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Human Neutrophil Chemotactic Response to Group A Streptococci: Bacteria-Mediated Interference with Complement-Derived Chemotactic Factors

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The influence of M protein on the capacity of group A streptococci to generate neutrophil chemotactic activity in normal human serum was examined. Incubation of serum with M^- bacteria for up to 10 min led to the production of chemotactic activity. In contrast, incubation of serum with M^+ bacteria did not elicit serum chemotactic activity over a 1-h period, even though complement was activated to completion. Further experiments revealed that both M^+ and M^- bacteria could inhibit the chemotactic activity of serum preexposed to zymosan. However, the M^+ bacteria possessed a 130-fold-greater inhibitory capacity in this regard than the M^- bacteria. This antichemotactic property was not detectable in the fluid phase of serum incubated with bacteria, thereby ruling out neutrophil-directed effects. Treatment of the bacteria with trypsin resulted in the release of the inhibitory molecule, suggesting that proteins are involved in its maintenance at the cell surface. However, the resistance of the chemotactic factor inactivator to pepsin and trypsin indicated that the protease-sensitive M protein was not involved. These results demonstrate a heretofore uncharacterized activity of group A streptococci that may contribute to virulence through modulation of the host chemotactic response.

The M protein of group A streptococci has long been associated with virulence in these organisms. Although its presence on the cell surface correlates with the ability of the bacterium to resist the host phagocytic defenses (13), the precise molecular mechanisms by which this ability is conferred remain unresolved. Recent studies have indicated that M^+ bacteria activate the alternate complement pathway less effectively than their M^- counterparts (4). Furthermore, M^+ cells are not opsonized effectively by complement-dependent means (19). This opsonic blockade is not, however, a direct result of lower levels of complement deposition since comparable amounts of surface-bound C3b stimulate only the phagocytosis of the M^- bacteria (9a).

Several pathogenic bacterial and fungal species activate the alternate complement pathway with the concomitant generation of serum chemotactic activity (14, 20, 22). The chemotactic response is considered to be responsible for the accumulation of phagocytic cells at sites of infection (26) and thus provides an important defense mechanism for the early elimination of invading microorganisms. Any means that a pathogen might have for preventing or depressing the chemotactic response would therefore contribute significantly to the virulence of the organism.

We investigated the hypothesis that a lower rate of complement activation by M^+ streptococci would be reflected in the subeffective generation of chemotactic stimuli. The data presented in this report show that M^+ and M^- bacteria differ quantitatively in their effect upon the appearance of complement-derived chemotactic activity. This difference is not related to different kinetics of complement activation; rather, it is ascribable to the concurrent inactivation of the generated chemotaxins.

MATERIALS AND METHODS

Bacterial cells. Strain S43, an M^+ type 6 group A streptococcus, and its M^- variant strain, S43G, are from the strain collection of the late Rebecca Lancefield, Rockefeller University, New York. For all experiments bacteria were grown to the exponential phase in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) supplemented with 2% neopeptone (Difco) and harvested by centrifugation at 13,000 × *g* for 10 min in the cold. The cells were washed twice in Hanks balanced salt solution containing 0.1% gelatin before use. Cell numbers were determined by optical density measurements. Bacteria suspended in phosphate-buffered saline (pH 7.4) at 5×10^8 cells per ml were killed by a 3-min exposure to irradiation from a

mercury vapor lamp (1,600 erg/s per cm^2). Under these conditions bacterial viability was reduced by approximately five orders of magnitude.

Enzymatic treatments. Trypsin and hyaluronidase digestions were performed essentially as described previously (2). Bacteria killed by UV light were incubated at 10^9 cells per ml in 0.067 M phosphate buffer (pH 8.0) containing 1.0 or 0.1 mg of trypsin (Sigma Chemical Co., St. Louis, Mo.) per ml or in phosphate-buffered saline (pH 7.4) containing 24 U of hyaluronidase (Sigma) per ml. Pronase treatment was in 0.067 M phosphate (pH 7.4) containing 1 mg of pronase (Calbiochem-Behring Corp., La Jolla, Calif.) per ml, and pepsin treatment was carried out in 0.067 M phosphate (pH 5.8) with 1 mg of pepsin (Sigma) per ml. Positive controls consisted of bacteria incubated in each buffer in the absence of enzyme. All incubations were performed at 37°C, after which the organisms were chilled on ice and washed twice in Hanks balanced salt solution–1% gelatin. Alternatively, soybean trypsin inhibitor (Sigma) was added in twofold excess to trypsin-digested cells before washing.

Leukocyte preparation. Leukocytes were collected by a standard procedure (8). Heparinized blood drawn from a healthy volunteer was mixed with 3.5% dextran (molecular weight, 200,000 to 300,000) (GIBCO Laboratories, Grand Island, N.Y.) in saline (3 ml per 10 ml of blood) and gravity sedimented at room temperature. The leukocyte-enriched plasma was collected and centrifuged at 220 × *g*. Contaminating erythrocytes were removed by hypotonic lysis in 0.87% NH_4Cl. Leukocytes were washed twice in Hanks balanced salt solution–0.1% gelatin and suspended for use at 3.5×10^7 polymorphonuclear leukocytes (PMNs) per ml in the chemotaxis assay medium.

Total complement assay. Hemolytic complement was determined by the method of Mayer (15). Sheep erythrocytes (Wilfer Laboratories, Stillwater, Minn.) were sensitized with an optimal amount of anti-sheep hemolysin (Cappel Laboratories, Downingtown, Pa.) (1/600 dilution). These cells were washed and suspended to a density of 5×10^8 cells per ml in gelatin veronal buffer containing Ca^{2+} and Mg^{2+}. Dilutions of test sera were mixed with the sensitized sheep erythrocytes, which were then incubated at 37°C for 60 min; 50% hemolytic complement values were calculated from the change in optical density of the supernatants.

Migration assays. An underagarose assay system for the measurement of chemotaxis as described by Nelson et al. (18) with modifications introduced by Chenoweth (6) and subsequently by Nelson et al. (16) was utilized for these studies. Each petri dish (60 by 15 mm) was pretreated with 1 ml of 0.5% gelatin (Difco) for 60 min at room temperature and then rinsed once with distilled water. After being dried, each dish was filled with 6 ml of an agarose mixture containing 1% agarose (Seakem Agarose; Marine Colloids, Rockland, Maine) and 0.5% gelatin in Eagle minimal essential medium (GIBCO Laboratories) buffered to pH 7.4 with 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (Sigma). Zymosan-activated serum (ZAS) was incubated in triplicate wells for 45 min at 37°C before the addition of 3.5×10^5 PMNs to each PMN well. Migrations were allowed to proceed in a moist atmosphere for 1.5 to 2.0 h at 37°C, after which the dishes were fixed overnight with 2.5% glutaraldehyde at 4°C. After development with Wright stain, the dishes were examined by projection upon a centimeter scale grid at 40× magnification. Migration was assessed in terms of the leading front, which was arbitrarily designated as the farthest tip of at least three cells, delimiting a continuous density of migrating cells. All values for directed migration are expressed in terms of the chemotactic differential, which is defined as the measured difference between directed migration and spontaneous migration values. The spontaneous migration controls consisted of heat-inactivated serum (56°C for 30 min) or buffer treated identically to the test samples. Data points represent the mean of triplicate determinations for a single experiment, and all experiments were performed at least twice.

Chemoattractants. For the preparation of ZAS, 1 volume of zymosan (Sigma) at 50 mg/ml in phosphate-buffered saline (pH 7.4) was added to 3 volumes of normal human serum. The mixture was rotated at 37°C for 30 min, whereupon the zymosan was removed by centrifugation at 15,000 × *g* for 2 min. The ZAS was then heated at 56°C for 30 min.

RESULTS

Bacteria-mediated generation of serum chemotactic activity and complement consumption. Microbial contact with serum has been demonstrated to cause the appearance of chemotactic activity in several systems, and evidence has indicated that this is a consequence of alternate complement pathway activation (3, 20, 22). Although group A streptococci possess complement-consuming capability (4), the potential of intact cells to produce serum-derived chemotactic activity has not been explored. To obtain information on this, M^+ and M^- bacteria were added to human serum lacking type-specific antibody to a density of 5×10^9 cells per ml, and the mixture was rotated at 37°C. Samples removed at different times were centrifuged at 15,000 rpm for 5 min at 4°C to remove bacteria, and the serum supernatants were subsequently heated at 56°C for 30 min to inactivate the residual potential for further complement consumption by the agarose in the assay system (21). M^+ cells were ineffective at generating chemotactic activity even after 60 min of incubation (Fig. 1). M^- cells, on the other hand, effectively induced chemotactic activity over the first 10 min, but longer incubation periods resulted in the loss of chemotactic activity.

The initial differences seen in the generation of chemotactic activity might have been the result of differential rates of complement consumption by M^+ and M^- bacteria (4). This possibility was examined by determining the total hemolytic complement activity for these same samples. The rate of complement consumption by M^+ cells was significantly lower than that by M^- cells, although by 1 h complete consumption had occurred in both cases (Fig. 2A and B). Whereas serum incubated with M^+

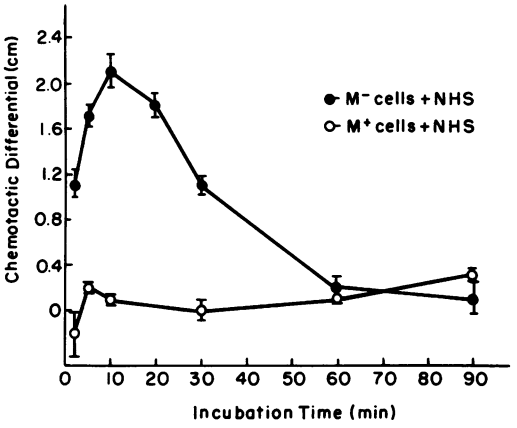

FIG. 1. Generation of chemotactic activity by M type 6 group A streptocci in normal human serum (NHS). M^+ or M^- bacteria (5.0×10^9 cells per ml) irradiated with UV light were incubated in serum at 37°C. Samples were removed at intervals, chilled to 4°C, and centrifuged at 15,000 × *g* for 5 min to remove cells. The supernatants were subsequently heated at 56°C for 30 min. Spontaneous-migration controls consisted of heat-inactivated serum treated in an identical manner. Data points represent the mean of triplicate determinations.

cells showed no chemotactic activity, serum incubated with M^- cells rapidly accumulated activity in parallel with the consumption of complement; this activity began to decay after 10 min and gradually disappeared over 60 min. In contrast, the chemotactic activity of serum incubated with zymosan (2×10^9 particles per ml) remained stable over the 30-min period during which the M^- bacteria demonstrated their effect (Fig. 2C).

Kinetics and dose-response relationships for the inhibition of ZAS chemotactic activity by intact bacteria. To examine the characteristics of the streptococcus-mediated abrogation of complement-derived chemotactic activity, M^+ amd M^- bacteria were tested for their effect upon serum chemotaxins pregenerated with zymosan. At high bacterial concentrations (5×10^9 cells per ml) ZAS chemotactic activity was largely lost within 5 to 10 min of incubation with either M^+ or M^- cells (Fig. 3A). The loss of chemotactic activity proceeded considerably faster with M^+ cells since there was an 80% inhibition by 2 min compared with a 40% inhibition for M^- cells. This difference was even more pronounced when ZAS was incubated with low bacterial concentrations (5×10^7 cells per ml); M^+ cells still destroyed chemotactic activity (80% inhibition by 10 min), whereas M^- cells were completely ineffective (Fig. 3B). ZAS incubated in the absence of bacteria retained full chemotactic activity for at least 60 min (Fig. 3), precluding the influence of spontaneous degradation of chemotactic activity in the responses observed with bacteria. A more quantitative comparison of the activity of these bacteria was accomplished by incubating ZAS with various concentrations of bacteria for 10 min. The results of this experiment (Fig. 4) demonstrate that the amount of ZAS inactivation was proportional to the bacterial dose. The 50% effective dose for inhibition, which is defined as the concentration of bacteria able to inhibit 50% of the control chemotactic activity, was used as a standard for comparison. By this criterion, we determined that M^+ bacteria were 130-fold more effective than M^- bacteria in inhibiting the chemotactic activity of ZAS.

Serum-derived chemotaxins as the target of the streptococcal inhibitor. The possibility was in-

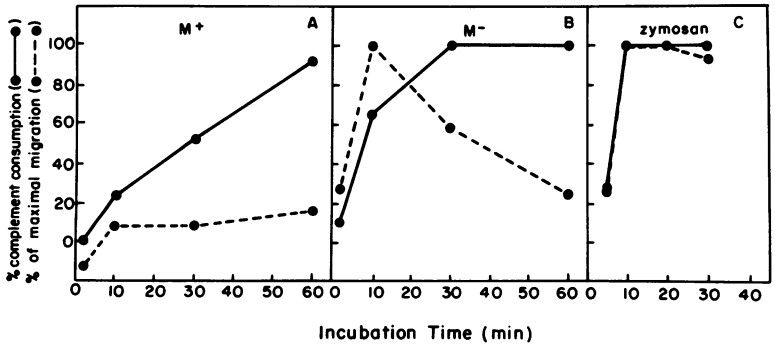

FIG. 2. Kinetics of complement consumption in normal human serum by M^+ (A) and M^- (B) bacteria (5×10^9 cells per ml). The 50% hemolytic component value for each sample is expressed as 100 minus the percent residual hemolytic activity, and each is compared with the percent of maximal migration determined for the same sample. Maximal migration refers to the peak migration occurring at 10 min of incubation. Migration data were obtained in triplicate, and 50% hemolytic complement values were from a single determination. The zymosan control determination (C) was performed in a separate experiment.

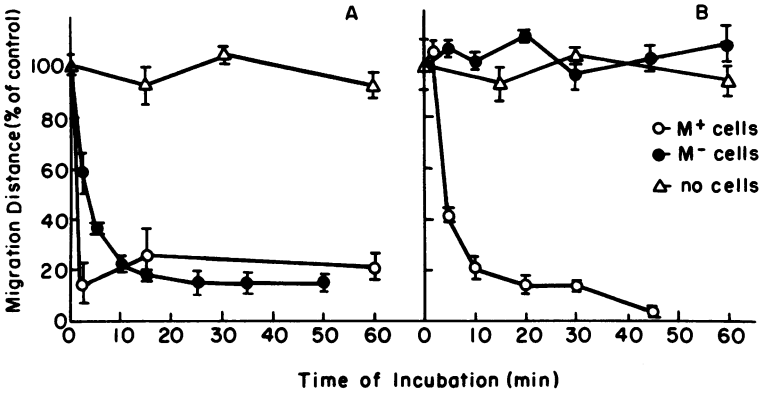

FIG. 3. Time course for the inhibition of ZAS chemotactic activity by M^+ and M^- bacteria. Bacteria were incubated in ZAS at either 5.0×10^9 cells per ml (A) or 5.0×10^7 cells per ml (B) at 37°C. Values for migration are expressed as a percentage of the control sample (no bacteria added). Data points represent the mean of triplicate determinations.

vestigated that the bacteria released an inhibitory activity into serum and that this soluble factor might act directly upon leukocytes alone to alter their responsiveness to a chemotactic gradient. To test this possibility, M^+ bacteria were incubated in heat-inactivated serum or ZAS at 5×10^9 cells per ml for 1 h at 37°C. Cells were removed by two centrifugations, and the serum supernatants were tested for inhibitory activity either by incubation with ZAS (Table 1) or by direct addition to the PMN well of the chemotaxis assay system (Table 2). Incubation of ZAS with heat-inactivated serum treated with M^+ bacteria had no effect upon the ability of the ZAS to induce a PMN directed migratory response (Table 1). Similarly, ZAS incubated with chemotactically inactive bacteria-treated ZAS was not significantly affected, suggesting that products of activated complement or other serum enzymatic systems are not involved in the disruption of chemotactic activity. These results indicate that soluble factors appearing in bacteria-treated sera do not contribute to chemotactic-factor inactivation and that these sera, when placed in the chemotactic factor well, do not inhibit the ability of PMNs to respond directionally (i.e., by diffusion to the PMN well). The effect of placing bacteria-treated sera directly into the PMN well is shown in Table 2. Neither heat-inactivated serum nor ZAS treated with M^+ bacteria was able to suppress the directed migratory response. As expected, the addition of untreated ZAS to the PMN well completely abrogated a chemotactic response to the same

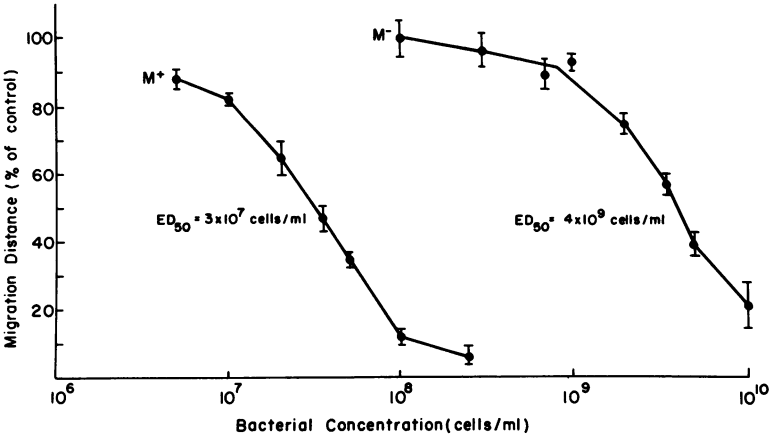

FIG. 4. Dose-response curves for the inhibition of ZAS chemotactic activity by M^+ and M^- bacteria. Bacteria were incubated in ZAS for 10 min at 37°C. Data points represent the mean of triplicate determinations. ED_{50}, 50% effective dose for inhibition.

TABLE 1. Effect of bacteria-treated sera[a] upon the chemotactic activity of ZAS

Addition to ZAS[b]	Directed migration (cm)[c]	% of control migration[d]
M^+ bacteria-treated, heat-inactivated serum	3.7 ± 0.2	106 ± 6
Heat-inactivated serum	3.5 ± 0.1	100 ± 3
M^+ bacteria-treated ZAS	3.0 ± 0.3	86 ± 9
M^+ bacteria	0.3 ± 0.3	9 ± 9

[a] Bacteria-treated sera were prepared by incubating M^+ streptococci at 5.0×10^9 cells per ml in either heat-inactivated serum or ZAS at 37°C for 60 min. This suspension was then centrifuged twice at 13,000 × *g* to remove bacterial cells.

[b] ZAS was incubated with an equal volume of bacteria-treated serum at 37°C for 60 min, and the mixture was then heated at 56°C for 30 min.

[c] Values for directed migration are expressed in terms of the chemotactic differential (chemotactic distance minus spontaneous distance). Each value represents the triplicate mean ± standard error of the mean.

[d] ZAS was diluted 1:1 with heat-inactivated serum was the control.

chemotaxin; this occurred through gradient abolition and probably also through deactivation of responsiveness (17). The results described above are consistent with the conclusion that the bacterial factor somehow alters serum chemotaxins rather than affects the responsiveness of PMNs.

TABLE 2. Effect of bacteria-treated sera[a] upon the chemotactic responsiveness of PMNs

Addition to PMN well[b]	Directed migration to ZAS (cm)[c]	% of control migration[d]
M^+ bacteria-treated, heat-inactivated serum	3.4 ± 0.1	106 ± 3
Heat-inactivated serum	3.2 ± 0.1	100 ± 3
M^+ bacteria-treated ZAS	3.0 ± 0.2	94 ± 6
ZAS	0.1 ± 0.1	3 ± 3

[a] Bacteria-treated sera were prepared by incubating M^+ streptococci at 5.0×10^9 cells per ml in either heat-inactivated serum or ZAS at 37°C for 60 min. This suspension was then centrifuged twice at 13,000 × *g* to remove bacterial cells.

[b] Five microliters of each test serum was added to five microliters of leukocyte suspension (7.0×10^7 PMNs per ml) before addition to the PMN well of the assay system.

[c] Values for directed migration are expressed in terms of the chemotactic differential (chemotactic distance minus spontaneous distance). Each value represents the triplicate mean ± standard error of the mean.

[d] Heat-inactivated serum added to the leukocyte suspension was the control.

TABLE 3. Proteolytic susceptibility of the cell-bound chemotactic factor inactivator activity

Treatment of bacteria[a]	Directed migration (cm)[b]	% Inhibitory activity[c]
Hyaluronidase	0.4 ± 0.2	89 ± 6
Trypsin	2.8 ± 0.2	15 ± 6
Pronase	2.9 ± 0.2	12 ± 6
Pepsin	0.9 ± 0.3	73 ± 9
Buffers[d]	0.5 ± 0.2	86 ± 6

[a] Bacterial cells at 10^9 cells per ml were killed by UV light, incubated for 20 min at 37°C in buffers containing the respective enzymes, and washed thoroughly at 4°C before being mixed with ZAS. Hyaluronidase was at 240 U/ml; trypsin, pronase, and pepsin were each at 1.0 mg/ml.

[b] Expressed as the chemotactic differential (chemotactic distance minus spontaneous distance) for ZAS incubated with 10^8 treated bacterial cells per ml for 30 min at 37°C. Each value represents the triplicate mean ± standard error of the mean.

[c] Expressed as 100 minus the percentage of control migration (ZAS without added bacteria).

[d] Since none of the different buffers used significantly altered the inhibitory activity of the bacteria, the value for directed migration represents the average value for these controls.

Proteolytic sensitivity of the streptococcal chemotactic factor inactivator. We undertook to determine (i) whether the chemotactic factor inactivator is located on the outer surface of the organism and (ii) whether this factor can be degraded or otherwise dissociated from the bacterium through the action of proteolytic enzymes. The M^+ bacteria treated with hyaluronidase retained their ability to inhibit the chemotactic activity of ZAS, precluding the role of capsular material (Table 3). Both trypsin and pronase, on the other hand, effectively removed the inhibitory capacity from these bacteria, causing a loss of 85 and 88%, respectively, of this capacity. The inhibitory activity of M^- bacteria was similarly susceptible to trypsin (data not shown). Because these proteolytic enzymes would not be expected to penetrate the cell membrane, we conclude that the chemotactic factor inactivator is exposed at the cell surface. This experiment did not distinguish between the proteolytic destruction of inactivator activity and its release from the cell surface. To answer this question the appearance of chemotactic factor inhibitory activity in the supernatants of trypsin-digested M^+ bacteria was examined. Figure 5 demonstrates a time-dependent accumulation of supernatant activity that initially corresponds with the disappearance of cell-associated inhibitory activity. In the absence of trypsin these changes did not occur. This result indicates that the functional activity of the che-

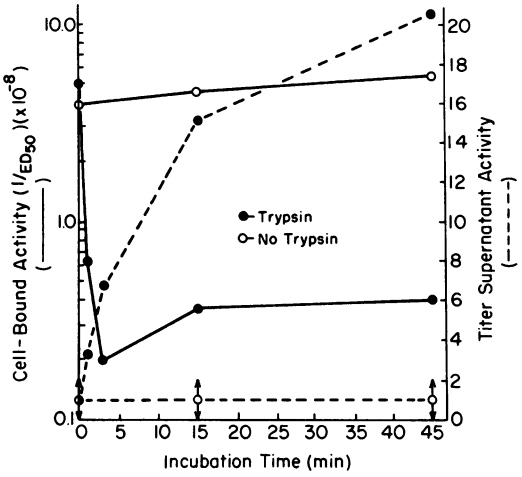

FIG. 5. Relationship between cell-bound and supernatant ZAS chemotactic factor inhibitory activity as a function of incubation time in the presence of trypsin. Living M^+ bacteria (10^{10} cells per ml) were incubated at 37°C with trypsin (0.1 mg/ml), and trypsin inhibitor was then added at various times. Samples were cooled to 0°C and centrifuged. The supernatants were collected for analysis, and the bacteria were washed twice in Hanks balanced salt solution–0.1% gelatin before use. Cell-associated activity is expressed as the reciprocal of the 50% effective dose for inhibition (ED_{50}). Values for supernatant activity represent the reciprocal of the dilution (in phosphate-buffered saline [pH 7.4]) which inhibited 50% of the ZAS chemotactic activity. Supernatant dilutions were assayed by incubation with an equal volume of ZAS for 30 min at 37°C followed directly by addition to the chemotaxis assay system.

motactic factor inactivator is unaffected by exposure to trypsin and suggests that release from the cell is brought about by cleavage of protein moieties that link the inactivator to the cell surface. The prolonged increase in supernatant activity that occurred without a further decrease in cell-bound activity might be due to continued production and secretion of inactivator or to the proteolytic activation of the molecule. In comparison with the effects of trypsin and pronase, pepsin was relatively ineffective in the release of chemotactic factor inhibitory activity (27%). This is in marked contrast with the potential of pepsin to release the M protein from streptococcal cells (2), suggesting that the M protein is not directly involved in the manifestation of inhibitory activity.

DISCUSSION

Although it has become axiomatic that M protein imparts to group A streptococci the ability to avoid phagocytosis, the contribution of other effects on phagocyte-mediated responses to streptococcal infection has received little attention. The mechanism by which phagocytic cells accumulate at the site of infection appears to be due to the ability of these cells to recognize and migrate directionally in response to gradients of specific chemotactic factors (26). Such chemotactic substances can derive from the pathogen itself or be produced as a consequence of contact of the invading organism with host tissues to activate the relevant pathways; these include the complement, coagulation, fibrinolytic, and kinin-forming pathways as well as the lipoxygenase transformations of arachidonic acid (10). Current evidence indicates that the major chemotactic factor of serum activated by the alternate complement pathway are the C5 cleavage products, C5a and $C5a_{des\ arg}$ (7). Interference with the formation or functional activity of these factors would provide a pathogen with a means of circumventing the host primary phagocytic defenses.

The original impetus for this study was to examine whether the relative inability of M^+ streptococci to activate complement (4) would be accompanied by a lower rate of appearance of chemotactic activity. This point, however, could not be resolved because of the discovery that these bacteria possess the capability of inactivating the chemotactic factors that have been generated. Indeed, the genetic expression of M protein was associated with a 130-fold-greater ability to inhibit the chemotactic activity of ZAS under standard conditions. It is noteworthy that at high cell densities M^- bacteria exhibit a similar inhibitory activity, the kinetics of which closely resemble that due to M^+ bacteria at low cell densities.

It was important to determine whether bacterial cell wall-bound factors or fluid-phase factors, the latter either released from the bacteria or generated by contact of bacterial constituents with serum, were responsible for the inhibition of chemotaxis. Such factors might either act upon the chemotaxins themselves or exert an effect directly upon the PMNs by diffusion through the gel matrix of the assay system. This question was addressed by testing heat-inactivated serum or ZAS that had undergone prior incubation with M^+ bacteria for the ability to cause chemotactic inhibition when included with either the chemotaxin (ZAS) or the leukocyte preparation. Since chemotaxis proceeded normally under either condition, we concluded that there was no contribution of soluble factors. This indicated that the target of chemotactic inhibition was the chemoattractant since the bacteria were never in direct contact with the PMNs. This conclusion was supported by the inability of bacteria-treated ZAS to inhibit migration when placed in the PMN well, where one

criterion for the functional presence of chemotaxin is the effectuation of this inhibition.

Although chemotactic factor inactivation was correlated with the expression of M protein, there is currently no direct evidence to suggest that this protein is itself a necessary element. Several factors are coproduced with M protein, including the M-associated protein (25) and the serum opacity factor (24); both are trypsin and pepsin sensitive (9, 25) and copurify with M protein obtained from Lancefield hot-acid extracts. Likewise, other unidentified cell wall components could distinguish M^+ and M^- cells. The loss of inhibitory activity from the cell after trypsin or pronase treatment indicates a participatory role for accessible cell surface proteins. Pepsin, on the other hand, was unable to effect such a removal. Since pepsin, but not trypsin, leaves the exterior fimbrial projections intact (2), it is likely that the activity is mediated by structures associated with the fimbriae. The chemotactic factor inactivator was trypsin resistant, appearing in active form in supernatants derived from the exposure of the bacteria to this enzyme. This result suggests that the inhibitory molecules are maintained in a cell-bound state through binding proteins (1). Alternatively, it is possible that a trypsin-labile domain of the inactivator molecule that is nonessential for functional activity may be responsible for attachment to the bacterium. The failure of pepsin to remove chemotactic factor inhibitory activity under conditions known to favor the removal of type-specific M protein (2) suggests that the inactivator is structurally independent of the M protein. In support of this conclusion is the relative resistance of the inactivator to trypsin degradation as compared with the highly trypsin-sensitive M protein (12). Moreover, preliminary experiments have failed to demonstrate the modulation of inhibitory function by type-specific antisera.

The mechanism by which chemotactic factor inactivation occurs has not been elucidated. One model holds that the proteins are enzymatically degraded or otherwise functionally altered. Precedents for this have mainly been in the context of fluid-phase mediators of host self-regulation; chemotactic factor inactivators have been isolated from normal human serum (3, 11), and neutral proteases from neutrophil azurophilic granules have been shown to degrade C5a (5, 27). An alternative explanation is that the chemotaxins are passively adsorbed to structures on the bacterial cell surface, thus being removed from participation in the formation of a chemotactic gradient. Investigations are currently in progress to distinguish between these possibilities. Substrate specificity and structural requirements for the interaction of the bacterial inactivator with chemotaxins can be probed by utilizing diverse chemotactic substances. Preliminary studies have indicated that several synthetic formylmethionyl oligopeptides are inactivated by M^+ bacteria (unpublished data). This inactivation appears to be less effective than that with ZAS and requires the presence of heat-inactivated serum. Since whole bacteria were used, it is possible that peptide inactivation occurs through the influence of a different system than with ZAS; this will be clarified when purified inactivator becomes available.

The ubiquity of chemotactic factor inactivator among different streptococcal strains cannot be assumed; however, two other bacterial strains, serotypes M4 and M49, possess levels of inactivator activity comparable to that of the M6 strain described here.

The contribution of chemotactic factor inactivation to the pathogenesis of group A streptococci remains, however, to be shown. It has been reported that the injection of M^+ bacteria into the rabbit peritoneal cavity elicits a markedly slower accumulation of PMN exudate cells than a similar injection of M^- bacteria (23). Thus, it is conceivable that this activity may play a role in the initial establishment of invading streptococci within the host tissues, perhaps by retarding the development of inflammatory conditions that may be unfavorable to bacterial survival.

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