Chemotaxis of Polymorphonuclear Leukocytes by Protein A of the Staphylococcus[1] (35073)

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The general sequence of cellular events involved in the acute inflammatory process has been studied extensively and appears to commence with margination and emigration of leukocytes from an intravascular location to the area of injury. Chemotaxis has been defined as "the specific and unidirectional migration of leukocytes towards an increasing concentration gradient of attractant" (1). Staphylococcal infections are generally characterized by extensive pus formation in tissues; this feature suggests active production of chemotactic factors by the staphylococcus *in vivo*. Recent work from this laboratory has shown that minute amounts of protein A, an outer component of the staphylococcus cell wall, is capable of activating the complement system (2) by its interesting direct reaction with the Fc portion of γ G globulin (3, 4). Protein A of the staphylococcus appears to react nonspecifically with human and all mammalian γ G globulins (5, 6). Chemotactic factors are induced by the sequences produced in the complement cascade (7–9). Since virtually all strains of *Staphylococcus aureus* contain protein A as a cell wall constituent (10, 11), generation of laudable pus in staphylococcal infections may be in part explained by the unique reactivity of γ G with protein A, activating the complement sequence and generating chemotaxis. This study was performed in an attempt to test these hypotheses *in vitro*.

Methods and Materials. The method of Boyden (12) as modified by Ward (13) constituted the *in vitro* chemotactic test system used in these experiments. This was accomplished with the Sykes–Moore tissue culture chambers (Bellco Biological Glassware and Equipment Inc., New Jersey) with centrally placed 5 μ pore size micropore filters (Millipore Filter Corporation, Bedford, Massachusetts). Fresh viable leukocytes were obtained from defibrinated venous blood utilizing a single donor. The erythrocytes were removed by sedimentation with a plasma expander, in this case Plasmagel (Laboratoire Roger Bellon, Neuilly, France) followed by paradoxical sedimentation (14). Fresh-frozen human serum obtained from a pool of three healthy human donors was the complement source. Most experiments were performed with protein A prepared by the method of Jensen (15) from the Cowan I strain *Staphylococcus aureus* (4, 5). Further purified protein A utilizing DEAE–Sephadex chromatography and Sephadex G-100 gel filtration (16) was used in a few instances. All final dilutions were made with medium 199.

Seven hundred fifty thousand leukocytes suspended in 1 ml of medium 199 and 0.2 ml of fresh human serum were placed in the upper chamber. The lower chamber contained 0.18 ml of fresh human serum and doses of protein A which varied from 2–200 μg diluted in medium 199 to final volume of 0.9 ml. The chambers were incubated at 37° for 3 hr; the filters were then removed, fixed in 95% ethanol, dehydrated and stained with Ehrlich's hematoxylin. Quantitation of the leukocytes that migrated through the filter was accomplished by routine microscopy at medium power (150×). The average of 20

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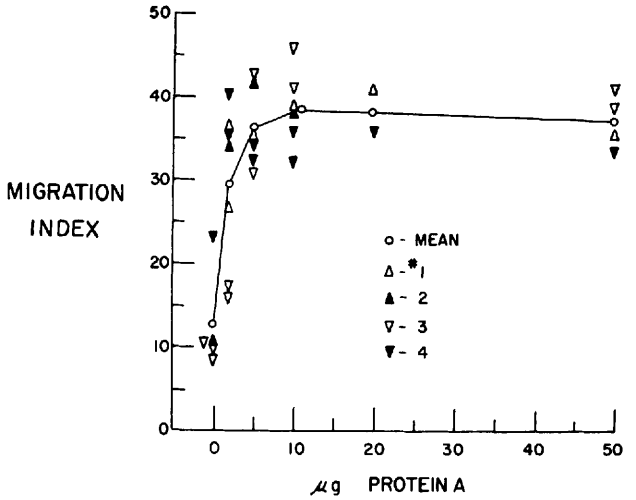

FIG. 1. Chemotactic dose response curve with protein A added to fresh human serum. The figure shows the results from four experiments. Controls containing fresh human serum alone are run in parallel with the tests containing protein A in doses from 2–50 μg. The means of the individual values show a peak response with 10 μg of protein A. No further increase of migration is noted with 20 and 50 μg of protein A.

microscopic fields of each filter represented the number of leukocytes showing chemotaxis; this is referred to as the migration index.

Parallel controls were included with each experiment and contained each of the foregoing materials with the exception of protein A. Additional controls included herein, but not performed with each experiment, were as follows: protein A at concentrations of 10 and 50 μg with heat-inactivated (56° × 60 min) serum; protein A alone without serum. Statistical analysis was accomplished by the Student *t* test.

Results. The data presented in Fig. 1 and Table I clearly show that in the presence of normal fresh human serum, protein A is highly chemotactic. Figure 1 illustrates the dose-response curve from the calculated mean of individual values of four separate experiments in which controls not containing protein A and tests containing protein A varying in final concentration from 2–50 μg were run in parallel. There are increased increments of chemotaxis from 2 μg to maximal chemotaxis at 10 μg. No further increase is noted at 20 and 50 μg of protein A. Table I presents the mean (± SD) and statistical analysis of the combined values of all experiments at each dose, including two runs with 200 μg dose of protein A and four additional separately run controls. It is evident that there is statistically significant chemotaxis over control values at all dose ranges of protein A ($p < .001$). The optimal dose, 10 μg is significantly higher than lowest dose, 2 μg ($p < .05$). In addition 200 μg of protein A shows a slight increment increase over the 50-μg dose (Table I).

Table II compares controls with fresh hu-

TABLE I. Chemotactic Response with Varying Doses of Protein A and Constant Amounts of Fresh Human Serum.

Protein A (μg)	Experiments (number)	Migration index (Means ± SD)	P^a
0 (controls)	9	10.8 ± 5.09	—
2	8	29.7 ± 8.98	<.001
5	7	36.1 ± 4.51	<.001
10	7	38.6 ± 4.24	<.001
20	3	38.6 ± 2.60	—
50	5	37.1 ± 2.87	<.001
200	2	52.2 ± 5.80	—

[a] Probability comparing the controls with each dose of Protein A.

TABLE II. Comparison of Controls Containing No Protein A, No Fresh Human Serum, or Protein A and Inactivated Human Serum.

Human serum	Protein A	Migration index
None	10 μg	11.1
Fresh, not inactivated	None	10.8
Inactivated[a]	10 μg	10.2
Inactivated[a]	50 μg	9.2

[a] Inactivated at 56° for 60 minutes.

man serum alone to controls with protein A alone and with heat-inactivated serum with protein A. There is no difference in migration index in these experiments showing that a heat-labile factor in fresh human serum is an absolute requirement for chemotaxis by protein A.

In separate experiments protein A, purified by DEAE–Sephadex chromatography and Sephadex G-100 gel filtration and added at doses of 10 and 50 μg did not increase chemotaxis above values obtained with the less purified product.

All experiments were run in duplicate and by the double-blind approach. There was no day-to-day variation in counts.

Discussion. Pus is the hallmark of staphylococcal infections; whether or not it be laudable is indeed a matter for debate. Our demonstration that an outer component of staphylococcal cell walls, protein A, is capable of inducing chemotaxis is of considerable interest. The increment in chemotaxis noted as the amount of protein A was increased from 2 to 200 μg/0.38 ml of fresh serum is in keeping with the quantitative aspects of complement activation by protein A already studied (2). No chemotaxis resulted when protein A was added to heat inactivated serum or when protein A was studied alone. Heat-labile factors, presumably complement, were necessary for chemotaxis produced by protein A. These results may indicate that laudable pus accumulates merely because of complement activation induced by the reaction between staphylococcal protein A and nonimmune γ G globulin present in excess in the blood and tissues of the infected host as

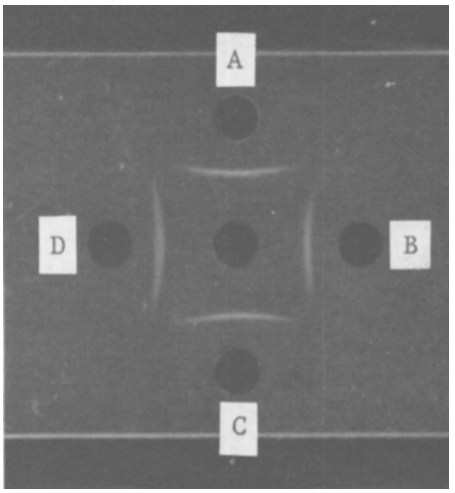

FIG. 2. Immunodiffusion in agar showing precipitin reaction between staphylococcal protein A and IgG myeloma globulins. The center cell contains protein A, 1 mg per ml. To well A was added myeloma serum HO, diluted 1/20. The isolated myeloma globulin from this serum, HO, was added to well C. Wells B and D contain isolated myeloma globulins, BO and LA at 3 mg per ml.

an innocent bystander. Figure 2 illustrates that protein A of the staphylococcus produces a direct precipitin reaction with myeloma globulins via this nonimmune mechanism.

Many bacterial products or substances have been shown to induce chemotaxis using the Boyden chamber system. Examples of such positive chemotactic responses have already been extensively studied as in the case of *E. coli* filtrates, tuberculoprotein, glycogen, and endotoxin preparations (17, 18, 19). The recent demonstration by Gewurz and co-workers of a more direct reaction between lipopolysaccharides of various gram-negative bacteria and the complement system (20–22) indicates that immune γ-globulin may not be a primary requirement for chemotaxis. In this sense it is analogous to the situation documented here for protein A of the staphylococcus where nonimmune IgG may be aggregated and rendered complement-activating. The short circuit (23) engendered by the reaction of staphylococcal protein A with the Fc portion of γ G globulin molecules—whether or not they have intrinsic antibody activity—may thus play a role

in the generation of pus in staphylococcal disease.

Summary. Protein A, a component of staphylococcal cell wall in the presence of fresh human serum is highly chemotactic *in vitro* for human polymorphonuclear leukocytes. Chemotaxis is dependent upon heat-labile factors, presumably complement within serum. Laudable pus in staphylococcal infection may, therefore, be the result of activation of chemotactic complement components generated by the reaction between protein A and nonimmune γ globulin.

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