

Interaction of Leukocyte Chemotactic Factors with the Cell Surface

I. CHEMOTACTIC FACTOR-INDUCED CHANGES IN HUMAN GRANULOCYTE SURFACE CHARGE

JOHN I. GALLIN, JOHN R. DUROCHER, and ALLEN P. KAPLAN

From the Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014, and the Division of Hematology, Walter Reed Army Institute of Research, Washington, District of Columbia 20012

ABSTRACT The negative surface charge of human granulocytes was diminished after incubation with the chemotactic factors C5a, dialyzable transfer factor, and the enzymes kallikrein and plasminogen activator. No such change was observed after incubation with human IgG, albumin, horseradish peroxidase, or a mixture of prekallikrein and plasminogen proactivator. Hydrocortisone inhibited the effect of C5a upon granulocyte surface charge and inhibited its chemotactic activity, suggesting that steroids act at the cell surface. The chemotactic inhibitors colchicine and cytochalasin B had no effect upon granulocyte surface charge, consistent with their presumed effect upon microtubules and microfilaments, respectively. The data suggest that the decrease in cell surface charge may be a prerequisite for normal cell movement.

INTRODUCTION

Considerable knowledge has been obtained in recent years concerning the identification of chemotactic factors capable of stimulating directional motion in leukocytes. However, little is known about how these chemotactic factors interact with the leukocyte to convert a chemical signal into directed mechanical work. It is generally agreed that leukocyte adhesiveness as well as mobilization of mechanical work elements (microtubules and microfilaments) are critical for the chemotactic process. Studies of the amebae and fibroblasts have shown that cell adhesiveness is dependent upon the cell surface charge (1, 2) and that a decrease in negative surface charge is associated with cell contrac-

tion while an increase in surface charge is correlated with cell expansion (2). The data presented in this report document that exposure of human granulocytes to three different chemotactic stimuli, including C5a (3), dialyzable transfer factor (4), and a mixture of kallikrein and plasminogen activator (5, 6) is associated with a decrease in the net cell surface charge. Concentrations of hydrocortisone that inhibit chemotaxis were found to block the C5a-induced change in surface charge, while cytochalasin B and colchicine, which also inhibit chemotaxis, have no effect upon the net cell surface charge.

METHODS

E. coli 0127: B8 lipopolysaccharide B (Difco Laboratories, Detroit, Mich.); ⁵¹Cr (Amersham/Searle Corp., Arlington Heights, Ill.); 5- μ m micropore filters (Millipore Corp., Bedford, Mass.); Gey's balanced salt solution (Microbiological Associates, Bethesda, Md.); hydrocortisone sodium succinate (preservative-free) (Upjohn Co., Kalamazoo, Mich.); colchicine (Sigma Chemical Co., St. Louis, Mo.); cytochalasin-B (Imperial Chemical Industries, Ltd., Pharmaceuticals Division, Macclesfield, Cheshire, England); dimethyl sulfoxide (DMSO) (Fisher Scientific Co., Silver Spring, Md.); bradykinin triacetate (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.); antisera to whole human serum, IgG, albumin, and B₂ glycoprotein I, (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.); goat antiserum to the fifth (C5) component of human complement (Meloy Laboratories, Inc., Springfield, Va.); enzodiffusion fibrin plates and streptokinase (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.); hexadimethrine bromide (Aldrich Chemical Co., Inc., Milwaukee, Wis.); crystallized human albumin (Miles Laboratories, Inc., Kankakee, Ill.); and horseradish peroxidase and pancreatic deoxyribonuclease (Worthington Biochemical Corp., Freehold, N. J.) were obtained as indicated.

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Preparation and assay of chemotactic factors

Chromatography on QAE¹ Sephadex (5), SP Sephadex (5) Sephadex G-150 (7), Sephadex G-100 (5), and alkaline disk gel electrophoresis (5) were performed as previously described. Kallikrein and plasminogen activator were concentrated by ultrafiltration through a UM-10 membrane (Amicon Corp. Lexington, Mass.) and C5a was concentrated with a UM-2 membrane. Dialyzable transfer factor was concentrated by lyophilization. Protein quantitation by weight was performed by Lowry, Rosebrough, Farr, and Randall (8) or micro-Kjeldahl analysis (9).

Kallikrein and plasminogen activator. A mixture of the chemotactic enzymes kallikrein and plasminogen activator were obtained as follows: 500 ml of human plasma was collected and sequentially fractionated by chromatography on QAE Sephadex, SP Sephadex, and Sephadex G-150 as previously described (7) to prepare the proenzymes prekallikrein and plasminogen proactivator. Each proenzyme contributed about half the total observed chemotactic activity. The Sephadex G-150 concentrate was then passed over an anti-IgG immunoabsorbant (10), before passage over an immunoabsorbant against B₂ glycoprotein I. A single band was observed upon alkaline disk gel electrophoresis at pH 9.3, and two bands were seen after sodium dodecyl sulfate (SDS) gel electrophoresis or isoelectric focusing in acrylamide gels representing each proenzyme. IgG utilized as a control in the chemotactic studies was purified from the Hageman factor substrates and B₂ glycoprotein I by concentrating the IgG peak from SP Sephadex and fractionating it on Sephadex G-150. The protein concentration of the prekallikrein-plasminogen proactivator mixture was set at 50 µg/ml and then activated by addition of 10% (vol/vol) purified Hageman factor fragments (25 µg/ml) and incubated for 1 h at 37°C and then overnight at 4°C.

Kallikrein was determined by incubation of 0.1 ml enzyme source with 0.2 ml heat-inactivated plasma, prepared as previously described (11) for 2 min at 37°C, and the bradykinin generated was determined by bioassay utilizing the isolated guinea pig ileum (11). Prekallikrein was assessed by incubation of 0.1 ml prekallikrein source with 0.1 ml Hageman factor fragments (25 µg/ml) for 5 min at 37°C. 0.2 ml heat-inactivated plasma was then added, the mixture incubated for 2 min at 37°C, and the bradykinin generated determined. Plasminogen activator¹ was assayed by incubating 20 µl of plasminogen activator source with 20 µl plasminogen (200 µg/ml) for 1 h at 37°C and the plasmin generated determined by a fibrin plate assay (10). Plasminogen proactivator was assayed by incubation of 10 µl plasminogen proactivator source with 10 µl Hageman factor fragments (25 µg/ml) for 10 min at 37°C. 20 µl plasminogen (200 µg/ml) was added, the mixture was incubated for 1 h at 37°C, and the plasmin generated determined.

Plasminogen was prepared by affinity chromatography of 100 µl of plasma with a lysine-Sepharose column and epsilon-aminocaproic acid elution, as described by Deutsch and Mertz (12). The plasminogen was then concentrated, dialyzed overnight at 4°C against 0.003 M PO₄ buffer, pH 8.0, containing 0.15 M NaCl, and further fractionated by Sephadex G-100 gel filtration (10). The plasminogen peak was pooled, concentrated, and adjusted to 200 µg/ml for routine

¹Abbreviations used in this paper: DMSO, dimethyl sulfoxide; LF, lower filter; QAE, quaternary amino ethyl; SDS, sodium dodecyl sulfate; SP, sulphopropyl.

use. Plasmin was assayed with Hyland fibrin plates, with a reference preparation of streptokinase-activated plasminogen, as previously described (10). Plasminogen was assayed by incubating 0.2 ml plasminogen source with 140 U streptokinase for 30 min at 37°C and the plasmin generated was determined by the fibrin plate assay.

Hageman factor fragments were purified by chromatography of plasma on QAE Sephadex, followed by rechromatography on QAE Sephadex, and further fractionation on SP Sephadex, Sephadex G-100, and elution from alkaline disk gels after electrophoresis at pH 9.3, as previously described (11, 13). After extensive dialysis against 0.003 M phosphate buffer, pH 8.0, containing 0.15 M NaCl, the fragments were concentrated to 25 µl/ml for routine use. When assayed functionally, 5 µl Hageman factor fragments generated 100 ng bradykinin after incubation with 0.2 ml fresh plasma for 2 min at 37°C.

C5a. The cleavage product of the 5th component of complement, C5a, was partially purified by fractionating 5 ml of endotoxin activated human serum (14) on a 5 × 100 column of Sephadex G-150. The major chemotactic peak eluted at 67% bed volume, corresponding to a mol wt of 15,500, and was heat-stable at 56°C, and the chemotactic activity was completely inhibited by goat antisera to human C5 (15). This peak was pooled and utilized at a protein concentration of 15 µg/ml (8). When examined at a protein concentration of 100 µg/ml, the major contaminant observed by immunoelectrophoresis against anti-whole human serum and alkaline disk gel electrophoresis was albumin. With a radial immunodiffusion assay to quantitate the albumin, it was estimated that 12–15% of the total protein content was C5a.

Transfer factor. A partially purified preparation of transfer factor was obtained by disruption of 3 × 10⁸ small lymphocytes by freezing and thawing, digestion with pancreatic deoxyribonuclease, and dialysis against pyrogen-free glass-distilled water for 48 h at 4°C (16). The dialysate was then fractionated by Sephadex G-25 gel filtration, as previously reported (4), and the column fractions containing both chemotactic factor and transfer factor activity were used. The percentage contamination of this preparation with other proteins or nucleic acids is unknown.

Granulocyte chemotaxis and determination of cell surface charge

Granulocyte-rich leukocyte preparations were obtained by dextran sedimentation of heparinized human blood from healthy subjects (14). Residual erythrocytes were removed by two cycles of hypotonic saline lysis and the resulting leukocytes (85% granulocytes, 15% mononuclear cells) were washed twice in modified Hanks' balanced salt solution (14). All experiments were completed within 5 h of obtaining the leukocytes.

Granulocyte chemotaxis. Granulocyte chemotaxis was evaluated by a chromium-51 radioassay, previously described (17). For this assay ⁵¹Cr-labeled leukocytes are placed in the upper compartment of a modified Boyden chamber, separated from a lower chamber by two 5 µm/Micropore filters. The ⁵¹Cr-labeled cells are suspended at a concentration of 2.3 × 10⁶ granulocytes/ml in Gey's balanced salt solution containing 2% bovine serum albumin, penicillin, and streptomycin. The chemotactic stimulus is added to the lower compartment. It has previously been shown that under these conditions only granulocytes migrate into the lower of the two filters and that the number of granulocytes entering the lower filter is proportional to the radioactivity incorpo-

rated into the lower filter (17). After adjusting for variability in specific activity and incorporation of the ^{51}Cr by the granulocytes, chemotaxis is expressed as corrected counts per minute in the lower filter (cor cpm LF). The chemotactic response for each experimental condition was the average of four chambers and mean of different experiments were compared with Student's *t* test.

Measurement of granulocyte surface charge. The surface charge of human leukocytes was measured by an electrophoretic mobility technique, previously described (18). For this method dextran-sedimented leukocytes were obtained as described above, washed thrice in modified Hanks' solution and once in 0.13 mM phosphate-buffered (pH 7.2) 5% sorbitol, and then exposed in suspension to the chemotactic stimulus for 1 h at 37°C at a concentration of 5×10^6 granulocytes/ml. The cells were then washed twice in the phosphate-sorbitol buffer and their electrophoretic mobility was determined with a Zeiss cytophorometer fitted with platinum electrodes (Carl Zeiss, Inc., New York). All determinations were made within five h after collecting the cells, at which time they excluded trypan blue dye, were capable of responding to a chemotactic stimulus, and were therefore considered viable. By this technique, the morphologic differences between granulocytes and small lymphocytes can be readily distinguished. All measurements were made in the frontal plane at 23°C. For each experimental point 20 determinations were made on 10 different cells, with the second measurement of each cell made after reversal of polarity. Granulocyte surface charge was calculated as previously described and expressed as $\mu\text{M}/\text{s}/\text{V}/\text{cm}$ (18). By this calculation a surface charge of 0 represents neutrality at a given pH, while numbers greater than 0 represent a net negative surface charge and those less than 0 represent a net positive charge.

Effect of inhibitors of chemotaxis upon C5a-induced changes of granulocyte surface charge. The following agents, which inhibit granulocyte chemotaxis, were evaluated for their effects on granulocyte surface charge: hydrocortisone sodium succinate, colchicine, and cytochalasin-B dissolved in 1.5 mg/ml DMSO. The granulocytes were incubated with each inhibitor for 30 min at 37°C, centrifuged, and then exposed to either buffer or C5a for the determination of chemotactic activity or change in granulocyte surface charge, as described above.

Statistical analyses. The means of different chemotactic experiments were compared with Student's *t* test. Surface charge experiments were compared by either the paired sample *t* test analysis of means or Student's *t* test of the differences between the experimental and control values.

RESULTS

The effect of the chemotactic factors, C5a, dialyzable transfer factor, and a mixture of kallikrein and plasminogen activator, upon granulocyte surface charge is shown in Fig. 1. Each chemotactic mixture was tested at a concentration of 15 $\mu\text{l}/\text{ml}$ and compared with the nonchemotactic proteins, human albumin, horseradish peroxidase, and human IgG, at the same concentration. In each individual experiment the granulocyte surface charge after exposure to chemotactic factors or nonchemotactic proteins is compared with the surface charge after exposure to buffer by connecting the mean value of 20 determinations on 10 cells after exposure to each agent. In every experiment with each chemotactic

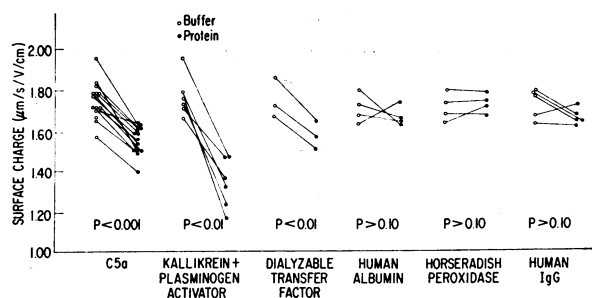


FIGURE 1 Effect of chemotactic factors and nonchemotactic proteins upon granulocyte surface charge. Each data point represents the mean surface charge of 20 determinations upon 10 different cells exposed to buffer control or experimental protein. The lines connect the mean values obtained for each experiment. *P* values represent the significance of differences between values obtained for buffer controls or experimental proteins (paired sample *t* test).

factor, there was a significant reduction in the granulocyte surface charge when compared to the buffer control ($P < 0.05$, Student's *t* test). The variability of absolute surface charge measurements among different cells was greater than the variability from day to day, in the same cell population. However, the percentage of chemotactic factor-induced decrease in granulocyte surface charge with different cell preparations was not significant. Horseradish peroxidase had no effect upon neutrophil surface charge ($P > 0.05$, Student's *t* test); however, human IgG and albumin produced small yet significant decreases in granulocyte surface charge in some experiments, but had either no effect or appeared to increase surface charge in others. When the data are examined collectively, the diminution in surface charge after exposure to each chemotactic factor compared to buffer was highly significant ($P < 0.01$, paired sample *t* test), but was not significant for the three control proteins ($P > 0.10$, paired sample *t* test). Table I shows the statistical comparison of surface charge (buffer minus experimental) of the mean differences observed after exposure to each chemotactic factor compared to each control protein. Significant differences were observed when C5a or the kallikrein-plasminogen activator mixture were compared with each control protein. The differences in the decrease of surface charge of granulocytes exposed to dialyzable transfer factor were significant compared to cells incubated with horseradish peroxidase ($P < 0.05$) but were not significant compared with cells exposed to IgG or human albumin ($P > 0.05$), probably because of the small sample size ($P < 0.10$).

A summary of the data comparing the chemotactic activity and surface charge effects of the above proteins is shown in Fig. 2. C5a, the kallikrein-plasminogen activator mixture, and dialyzable transfer factor each

TABLE I
Specificity of Chemotactic Molecules for Inducing Changes in Granulocyte Surface Charge

Chemotactic factor	Nonchemotactic protein		
	Human albumin	Horseradish peroxidase	IgG
	<i>P values</i>		
C5a	<0.01	<0.01	<0.01
Kallikrein	<0.01	<0.02	<0.01
Dialyzable transfer factor	<0.20	<0.01	<0.10
	>0.10		>0.05

P values, Student's *t* test: comparison of the differences between buffer and experimental for nonchemotactic and chemotactic proteins.

possessed significant chemotactic activity and each diminished the negativity of the cell surface charge when compared with the control proteins, as indicated above. It is noteworthy that dialyzable transfer factor prepared from leukocytes obtained from patients with the Sézary syndrome had no chemotactic activity (17), and also had no effect on granulocyte surface charge.

Demonstration of chemotactic activity and the ability to induce changes in granulocyte surface charge by the conversion of prekallikrein and plasminogen proactivator

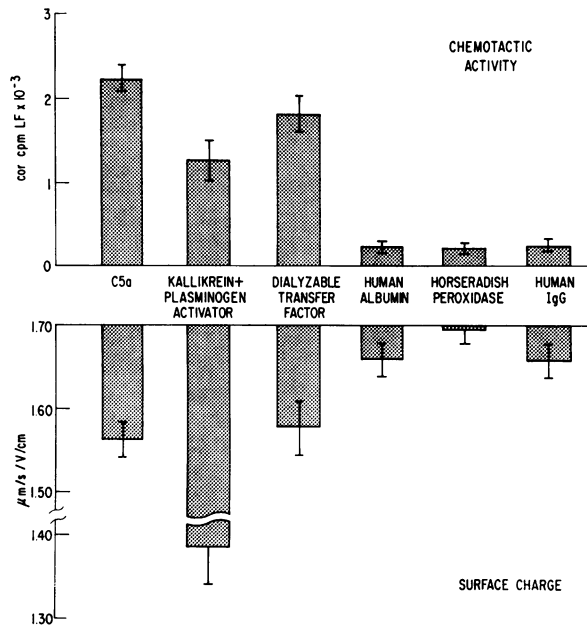


FIGURE 2 Granulocyte chemotactic activity (upper panel) and effect on surface charge (lower panel) of chemotactic factors and nonchemotactic proteins. The bars denote the mean \pm SE of five experiments with each protein for chemotactic activity and the mean \pm SE of the changes in surface charges shown in Fig. 1.

tor to kallikrein and plasminogen activator. A highly purified mixture of prekallikrein and plasminogen proactivator, free of B₂ glycoprotein 1 and IgG, had a small amount of chemotactic activity and induced small decreases in granulocyte surface charge (Table II), reflecting the presence of kallikrein and plasminogen activator in the preparation. Conversion of the prekallikrein-plasminogen proactivator mixture to the active enzymes by incubation with Hageman factor fragments resulted in the generation of significant chemotactic activity, as well as the ability to induce significant reductions in granulocyte surface charge. Hageman factor fragments in buffer had no effect upon granulocyte surface charge.

Kallikrein and plasminogen activator obtained from the SP Sephadex step were partially separated from each other by chromatography on Sephadex G-150 (Fig. 3), and the fractions were tested for chemotactic activity and their ability to modify granulocyte surface charge. As shown in the middle panel of Fig. 3, two peaks of chemotactic activity were noted, corresponding to kallikrein and plasminogen activator. The fractions containing chemotactic activity also produced significant changes in granulocyte surface charge (lower panel, Fig. 3). A small peak of surface charge activity was also noted in fractions containing IgG; however, it did not coincide with the IgG peak and could not be identified.

Granulocyte chemotactic activity and surface charge changes present in G-150 Sephadex fractions of endotoxin-activated serum. Assay of the fractions obtained after Sephadex G-150 gel filtration of unactivated serum revealed minimal chemotactic activity and little change in surface charge (left-hand panels, Fig. 4). However, assay of the fractions obtained after Sephadex G-150

TABLE II
Granulocyte Chemotactic Activity and Surface Charge Changes by Conversion of a Prekallikrein-Plasminogen Proactivator Mixture* to Kallikrein and Plasminogen Activator

Stimulus	Chemotaxis	Surface charge
	cor cpm LF	µm ² /V/cm
Buffer	146 \pm 23	1.80 \pm 0.02
Prekallikrein-plasminogen proactivator	286 \pm 18	1.76 \pm 0.03
Hageman factor fragments + buffer	120 \pm 15	1.83 \pm 0.02
Prekallikrein-plasminogen proactivator + Hageman factor fragments	1,010 \pm 89†	1.66 \pm 0.03‡

* The concentration of prekallikrein and plasminogen proactivator utilized in these experiments was 5 µg/ml.

† Significantly different from prekallikrein + buffer (*P* < 0.01, Student's *t* test).

gel filtration of serum activated with *E. coli* endotoxin (right panel, Fig. 4) resulted in a large peak of chemotactic activity, with an estimated mol wt of 15,500, which was identified as C5a. Chemotactic activity was also detected in fractions 64–74, which contained both

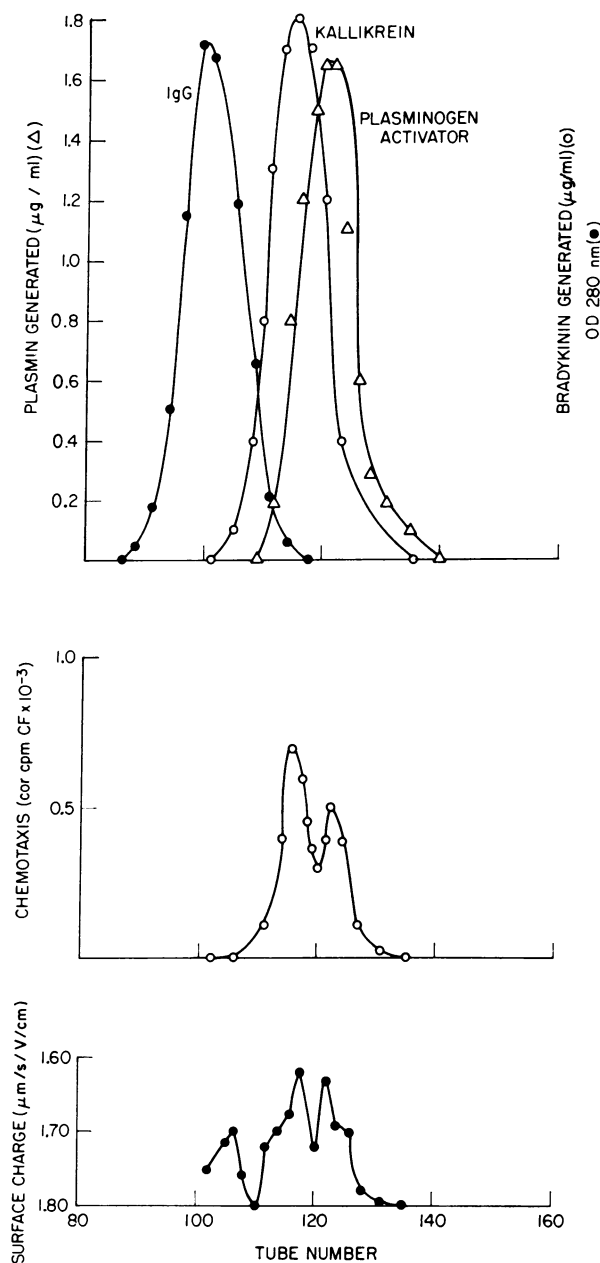


FIGURE 3 Sephadex, G-150 chromatography of a kallikrein and plasminogen activator mixture obtained from SP Sephadex (upper panel). The middle panel contains the assay of these column fractions for chemotactic activity, and the lower panel shows the effect of the various column fractions upon granulocyte surface charge. (Note inverted scale for surface charge).

TABLE III
Specificity of Chemotactic Factor-Induced Surface Charge Changes to Chemotactically Responsive Cells

Cell*	Surface charge†	
	Buffer	C5a
	$\mu\text{m}^2/\text{s}/\text{V}/\text{cm}$	
Granulocyte	1.76 ± 0.03	1.58 ± 0.04
Small lymphocyte	1.85 ± 0.04	1.77 ± 0.02
Erythrocyte	2.04 ± 0.04	2.07 ± 0.03

* All cells from one individual, dextran sedimentation.

† Mean \pm SR.

kallikrein and plasminogen activator activity. It is apparent in Fig. 4 (bottom right panel) that the major change in granulocyte surface charge was associated with fractions containing C5a, while other fractions that contained chemotactic activity also reduced the granulocyte surface charge. Small changes in the granulocyte surface charge were also induced by some column fractions in which chemotactic activity was not detected; we have not identified what, if any, biological activity these fractions have on the leukocyte. However, these data demonstrate that molecules other than chemotactic factors can induce cell surface charge changes.

The fractions eluted from the G-150 Sephadex column were uniformly contaminated with trace amounts of endotoxin (2–5 $\mu\text{g}/\text{ml}$) (limulus assay, courtesy of Dr. Ronald Elin). A dose-response study using endotoxin (0–30 $\mu\text{g}/\text{ml}$) failed to demonstrate any endotoxin-induced effects on granulocyte surface charge. The G-150 fractions that contained C5a (fractions 108–126, Fig. 4, lower right) were pooled, concentrated to 100 $\mu\text{g}/\text{ml}$ protein, and then tested in varying dilutions for chemotactic activity and the ability to induce surface charge changes. As shown in Fig. 5, varying concentrations of C5a induced a log-linear increase in the chemotactic response and a related log-linear decrease in the net negative granulocyte surface charge. The magnitude of the decrease in surface charge was proportional to the granulocyte-chemotactic response.

Specificity of chemotactic factor-induced surface charge changes to chemotactically responsive cells. C5a does not appear to have chemotactic activity for human lymphocytes and has no known effect on erythrocytes. We therefore studied the effect of C5a on the surface charge of these cells to determine whether the effects noted with granulocytes were restricted to chemotactically responsive cells or also occur in chemotactically unresponsive cells. As shown in Table III, a significant reduction in surface charge ($P < 0.01$) resulted when neutrophils were treated with C5a ($P < 0.001$). A small reduction in the surface charge of lymphocytes

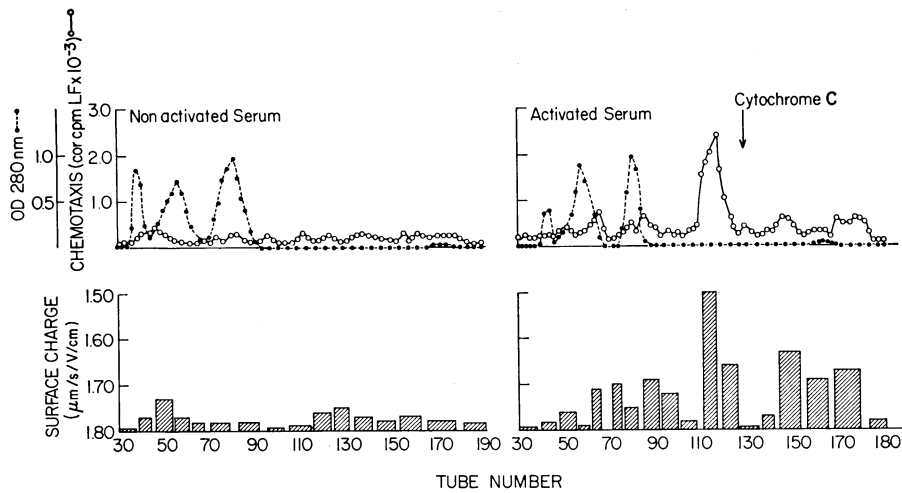


FIGURE 4 G-150 Sephadex chromatography of nonactivated (left) and endotoxin-activated (right) human serum. The upper panels show absorbance at 280 nm (closed circles) and chemotactic activity (open circles) while the bars in the lower panels represent the mean granulocyte surface charge after incubation of the indicated fractions with granulocytes (20 determinations upon 10 cells) (note inverted scale for surface charge).

was also noted ($0.05 > P > 0.01$); however, the change of surface charge after incubation of granulocytes with C5a was significantly larger than the change observed

in the small lymphocyte ($P < 0.05$). C5a had no effect on the surface charge of erythrocytes ($P > 0.05$).

Effect of inhibitors of chemotaxis upon the ability of C5a to induce surface charge changes. A variety of agents that inhibit the cellular response to chemotactic factors were evaluated for their effect upon leukocyte surface charge, as shown in Table IV. The drugs colchicine, cytochalasin B, and hydrocortisone were added at concentrations that inhibited chemotaxis (19). None of these inhibitors had a direct effect upon granulocyte surface charge, and the DMSO in which the cytochala-

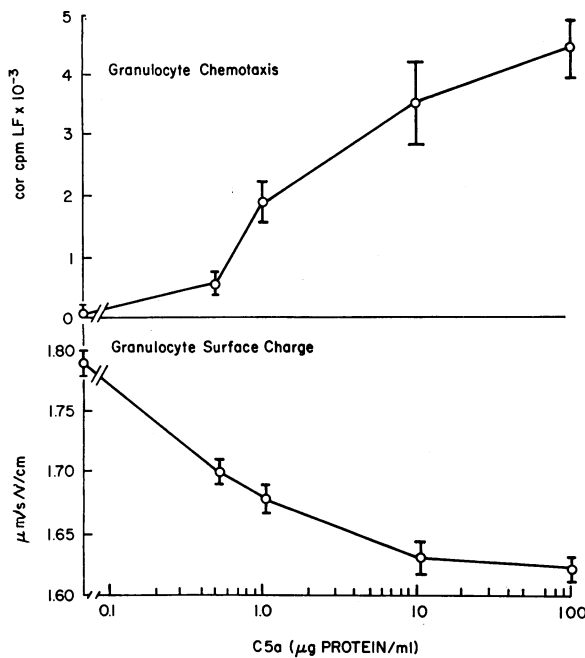


FIGURE 5 Determination of the granulocyte chemotactic activity of increasing concentrations of C5a (upper panel) and the effect of these concentrations of C5a upon granulocyte surface charge (lower panel). The bars denote the SEM of four determinations of chemotaxis and 20 determinations on 10 cells of the granulocyte surface charge.

TABLE IV

Effect of Inhibitors of Granulocyte Chemotaxis on C5a-Induced Surface Charge Changes

Drug	Chemotaxis*	Surface charge*
	<i>cor cpm LF</i>	$\mu\text{m/s/V/cm}$
None		
+buffer	180±26	1.75±0.03
+C5a	2,280±210	1.58±0.02
Colchicine (4.0×10^{-4} M)		
+buffer	168±23	1.71±0.03
+C5a	633±75	1.64±0.02
Cytochalasin (3.0 µg/ml)		
+buffer	162±46	1.76±0.04
+C5a	210±22	1.62±0.02
Hydrocortisone sodium succinate (0.5 mg/ml)		
+buffer	135±26	1.77±0.04
+C5a	960±78	1.82±0.03

* Mean ±SE, three experiments.

sin B was dissolved had no effect upon the cell surface charge or the chemotactic response. Colchicine and cytochalasin B did not modify the ability of C5a to reduce the cell surface charge significantly ($P > 0.05$). In contrast, pretreatment of granulocytes with hydrocortisone sodium succinate blocked the ability of C5a to reduce the granulocyte surface charge. The effect could not be reversed by extensive washing of the cells after pretreatment with this agent.

DISCUSSION

The reduction of granulocyte surface charge observed upon exposure of human granulocytes to the chemotactic factors C5a, kallikrein, and plasminogen activator, and dialyzable transfer factor, and the lack of such effects with the nonchemotactic proteins, prekallikrein and plasminogen proactivator, human albumin, human IgG, and horseradish peroxidase, demonstrate a high degree of association between surface charge changes and the chemotactic process. The apparent discrepancy in the magnitude of the chemotactic response and change in surface charge, noted with the different chemotactic factors studied, may be related to different biochemical properties of the chemotactic factors. Kallikrein and plasminogen activator are enzymes whose chemotactic properties are dependent upon the integrity of their active site (5, 6, 20), while C5a and dialyzable transfer factor have no known enzymatic activity. These factors may therefore initiate chemotaxis by different mechanisms.

Although the observed chemotactic factor-induced change in granulocyte surface charge may be a consequence of the chemotactic process, studies on other motile cells suggest that the decreased surface charge is a prerequisite for directed locomotion. Treatment of rat macrophages with polyelectrolytes to diminish their surface charges has been shown to facilitate attachment of particles during phagocytosis (21). The inverse correlation between cell adhesiveness compared to the negativity of surface charge in the amoebae (2), the association of cell contraction and expansion with decreases or increases in surface charge, respectively (2), and the demonstration of high negative surface charge, poor distensibility, low cell adhesiveness, and poor pseudopod formation in bone marrow granulocyte precursor cells (22) support the concept that an appropriate alteration in surface charge is critical for human neutrophils to respond to a chemotactic stimulus.

The mechanism for the modification of surface charge of granulocytes exposed to chemotactic factors has not been determined. It appears unlikely that the alteration in granulocyte shape previously described in cells exposed to chemotactic factors (19) causes the decreased surface charge, since no change in surface charge is

associated with crenation of erythrocytes² or sickling (23). We were not able to detect a release of sialic acid to the surrounding media after granulocytes were exposed to C5a,³ which argues against displacement of such anionic moieties from the membrane surface. An involution of the membrane or unfolding of inner membrane cationic sites consequent to the interaction of granulocytes with chemotactic factors could explain the reduced surface charge. The chemotactic molecules C5a and kallikrein are each positively charged and the decrease in surface charge could simply reflect masking of negatively charged membrane receptors by the positively charged chemotactic factor; however, the chemotactic factor in dialyzable transfer factor is a negatively charged molecule,⁴ which argues against this explanation. Alternatively the chemotactic factors C5a, kallikrein, and dialyzable transfer factor have recently been shown to induce a net calcium release from granulocytes into the surrounding media (19). Neutralization of negative membrane surface charges by the local ionized calcium might also explain the observed phenomenon (24).

The inability of colchicine or cytochalasin B to prevent C5a-induced surface charge changes at concentrations that inhibited chemotaxis is compatible with the presumed site of action of these agents upon microtubules (25, 26) and microfilaments (27), respectively, and suggests that these agents do not inhibit chemotaxis by their effects on the cell surface. The mechanism by which steroids inhibit chemotaxis is not known. However, the inhibition of C5a-induced reduction of granulocyte surface charge by hydrocortisone suggests that steroids may interact with the cell surface, perhaps by binding and preventing proper C5a neutrophil interaction.

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² Durocher, J. R. Unpublished observation.

³ Gallin, J. I., and J. R. Durocher. Unpublished observations.

⁴ Kirkpatrick, C. H., J. I. Gallin, and A. P. Kaplan. Unpublished observations.

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