Acquired Granulocyte Abnormality During Drug Allergic Reactions: Possible Role of Complement Activation

By Timothy K. Bowers, Philip R. Craddock, and Harry S. Jacob

A profound defect in granulocyte chemotaxis was documented in an otherwise healthy 21-yr-old man who failed to localize granulocytes to an area of cellulitis during an allergic reaction to cephalothin. During the period of drug allergy, characterized by urticaria, eosinophilia, and profound hypocomplementemia, in vitro migration of the patient's granulocytes in the Boyden chamber was markedly impaired. Although devoid of hemolytic complement activity, the patient's serum possessed supranormal chemotactic activity, even following heat inactivation, suggesting the presence of chemotactically active complement split products. Chemotactic function improved concomitantly with steroid therapy and normalization of serum complement levels, and was entirely normal following clinical recovery and cessation of steroid therapy. The chemotactic abnormality noted in the patient's cells was reproduced in normal granulocytes by preincubation either with patient serum or with cobra venom-activated fresh (but not heated) normal serum, suggesting that in vivo exposure of granulocytes to activated complement was responsible for the patient's abnormal chemotactic response. This mechanism may contribute to the increased infection propensity noted in other conditions characterized by in vivo complement activation, such as rheumatoid arthritis and systemic lupus erythematosus.

POLYMORPHONUCLEAR NEUTROPHILS perceive and move actively toward increasing concentrations of various substances, including the complement components C3a, C5a, and C567 and certain soluble bacterial products. This active locomotion (chemotaxis) is crucial in localizing infections in that it serves to attract and accumulate phagocytes at infection sites with the resulting generation of pus.

Since 1963, when Boyden introduced his micropore filter technique for the study in vitro of chemotaxis, acquired abnormalities of granulocyte chemotaxis associated with increased incidence of infection have been described in various clinical situations, including hypophosphatemia, diabetes mellitus, and corticosteroid administration. Mowat and Baum studied the chemotactic response in patients with rheumatoid arthritis and found it variably impaired. Intriguingly, chemotactic efficiency closely correlated with serum complement levels; the patients with the lowest complement values had the lowest chemotactic indices.

We present here studies in a patient with an allergic drug reaction and associated transient hypocomplementemia, whose granulocyte chemotactic function was markedly impaired when studied in vitro. An analogous impairment in vivo...
seemed likely in that prolonged cellulitis resisted localization or progression to abscess formation. Following drug withdrawal and corticosteroid therapy, cellulitis rapidly disappeared, while complement levels and the chemotactic index normalized concomitantly.

CASE REPORT

An otherwise healthy 21-yr-old male noted tenderness and swelling in the right anterior cervical triangle associated with sore throat and fever. Oral clindamycin was prescribed, and, when there was no response, he was hospitalized and given intravenous cephalothin. Two days later, an intensely pruritic urticarial eruption involving the entire body developed, and he was transferred to the University of Minnesota Hospitals.

On examination, the patient appeared acutely ill. The blood pressure was 100/60, the pulse 100, and the temperature 38.2°C. An erythematous urticarial rash covered the entire body; the throat was devoid of exudate or erythema. There was a nonfluctuant 5 x 7-cm area of erythema, induration, and tenderness in the right anterior cervical triangle, and several small shotty anterior cervical lymph nodes were palpable. A grade 2/6 nonradiating systolic ejection murmur was audible at the left sternal border. The remainder of the physical examination was normal.

The hemoglobin was 13.1 g/100 ml, hematocrit 40%, and white cell count 16,800 with 90% neutrophils and 7% eosinophils. Total hemolytic complement (CH 50) was undetectable (normal = 30-80 hemolytic units/ml). The ASO titer was less than 100, and the heterophile screening test was negative. The serum histamine level (kindly performed by Dr. Malcolm Blumenthal) was 30 ng/ml. The platelet count was 58,000.

Despite intravenous oxacillin, fever continued to 39°C; 1 day following admission, the neck lesion was surgically explored. The gross findings suggested cellulitis without abscess formation. Gram stains revealed neither organisms nor leukocytes, and cultures were sterile. Lymph nodes removed from the involved area were hyperplastic, but otherwise normal.

All antibiotics were discontinued 5 days following admission, but fever continued. On the seventh day, prednisone, 25 mg every 6 hr, was begun. On the eighth day the CH 50 was normal, and 1 day thereafter the temperature was normal, the rash resolving, and the platelet count rising. Prednisone dosages were tapered beginning on the 13th day, and the CH 50 remained normal thereafter. The patient recovered uneventfully.

MATERIALS AND METHODS

Leukocyte suspensions from the patient and several healthy laboratory personnel with previously confirmed normal chemotactic efficiency were prepared from heparinized venous blood by dextran sedimentation as previously described.8 The leukocyte-rich supernatant fluid was expressed and centrifuged in plastic tubes at 450 g for 5 min. After resuspension of the residual cell buttons in Hank’s balanced salt solution, neutrophils were counted manually in Türk’s solution. Following repeat centrifugation at 450 g for 5 min at room temperature, the leukocytes were re-suspended in Hank’s solution containing human albumin (0.5 g/dl) to a final concentration of 10 x 10⁶ neutrophils per milliliter.

Chemotaxis was measured by a slight modification of Boyden’s method.7 Briefly, into the lower portion of plastic Boyden chambers, the chemotactic factor was instilled, and into the upper, 2 x 10⁶ neutrophils (0.2 ml). A millipore filter of 3.0-μm mean pore size divided the chamber. In each study, duplicate series of chambers were run; one series utilized as the chemotactic factor supernatant fluid from an overnight growth of Escherichia coli, diluted to 2%-5% in Hank’s balanced salt solution containing human albumin (0.5%).8 The other series utilized activated complement components prepared by preincubating fresh normal serum with purified anticomplementary factor of Naja naja cobra venom,9 followed by dilution in Hank’s solution to 0.5%-2.0%. The chemotactic activity of both systems was verified with control leukocytes, which were assayed in parallel with chambers containing patient leukocytes. After loading, the chambers were placed in a humidified incubator for 2 hr at 37°C. The filters were then removed, stained with Ehrlich’s acid alum hematoxylin, dried with ethanol, and clarified with xylene. After mounting under a cover slip, the number of neutrophils migrating to the distal side of the filter was counted by light microscopy in ten high power fields (HPF). Each study was
run with quadruplicate chambers, and the mean cell counts from chambers containing patient leukocytes were expressed as a percentage of the mean counts of quadruplicate chambers with control leukocytes, run in parallel under identical conditions.

The chemotactic activity of the patient's serum (stored frozen at −70°C) was assayed by placing a 1:10 dilution of this serum, either fresh or heated at 56°C for 30 min, in the bottom of the Boyden chamber, and $2 \times 10^6$ normal granulocytes in the top portion. Control chambers were run utilizing a similar dilution of normal serum, fresh or heat inactivated.

The effect of preexposing granulocytes to activated complement or to patient serum was studied utilizing cell suspensions from healthy normal donors. Leukocyte suspensions were preincubated for 30 min at 37°C in various dilutions (0.25%–2.0%) of cobra venom-activated normal serum, or undiluted, fresh patient serum, prior to being washed twice and placed in the top portion of the Boyden chambers. Control leukocytes were similarly treated with equal dilutions of normal serum heated at 56°C for 30 min to inactivate complement prior to treatment with cobra venom, or with fresh undiluted normal serum.

As additional controls, we also measured the chemotactic response of granulocytes from five patients with localized infections, receiving cephalothin (two patients), oxacillin, nafcillin, or penicillin-G (one patient each), with no evidence of drug allergy.

Granulocyte viability as assessed by trypan blue exclusion was in excess of 99% in all studies.

RESULTS

Patient's granulocytes. When initially studied during the period of hypo-complementemia and prior to steroid therapy, the chemotactic response of the patient's cells was markedly impaired (Fig. 1); i.e., when studied with complement chemotactic factors they migrated roughly one-third as well as normal leukocytes and, more strikingly, were almost incapable of response toward E. coli factor (13% of normal). When restudied after 5 days of steroid therapy, when the serum complement had returned to normal, response toward the complement chemotactic factors was in the normal range, and that toward E. coli factor was moderately impaired (63% of normal). When finally studied, 10 days after cessation of steroid therapy, the response toward both chemoattractants was well within the normal range.

Chemotactic activity of patient serum. The chemotactic potency of the patient's serum was markedly increased over that of normal serum, whether fresh [137 ± 33 (mean ± SD) versus 40 ± 4 cells/10HPF] or heated [98 ± 9 versus 27 ± 2 cells/10HPF].

![Fig. 1. Chemotactic response of patient granulocytes. Mean ± SE of quadruplicate determinations. Prior to steroid therapy (day 1), migration toward both complement- (circles) and E. coli–derived (triangles) chemoattractants was markedly impaired. On steroids (day 6), migration toward complement was normal and toward E. coli was moderately impaired. Following recovery (day 32), chemotactic response was entirely normal.](image-url)
Chemotaxis toward:
Activated C' Bacterial Supernatant

Fig. 2. Chemotactic response of normal granulocytes preincubated with cobra venom-activated complement. Each point represents the mean of four determinations on one subject. Chemotaxis toward complement (A) is inhibited by prior incubation of the granulocytes with 0.5% and 2.0% activated serum. Chemotaxis toward bacterial supernatant (B) is inhibited only at the higher level; p values calculated by unpaired t test.

**Chemotactic response of normal granulocytes preexposed to patient serum.** Preincubation of normal granulocytes with patient serum resulted in a marked inhibition of complement-induced chemotaxis [chemotactic response equal to 25±9% (SD) of control granulocytes similarly exposed to normal serum], but no inhibition of bacterial factor induced chemotaxis (response of 160±25% of control).

**Chemotactic response of normal granulocytes preexposed to cobra venom-activated complement.** The effect of preincubation of normal cells with cobra venom-activated normal serum was concentration-dependent (Fig. 2). Exposure to relatively low levels of activated complement (0.5% dilution of cobra venom-activated serum) resulted in a moderate impairment of response toward complement, but an entirely normal response toward *E. coli* factor, while preexposure to higher levels (2.0% dilution of activated serum), led to impairment of response toward both chemotactic factors. Preincubation with serum heated at 56°C for 30 min prior to activation with cobra venom did not affect granulocyte chemotaxis.

Granulocytes from infected patients receiving cephalothin or other penicillin-related antibiotics without allergic reactions migrated normally toward both complement (102±5% of control, range 93%-108%) and bacterial chemotactants (98±10% of control, range 88%-110%).

**DISCUSSION**

This patient's prolonged period of cellulitis with neither resolution nor progression to abscess formation, together with the lack of granulocytes in the lesion, led us to suspect that a defect in chemotaxis was present. Indeed, such a defect could be verified by studies in vitro in Boyden chambers. Moreover,
during the period of impaired chemotaxis, the patient's serum contained a specific inhibitor of complement-induced chemotaxis. We suggest that the likely pathophysiologic sequence of events producing this chemotactic defect was: bacterial pharyngitis and cervical adenitis → allergic reaction to cephalothin, with immune complex activation of complement (and associated urticarial rash, thrombocytopenia, and eosinophilia) → complement-induced granulocyte paralysis.

Several lines of evidence (albeit indirect) support this hypothesis. Because of antibiotic therapy prior to referral, bacterial infection was never proven; however, clinically and surgically, the neck lesion appeared to be an area of cellulitis, but lacked the expected polymorphonuclear infiltrate. Drug allergy, manifest by urticaria and eosinophilia, seemed evident.

The chemotactic response in vitro of the patient's granulocytes during the hypocomplementemic period was clearly abnormal, and recovery of chemotaxis coincided with recovery of serum complement levels.

Acquired, reversible depletion of serum complement could theoretically result either from decreased synthesis (clearly demonstrated only in severe liver disease), or from increased catabolism, i.e., with activation of the complement cascade. That the latter mechanism indeed led to the profound hypocomplementemia of our patient was suggested by the increased chemotactic activity of his serum (even when heated) at a time when the hemolytic activity was markedly depressed. This apparent disparity could be explained by complement activation and consumption with subsequent formation of chemotactically active "split products" (C3a, C5a, C567) that, once formed, were heat stable. Thus, normal serum, activated by zymosan or cobra venom, was devoid of hemolytic activity, but was a potent chemoattractant, even when heated.

Others have shown that preexposure of granulocytes to activated complement impairs migration toward complement chemoattractants. We have confirmed this finding and extended it, showing that exposure to high (but not low) concentrations of activated complement impairs migration toward bacterial chemoattractants as well.

That the inhibitor of chemotaxis present in the patient's serum was, indeed, activated complement was suggested by the specific inhibition of complement-induced chemotaxis. We hypothesize that duration of exposure to activated complement may lead to a differential effect on subsequent chemotaxis analogous to the concentration effect, with brief exposure, or low concentrations, inhibiting only complement-induced chemotaxis, and longer exposure (i.e., in vivo) or higher concentrations inhibiting bacterial factor induced chemotaxis as well.

Several alternative explanations of this patient's transient chemotactic defect must be considered. Infection per se can be excluded, as Hill et al. have shown that patients with active but localized bacterial infections demonstrate supernormal granulocyte chemotaxis. We have studied patients receiving cephalothin and other penicillin-related drugs, and have found a normal chemotactic response, excluding a direct drug effect. A third potential mechanism, phagocytosis by granulocytes of antigen-antibody complexes, is unlikely, since penicillin-antibody complexes are soluble and hence not subject to phagocytosis. Finally,
a histamine effect is virtually excluded by a serum level well below the concentration necessary to inhibit chemotaxis.\textsuperscript{16}

Since exposure in vitro of granulocytes to activated C clearly may inhibit the subsequent chemotactic response to both C-derived\textsuperscript{11,12,13} and bacterially derived (present study) chemoattractants in the Boyden chamber system, it seems plausible that an analogous situation could arise in vivo. We believe that the correlation of chemotactic response and complement levels in patients with rheumatoid arthritis,\textsuperscript{3} the recently described chemotactic defect in patients with exacerbations of systemic lupus erythematosus and hypocomplementemia,\textsuperscript{17} and the chemotactic defect found in our patient, all represent manifestations of this phenomenon.

REFERENCES

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