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Influence of Serum-Derived Chemotactic Factors and Bacterial Products on Human Neutrophil Chemotaxis

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The chemotaxis of neutrophils has been shown to be modulated by serum factors, tissue factors, bacterial products, and a host of other substances. In vivo, these factors may act in concert with each other to modify neutrophil movement. We examined the effect of aggregated gamma globulin-activated serum (AS), bacterial factors, and endotoxin either alone or in combination with each other, on human neutrophil chemotaxis. Exposure of neutrophils to AS resulted in deactivation to AS but not to *Escherichia coli* or *Staphylococcus epidermis* culture filtrate. Exposure of neutrophils to *S. epidermis* or *E. coli* CF or *E. coli* endotoxin resulted in deactivation to AS or C5a but not to *E. coli* or *S. epidermis* culture filtrate. Addition of endotoxin to AS or C5a resulted in inhibition of chemotaxis by untreated neutrophils toward this combination as compared with AS alone. These results suggest that separate mechanisms may be involved when serum or bacterial chemotactic factors initiate human neutrophil chemotaxis. Furthermore, the potent but specific inhibitory effect of endotoxin on chemotaxis toward AS may be of clinical significance.

Chemotaxis of neutrophils is important for controlling microbial infection in man. Recent studies have led to a greater understanding of this process in both animals and man. Indeed, several abnormalities of neutrophil chemotaxis associated with an increased susceptibility to severe and recurrent infection have been described recently (reviewed in reference 15). Neutrophil chemotaxis has been shown to be modulated by serum factors, tissue factors, bacterial products, and many other substances (29). In vivo, many of these factors may act alone or in concert with each other to modify cell movement.

Complement factors, generated when immune complexes are added to rabbit serum, are not only chemotactic for rabbit neutrophils but can also deactivate rabbit neutrophils (26). By this, it is meant that rabbit neutrophils exposed to one chemotactic factor lose the ability to migrate toward the same or toward a different chemotactic factor. In their studies, Ward and Becker (25, 26) demonstrated that rabbit serum, activated with immune complexes as well as C5a or $C\overline{567}$, could deactivate rabbit neutrophils in such a way that the neutrophils no longer migrated when exposed to complement-derived chemotactic factors or to chemotactic factors derived from *Escherichia coli*. By contrast, the chemotactic factor derived from *E. coli* could not deactivate neutrophils to either the same *E. coli*-derived chemotactic factor or to serum-derived chemotactic factors.

Recently, Van Epps has demonstrated that important differences exist among species with respect to neutrophil movement (23). Specifically, the chemotaxis of human neutrophils could be suppressed completely by streptolysin O, whereas the chemotaxis of rabbit neutrophils was unaffected. In addition, chemotaxis of human neutrophils, unlike rabbit neutrophils, has been shown to be inhibited by colchicine (3, 4, 18). Because of these important species differences, we have examined the effects of serum chemotactic factors and bacterial factors, either alone or in combination with each other, on human neutrophil chemotaxis in vitro using the recently described (6, 11, 17) agarose assay for chemotaxis. Our results suggest that exposure of human neutrophils to serum-derived chemotactic factors and bacterial-derived chemotactic factors impairs their subsequent chemotactic responses only to serum-derived chemotactic factors but not to bacterial-derived chemotactic factors.

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MATERIALS AND METHODS

Preparation of cells. Blood was drawn from healthy human volunteers into sterile disposable

syringes containing 10 U of sodium heparin per ml of blood. Erythrocytes were sedimented by gravity at room temperature. The leukocyte-rich supernatant was removed and centrifuged at 200 × *g* for 5 min. The cell pellet was washed and resuspended to a concentration of 8×10^7 neutrophils per ml in M199 (GIBCO, Grand Island, N.Y.) containing 50 U of penicillin per ml and 50 μg of streptomycin per ml (GIBCO, Grand Island, N.Y.). Leukocyte suspensions prepared in this way contained approximately 70% neutrophils, 30% mononuclear cells, occasional platelets, and erythrocytes. For some experiments, neutrophils were obtained by Ficoll-Hypaque separation as described by Boyum (2). Leukocyte suspensions prepared in this way contained greater than 95% neutrophils. However, neutrophils tended to clump excessively after this procedure, and their chemotactic response to all chemotactic substances was diminished.

Preparation of agarose plates. The agarose plates were prepared as described by Nelson et al. (17), with minor modifications. Briefly, agarose (Fisher Scientific, Toronto, Canada) was dissolved in boiling, sterile distilled water, cooled to 48°C, and then made up to a final concentration of 0.75% in M199 containing 10% heat-inactivated AB serum. From this solution, maintained at 48°C, 7.5 ml was added to plastic tissue culture dishes (10 by 15 mm) (Falcon, Oxnard, Calif.) and allowed to harden. A template was used to cut wells 2.4 mm in diameter and 2.4 mm apart in rows of three wells, as described (17).

Preparation of chemotactic factors. Heat-aggregated human gamma globulin (HAHG) was prepared by dialyzing immune serum globulin (Connaught Laboratories, Toronto, Canada) against saline for 20 h, and then heating at 63°C for 20 min. This suspension was blended in a Vortex mixer, sonicated, and stored in small aliquots at −70°C for future use. Serum-derived chemotactic factors were prepared from human AB serum (stored at −70°C) by adding 300 μg of HAHG per ml of AB serum and incubating the mixture at 37°C for 60 min. This will be referred to as activated serum (AS). In some experiments the larger aggregates were removed from the activated AB serum by centrifugation at 40,000 rpm for 40 min. Partially purified C5a was prepared by gel filtration of the AS on Sephadex G75 as described by Gallin and Rosenthal (7). Assay of total hemolytic complement was performed as previously described (12). *E. coli* K-88, K-12 AC (kindly supplied by C. Gylles, Guelph, Ontario) and *Staphylococcus epidermis* (a human isolate) were grown in M199 for 24 h. The filtrates (Millipore Corp., 0.45-μm-pore size filter) of these cultures were stored in small aliquots at −70°C for future use.

Leukocyte chemotaxis assay. Ten microliters of leukocyte suspension (8×10^5 neutrophils) was placed into the center well of each row of three wells. Ten microliters of the chemotactic substance was placed into the peripheral well of each row. M199 was placed into the central (control) well when bacterial culture filtrates were tested, and heat-inactivated AB serum treated with HAHG was placed into the central (control) well when AS was tested. The tissue culture dishes were incubated for 2.5 h at 37°C in a humidified 5% CO_2 and air incubator. After the incubation, the dishes were flooded with methanol for 10 min. After this, the methanol was removed and replaced with 40% formalin for 10 min. After this, the formalin was removed and the dishes were frozen at −70°C overnight, and then the agarose was carefully lifted off as it began to thaw. The fixed cells were stained for 10 min with Giemsa.

The migration patterns were magnified 45 times and projected onto a white background with a projecting microscope. Quantitation of migration was done by measuring the linear distance in centimeters that the cells had moved from the margin of the well toward the chemotactic factor well (distance A) and by measuring the linear distance that the cells had moved from the opposite margin of the well toward the control well (distance B). A chemotactic differential (CD) was calculated by subtracting distance B (toward the control) from distance A (toward the chemotactic well) and expressed as mean ± standard error of the mean (SEM).

Pretreatment of cells. In some experiments, leukocytes (8×10^7 neutrophils per ml) were incubated for 20 min at 37°C with an equal volume of AS, *E. coli* culture filtrate (CF), *S. epidermis* CF, or varying concentrations of *E. coli* 0111-B4 endotoxin (Difco, Detroit, Michigan). The control cells were incubated with an equal volume of M199 or heat-inactivated AB serum treated with HAHG. After incubation, the cells were washed twice with 75 volumes of M199 and resuspended in a volume of M199 containing 15% heat-inactivated AB serum to contain 8×10^7 neutrophils per ml.

Hexose monophosphate shunt activity (HMPS). HMPS was assessed for leukocytes at rest, and during phagocytosis of latex particles (Difco, 0.81 μm), by measuring the generation of $^{14}CO_2$ from 1-[^{14}C]D-glucose as previously described (17).

RESULTS

Patterns of cell migration. The typical patterns of neutrophil migration toward AS and *S. epidermis* CF are shown in Fig. 1a and b. These patterns were obtained by adding 10 μl of the leukocyte suspension (8×10^5 neutrophils) to the center well. In Fig. 1a, AS was placed into the peripheral well and heat-inactivated AB serum treated with HAHG for 60 min was placed into the central (control) well. Heat-inactivated AB serum was necessary because the complement system of normal AB serum was activated by the agarose. In Fig. 1b, *S. epidermis* CF was placed into the peripheral well and M199 was placed into the central (control) well.

A 2.5-h incubation period was found to be optimal for assessing chemotaxis. A "rocket" pattern (Fig. 1a) was characteristic of migration toward serum-derived chemotactic factors. A "blunted" pattern (Fig. 1b) was typical of migration towards *S. epidermis* or *E. coli* CF.

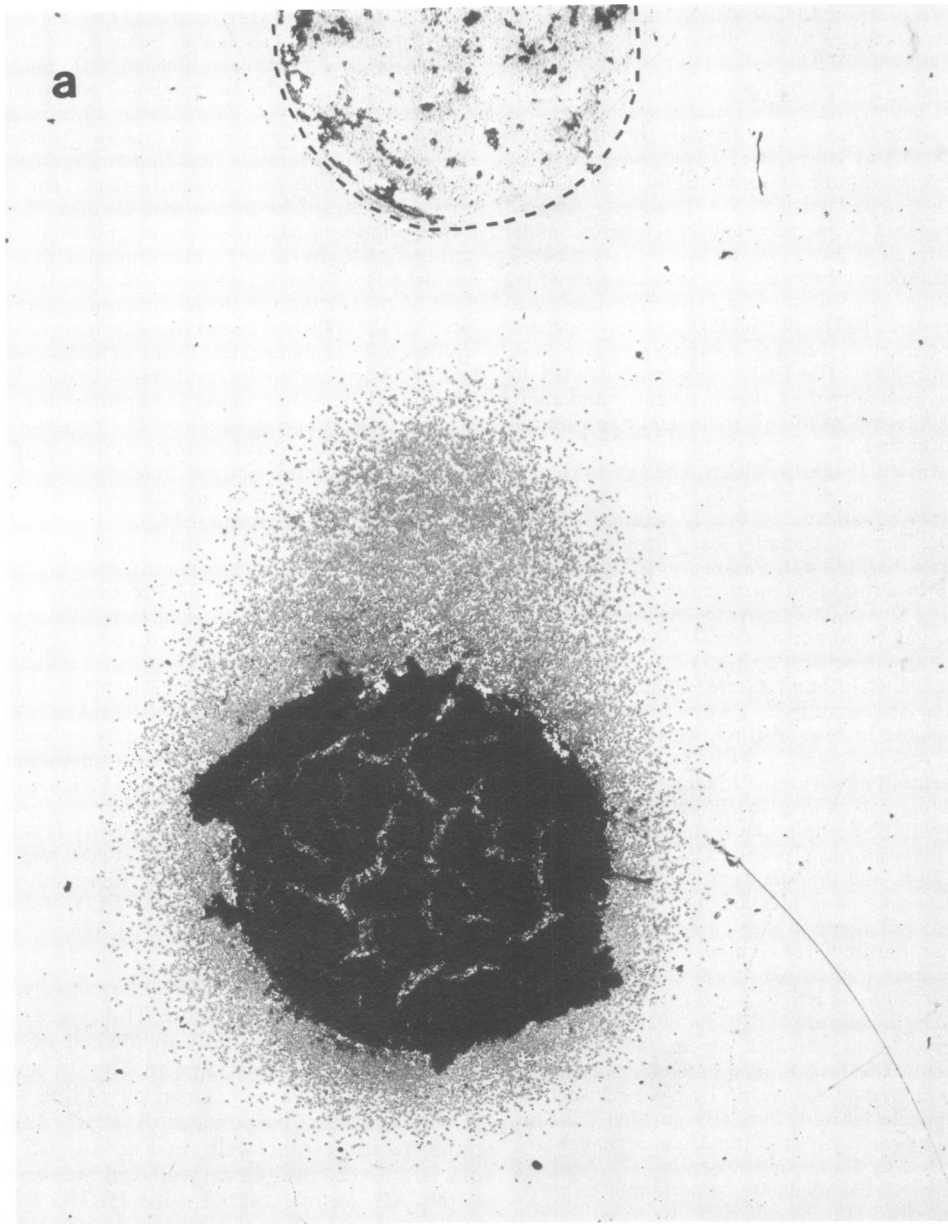

FIG. 1. *Migration patterns of the neutrophils towards aggregated gamma globulin AS in the top well (a) and towards Staphylococcus epidermis CF in the top well (b). Neutrophils, 8 × 10⁵, were placed into the center well. Heat-inactivated AB serum treated with aggregated gamma-globulin was in the control well at bottom (a), and medium 199 was in the control well at bottom (b). (×20).*

The CD obtained in this way was highly reproducible: chemotaxis toward AS was CD = 6.61 ± 0.26, chemotaxis toward *E. coli* CF was CD = 4.74 ± 0.09, and chemotaxis toward *S. epidermis* CF was CD = 4.49 ± 0.15. Neutrophil migration toward the control well was very consistent (2.82 ± 0.1 cm), except for those experiments in which neutrophils had been preincubated with AS (1.20 ± 0.24 cm).

Nature of serum chemotactic factors. Experiments were performed to assess the nature of chemotactic activity generated in serum

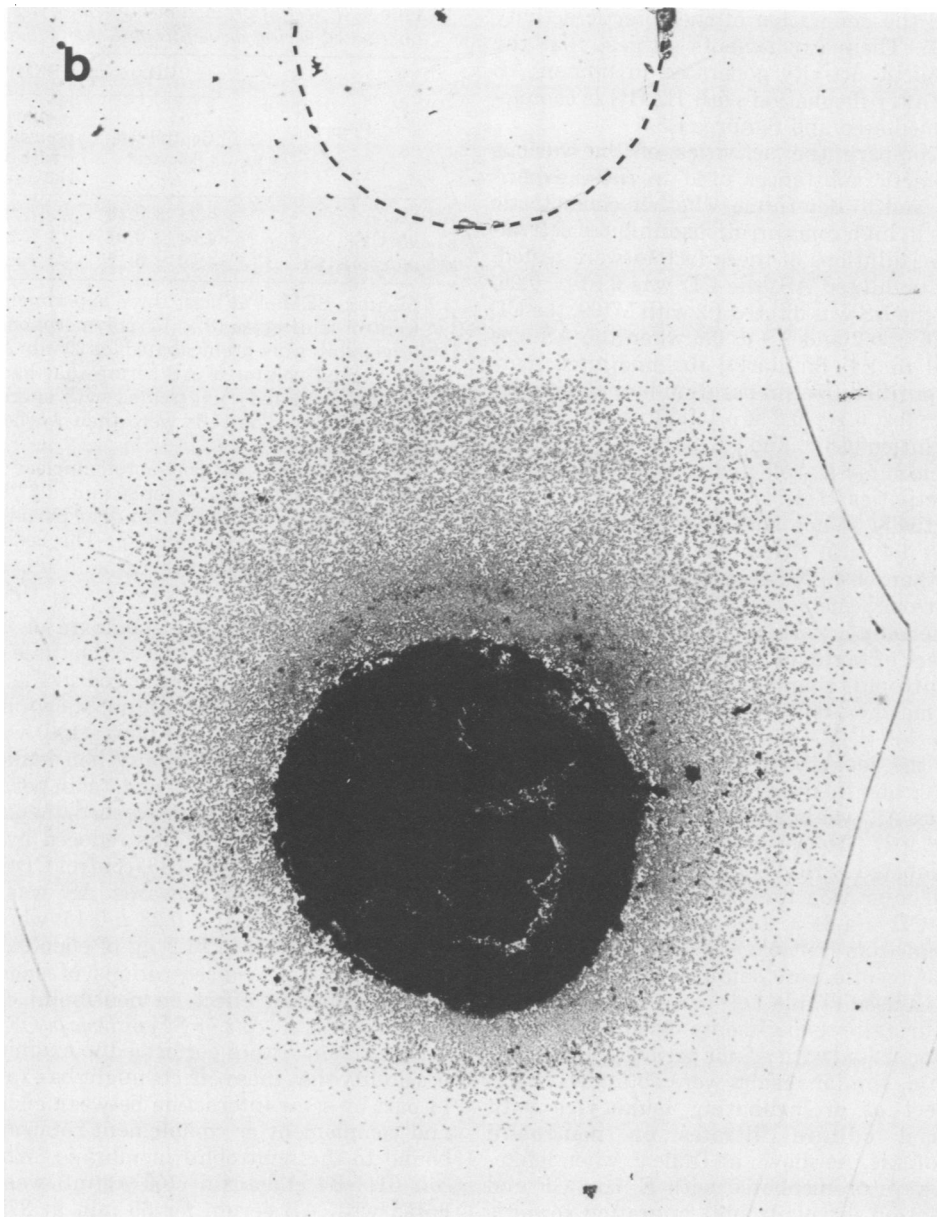

FIG. 1. *Continued*

after incubation with HAHG. When normal AB serum was first heated at 56°C for 30 min and then incubated with HAHG, no chemotactic activity could be generated. The generation of chemotactic activity by HAHG could be prevented by the addition of 10 mM ethylenediaminetetraacetic acid. Full chemotactic activity could be generated in serum deficient in the second component of complement (C2) (gift from Erwin Gelfand). However, absorption of this C2-deficient serum with rabbit anti-human factor B (gift from Chester Alper) completely blocked the generation of chemotactic activity by HAHG. This antiserum did not alter the capacity of normal AB serum to generate chemotactic activity. Finally, absorption of AB serum with rabbit antihuman C5 (gift from Chester Alper), before activation with HAHG, in-

hibited the generation of chemotactic activity by 85%. These experiments suggest that the chemotactic activity generated in human AB serum after incubation with HAHG is complement mediated and requires C5.

To compare the activities of the various chemotactic substances used in these experiments and to determine whether chemotactic factors in high concentrations inhibited cell migration, dilutions of these factors were tested. Using undiluted AS, the CD was 6.61 ± 0.26. When the AS was diluted 1:2 with M199, the CD was 5.6 ± 0.2 and 2.9 ± 0.6 when the AS was diluted to 1:4. Similarly, the undiluted, partially purified C5a preparation had an activity of 7.2 ± 0.2, 6.1 ± 0.2 at a 1:4 dilution, 4.9 ± 0.2 at a dilution of 1:8, and 3.0 at a dilution of 1:12. The undiluted *E. coli* and *S. epidermidis* CFs had activities of 4.74 ± 0.1 and 4.49 ± 0.15, respectively. When both culture filtrates were diluted 1:4 with M199, the CD decreased by more than 50%. No dose-dependent inhibition of neutrophil chemotaxis was observed to any of the chemotactic factors.

Effect of preincubating leukocytes with AS on neutrophil chemotaxis. As shown in Table 1, normal neutrophils migrated vigorously towards AS (CD = 6.11 ± 0.26). By contrast, when the leukocytes had been preincubated with AS and then tested, neutrophil migration towards AS was markedly diminished (CD = 0.87 ± 0.27). However, when leukocytes were preincubated with AS and then tested for neutrophil migration towards *E. coli* CF, chemotaxis (CD = 4.84 ± 0.29) was not significantly different from control cells (CD = 4.8 ± 0.01). Similar results were obtained when *S. epidermis* was used (Table 1).

In separate experiments when leukocytes were incubated with AS for periods as short as 1 to 2 min, similar results were obtained.

Effect of preincubating leukocytes with bacterial culture filtrates on neutrophil chemotaxis. As shown in Table 2, when leukocytes were preincubated with *E. coli* CF and then tested for neutrophil migration towards AS, their response was markedly diminished (CD = 0.2 ± 0.11). However, when leukocytes were preincubated with *E. coli* CF and then tested for neutrophil migration, the chemotactic differential towards *E. coli* CF (CD = 3.95 ± 0.12) was only slightly less than that of control leukocytes (CD = 4.74 ± 0.1). Neutrophil migration towards *S. epidermis* CF was not significantly influenced by *E. coli* CF preincubation (CD = 4.65 ± 0.16).

Effect of preincubating leukocytes with endotoxin on subsequent neutrophil chemotaxis. The effect of preincubating leukocytes with varying concentrations of commercially prepared *E. coli* 0111-B4 endotoxin on subsequent neutrophil chemotaxis to the three chemotactic substances is summarized in Fig. 2. Of importance was the finding (not shown in Fig. 2) that endotoxin (0.1 to 1,000 μg/ml) was not chemotactic for neutrophils. When leukocytes were exposed to 0.02 μg of endotoxin per ml for 20 min at 37°C and then washed, neutrophil chemotaxis towards AS was reduced by more than 50% (CD = 2.99 ± 0.38, control CD = 6.61 ± 0.26). Chemotaxis towards AS was completely abolished (CD = 0.28 ± 0.15) when leukocytes were exposed to 2 μg of endotoxin per ml. These same concentrations of endotoxin had no apparent effect on neutrophil chemotaxis towards *E. coli* or *S. epidermis* CF.

Experiments were performed to examine the possibility that these effects might be explained in part by some interaction between endotoxin and complement or complement component(s) bound to the neutrophil membrane. When *E. coli* 0111-B4 endotoxin (100 μg/ml) was incubated with AB serum for 60 min at 37°C, no depletion of hemolytic complement activity was detected. When the leukocyte suspension was incubated with endotoxin for 20 min in the presence of 10 mM ethylenediaminetetraacetic acid in calcium- and magnesium-free Hanks balanced salt solution, neutrophil chemotaxis towards AS was still suppressed. The effect of endotoxin could not be reversed by exhaustive washing. Finally, when mononuclear cells were removed by Ficoll-Hypaque density gradient from the leukocyte suspension, the capacity of endotoxin to inhibit neutrophil chemotaxis towards AS was unchanged.

Chemotaxis of neutrophils towards chemo-

TABLE 1. *Effect of preincubating leukocytes with activated serum on neutrophil chemotaxis*

Prepn	CD (mean ± SEM)[a]	
	Control leukocytes[b]	Leukocytes preincubated with activated serum[c]
AS	6.11 ± 0.26	0.87 ± 0.27
E. coli CF	4.84 ± 0.01	4.8 ± 0.29
S. epidermis CF	4.40 ± 0.17	5.4 ± 0.11

[a] Mean ± SEM of at least three experiments.

[b] Control leukocytes (8×10^7 polymorphonuclear leukocytes/ml) were preincubated for 20 min at 37°C with an equal volume of AB serum that had been heat inactivated and then treated with aggregated gamma-globulin. The cells were then washed and tested.

[c] Leukocytes (8×10^7 polymorphonuclear leukocytes/ml) were preincubated for 20 min at 37°C with an equal volume of AB serum that had been treated with aggregated gamma-globulin. The cells were then washed and tested.

TABLE 2. *Effect of preincubating leukocytes with bacterial CFs on neutrophil chemotaxis*

Prepn	CD (mean ± SEM)[a]		
	Control leukocytes[b]	Leukocytes preincubated[c] with CF from:	
		E. coli	*S. epidermis*
AS	6.61 ± 0.26	0.2 ± 0.11	0.81 ± 0.21
E. coli CF	4.74 ± 0.09	3.95 ± 0.12	4.0 ± 0.08
S. epidermis CF	4.49 ± 0.15	4.65 ± 0.16	4.91 ± 0.19

[a] Mean ± SEM of at least three experiments.

[b] Control leukocytes (8×10^7 polymorphonuclear leukocytes/ml) were preincubated for 20 min at 37°C with an equal volume of medium 199, and then washed and tested.

[c] Leukocytes (8×10^7 polymorphonuclear leukocytes/ml) were preincubated for 20 min at 37°C with an equal volume of *E. coli* culture filtrate or *S. epidermis* culture filtrate, and then washed and tested.

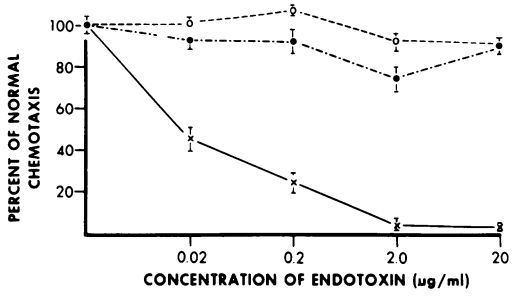

FIG. 2. *Effect of preincubation of leukocytes with E. coli endotoxin on neutrophil chemotaxis. Leukocytes (4×10^7 polymorphonuclear leukocytes/ml) were preincubated for 20 min at 37°C with varying concentrations of E. coli endotoxin, and then washed and tested for neutrophil chemotaxis towards AS (×), E. coli CF (○), or S. epidermis CF (●). Values are means ± 1 SEM.*

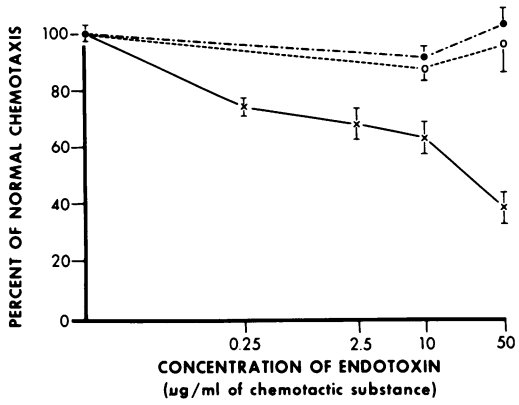

FIG. 3. *Effect of combining E. coli endotoxin with chemotactic substances on neutrophil chemotaxis. The percentage of normal chemotaxis of neutrophils towards AS containing endotoxin (0 to 50 µg/ml) (×), towards E. coli CF containing endotoxin (0 to 50 µg/ml) (○), and S. epidermis CF containing endotoxin (0 to 50 µg/ml) (●) is plotted. Values are means ± 1 SEM.*

tactic substances containing endotoxin. In separate experiments, the three chemotactic factors were mixed with endotoxin and placed into the chemotactic well. As can be seen from Fig. 3, when AS was the chemotactic substance, the addition of 0.25 µg of endotoxin per ml of AS reduced chemotaxis by 26%. Higher concentrations of endotoxin resulted in greater suppression of chemotaxis to a maximum of 62%. By contrast, the addition of endotoxin to either *E. coli* CF or *S. epidermis* CF did not impair chemotaxis towards these combinations.

Effect of preincubating leukocytes with endotoxin or *S. epidermis* CF on neutrophil HMPS. The effect of endotoxin and *S. epidermis* CF on neutrophil HMPS activity was investigated. Endotoxin in a concentration of 0.25 µg/ml was chosen, since this concentration consistently inhibited neutrophil chemotaxis to AS. Under these experimental conditions, endotoxin did not significantly alter neutrophil resting HMPS activity (143 ± 28% SEM of control). Furthermore, 0.2 µg of endotoxin per ml did not affect the ability of neutrophils to increase HMPS activity after ingestion of latex particles (experimental stimulated 125 ± 9.9% SEM of control stimulated). Similarly, *S. epidermis* CF preincubation did not alter the resting or stimulated HMPS activity of neutrophils (102 ± 14.3 and 92 ± 4% of the control).

DISCUSSION

With the agarose chemotaxis assay, we were able to rapidly and reproducibly quantitate neutrophil chemotaxis towards serum- and bacterial-derived chemotactic factors. The difference in migration patterns towards AS and bacterial CFs ("rocket" versus "blunted," Fig. 1a and b) may be related in part to the difference in molecular weight and diffusion rates of these chemotactic substances. The molecular weight of C5a in AS is about 15,000 (20), whereas *E. coli*-derived chemotactic factor(s) is less than 2,000 (19).

The results of our experiments using sera depleted of factor B or C2 suggest that activa-

tion of either the classical and/or the alternate complement pathways by HAHG can generate similar quantities of this chemotactic activity. The results using C5-depleted serum suggest that this chemotactic activity is C5 dependent and may be either C5a or C567 or both (14, 20, 25, 28). The non-C5-dependent chemotactic factors such as C3a, kallikrein, and plasminogen activator (reviewed in references 13 and 24) may be responsible for the slight (<15%) chemotactic activity that can be generated in C5-depleted serum. This conclusion is supported by the observation that gel filtration of AS on Sephadex G-75 yields one major peak of chemotactic activity in the 12- to 15,000-molecular-weight region, suggesting the presence and activity of C5a. Similar results were reported by Gallin (7) using endotoxin-activated serum.

It has been observed previously that the exposure of rabbit neutrophils to a chemotactic factor could influence subsequent chemotactic activity (1, 25, 26). Rabbit neutrophils that had been exposed to rabbit AS lost the ability to respond via chemotaxis, i.e., they were deactivated, to AS as well as to C5a, C567, or chemotactic factors derived from *E. coli*. We have examined the effect of preincubating human leukocytes with AS on subsequent neutrophil chemotaxis. This treatment resulted in deactivation of the neutrophils to AS, but no effect was observed on the chemotactic response to bacterial-derived chemotactic factors (Table 1). Furthermore, qualitatively similar results were obtained using a partially purified preparation of C5a. Craddock et al. (5) have very recently reported similar results using serum activated by cobra venom factor. When human neutrophils were preincubated with either *E. coli* or *S. epidermis* CF (Table 2), deactivation was again observed, but only to AS. The chemotaxis towards both bacterial CFs remained vigorous.

The mechanism by which *E. coli* CF and *S. epidermis* CF deactivate neutrophils to AS is unknown. Since the *E. coli* CF likely contains endotoxin in addition to chemotactic factors, we investigated whether preincubation of the leukocytes with *E. coli* endotoxin influenced neutrophil chemotaxis. Pretreatment with endotoxin, which itself was not chemotactic, markedly suppressed chemotaxis to AS (Fig. 2) and to C5a (not shown). Of importance was the finding that endotoxin had no apparent affect on neutrophil chemotaxis towards *E. coli* or towards *S. epidermis* CF. In preliminary experiments, smaller concentrations of a highly purified preparation of *E. coli* endotoxin (a gift from Kenneth Johnson) gave similar results to the commercial preparation of endotoxin. Specifically, inhibition of chemotaxis to AS could be achieved with less than 0.1 ng of purified endotoxin per ml. Neither endotoxin preparation affected the hexose monophosphate pathway activity of the leukocytes either at rest or after particle ingestion at the concentrations required to inhibit neutrophil chemotaxis. The viability of the cells was also preserved as judged by trypan blue exclusion. In preliminary experiments, when the *E. coli* CF was subjected to ultrafiltration, the deactivating principle was found in the high-molecular-weight (greater than 100,000) fraction and not in the low-molecular-weight fraction, which contained the chemotactic activity. This may indicate that the "deactivation" by *E. coli* CF is not due to the chemotactic factor at all but rather, to some other bacterial substance capable of specifically inhibiting chemotaxis towards AS or C5a.

In contrast to our results with human neutrophils, Ward and Becker reported that *E. coli* CF was unable to deactivate rabbit neutrophils to AS, C5a, or *E. coli* CF (1, 25, 26). These experiments with rabbit neutrophils were done using the Boyden chamber to measure chemotaxis. To determine whether this discrepancy in the behavior of rabbit and human neutrophils was due to the different assay systems used, or to a species difference, we performed two experiments using rabbit peripheral blood neutrophils and the agarose chemotaxis assay. In these experiments, the same results were obtained with the agarose chemotaxis assay, as had been reported with the Boyden chamber assay. It would appear that our results are not due to the use of a different assay system and, therefore, the mechanisms involved in deactivation of human and rabbit neutrophils may not be the same.

The deactivation of rabbit neutrophils was attributed in part to the ability of chemotactic factors to activate membrane serine esterases. Ward and Becker (1, 26, 27) described two membrane serine esterases that were important for chemotaxis. One of these, present in an inactive form (proesterase 1), was activated by C3a, C5a, or C567. The activation of proesterase 1 was related to the ability of C3a, C5a, or C567 to deactivate rabbit neutrophils to all chemotactic factors. In contrast, no activation of proesterase 1 was detected when the neutrophils were exposed to *E. coli*-derived chemotactic factors. Our results suggest that human neutrophil chemotaxis is initiated or controlled differently from rabbit neutrophil chemotaxis. The human neutrophil may have separate sig-

nals for initiating chemotaxis towards AS or C5a (not shown) and *E. coli* or *S. epidermis* CF, suggesting perhaps the presence of more than one activatable proesterase. A difference between human and rabbit neutrophils has also been suggested by Van Epps et al. (23), when they showed that streptolysin O treatment could impair human but not rabbit neutrophil chemotaxis. Furthermore, colchicine inhibits the migration of human but not rabbit neutrophils (3, 4, 18). Lastly, in our experiments with rabbit neutrophils, we noted that the chemotaxis of these cells was unaffected by 2 μg of endotoxin per ml whereas human neutrophils became unresponsive to AS after this treatment.

This mechanism(s) by which endotoxin caused the inhibition of chemotaxis is unknown. Monocytes and lymphocytes were not required for the effect. The complement system did not seem to be involved, since *E. coli* 0111-B4 endotoxin did not activate human complement (16; unpublished observations). The report by Goetzl and Austen (9) that a neutrophil-immobilizing factor (NIF) was released from leukocytes exposed to endotoxin may be relevant to our observations. However, for optimal NIF release, they incubated the leukocytes from 1 to 4 h with endotoxin, and after washing, the same leukocytes were incubated for a similar length of time in medium deficient in potassium. It was during the second incubation that most of the NIF was released. Our conditions allowed only 20 min for the interaction between endotoxin and the leukocytes. Even 2 to 4 min was sufficient to produce the effect. Furthermore, leukocytes that had been exposed to endotoxin, washed, and then added to normal leukocytes, did not suppress the chemotaxis of the normal neutrophils.

It has been shown that endotoxin binds to neutrophils (8, 21). Our results do not eliminate the possibility that endotoxin blocks the sites of attachment of C5a or that the bound endotoxin alters the metabolic processes of the cell so that it becomes refractory to AS and C5a. The endotoxin may even inactivate or interfere with the activation of the proesterase that is responsive to C5a.

The experiments reported here demonstrate that the chemotactic response of human neutrophils to serum-derived chemotactic factors can be influenced significantly by endotoxin and bacterial products released into liquid culture media. The finding of impaired chemotaxis towards a mixture of endotoxin and AS or C5a as compared with AS or C5a alone may suggest that this mechanism plays a role in vivo. This suggestion is strengthened by the recent report of a transient decrease in neutrophil chemotaxis towards serum-derived chemotactic factors, after endotoxin injection into man (22).

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