

THE RELATIONSHIP OF THE CHEMOTACTIC BEHAVIOR
OF THE COMPLEMENT-DERIVED FACTORS, C3a, C5a, AND
C567, AND A BACTERIAL CHEMOTACTIC FACTOR TO
THEIR ABILITY TO ACTIVATE THE PROESTERASE 1 OF
RABBIT POLYMORPHONUCLEAR LEUKOCYTES*

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The high molecular weight chemotactic factor, C567, derived from the fifth, sixth, and seventh components of complement, induces chemotaxis in rabbit polymorphonuclear leukocytes by activating a serine esterase (1, 2).¹ This esterase is capable of splitting acetyl DL-phenylalanine β -naphthyl ester; it exists in or on the polymorphonuclear leukocyte in an inert form termed proesterase 1; the active form is known as esterase 1 (3, 4).

C567 incubated with the rabbit polymorphonuclear leukocyte changes the cell in such a manner that it no longer is capable of giving a chemotactic response; this specific decrease in chemotactic responsiveness is called "deactivation" (2). The activation of proesterase 1 is involved in the deactivation by C567 (2). If a cell is deactivated by C567 it will not respond chemotactically to the other complement-derived chemotactic factors (C3a, C5a) nor to bacterial chemotactic factors; that is, it is deactivated to all of them (5). Similarly, if a cell is deactivated by C3a or C5a, it is also deactivated to all the others including the bacterial factors. However, incubation of rabbit polymorphonuclear leukocytes with bacterial factor does not induce deactivation to either the bacterial factor not to any of the complement-derived factors (5).

Previous work on the involvement of proesterase 1 in the chemotaxis of the polymorphonuclear leukocyte and its activation had been done only with C567. Thus, the question remained as to whether the other chemotactic factors, C3a and C5a, and bacterial factors induce chemotaxis in the neutrophil by activating proesterase 1. The work to be described was undertaken in order to answer this question.

In this study, it is shown that the *p*-nitrophenyl ethyl phosphonate esters give the same kind of what we have called (1) chemotactic factor-dependent inhibition of chemotaxis when C3a, C5a, or a bacterial agent is the chemotactic factor as when C567 is employed. This pattern and kind of inhibition indicates a requirement for the activation of proesterase 1 in the chemotactic response to C567 (1) and *pari passu* indicates the same sort of requirement in the chemo-

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¹ Whether C567 transforms proesterase 1 to esterase 1 directly or indirectly is not known. In what follows, the term "activation" will not necessarily imply direct activation.

tactic response to C5a and the bacterial factor. However, when the activation of proesterase 1 was directly tested by measuring the acetyl DL-phenylalanine β -naphthyl esterase activity of leukocytes with and without contact with chemotactic factor, it was found that a measurable activation of proesterase 1 could be demonstrated in cells treated with C3a, C5a, or C567 but not with bacterial factor. The implications of these observations in regard to enzymatic mechanisms associated with chemotaxis will be presented.

Materials and Methods

Buffers.—Medium 199 (Baltimore Biological Laboratories, Cockeysville, Md.) buffered with 0.02 M tris (hydroxymethyl) aminomethane (Tris) (Sigma Chemical Co., St. Louis, Mo.) was used for deactivation studies. Hanks' buffered salt solution with 2 mg/ml added glucose was used for chemotactic studies where no preincubations of cells was involved. Unpublished work has shown that cells incubated at room temperature before chemotactic assay retained their chemotactic responsiveness better in Medium 199 than in Hanks' buffer. However, the chemotactic responsiveness of cells which were not preincubated was essentially the same in either buffer. In all cases, chemotaxis was performed with cells suspended in 0.1% bovine serum albumin (BSA)² (fraction V, Pentex Biochemical, Kankakee, Ill.). The buffer for esterase assays was TGS buffer, Tris 0.02 M, pH 7.3, in 0.15 M saline with 0.1% added gelatin.

Phosphonates.—The *p*-nitrophenyl ethyl chloroalkylphosphonates and the *p*-nitrophenyl alkylphosphonates were the same as described previously (1). They were dissolved in acetone as a 0.1 M stock solution. The phosphonates were diluted to 0.67 mM in Hanks' buffer just before use, and then an equal volume of inhibitor and cells were added, the mixture was immediately placed in the upper compartment of the chemotaxis chamber after the chemotactic agent had been added in the lower compartment. The chamber was then placed at 37°C as rapidly as possible. Cells suspended in Hanks' buffer containing 0.33% acetone were used as controls. Previous work had shown that 0.5% acetone had no effect on the chemotactic responsiveness of polymorphonuclear leukocytes to C567 (1) and preliminary work confirmed an absence of an effect of 0.5% acetone on the responsiveness of the cells to C3a, C5a, and bacterial factor.

Chemotactic Factors.—The C3a and C5a were prepared by the digestion of highly purified human C3 and C5 with twice-crystallized trypsin (6). The trypsin was 4% by weight of the C3 and 2% by weight of the C5. In each case, the complement components were incubated with the trypsin for 15 min at room temperature. The reaction was stopped by the addition of a four times greater weight than the added trypsin of crystalline soybean trypsin inhibitor. The amount of C3a or C5a employed in any experiment is reported in terms of the amount of C3 or C5, respectively, used in preparation of the given fragment. The control was C3a or C5 incubated for 15 min to which trypsin was added after the soybean trypsin inhibitor.

The bacterial factor was a crude culture filtrate of *Escherichia coli* prepared as described in reference 7.

The C567 was a euglobulin preparation from human serum activated by sensitized sheep erythrocyte stromata containing the first five components of rabbit complement as described in reference 8. The nonactivated C567 was simply the same euglobulin which had not incubated with sensitized sheep stromata.

Polymorphonuclear Leukocytes.—The rabbit peritoneal polymorphonuclear leukocytes were obtained in primed animals by the injection of 0.5–1 liter of 0.02% glycogen in sterile saline. The peritoneal exudate was obtained from the rabbits 12 hr later. The polymorphonuclear leukocytes made up 85–95% of the cells of the exudate.

² Abbreviations used in this paper: BSA, bovine serum albumin; TGS, 0.02 M Tris, pH 7.3, in 0.15 M saline with 0.1% added gelatin.

Cells for esterase assay were obtained by bleeding the rabbit from the central ear artery into 1/40 mg the volume of 0.15 M ethylenediaminetetraacetate (EDTA), pH 7.3. The white cells were largely freed from the red cells by mixing 1 volume of blood with 1 volume of 2% gelatin in 0.15 M saline. The mixture was incubated at 37°C for 45 min, and the supernatant drawn off and centrifuged at room temperature for 10 min at 600 g. The cells were then suspended in 5 ml of Medium 199 and treated at room temperature for 15 min with *p*-nitrophenyl ethyl 5-chloropentylphosphonate at a final concentration of 5×10^{-5} M in order to destroy all of the already activated esterase 1 and esterase 2 (2, 3). They were then washed twice with 200 ml of TGS and suspended in Medium 199 containing 0.1% bovine serum albumin at a concentration of 2×10^7 polymorphonuclear leukocytes/ml.

The leukocyte suspension contained variable numbers of red cells; the white cells were in the same proportions as in the original blood. Extensive work reported previously (4) showed the esterase activity was attributable only to the polymorphonuclear leukocytes of the mixture so no further purification was attempted.

Chemotactic Assay.—This was performed as described previously (1).

Esterase Assay.—The assay for esterase activity was somewhat modified from that described previously (4). To 0.1 ml of cells containing 2×10^6 polymorphonuclear leukocytes obtained from the blood was added 0.4 ml of substrate (8 mg of acetyl DL-phenylalanine β -naphthyl ester (Schwarz Bio Research Inc., Orangeburg, N.Y.) dissolved in 3 ml dimethylformamide and 6 ml of acetone and diluted 1:10 in TGS). The mixture was incubated at 37°C in a shaking water bath. After 45 min the reaction was stopped by the addition of 1 ml of acetone. The precipitated protein and cellular debris were centrifuged off and to the acetone supernatant was added 0.1 ml of diazonium coupling reagent (11.5 ml fast scarlet in 5 ml of water). Any turbidity which developed was removed by centrifugation. The orange-red color which formed as a product of the coupling of the liberated β -naphthol with the diazonium salt was read at 485 nm in a Gilford 300-N spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, Ohio).

RESULTS

Phosphonate Inhibition.—The results of studies of the chemotactic factor-dependent inhibition by the series of *p*-nitrophenyl ethyl chloroalkylphosphonates and *p*-nitrophenyl ethyl alkylphosphonates are seen in Fig. 1. As is evident from Fig. 1, when either C3a, C5a, or bacterial factor are used as chemotactic agents, the peak inhibitory activity in the chloroalkylphosphonate series is shown by the *p*-nitrophenyl ethyl 4-chlorobutylphosphonate and by the *p*-nitrophenyl ethyl butylphosphonate in the alkylphosphonate series. Two other experiments not shown here carried out with both C5a and bacterial factor as the chemotactic agents gave the same results as seen in Fig. 1.

The structure activity relationships (inhibition profiles) seen in Fig. 1 are the same as found when these same series of phosphonates are used to inhibit the chemotactic response to C $\overline{567}$ (1). Such inhibition profiles have been shown to be due to the inhibition of the activatable esterase of chemotaxis (proesterase 1) (1-4).

Activation of Proesterase 1.—Numerous attempts failed to demonstrate an increase in acetyl DL-phenylalanine β -naphthyl esterase activity of peripheral blood polymorphonuclear leukocytes after incubation with varying dilutions of bacterial factor. In these experiments, 0.1 ml of blood leukocytes treated with phosphonate as described, and containing 2.0×10^6 polymorphonuclear leuko-

cytes were incubated at room temperature with varying dilutions of either bacterial factor, C567, or the nonactivated C567. At the end of 20 min, 2 ml of ice-cold Medium 199 with 0.1% BSA was added, the tubes were centrifuged in the cold and the supernatant discarded. The cells were resuspended in 0.1 ml of Medium 199 containing 0.1% BSA and then 0.4 ml substrate was added and the assay for esterase conducted as described.

Table I shows the results of two such experiments in which the dilutions of bacterial factor were varied over a 160-fold range. In no instance was there any evidence of esterase activation by the bacterial factor. In neither instance could this be attributed to a lack of proesterase 1 in the cells used or an inherent inabil-

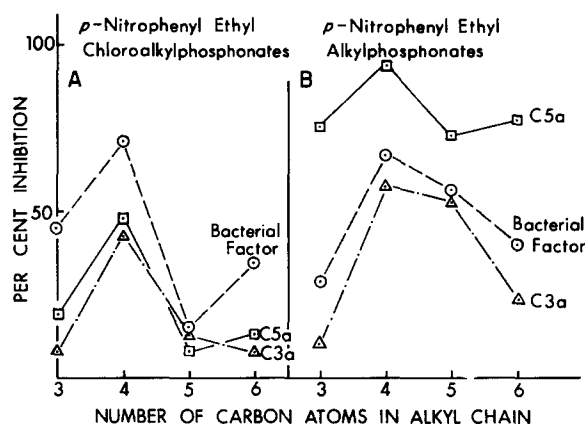


FIG. 1. Profiles of chemotactic factor-dependent inhibition by *p*-nitrophenyl ethyl alkylphosphonates and *p*-nitrophenyl ethyl chloroalkylphosphonates using either C3a (200 $\mu\text{g}/\text{ml}$), C5a (10 $\mu\text{g}/\text{ml}$), or bacterial factor (1/100) as the chemotactic stimulant.

ity of the proesterase to be activated. C567 used at a concentration previously found to give chemotactic deactivation gave significant esterase activation (Table I) of the same cells and under the same circumstances where the bacterial factor failed to give demonstrable activation

In the experiments of Table I, although testing of the bacterial factor for its ability to activate proesterase 1 of the leukocyte was done over a wide range of concentrations, only one time and temperature of incubation, 20 min and room temperature, respectively, were employed. It was therefore considered possible that the use of other time intervals or other temperatures of incubation might give different results. To test this possibility, experiments were carried out as described for the studies recorded in Table I except that 0.2 ml volumes of buffer of 1:20 or 1:100 dilutions of bacterial factor added to 0.1 ml of rabbit blood polymorphonuclear leukocytes ($2 \times 10^7/\text{ml}$ polymorphonuclear leukocytes) were incubated for either 5, 20, 40, or 60 min at room temperature or for 20 min at 37°C, 30°C, or room temperature.

As seen in Table II, varying the time or temperature of incubation of bacterial factor with cells still did not result in any demonstrable activation of proesterase 1.

The same experiments employing C5a as the activating agent (Table III) gave distinctly different results than when bacterial factor was used. The experiments were carried out essentially as described using the bacterial factor. However, since we had previously shown with C567 that the activation of proesterase 1 was demonstrable only when the extent of chemotactic deactivation was 50% or greater (4), it was felt to be of interest to see if this same relationship held

TABLE I
Comparison of Esterase Activation by C567 and by Bacterial Factor

Experiment	Substance added to cells	Esterase activity OD at 485 nm \pm SD	Δ OD
A	0.4 ml buffer	0.307 \pm 0.022 (N = 12)	
	0.4 ml C567	0.345 \pm 0.0035 (N = 2)	0.038*
	0.4 ml C567	0.317 \pm 0.017 (N = 2)	0.010
	0.4 ml bacterial factor 1:5	0.283 \pm 0.00424 (N = 2)	-0.024
	0.1 ml " " 1:5	0.317 \pm 0.013 (N = 2)	0.010
	0.4 ml " " 1:100	0.289 \pm 0.023 (N = 2)	-0.018
B	0.4 ml buffer	0.249 \pm 0.0205 (N = 12)	
	0.4 ml C567	0.308 \pm 0.016 (N = 2)	0.059‡
	0.4 ml C567	0.260 \pm 0.064 (N = 2)	0.011
	0.4 ml bacterial factor 1:100	0.241 \pm 0.0028 (N = 2)	-0.008
	0.2 ml " " "	0.234 \pm 0.025 (N = 2)	-0.015
	0.1 ml " " "	0.255 \pm 0.028 (N = 2)	0.006
	0.05 ml " " "	0.247 \pm 0.0071 (N = 2)	-0.002

* Difference is statistically significant, $P < 0.05$.

‡ Difference is statistically highly significant, $P < 0.01$.

when activation was attempted with a complement-derived factor other than C567. Therefore, the procedure was slightly modified so that the cells could be tested both for proesterase activation and chemotactic deactivation. For this purpose, 0.2 ml of peripheral blood leukocytes containing 4×10^6 polymorphonuclear leukocytes were incubated for 20 min with the agents given in Table III. After the cells were washed they were suspended in 0.2 ml of Medium 199 with added 0.1% BSA. Of the 0.2 ml of cell suspension, 0.1 ml was employed for the assay of residual chemotactic responsiveness (deactivation) using a 1:100 dilution of bacterial factor and the other 0.1 ml was tested for esterase activity.

As is evident from Table III, significant esterase activation was achieved by C5a in both experiments. The activation was specific; equivalent amounts of C5 from which the C5a was produced failed to activate. Those cells which showed

significant esterase activation also showed concomitant deactivation. As mentioned, in previous work it had been found that unless the C567 deactivated the cells to the extent of 50% or more, it was not possible to discern significant activation of esterase (4). The results of Table III confirm this. Incubation with 50 μg equivalent of C5a resulted in no deactivation (experiment A) and no significant degree of esterase activation was found. However, 100 μg equivalent

TABLE II

Effect of Variations in the Time and Temperature of Incubation of Bacterial Factor with Rabbit Blood Polymorphonuclear Leukocytes on the Activation of Proesterase I

Experi- mental	Time of incuba- tion		Esterase activity \pm SD OD 485 nm
	<i>min</i>		
A	5	0.2 ml buffer	0.145 \pm 0.017 (N = 4)
		0.2 ml 1:100 bacterial factor	0.125 \pm 0.018 (N = 2)
		0.2 ml 1:20 " "	0.153 \pm 0.0014 (N = 2)
	20	0.2 ml buffer	0.162 \pm 0.011 (N = 4)
		0.2 ml 1:100 bacterial factor	0.147 \pm 0.000 (N = 2)
		0.2 ml 1:20 " "	0.163 \pm 0.019 (N = 2)
	40	0.2 ml buffer	0.153 \pm 0.015 (N = 4)
		0.2 ml 1:100 bacterial factor	0.145 \pm 0.020 (N = 2)
		0.2 ml 1:20 " "	0.153 \pm 0.025 (N = 2)
	60	0.2 ml buffer	0.177 \pm 0.024 (N = 4)
		0.2 ml 1:100 bacterial factor	0.172 \pm 0.006 (N = 2)
		0.2 ml 1:20 " "	0.174 \pm 0.007 (N = 2)
	<u>Temperature of incubation</u>		
B	Room tem- perature	0.2 ml buffer	0.262 \pm 0.019 (N = 4)
		0.2 ml 1:100 bacterial factor	0.264 \pm 0.006 (N = 2)
		0.2 ml 1:20 " "	0.264 \pm 0.020 (N = 2)
	30°C	0.2 ml buffer	0.282 \pm 0.028 (N = 4)
		0.2 ml 1:100 bacterial factor	0.263 \pm 0.006 (N = 2)
	37°C	0.2 ml 1:20 " "	0.304 \pm 0.039 (N = 2)
		0.2 ml buffer	0.295 \pm 0.054 (N = 4)
		0.2 ml 1:100 bacterial factor	0.280 \pm 0.034 (N = 2)
		0.2 ml 1:20 " "	0.248 \pm 0.019 (N = 2)

of C5a produced cells which were 57% deactivated and showed significant esterase activation. In experiment B, incubation of the cells with 100 μg equivalent of C5a resulted in only 35% deactivation and no significant proesterase activation, whereas, 200 μg equivalent of C5a gave cells which were 62% deactivated and exhibited significant proesterase activation. Table III also shows the sort of variation that was seen from one experiment to another. The cells used in experiment A required only half the amount of C5a to give the same degree of deactivation and esterase activation as was necessary with the cells of experiment B.

In another experiment not shown here, 200 μg equivalent of C5a gave no deactivation and no esterase activation. The reason for this variability is unknown, but was also seen previously when C567 was the chemotactic agent used (4).

C3a was also tested for its ability to give activation of proesterase 1 by adding varying volumes of C3a, C3, or buffer to 0.1 ml of 2×10^6 peripheral blood polymorphonuclear leukocytes in Medium 199 containing 0.1% BSA and incubating for 20 min at room temperature. The cells were then treated, centrifuged, and tested for esterase activation as described. From the results seen in Table IV it

TABLE III
Activation of Proesterase 1 and Chemotactic Deactivation by C5a

Experiment	Substance added to cells	Esterase activity (OD at 485 nm \pm SD)	Δ OD	Chemotactic activity (cells/3HPP)	Deactivation§
A	Buffer	0.249 \pm 0.0084 (N = 6)		109	%
	50 μg C5a*	0.248 \pm 0.015 (N = 2)	-0.001	116	0
	50 μg C5	0.265 \pm 0.013 (N = 2)	0.017	137	0
	100 μg C5a	0.284 \pm 0.0085 (N = 2)	0.035†	47	57
	100 μg C5	0.247 \pm 0.0042 (N = 2)	-0.002	129	0
B	Buffer	0.264 \pm 0.0075 (N = 6)		110	
	100 μg C5a	0.272 \pm 0.016 (N = 2)	0.008	71	35
	100 μg C5	0.246 \pm 0.012 (N = 2)	-0.018	135	0
	200 μg C5a	0.295 \pm 0.023 (N = 2)	0.031†	42	62
	200 μg C5	0.260 \pm 0.011 (N = 2)	-0.004	113	0

* The amount of C5a is given in terms of the amount of C5 from which the C5a was produced.

† Difference from mean esterase activity of buffer control is statistically significant, $P < 0.01$.

(chemotactic activity of control cells - chemotactic activity of treated cells)

§ Per cent deactivation = $100 \frac{\text{chemotactic activity of control cells} - \text{chemotactic activity of treated cells}}{\text{chemotactic activity of control cells}}$.

is evident that a sufficiently high concentration of C3a induced significant activation of proesterase 1.

The Chemotactic Activity of C567, C5a, and C3a Relative to Bacterial Factor.—The proesterase 1 content of rabbit peripheral blood polymorphonuclear leukocytes is higher than that of the corresponding peritoneal leukocytes and this is associated with a higher chemotactic activity of the blood leukocytes compared to the peritoneal cells (4). On this basis, if the bacterial factor activates less proesterase 1 than does C3a, C5a, or C567 as suggested by the results described so far, it also might give less maximal chemotactic activity than the three complement-derived chemotactic factors. To explore this possibility, rabbit peritoneal polymorphonuclear leukocytes were suspended in 0.1% BSA in Hanks'

buffer at a concentration of 3.0×10^6 /ml and tested for chemotactic activity in the usual way with varying concentrations of C3a, C5a, C567, and bacterial factor. The results are seen in Figs. 2 and 3.

The plateau in chemotactic activity from a $1/20$ dilution of bacterial factor to a $1/100$ dilution evident in Figs. 2 and 3 has been seen repeatedly, although the level of maximum activity attained varies from experiment to experiment.

TABLE IV
Activation of Proesterase 1 by C3a

Substance added to cells	Esterase activity (OD at 485 nm \pm sd)	Δ OD
Buffer	0.188 \pm 0.0165	
160 μ g C3a*	0.184 \pm 0.012	0
160 μ g C3	0.174 \pm 0.0078	
500 μ g C3a	0.198 \pm 0.0014	0.010
500 μ g C3	0.182 \pm 0.0084	
1000 μ g C3a	0.215 \pm 0.0063	0.027 \ddagger
1000 μ g C3	0.172 \pm 0.0019	

*The amount of C3a is given in terms of the amount of C3 from which the C3a was produced.

\ddagger Difference from mean esterase activity of buffer control is statistically significant, $P < 0.05$.

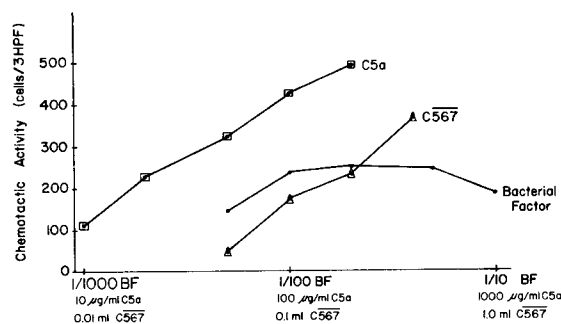


FIG. 2. Comparison of the chemotactic activity of C5a, C567, and bacterial factor (BF).

Whether the inhibition at higher concentrations of bacterial factor is due to an impurity present in the crude culture filtrate which is the source of the bacterial factor is not known. However, Dr. P. A. Ward in a personal communication has stated that he has found the same inhibition using highly purified factor suggesting it may be an intrinsic property of the factor. With either C5a or C567 (Fig. 2) no such plateau of inhibition was evident. In view of the purpose of the experiments, the most important point, however, is that the maximum activity attainable by the bacterial factor is distinctly less than the maximal activity attained by either C567 or C5a (Fig. 2). The same results were obtained

in another experiment not shown here, in which the chemotactic activity of bacterial factor and C5a were compared. Here also a plateau of chemotactic activity was evident with the bacterial factor; however, in this experiment the activity given by 100 μg equivalent of C5 was distinctly greater than that given by 200 μg equivalent of C5a (496 cells/3HPF compared to 237 cells/3HPF). Nevertheless, the maximal activity given by the bacterial factor was even less, 183 cells/3HPF.

The chemotactic activity of C3a was compared in the same way with that of the bacterial factor with the same results. As seen in Fig. 3, the maximum activity of the bacterial factor was reached between a $1/20$ and $1/100$ dilution.

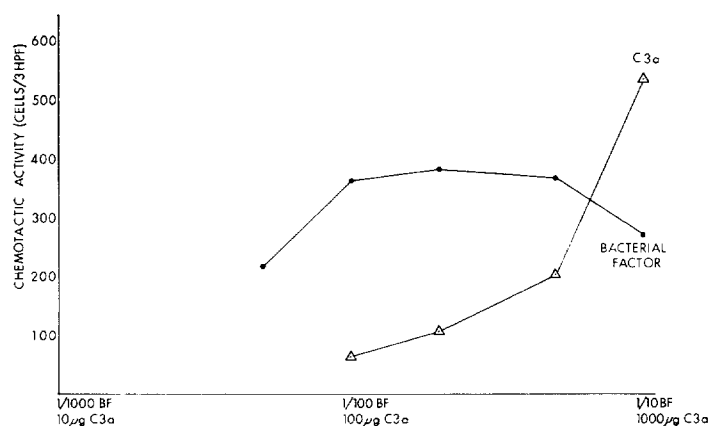


FIG. 3. Comparison of the chemotactic activity of C3a and bacterial factor (BF).

This activity was distinctly less than that reached by 1000 $\mu\text{g}/\text{ml}$ equivalent of C3a, the highest concentration of C3a tested. The larger amounts of C3a than C5a (expressed in amounts of the complement component used to produce the fragment) required to give chemotactic activity greater than the maximal activity of the bacterial factor (Fig. 2 compared with Fig. 3) mirrors the larger amounts of C3a required to activate proesterase 1 (Table III compared with Table IV).

DISCUSSION

The profiles of inhibition by the *p*-nitrophenyl ethyl alkylphosphonates and the *p*-nitrophenyl ethyl chloroalkylphosphonates when C3a, C5a, and the bacterial factor are used as the chemotactic agents are the same as those found when chemotaxis is induced by C567 (1), or in the prevention of deactivation by C567 (2) or in the inhibition of esterase 1 (3, 4). The essential identity of the inhibition profiles given by the two series of phosphonates under all of these circumstances implies that like the chemotactic response to C567, chemotaxis induced by C3a,

C5a, or bacterial factor involves, as a necessary step, an activation of proesterase 1. The finding that C5a and C3a activate proesterase 1, as measured by the increase of acetyl DL-phenylalanine β -naphthyl esterase activity (Tables III and IV), is direct confirmation of this conclusion as far as C3a and C5a are concerned. However, this conclusion is seemingly contradicted when bacterial factor is employed since under no circumstance could activation of proesterase 1 be demonstrated by direct measurement of acetyl DL-phenylalanine β -naphthyl esterase activity using bacterial factor as the stimulus (Tables I and II).

The hypothesis best explaining this seeming contradiction is that the activation of proesterase 1 of the polymorphonuclear leukocyte is an essential step in the chemotactic response to all the chemotactic agents, C567, C5a, C3a, and the bacterial factor. However, there are quantitative differences in the ability of these substances to activate proesterase 1. The bacterial factor, for reasons unknown, activates a much smaller portion of the proesterase 1 of the cell than do the complement-derived factors. The amount activated by the bacterial factor is sufficient for the cell to respond chemotactically but insufficient to be detectable by direct biochemical assay.

This hypothesis not only explains the observations recorded here but also explains the observations of Ward on the inability of bacterial factor to deactivate to itself or the other complement-derived factors, although the complement-derived factors can deactivate to themselves, to each other, and to the bacterial factor (5). As already demonstrated, activation of proesterase 1 is a necessary step in deactivation (2). Since, by hypothesis, bacterial factor activates only a very small proportion of proesterase 1 when incubated with the leukocyte, it always leaves enough proesterase 1 so that sufficient is available for a chemotactic response to either fresh bacterial factor, or to one or another of the complement-derived factors. Thus, it neither deactivates to itself nor to the other factors. Yet, the activation of proesterase 1 is a required step in the chemotactic response of the cell to bacterial factor as well as to the complement-derived factors (Fig. 1 and reference 1). If sufficient proesterase 1 is used up by prior contact with one or another of the complement-derived factors, the cell will not have sufficient proesterase 1 to be activated by either the bacterial factors or the other factors. Thus, the cell deactivated to one of the complement-derived factors will not only be deactivated to itself and to the other factors, but to the bacterial factor as well.

The quantitative difference in the response of the proesterase 1 of blood polymorphonuclear leukocyte to the bacterial factor and to the other factors is analogous to the difference in the response of the proesterase 1 of the blood and peritoneal polymorphonuclear leukocyte to C567. The chemotactic response of the peritoneal leukocyte to C567 operates through activation of proesterase 1 as shown by the inhibition profiles with the phosphonate esters (1). Yet, no direct biochemical activation of proesterase 1 by measurement of acetyl DL-phenyl-

alanine β -naphthyl esterase activity can be demonstrated with these cells; it is necessary to test the peripheral blood leukocytes in order to show activation by direct biochemical measurement. Although the two situations are analogous they are not identical. The bacterial factor activates only a small proportion of the proesterase 1 on the cell whether it is a peripheral blood leukocyte or a peritoneal leukocyte. C567, on the other hand, presumably activates a high proportion of the proesterase on the cells from the two sources, but the peritoneal cell has distinctly less proesterase 1 than the peripheral blood leukocyte.

As shown previously, the presence of a larger amount of proesterase 1 on the blood polymorphonuclear leukocyte than on the peritoneal leukocyte that is capable of being activated is associated with a greater degree of chemotactic activity of the former compared with the latter (4). This association is also present when the chemotactic activity of bacterial factor and of C3a, C5a, and C567 are compared (Tables I, III, and IV and Figs. 2 and 3). As is evident from Figs. 2 and 3, the maximal chemotactic activity attainable with bacterial factor is distinctly less than that obtained with any of the three complement-derived factors. Why there should be inhibition at the higher concentrations of bacterial factor, whether it is due to an inhibitor present in the crude culture filtrate or is an intrinsic property of the bacterial factor, is not known. The results obtained here suggest that, whatever the reason, the lower maximal activity of the bacterial factor compared with the activity attainable by C3a, C5a, or C567 is due to the lesser degree of activation of proesterase 1, induced by the bacterial factor. Thus, the results obtained in this work not only indicate that the activation of proesterase 1 is a general requirement for chemotactic activity with the known macromolecular agents, but suggest that under several different circumstances the level of chemotactic activity attained is related to the degree of such activation.

SUMMARY AND CONCLUSIONS

The inhibition profiles obtained when a series of *p*-nitrophenyl ethyl alkylphosphonates and of *p*-nitrophenyl ethyl chloroalkylphosphonates were used to interfere with the chemotactic activity of polymorphonuclear leukocytes stimulated by C3a, C5a, and bacterial factor were the same as found previously when C567 was the chemotactic agent. This indicates that as in the chemotactic activity induced by C567, an obligatory step in the chemotaxis caused by C3a, C5a, and bacterial factor is the activation of proesterase 1 of the rabbit polymorphonuclear leukocyte.

C5a and C3a activate proesterase 1 of peripheral blood polymorphonuclear leukocytes as measured by the increase of acetyl DL-phenylalanine β -naphthyl esterase activity. Attempts to detect in a like manner the proesterase 1 of the same leukocytes using bacterial factor under varying circumstances have consistently failed. It is concluded that bacterial factor, for unknown reasons, is

unable to activate proesterase 1 to the same extent as the complement-derived chemotactic factors.

The hypothesis of there being a quantitative difference in the ability of bacterial factor to activate proesterase 1 compared with the complement-derived factors explains the previous observations that bacterial factor can not deactivate to itself or to the complement-derived factors, although these latter factors can deactivate to themselves, to each other, and to the bacterial factor.

The quantitative difference in the ability of bacterial factor to activate proesterase 1 compared to the complement-derived factors is also associated with and explains the finding that the maximal chemotactic activity attainable when bacterial factor is the chemotactic agent is distinctly less than that obtained using either C3a, C5a, or C567.

These results indicate that the activation of proesterase 1 is a general requirement for the chemotactic activity of rabbit polymorphonuclear leukocytes with known macromolecular chemotactic agents and suggest that under several different circumstances the level of chemotactic activity attained is related to the degree of such activation.

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