Neutrophil Chemotaxis in Patients with *Staphylococcus aureus* Furunculosis

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Neutrophil chemotaxis was evaluated in patients with staphylococcal furunculosis using a modified Boyden chamber assay. Neutrophil chemotactic response to *Staphylococcus aureus*-derived chemotactic factor was compared with response to *Escherichia coli*-derived chemotactic factor and zymosan-activated serum. Twenty-one patients with active furunculosis were compared with 29 patients with a history of furunculosis but no recent infection and with 29 healthy control subjects. Chemotactic response to the staphylococcal chemotactic factor was significantly higher in patients with active furunculosis (mean 61.6) than in patients with a history of furunculosis (mean 36.4) or controls (mean 31.4), P < 0.001. Neutrophils from patients with active staphylococcal infections also had higher chemotactic activity toward *E. coli* chemotactic factor, but not significantly so (P = 0.09). Chemotactic response to zymosan-activated serum and background neutrophil motility was comparable among the three groups. The increased neutrophil chemotactic response of patients with active infection to bacterial factors, but not zymosan-activated serum, may represent a specific neutrophil response to products of infecting organisms. The differential response of the patients' neutrophils to these attractants supports evidence for the presence of separate categories of chemotaxin receptor on the surface of neutrophils.

Most normal individuals are colonized with *Staphylococcus aureus* at one time or another, but relatively few develop staphylococcal infections. The propensity for certain individuals to develop recurrent staphylococcal furunculosis is not fully understood. Factors such as inoculum size, virulence of the organism, and integrity of skin and mucosal barriers may be important in some instances, but as in any type of recurrent infection, an abnormality of the immune response must be suspected. Since recurrent staphylococcal infection has been associated with decreased neutrophil chemotaxis in such disorders as the hyperimmunoglobulinemia E and Chediak-Higashi syndromes, a prospective study of neutrophil chemotaxis was performed in patients with recurrent staphylococcal furunculosis (2, 5, 10, 12).

A *S. aureus*-derived chemotactic factor was employed as a chemotaxin in this study since *S. aureus* was the primary infecting agent in these patients. In addition, two standard attractants, *Escherichia coli* chemotactic factor and zymosan-activated serum, were used.

MATERIALS AND METHODS

Patient selection. All patients included in the study had recurrent staphylococcal furunculosis as defined by three or more episodes of purulent lesions involving skin and subcutaneous tissue that were greater than 1 cm in diameter and culture positive for *S. aureus*. Patients were divided into two study groups based on the presence or absence of infection near the time of evaluation. The first group consisted of 21 patients with active staphylococcal furunculosis within 2 weeks of study. The second group consisted of 29 patients with a history of recurrent staphylococcal furunculosis but no infection within 4 weeks of study. Three patients were studied longitudinally, both with and without infection, and therefore appear in both groups. Eight of the patients with active furunculosis had known underlying diseases. Six had hyperimmunoglobulinemia E syndrome, one had chronic granulomatous disease, and one had Wiskott-Aldrich syndrome. Six patients with past furunculosis had underlying diseases. Three had hyperimmunoglobulinemia E syndrome, two had chronic granulomatous disease, and one had Wiskott-Aldrich syndrome. The rest of the patients in the two groups had no known underlying disease which might account for their recurrent staphylococcal infections. Controls consisted of 29 healthy laboratory personnel.

Leukocyte preparation. Leukocytes were prepared by dextran sedimentation of heparinized venous blood (10 U of heparin per ml of whole blood) using methods previously described (3). After washing, leukocytes were suspended at a concentration of 2 × 10⁶ polymorphonuclear leukocytes per ml in Hanks balanced salt solution with 10% autologous plasma.

Chemotactic factor preparation. Bacterial chemotactic factors were prepared from *S. aureus*...
502A and a laboratory strain of *E. coli* provided by Peter Ward. Cultures were maintained on nutrient agar at 4°C. One loopful of bacteria was inoculated into 100 ml of tissue culture medium 199 (Microbiological Associates). After incubation at 37°C for 24 h, the suspension was centrifuged and the supernatant was filtered. Aliquots were stored at −70°C. The *S. aureus* supernatant was diluted 1:1 in Hanks balanced salt solution, and the *E. coli* supernatant was diluted 1:20 in Hanks balanced salt solution for use as chemotactants.

Zymosan-activated serum was prepared as previously described (3). The activated serum was diluted 1:10 for use as a chemotactrant.

**Neutrophil chemotaxis assay.** Neutrophil chemotaxis was measured using a previously described modification of the Boyden chamber technique with membrane filters (5 μm pore size; Millipore Corp.) (3). Briefly, 0.4 ml of the leukocyte suspension was added to the upper compartment of a modified Boyden chamber (Neuroprobe, Inc.). The chemotaxin was added to the lower compartment in the concentrations stated above. Hanks balanced salt solution alone was used in the lower compartment to assess background neutrophil motility. After 2 h of incubation at 37°C, filters were removed from the chambers, fixed, stained, dehydrated, and mounted. Chemotactic activity was quantitated by determining the number of neutrophils adhering to the chemotactrant side of the filter inside a grid (5 by 5 mm) in 10 randomly selected high-power fields. Results represent the average of triplicate determinations.

**RESULTS**

The neutrophil chemotactic response to the staphyloccocal-derived chemotactic factor is shown in Fig. 1. Chemotactic response to the staphyloccocal chemotactic factor was significantly higher in patients with active staphyloccocal infection within 2 weeks of study (mean 61.6) than in patients with past infections (mean 36.4) and control subjects (mean 31.4; *P* < 0.001). Patients with active staphyloccocal infection also had higher chemotactic response to the *E. coli*-derived factor than the other two groups (mean 95.0, versus 75.5 for patients with past infection and 72.1 for controls), but this difference is not statistically significant (*P* = 0.09) (Fig. 2).

In contrast, there was no difference in chemotactic response to zymosan-activated serum. Mean chemotactic activity in patients with active infection was comparable to that of controls and patients with a history of infections. The means were 114.4, 116.9, and 92.0, respectively (Fig. 3). There was no significant difference in background neutrophil motility among the three groups (data not shown).

When the chemotactic results were analyzed according to age, sex, antibiotic use, and underlying disease, no significant difference in mean values was found. One of the patients with the hyperimmunoglobulinemia E syndrome and active staphyloccocal infection had a pronounced defect to all chemotaxins, but the rest of the patients with this syndrome had chemotactic activity comparable to that of others in their respective groups.

Three patients were studied both during active staphyloccocal infection and at least 4 weeks after infection. In each of the three patients, chemotactic activity toward the *S. aureus* chemotactic factor was elevated during active staphyloccocal infection but returned to normal upon resolution of the infection (Fig. 4).

Neutrophil chemotaxis has been evaluated in patients with acute "non-staphyloccocal" infections, primarily *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Mean chemotactic response to the staphyloccocal factor (29.7, range 10 to 43) was comparable to that of controls (31.4) and patients with past staphyloccocal infections (36.4), but was lower than patients
with active staphylococcal furunculosis (61.6). There was no difference in chemotaxis toward the E. coli factor of zymosan-activated serum between this group of patients and patients with staphylococcal infections.

To determine if the increased response of neutrophils to the staphylococcal factor was due to the presence of a plasma factor, washed control neutrophils were preincubated (15 min, 37°C) with plasma from patients with a hyperactive response to this chemotxin. The resulting chemotactic activity to the S. aureus-derived factor was comparable to that obtained when control cells were suspended in control plasma (data now shown). This suggests that the hyperactive patients chemotactic response to the staphylococcal factor was a cellular phenomenon and was not plasma mediated.

**DISCUSSION**

Patients with active staphylococcal furunculosis were found to have increased neutrophil chemotactic activity toward bacteria-derived chemotaxins but normal chemotactic activity toward zymosan-activated serum. Patients with a past history of staphylococcal furunculosis but no recent infection and patients with active non-staphylococcal infections also had normal neutrophil chemotaxis to all attractants.

Hill et al. reported increased chemotactic activity to E. coli chemotactic factor in 25 patients with active bacterial infections due to several different bacterial species (9). The hyperresponsiveness in these patients, as in ours, was due to a cellular neutrophil phenomenon, i.e., was not plasma mediated, and resolution of infection correlated with return of chemotaxis to normal. Although the increase in chemotactic activity to the E. coli factor in the present study was not statistically significant, there was a more pronounced hyperresponsiveness to the staphylococcal factor. This finding in patients with active staphylococcal disease may reflect some specificity of the neutrophil response for infecting organisms. That this difference in response may be a consequence of antimicrobial therapy is unlikely since penicillin was the antimicrobial agent used in the majority of patients and penicillins have not been shown to affect chemotactic responsiveness of human neutrophils in our lab.

**FIG. 2. Neutrophil chemotactic response to E. coli-derived chemotactic factor (mean ± standard error).**

**FIG. 3. Neutrophil chemotactic response to zymosan-activated serum (mean ± standard error).**
with ylococcal-derived chemotactic makes leukocytes (11, 13, 16, 17).

Furthermore, neutrophil chemotaxis was not increased in patients with active non-staphylococcal infections.

The wide range of neutrophil chemotaxis in controls and patient groups to the three chemotactants makes it difficult to draw firm conclusions; however, the increased activity in patients with active staphylococcal infection toward the staphylococcal factor is statistically significant.

Products from several species of bacteria have been demonstrated to be active chemotaxis for leukocytes (11, 13, 16, 17). In addition to chemotaxis, bacteria can also produce chemotaxigenic substances which activate serum causing the release of chemotaxins (6, 7, 8, 15). Studies have shown that crude suspensions of S. aureus, purified staphylococcal cell wall preparations, peptidoglycan, protein A, and enterotoxin B are chemotaxigenic (6, 7, 8, 14a, 15). Experiments using heated serum and anti-C5a suggest that the active chemotaxin produced by these staphylococcal products is C5a (14a). The increased chemotactic response seen in patients with active S. aureus infection was toward a staphylococcal chemotaxin rather than zymo-

san-activated serum which contains the same kind of product, C5a, produced by staphylococcal chemotaxigenes.

In separate studies, Aswanikumar et al. and Chenoweth et al. have shown that the receptor on the neutrophil surface for chemotaxins related to the E. coli chemotactic factor is distinct from that for C5a (1, 4, 14). Our finding an increase in chemotactic response to one factor, that derived from S. aureus, but not to zymosan-activated serum in a group of patients is consistent with the presence of separate receptors on the surface of the neutrophil for different attractants. Furthermore, it suggests that clinical circumstances may dictate which neutrophil receptor is activated.

It is tempting to postulate that hyperactive neutrophil response to the staphylococcal chemotactic factor may be responsible for the large pus-filled lesions in patients with active staphylococcal infection. It is possible, however, that it represents the normal specific host response to bacterial invasion.

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LITERATURE CITED

1008 CATES AND QUIE


