demonstrated chemotactic complement split products (e.g., C3a, C5a) present in psoriatic scales [1–3]. Further, serine esterases capable of activating the fifth component into a chemoattractant were found in psoriatic stratum corneum [4]. Recently it was shown that the chemotactic activity (CA) of PMNs in psoriasis is greatly increased as compared to nonpsoriatic controls, when in the Boyden chamber the cells were confronted with autologous serum [5]. Evidence was presented indicating that fresh sera contained increased chemoattractant activities after incubation with migrating PMNs [5]. These results suggest that in serum chemoattractants are generated in the presence of PMNs.

In the present study the ability of serum from psoriasis to affect chemotactic responses of PMNs is further examined with respect to the state of the disease, its duration and the extent of skin involvement.

The results demonstrate the presence of a circulating inhibitor of PMN chemotaxis in long standing psoriasis as well as in inflammatory diseases in which PMN play a major role.

MATERIALS AND METHODS

Patients

All patients investigated were hospitalized and agreed to the proposed investigations by written consent. All blood samples were taken before any therapy was started. Biopsies were taken in most of these dermatologic patients confirming the clinical diagnosis.

Sera from patients with rheumatic fever (RF) and chronic glomerulonephritis (CG) were kindly provided by Dr. M. Gross, I. Department of Internal Medicine, University of Kiel. These patients were regularly checked for disease activity. All suffered from progressive joint or kidney disease for more than 3 yr.

For controls healthy persons (medical personnel, students) were selected. They were 21 to 64 yr of age.

Preparation of PMNs

PMNs were isolated by a modification of the method described by Henson [6]. Venous blood from healthy nonpsoriatic donors was drawn into 1/6 vol of acid citrate dextrose (0.085 mol Na-citrate, 0.065 mol H-citrate, 2% w/v Dextran T70, Pharmacia), placed in disposable plastic tubes and centrifuged (20 min; 500 g). The supernatant, the buffy coat, and the upper portion of the erythrocyte sediment were removed.

The remaining erythrocytes and PMN containing sediment were gently mixed with 1-1.5 vol of 2.5% gelatin (Merck) in 0.9% NaCl. The cell suspension was allowed to sediment in a waterbath at 37°C for 30 min. The PMN-containing supernatant above the erythrocytes was carefully removed and centrifuged at 20°C (10 min; 400 g). Contaminating erythrocytes were lysed with 0.85% (w/v) NH4Cl in distilled water. The PMN-suspension was centrifuged at 4°C (10 min; 150 g) and washed twice with cold medium TC 199. The final cell preparation contained more than 90% neutrophils with a viability of greater than 95% as assessed by trypan blue exclusion.

Chemotaxis Assay

50 μl heat treated (30 min/56°C) serum was diluted with 400 μl medium TC 199 and 50 μl phorbol myristate acetate (12-0-tetradecanoyl-phorbol-13-acetate, PMA, Sigma) in buffer pH 6.5. This was prepared just before use by dilution of a dimethylsulphoxide stock solution of PMA (1.0 mg/ml) with acid sodium acetate buffer, pH 6.5, to a final concentration of 10 ng/ml. The PMA stock solution was kept frozen between the experiments [7].

Diluted PMA-serum was placed in the lower compartment (150 μl) of a modified Boyden chamber of the blind well type. The compartments of the chamber were separated by a membrane filter (cellulose nitrate filter, pore size 5.0 μm, Sartorius). The upper compartments were filled with 100 μl of the PMN-suspension (3 x 10⁶ cells /ml). Chambers were incubated for 2.5 hr at 37°C.

Thereafter the filters were removed, fixed with methanol, stained with hematoxylin, cleared with xylene and mounted on glass slides.

All cells present on the lowest plane of the filter were counted (magnification 500 ×). Five randomly selected fields were counted and the average number of cells per high power field (HPF) was expressed as the average of duplicate or triplicate filters. Casein (“Hammansten,” Merck, 4 mg/ml TC 199) was used as a standard chemoattractant for every PMN preparation. The range of cells per HPF was 20-40.
The chemotactic activity of the specimens under study was expressed as the percentage of chemotactic activity of PMN against casein (= relative chemotactic activity, RCA) [5] according to the formula:

$$\text{RCA} = \frac{\text{mean number of PMNs per high power field}}{\text{mean of casein control per high power field}} \times 100$$

**Gel Chromatography**

1.0 ml of fresh serum (stored below -70°C) was chromatographed by using Sephacryl S-300 superfine (Pharmacia) in Dulbecco's phosphate buffered saline (PBS). The dimensions of the column were 1.5 × 50 cm and the flow rates were kept at 5.0 ml per hr resulting in 5.0 ml fractions. Eluted fractions were measured for protein by optical density at 280 nm.

In the chemotaxis assay PMA was added to each fraction at a final concentration of 1 ng/ml.

In order to obtain an estimate of the molecular weight of the chemotaxis inhibitor the column chromatography (Sephacryl S-300 superfine) was calibrated using solutions of blue dextran 2,000, thyroglobulin (669,000), ferritin (440,000), catalase (232,000), aldolase (158,000) and cytochrome C (12,400). Void volumes (V0) were determined by means of blue dextran 2,000 and the Ve/Vo was calculated by the method of Andrews [8]. For determination of IgA the column fractions were 5-fold concentrated (Millipore Ultrafilters, exclusion limit: 10,000 Daltons) and examined by the method of Mancini, Carbonara, and Heremans [9] using Hyland Immunoplates (Hyland Travenuol GmbH).

**Dose-Response of Different PMA Concentrations on Chemotaxis with Serum**

Into duplicate vials containing 50 μl heat-inactivated (56°C/30 min) serum 400 μl of TC 199 and 50 μl of a freshly prepared PMA-solution (in 0.1 M acetic acid sodium acetate buffer, pH 6.5) were added at a desired final concentration of 0.1, 0.5, 1, 5, 10, and 100 ng/ml.

**Determination of IgA**

Serum IgA was determined by the method of Mancini, Carbonara, and Heremans [9] using Hyland Immunoplates (Hyland Travenuol GmbH). The concentrations are given in ng/dl (WHO values).

**RESULTS**

**Potentiation of CA by Phorbol-Myristate-Acetate (PMA)**

Addition of PMA at various concentrations to the serum resulted in dose-related potentiation of CA (Fig 1). Maximum increases in CA were present at a concentration of 1 ng PMA/ml, with higher concentration of PMA no further increase of CA was seen, instead, fewer PMNs migrated through the filters.

**Fig 1. Relative chemotactic activity (RCA, ordinate) of normal human serum (diluted 1:10 with TC 199) in the presence of various concentrations of PMA (abscissa). Peak chemotactic potentiation by PMA is seen at a concentration of 1 ng/ml. This dose is used in all subsequent experiments of PMA chemotaxis.**

At a concentration of 10 ng/ml which has been shown to stimulate the release of PMN lysosomal enzymes [10] chemotactic migration was greatly reduced (Fig 1). Reproducibility proved to be adequate in this system. For these reasons a concentration of 1 ng/ml PMA was used in all subsequent experiments. By the addition of PMA to the upper and lower chamber the possibility that this potentiation of chemotaxis was in fact caused by increased chemokinesis could be excluded.

**Chemotactic Activity of Sera from Patients with psoriasis**

A total of 58 sera from patients with different forms of psoriasis was analyzed (Fig 2). In all cases the presence of PMA augmented the chemoattractant potency of the sera against normal PMNs. No difference was found in the average RCA between psoriatic and nonpsoriatic sera. This confirms previous observations obtained with fresh as well as heatinactivated sera without the use of PMA [5]. In psoriasis wide variations were seen as compared to the control group (Fig 2). Eighteen sera demonstrated very low CA (below 100% RCA) whereas 19 sera showed highly increased chemotactic activity with RCA values above 300%. In the remaining 21 sera no alterations of PMA induced CA were detected (Fig 2).

Further examination of 10 randomly selected sera of each of the 2 groups with chemotactic abnormalities is presented on Tables I, II. Whereas the age of the patients and the history of psoriasis did not vary significantly in the 2 groups, patients with low RCA nearly constantly suffered from the persistent stationary type of psoriasis covering wide areas of the body (more than 30-50%, Table I). On the other hand high serum chemotactic activity correlated with the presence of relapsing psoriasis predominantly of the guttate type (Table II). Also the skin was affected to a lesser extent as compared to the former group.

**Fig 2. Relative chemotactic activity (RCA, ordinate) of heat-inactivated serum from patients with various forms of psoriasis. Note wide variations of RCA data especially in the psoriatic group. Single dots represent average values of duplicate chemotaxis assays. No difference is found between psoriatic and nonpsoriatic sera. PMA (1 ng/ml) was used for potentiation of chemotaxis.**
In view of the fact that PMN chemotaxis and random migration is reported to be inhibited by IgA myeloma sera in contrast to IgG myeloma sera [11] the levels of IgA were quantitatively determined by using radial immunodiffusion. Average values for normal healthy persons were 164 ± 76 mg/dl. In the control persons examined during this study the average IgA serum levels were close to given standard data. In psoriasis IgA levels averaged 357.8 ± 113.7 mg/dl in the low responder group whereas sera with increased RCA showed low levels of IgA (134.9 ± 29.0 mg/dl). The differences between the 2 IgA levels were significant (p < 0.001).

Comparison with Other Inflammatory Diseases

In Table III various inflammatory skin diseases which were examined for serum chemotactic activity are reported. These include pyoderma gangrenosum, atopic dermatitis, chronic mucocutaneous candidiasis, one patient with severe fistulating acne conglobata of the axilla and genito-crural region and a patient with Sweet syndrome.

As can be seen inhibitory activity of serum chemotaxis like that in psoriasis is present in a group of patients with pyoderma gangrenosum, the patient with acne conglobata and the patient with Sweet syndrome. The patients with atopic dermatitis showed slightly increased chemotactic activities. IgA levels paralleled the presence of serum inhibition of chemotaxis, the highest IgA value was found in the acnec patient. Low IgA levels were seen in the atopic group (Table III).

For comparison RCA was determined in 10 patients with chronic arthritis following rheumatic fever and 10 patients with chronic glomerulonephritis (data not shown). In addition, 30 healthy persons served as controls. The results revealed abnormally low RCA in 5 patients with arthritis and one patient with nephritis. IgA levels were not measured except for the controls (164 ± 76 mg/dl).

Effects of Serum Dilution upon PMN Chemotaxis

Serum taken from a patient with long-standing wide-spread psoriasis was serially diluted with TC 199 after heat inactivation and assayed for RCA in the presence of PMA. Starting at a dilution of 1:32 of this patient’s serum the inhibition of PMN migration is gradually lessened (Fig 3). As can be seen on the same graph the control serum exerts inhibitory effects when applied after 1:2 and 1:4 dilution (Fig 3). PMN chemotactic migration is highest when heat inactivated control serum is diluted 1:8 to 1:64 in TC 199 and gradually falls off at further dilution steps.
Further Characterization of the Inhibitory Component(s) Present in Low Responder Sera

Subsequently 10 sera of the low responder group were chromatographed using Sephacryl S-300-column chromatography and the RCA of the eluent fractions were measured. Inhibition of RCA was seen with fraction number 9 to 14 (Fig 4) while the rest revealed no major inhibitory activity. The main inhibitory fractions eluted with an approximate molecular weight of 200,000 Daltons.

As revealed by the Mancini technique IgA was present in fraction number 9 to 13 only with a major peak in fraction number 11 (data not shown). These results indicate, that the inhibitory compound may be IgA.

In addition to sera from patients with chronic stationary psoriasis (low responders of chemotaxis) serum from 4 healthy control persons the patient with acne conglobata as well as a patient with pyoderma gangrenosum were chromatographed in this manner. In all chromatograms except for the controls defined fractions with strong (80-100%) inhibition of chemotaxis were eluted. The inhibitory peaks were of the same molecular weight range (ca.200,000 Daltons).

DISCUSSION

In studies reported recently a highly increased chemotactic activity was noted when PMNs from patients with psoriasis were confronted with autologous sera (fresh as well as heat inactivated) [5]. Since wide variations from patient to patient were seen attempts were made to more closely examine the chemotactic activity of serum under standardized conditions.

These included the use of PMA, a known tumor promotor which recently has been shown to elicit membrane responses in human PMN [12]. White and Estensen demonstrated the selective release of enzymes of the secondary (specific) granules, when the cells were incubated with PMA at a concentration of 10 ng/ml [10]. By adding PMA at concentrations of 1.0 ng and 10 ng/ml to E. coli bacterial filtrate the chemotactic activity increased up to 700% [7]. This potentiation of leukotaxis by PMA is thought to derive from the stimulatory effect of PMA on cGMP levels already induced by the chemoattractant [13].

In the present study the addition of various concentrations of PMA to serum is followed by strong potentiation of RCA (Fig 1). This result confirms the findings of Estensen et al [7] using a different chemoattractant. As shown below the potentiation of RCA allows more precise discrimination between chemoattractant potencies of serum.

Concentrating upon psoriatic sera we now observed that a proportion of patients (approximately 30% of the present patient grouping) shows abnormal chemotactic activities which range from nearly complete absence of migrating cells to greatly increased chemotactic activity. Analysis of the low responder sera as compared to high responders (Table I, II) leads to the notion, that patients with the persistent, stationary form of psoriasis are among the low responders. On the other hand patients with recent onset of psoriasis or recent relapses show high serum chemotactic activity. In addition, the extent of skin
involvement in both groups differs greatly with the low responder group showing considerably more skin affected (50% surface covered) as compared to the high responder group.

Interestingly the inhibitory effect on the migration of normal PMNs was also present in PG, severe acne conglobata and several patients with arthritis. Patients with atopic dermatitis or glomerulonephritis were included in this study as representatives of chronic inflammatory diseases without the predominant involvement of neutrophils. Since no inhibition of RCA was found in the latter diseases, our results indicate, that the inhibition of RCA may be related to tissue alterations in which PMN play a major role.

Furthermore our data suggest a close correlation between the levels of circulating IgA and chemotactic inhibition in psoriasis as well as in other neutrophil related dermatoses (Fig 5). As an example in patients with chronic stationary psoriasis IgA values were increased ranging from 224 to 575 mg/dl (Table II). This is in accordance with the findings of Margheschi and Braun-Falco [14] and more recently Guilhou et al [15] who found elevated IgA levels in generalized psoriasis. As shown (Table I) these patients demonstrate strong inhibition of RCA. In contrast patients with increased RCA (Table II) showed average IgA levels of 134.9 mg/dl.

This correlation between the ability of heat inactivated serum to inhibit the migratory response of normal PMNs and the serum level of IgA, also seen in PG as well as in the patient with acne conglobata and the case of Sweet syndrome (Table III) may be a secondary phenomenon due to a prolonged neutrophil related inflammatory response. Recent studies have shown that isolated IgA paraproteins inhibit neutrophil chemotaxis [11], bactericidal activity [16] and normal serum IgA inhibits phagocytosis of C. albicans [17]. The polymeric (aggregated) forms of IgA appear to be responsible for most of these effects on PMN function [11]. Kemp, Cripps, and Brown observed that isolated IgA from normal human serum inhibits both random as well as chemotactic migration of human PMN [18]. It is felt that the inhibitory activity may be due to the aggregated forms of IgA as produced during the isolation procedure. Native monomeric IgA present in normal serum is found not to inhibit PMN migration [18]. It has been postulated that polyclonal IgA binds to IgA receptors on the PMN [11,19] and that chemotaxis is suppressed by the Fc receptor binding of IgA [16].

The present results indicate IgA-associated inhibition of chemotaxis. This inhibition is shown to be increased in diseases, in which PMNs play a major role. The inhibiting principle consistently showed a molecular weight of approximately 200,000 Daltons, which is close to the mol. weight of IgA. By radial immunodiffusion it was demonstrate that this immunoglobulin was present only in the inhibitory fractions. Therefore it appears that the serum factor responsible for the inhibition of human neutrophil chemotaxis is IgA. Further confirmation will be obtained by affinity chromatography, which is in progress.

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REFERENCES