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Chemotactic Deactivation of Human Neutrophils: Evidence for Nonspecific and Specific Components

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Human polymorphonuclear neutrophils have been preexposed to activated complement as zymosan-activated serum (ZAS) or to the chemotactic oligopeptide *N*-formyl methionylphenylalanine (F-Met-Phe). Spontaneous migration and chemotactic responses toward the deactivating and other cytotaxins were monitored after washing and resuspension of cells in cytotaxin-free medium. Two patterns of deactivation were observed. Preexposure of the leukocytes to high doses of ZAS or F-Met-Phe decreased all subsequent migratory responses. Preexposure of the leukocytes to lower doses of ZAS or F-Met-Phe decreased only a subsequent chemotactic response to the deactivating cytotaxin. These results suggest two mechanisms, or components, of chemotactic deactivation.

Chemotactic deactivation is a term introduced by Ward and Becker a decade ago to describe the effect of preexposure of rabbit peritoneal neutrophils to activated complement on a subsequent chemotactic response (14). They observed that preincubation of these neutrophils with activated complement significantly reduced subsequent chemotactic responses to activated complement and bacterial cytotaxins. Since that time others have demonstrated that human peripheral blood neutrophils (3, 6, 8; R. D. Nelson, R. T. McCormack, V. D. Fiegel, M. Herron, R. L. Simmons, and P. G. Quie, submitted for publication) and eosinophils (16) are similarly affected by preexposure to cytotaxin. In these latter studies it has also been shown that neutrophil spontaneous migration as well as chemotaxis is reduced as a consequence of this treatment (6, 8; Nelson et al., submitted for publication). A contribution of loss of spontaneous mobility to loss of a chemotactic response becomes an important consideration in understanding the mechanism of chemotactic deactivation.

We have studied the relationship of loss of spontaneous and chemotactic migratory functions after deactivation. This report describes experiments which separate the deactivation phenomenon into two separate components. One component is nonspecific, affecting spontaneous migration and chemotactic responses to multiple cytotaxins. Another component is specific, affecting only a chemotactic response to the deactivating cytotaxin.

MATERIALS AND METHODS

Preparation of cells. Blood was drawn from healthy donors and patients in heparinized syringes, and polymorphonuclear neutrophils (PMN) were isolated as previously described (9).

Measurement of PMN chemotaxis and spontaneous migration. The chemotactic and spontaneous migratory functions of purified populations of PMN were assessed by the chemotaxis-under-agarose method as previously described (9), with the following exceptions. To increase migration distances and densities of the migration patterns to improve test replication, the cell number was increased to 5×10^5 per well. Furthermore, to eliminate all possibility that a rapidly developing gradient of attractant could pass the central well containing cells to influence the migration of cells on the "B" side of the migration pattern, the method for assessing spontaneous migration was altered. This function was measured instead by considering the distance of migration of cells placed in separate wells toward wells containing tissue culture medium. The chemotactic agents, zymosan-activated serum (ZAS) and cell-free supernatant from a culture of *Escherichia coli* (BFE), were prepared as described by Ward et al. (16, 17). Both agents were used as attractants without dilution. *N*-formyl methionylphenylalanine(F-Met-Phe) was obtained commercially (Andrulis Research Corp., Bethesda, Md.) and used as an attractant (13) at a concentration of 1.5×10^{-4} M.

Deactivation methodology. Activated complement-mediated deactivation was carried out by preincubation of 2×10^6 PMN for 20 min at 37°C per 0.1 ml of ZAS diluted to concentrations of 50 to 10% (vol/vol) with minimal essential medium (MEM) supplemented with glutamine (2 mM), penicillin (100 U/ml), and

streptomycin (100 μg/ml) (all from Grand Island Biological Co., Grand Island, N.Y.). Control populations were preincubated under identical conditions in MEM alone. Use of serum heated at 56°C for 30 min and diluted to a concentration of 50% as the control medium was observed to result in slight stimulation of the PMN migratory functions. Consequently, MEM was chosen as the appropriate control medium. Washing of the preincubated cell populations was done by first adding 3 ml of MEM to each treated population. After sedimentation of the cells by centrifugation at 200 × *g* for 10 min, the supernatant fluid was decanted and the cells were resuspended in 2 ml of MEM for counting. After counting, the cells were again sedimented by centrifugation, the supernatant fluid was decanted, and the cells were resuspended in MEM for plating at a concentration of 5×10^7/ml. Deactivation mediated by F-Met-Phe solubilized in MEM at concentrations of 1.5×10^{-4} to 1.5×10^{-6} M was carried out by the same protocol described for ZAS.

All tests for spontaneous migration and chemotaxis were conducted in triplicate. Deactivation has been assessed by comparing the migration distances for chemotaxis or spontaneous migration of cells preincubated with cytotaxin with the distances for these respective functions of cells preincubated in MEM and expressed as percent decrease in migration distance. Results are expressed as mean percent decrease in migratory response ± standard error of the mean.

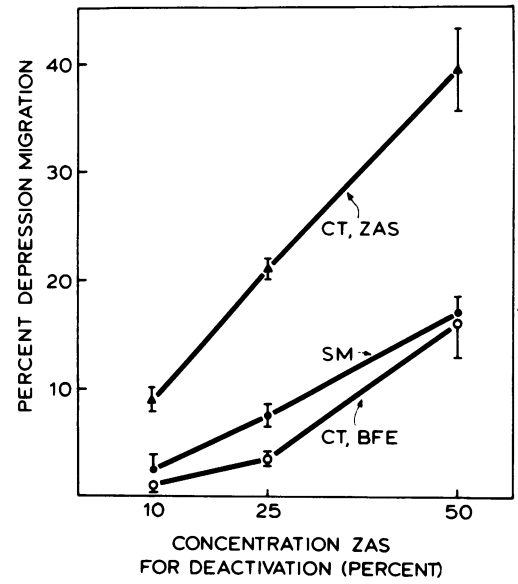

FIG. 1. *Titration of the effect of ZAS as a deactivating agent. Subsequent spontaneous migratory and chemotactic responses to ZAS and BFE were monitored. Data derived from four experiments are presented as mean percent depression of migration ± standard error of the mean.*

RESULTS

In initial studies, we tested the effect of preincubation of PMN in decreasing concentrations of ZAS on their ability to migrate spontaneously and chemotactically towards ZAS, BFE, or F-Met-Phe (Fig. 1). Preexposure of PMN to 50% ZAS was observed to reduce a subsequent chemotactic response of these cells to undiluted ZAS by an average 38%. This deactivation protocol was also observed to significantly reduce both spontaneous migration and chemotaxis toward BFE by approximately 14%. Preexposure of PMN to 25% ZAS had lesser effects on these migratory functions, reducing them by averages of 21, 8, and 4%, respectively. Preexposure of PMN to 10% ZAS, however, produced a different pattern of inhibition of these migratory functions. Whereas a chemotactic response to ZAS was inhibited by an average 10%, spontaneous migration and a chemotactic response to BFE were not significantly affected by this treatment. In other experiments the pattern of subsequent chemotactic responses to F-Met-Phe has mimicked that to BFE (data not shown).

Our use of activated complement as ZAS makes it impossible to identify the specific complement component responsible for the effects observed. Furthermore, the possibility that other serum factors may modulate these effects cannot be excluded. Therefore, to assess the role of a purified cytotaxin as a deactivating agent, we have conducted parallel experiments using the synthetic cytotaxin F-Met-Phe. Data presented in Fig. 2 illustrate the results obtained in three such experiments.

Preexposure of PMN to 1.5×10^{-4} F-Met-Phe was observed to reduce a subsequent chemotactic response of the treated cells to F-Met-Phe by an average of 57%. This deactivation protocol also reduced spontaneous migration and chemotaxis toward ZAS and BFE by averages of 16, 17, and 9%, respectively. Preexposure of PMN to 7.5×10^{-5} M F-Met-Phe had lesser, but significant, inhibitory effects on all four migratory functions, reducing them by averages of 37, 7, 7, and 2%, respectively. Preexposure of PMN to 1.5×10^{-5} M F-Met-Phe, however, reduced a subsequent chemotactic response of the treated cells to F-Met-Phe by an average of 20%, but did not significantly affect the other migratory functions tested. Preexposure to PMN To 7.5×10^{-6} M F-Met-Phe had a similar specific inhibitory effect on subsequent chemotaxis toward F-Met-Phe, reducing the response by an average of 6%

DISCUSSION

Our results demonstrate that preexposure of human neutrophils to high doses of cytotaxin reduces a subsequent chemotactic response to the deactivating agent, spontaneous migration,

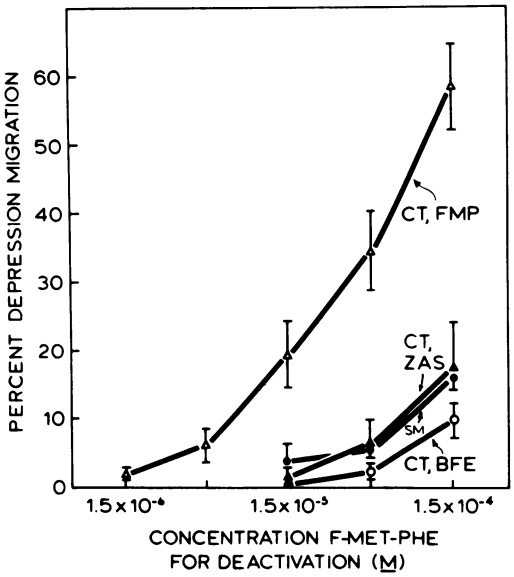

FIG. 2. *Titration of the effect of F-Met-Phe (FMP) as a deactivating agent. Subsequent spontaneous migration and chemotactic responses to FMP, ZAS, and BFE were monitored. Data derived from three experiments are presented as mean percent depression of migration ± standard error of the mean.*

and chemotaxis toward other chemotactic factors. Concentrations of 25 to 50% ZAS or of 7.5×10^{-5} to 1.5×10^{-4} M F-Met-Phe produced this effect. With both deactivating agents subsequent chemotactic responses to the deactivating cytotaxin was reduced to the greatest degree. Spontaneous migration and chemotactic responses to other cytotaxins were reduced to lesser, but similar, degrees. Preexposure of neutrophils to lower doses of cytotaxin consistently reduced only a subsequent chemotactic response to the deactivating cytotaxin. Concentrations of less than 25% activated serum and of less than 7.5×10^{-5} M F-Met-Phe produced this effect.

These results indicate that a part of the loss of the ability of the treated leukocytes to respond chemotactically to the deactivating cytotaxin is due to a decrease in their spontaneous mobility. This loss of spontaneous mobility may, however, account totally for the decrease in chemotactic responses observed for the other cytotaxins. The reduced chemotactic response to the deactivating cytotaxin seen after exposure to lower doses of cytotaxin cannot be accounted for by an effect on spontaneous mobility. Thus, two components of deactivation are suggested: a nonspecific component, induced by high doses of cytotaxin, affecting all subsequent migratory responses; and a specific component, induced by low doses of cytotaxins, affecting only a subsequent chemotactic response to the deactivating cytotaxin.

The mechanism of chemotactic deactivation remains undefined at this time. The studies of rabbit neutrophil deactivation by Ward and Becker have led to the identification of at least two esterase enzymes which are involved in the leukocyte chemotactic response (14). Based upon these studies, Ward and Becker have proposed several possible mechanisms to account for complement-mediated deactivation of neutrophils (15). One possibility is that receptors for activated complement components become saturated to block subsequent interactions of cytotaxin with the cell. A second possibility is that the amount of proesterase 1 available on or within the neutrophil is limited and that esterase 1 is particularly labile. On preincubation of cells with activated complement, the active enzyme pool may be depleted and the potential for generation of additional esterase 1 activity may be lost to effect chemotactic deactivation. A third alternative is that preexposure of cells to activated complement results in utilization and depletion of a cofactor or substrate required for the leukocyte chemotactic response.

The two components of deactivation demonstrated in this report are difficult to explain on the basis of a single mechanism. We propose, rather, that two mechanisms are operative. We suggest that the nonspecific component of deactivation is due to autoxidative reactions (1, 2, 7, 11; Nelson et al., submitted for publication), aggregation, and increased adherence properties of the treated cells (4, 10) and/or to polymerization of cytoplasmic microtubules and microfilaments (5). We suggest that the specific component of deactivation is due to loss of specific receptor activity by simple binding of the deactivating cytotaxin to a number of specific membrane receptor sites and/or by endocytosis of the cytotaxin-receptor complex. Since an identity of receptors for certain bacterial cytotaxins and the synthetic *N*-formylmethionine oligopeptides is possible (12), our failure to observe that preexposure of neutrophils to F-Met-Phe inhibits subsequent chemotactic responses to both F-Met-Phe and BFE in a similar pattern suggests that the active chemotactic agents in the culture supernatant used are not homologous to F-Met-Phe.

We believe that the results reported herein provide a feasible explanation for the multiple effects of preexposure of leukocytes to cytotaxin which have been reported. We are currently pursuing explanations for the separate nonspecific and specific consequences of such treatment.

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