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Immunology

Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes

(leukocyte chemotaxis/anaphylatoxin/complement/C5a derivatives)

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ABSTRACT **Human C5a, a complement-derived anaphylatoxin, is a potent mediator of human leukocyte chemotaxis. Using a homogeneous preparation of C5a that was ^{125}I-labeled, we have demonstrated the presence of a specific cellular receptor for this glycoprotein on intact human polymorphonuclear leukocytes. Cellular uptake of the radiolabeled ligand occurred rapidly and the rate of dissociation was extremely slow. Cellular binding was saturable with respect to ^{125}I-labeled C5a, and half-saturation occurred at a concentration of $3–7 \times 10^{-9}$ M. The number of C5a binding sites per cell was estimated as $1–3 \times 10^5$. The ligand (C5a) displays specific structural features that are required for binding because analogs of C5a such as $C5a_{des\ Arg}$ or a yeast carboxypeptidase-digested C5a derivative C5a-(1-69) inhibited the binding but C3a anaphylatoxin, which resembles C5a chemically, did not. Both C5a-mediated leukocyte chemotaxis and C5a-induced lysosomal enzyme release from cytochalasin B-treated cells closely paralleled uptake of the ligand, clearly indicating that it is a receptor–C5a interaction that leads to stimulation of these cellular responses.**

Human C5a anaphylatoxin is a glycoprotein consisting of 74 amino acid residues and an oligosaccharide unit of approximately $3000M_r$ (1). During complement activation, the C5a fragment is selectively cleaved from the fifth component of complement (C5). C5a possesses the spasmogenic properties of an anaphylatoxin and is also a potent mediator of human polymorphonuclear leukocyte (PMN) chemotaxis (2).

Procedures for isolating homogeneous human C5a (3), the serum carboxypeptidase B-digested derivative $C5a_{des\ Arg}$ (1), and human C3a anaphylatoxin (4) have been described. The amino acid sequence of these factors is now established (1, 5), and the availability of purified factors provided the opportunity to conduct quantitative studies of their biological activities. These studies clearly showed that nanomolar concentrations of C5a and $C5a_{des\ Arg}$ are sufficient to promote human PMN chemotaxis and that human C3a is totally devoid of chemotactic activity (6, 7). Similar results were obtained when these purified factors were assessed for their ability to induce lysosomal enzyme release from cytochalasin B-treated PMNs, although C3a was noted to possess minimal activity at increased concentrations (8).

These studies and others (9) suggested that C5a elicits varied PMN biological responses by acting through a specific cellular receptor. With purified human C5a available, we conducted experiments to determine if such a receptor actually exists on intact human PMNs and, if so, to provide an initial characterization of this receptor.

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MATERIALS AND METHODS

Preparation of Human C5a, C5a Derivatives, and C3a. Human C5a was isolated from yeast cell-activated serum containing 1 M ε-aminocaproic acid (EACA) according to the method of Fernandez and Hugli (3). Human $C5a_{des\ Arg}$ was prepared directly from yeast cell-activated serum without EACA (1). An additional C5a derivative, C5a-(1-69), resulted from digesting $C5a_{des\ Arg}$ with yeast carboxypeptidase (800:1, wt/wt) for 3.5 hr at 37° in 0.1 M sodium acetate (pH 5.5). Human C3a was purified from inulin-activated serum containing 1 M EACA according to the procedure of Hugli *et al.* (4). All polypeptides were quantitated by total amino acid analysis after acid hydrolysis (10, 11), by using a Beckman amino acid analyzer (model 121 M).

Human C5a was labeled with ^{125}I by the method of Bolton and Hunter (12). Typically, 5–10 μg of the protein in 0.1 M sodium borate (pH 8.5) was added to 200–400 μCi of Bolton–Hunter reagent (*N*-succinimidyl 3-(4-hydroxy-5-[^{125}I]iodophenyl) propionate; Amersham/Searle) and allowed to react for 30 min at 4°. The reaction mixture was dialyzed against Hanks' balanced salt solution at 4° until the dialysate was free of ^{125}I. Analysis of these preparations by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (1) showed >98% of the radiolabel was incorporated into C5a. Radiolabeled preparations were quantitated according to the principles of competitive binding assays (13). In our case, precisely known amounts of unlabeled C5a served as the competing ligand for the labeled polypeptide and purified PMNs served as the ligand acceptor. Specific activities of the various radiolabeled ligand preparations ranged from 8 to 12 μCi/μg.

Preparation of Human Peripheral Blood Cells. PMNs were prepared from heparin-anticoagulated (10 units/ml) venous blood obtained from healthy male volunteers as described by Böyum (14). Residual erythrocytes were removed by combined dextran T-500 sedimentation and hypotonic lysis (15). Leukocytes were harvested by centrifugation at $150 \times g$ for 10 min and washed twice with an appropriate buffer (see below). Average preparations contained 95–97% PMNs, 2–4% eosinophils, and 0–2% mononuclear cells. Cell viability, as judged by trypan blue exclusion, was 97 ± 2% (mean ± SD).

For determination of PMN chemotaxis, cells were suspended at a density of 2.5×10^7 PMN per ml in basal medium (Eagle's) supplemented with glutamine (2.9 mg/ml) and $NaHCO_3$ (1.4

Abbreviations: PMN, polymorphonuclear leukocyte; EACA, ε-aminocaproic acid; C5a-(1-69), a polypeptide consisting of the NH_2-terminal 69 residues of human C5a; ^{125}I-C5a, ^{125}I-labeled C5a; ED_{50}, the concentration of ligand required to provide half-maximal ligand uptake; ED_{99}, the concentration of ligand required to provide 99% of the maximal ligand uptake.

mg/ml). For studies of lysosomal enzyme release, PMNs were suspended in balanced salt solution at a density of 4×10^6 cells per ml. For the cellular binding assay, PMNs were used at a density of $2–3 \times 10^7$ cells per ml in balanced salt solution containing 0.5% gelatin.

Human erythrocytes were obtained by dextran sedimentation. After removal of the leukocyte-rich supernatant and upper 2 cm of erythrocytes, the remaining erythrocytes were extensively washed with balanced salt solution to ensure removal of leukocytes. The washed cells were then suspended at a density of 1×10^8 cells per ml in balanced salt solution/0.5% gelatin. Transformed lymphocytes (WI-L2) grown in continuous culture were kindly provided by N. Cooper (Scripps Clinic). Culture fluid was decanted after pelleting the cells by centrifugation and the cells were resuspended in balanced salt solution/0.5% gelatin at a density of 2×10^7 cells per ml.

C5a Binding Assay. Samples of freshly prepared human PMNs ($0.9–1.5 \times 10^6$ cells) were incubated in the assay buffer (balanced salt solution/0.5% gelatin) in 1.5-ml conical polypropylene tubes with various amounts of ^{125}I-labeled C5a (^{125}I-C5a) in a final volume of 0.1 ml. After an appropriate interval at 24°, cells were sedimented by centrifugation at $11,000 \times g$ (Beckman Microfuge B) for 30 sec. After centrifugation, one-half of the cell-free supernatant solution (50 μl) was transferred to a separate tube. The paired samples, consisting of pelleted cells with residual supernatant (P) and one-half the supernate (S), were then assayed for radioactivity in a Packard Auto-Gamma scintillation spectrometer. After correction for background radioactivity, the amount of cell-bound radiolabeled C5a was calculated from the formula:

$$\% \text{ bound} = \left(1 - \frac{2 \times \text{cpm (S)}}{\text{cpm (S)} + \text{cpm (P)}}\right) \times 100.$$

Measurement of PMN Biological Functions. PMN chemotaxis was assessed by a serum-free modification of the "chemotaxis under agarose" method (16, 17). Results of these studies were expressed as the "chemotactic index"—i.e., the distance of cellular migration in response to a chemotactic stimulus (A) divided by the distance migrated in response to a buffer control (random migration, B). C5a-induced β-glucuronidase release from cytochalasin B-treated cells was examined by published methods (9, 15). Extracellular β-glucuronidase levels were determined, after incubation at 37° for 20 hr, by the method of Brittinger *et al.* (18) with phenolphthalein glucuronidate as substrate. Results were expressed as μg of phenolphthalein liberated per hour of incubation.

RESULTS

Comments on Binding Assay. Throughout these studies, cell-bound radiolabeled ligand was quantitated by determining the amount of ^{125}I-C5a remaining free in solution, as opposed to a measurement of the cell-bound factor. This technique was selected to ensure a continuous equilibrium between cells and ligand throughout and to minimize dissociation of the cell-bound polypeptide which would result in an underestimation of the degree of ligand–cell interaction. Subsequent assessment showed that the alternative approach—i.e., direct measurement of cell-bound ligand after removing the unbound factor by rapid washing—gave comparable results for ^{125}I-C5a cellular uptake.

In all cases, the binding data were corrected for nonspecific binding of the positively charged radiolabeled C5a to the surface of incubation tubes and pipette tips used for transfers of solutions. Nonspecific binding, as determined with acellular samples containing between 0.1 nM and 1 μM ^{125}I-C5a, was typically $0.8 \pm 1.0\%$ (mean ± SD; $N = 16$).

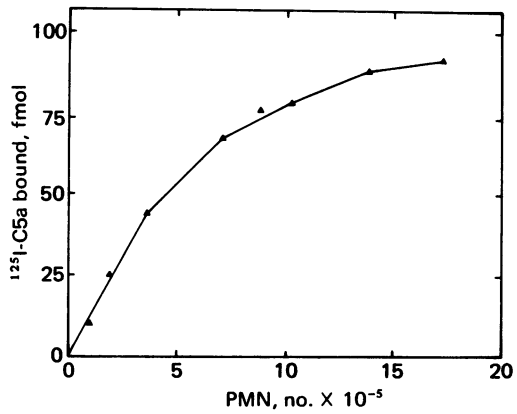

FIG. 1. Various quantities of human PMNs were incubated with ^{125}I-C5a at a final total concentration of 3 nM. Incubations were carried out at 24° for 45 min. The quantity of bound ligand shown for each point is the mean of triplicate determinations.

As shown in Fig. 1, at a fixed ligand concentration, the quantity of bound ligand was not linearly dependent on the number of PMNs added. With greater than 4×10^5 PMNs in the incubation mixture, the amount of cell-bound ligand decreased compared to quantities expected from a linear extrapolation. This deviation became more pronounced as cell density increased. In order to provide a sufficient number of cells for readily measurable ligand uptake, $0.8–1.2 \times 10^6$ PMNs were usually used in each incubation mixture. However, because of this nonlinear behavior, the actual number of cells present in any given experiment is specified.

Rate of Association and Dissociation of ^{125}I-C5a with Human PMN. As depicted in Fig. 2, ligand binding by intact PMNs was rapid and essentially complete within 3–5 min at 24°. Equilibrium between bound and unbound ^{125}I-C5a was established after 15 min and was maintained until 90 min after addition. There was no evidence of spontaneous dissociation, degradation, or additional uptake of the polypeptide during this time. Similar rates of ligand uptake were observed with ^{125}I-C5a at total concentrations ranging from 1 to 30 nM.

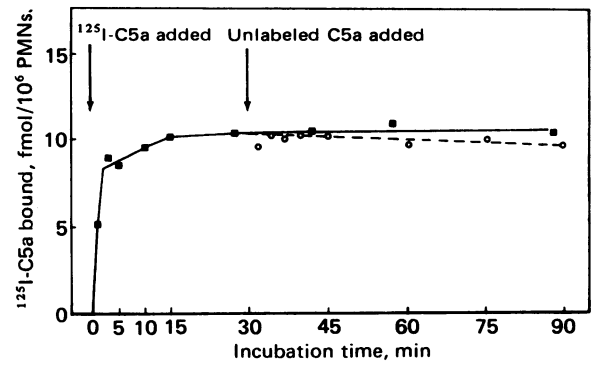

FIG. 2. Rate of association and dissociation of ^{125}I-C5a with human PMNs at 24°. Cellular uptake was initiated by the addition of ^{125}I-C5a to a final total concentration of 2 ng/ml (0.24 nM) at zero time (arrow). At various times, 100-μl samples (containing 10^6 PMNs) were removed from the incubation mixture and the quantity of cell-bound ligand was determined. After 30-min of incubation (arrow), the reaction mixture was divided into two portions; one was diluted with 100 μl of Hanks' balanced salt solution/0.5% gelatin as a control and the other was mixed with 100 μl of unlabeled C5a (final total concentration, 2 μg/ml or 0.24 μM). Portions of each mixture were then removed at intervals for determination of the bound radiolabeled ligand. ■, ^{125}I-C5a bound in the absence of C5a O, ^{125}I-C5a bound in the presence of 1000-fold molar excess unlabeled C5a.

In addition, uptake was examined at 4° and 37°. Although the initial rates of uptake at these temperatures corresponded to those at 24°, only about 50–60% as much of the ligand was bound at 4° and ligand binding was slightly enhanced (110–120% of that at 24°) at 37°.

To assess the dissociation rate of cell-bound ligand, a 1000-fold molar excess of unlabeled polypeptide was added to the incubation mixture after equilibrium was reached. Over the next 60 min, displacement of the radiolabeled ligand was extremely slow, with about 93% of the ^{125}I-C5a remaining cell-bound after this time. The slow dissociation of the ligand-cell complex was also substantiated in a separate experiment. Cells were exposed to ^{125}I-C5a at a total concentration of 50 ng/ml (6 nM) and the amount of ligand was determined after 45 min of incubation at 24°. Unbound ^{125}I-C5a was removed by suspending the PMNs three times in 4 ml of cold balanced salt solutions and pelleting by centrifugation over a period of 20 min. Final quantitation of the residual cell-bound ^{125}I-C5a revealed that 96.9 ± 3.1% (mean ± SD; N = 6) of the ligand originally bound remained cell-associated.

Concentration-Dependency of ^{125}I-C5a Binding to PMNs. To determine if a specific saturable receptor for C5a existed on human PMNs, the degree of ^{125}I-C5a binding at various ligand concentrations was examined (Fig. 3). Different quantities of the radiolabeled polypeptide were incubated with 1.4×10^6 PMNs for 45 min at 24°; after equilibration of the ligand with cells, the amount of cell-bound ^{125}I-C5a was determined. Under these conditions, 3.7% of the ligand was bound at the highest (0.12 μM) and 43% at the lowest (60 pm) concentrations. At ^{125}I-C5a concentrations of 250 ng/ml (30 nM) or greater, a constant amount of ligand uptake was found, suggesting saturation of PMN receptors. There was no statistically significant nonspecific binding (i.e., linear uptake) of the ligand to PMNs when the uptake data were analyzed by a computerized adaptation of the analysis suggested by Colquhoun *et al.* (19).

Because prior studies had shown an extremely slow rate of dissociation of cell-bound ligand, the typical Scatchard plot (20) analysis was deemed inappropriate. The receptor's affinity for the ligand and the total number of cellular receptors were estimated by a computer-assisted probit analysis of the uptake data (21). Under the stated conditions, considering the total ligand concentration, the concentration of ligand required to provide half-maximal (ED_{50}) cellular binding of ^{125}I-C5a was 44.2 ng/ml (95% confidence limits, 31.8–61.6 ng/ml) or 5.3 nM (95% confidence limits, 3.8–7.4 nM). If only the concentration of the free ligand was considered in such calculations, the ED_{50} was estimated to be 3.3 nM (95% confidence limits, 2.4–4.6 nM). Correlation coefficients of 0.978 and 0.982 were obtained for calculations, respectively. An ED_{99} of 515 ng/ml (95% confidence limits, 348–800 ng/ml) or 62 nM (95% confidence limits, 42–96 nM) was also calculated. Therefore, based on the extent of ligand uptake at saturating concentrations, an average number of receptors per cell was approximately $1.6–2.2 \times 10^5$.

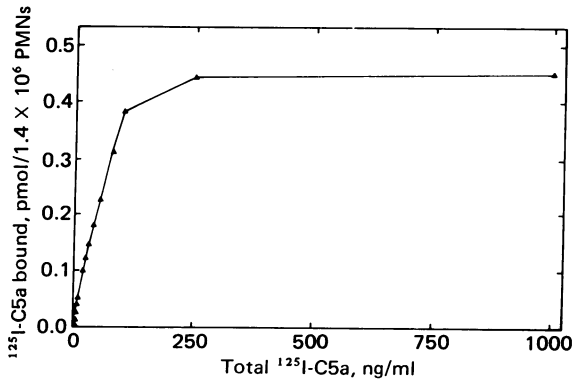

FIG. 3. Uptake of ^{125}I-C5a by human PMNs at various concentrations of ligand. Human PMNs (1.4×10^6) were incubated with varying amounts of ^{125}I-C5a in a total volume of 0.10 ml for 45 min at 24°. The amount of cell-bound ligand is shown as points that represent the mean values of triplicate determinations.

The specificity of ^{125}I-C5a binding to PMNs was further substantiated by the fact that human erythrocytes and cultured lymphocytes (WI-L2) both failed to bind the glycopeptide over the same ligand concentration range.

Effects of Other Factors on ^{125}I-C5a Binding to PMNs. Binding of ^{125}I-C5a to PMNs was next assessed for specificity, and the nature of the receptor was also studied by using the unlabeled structural analogs $C5a_{des\ Arg}$ and C5a-(1-69) in two different inhibition assays. The C3a anaphylatoxin and its des Arg derivative were included because of their chemical similarity to C5a. First, PMNs were incubated with the potential inhibitory factors for 15 min at 24° before the addition of various amounts of ^{125}I-C5a. After the radiolabeled ligand reached equilibrium with the cells, the quantity of bound ^{125}I-C5a was determined. This experimental design permitted potential competitors of ^{125}I-C5a binding to obstruct binding to the receptor or occupy it while avoiding direct competition with C5a which had previously been shown to be avidly bound to the cells. Second, mixtures containing various concentrations of the potential inhibitor and radiolabeled C5a were added to PMNs. The binding of ^{125}I-C5a to PMNs under equilibrium conditions (30 min at 24°) was then measured after simultaneous exposure of the cells to both the ligand and other potential inhibitory factors.

As shown in Fig. 4, when the cells were incubated with unlabeled C5a at a concentration of 100 ng/ml (12 nM) for 15 min before the addition of the radiolabeled ligand, binding of ^{125}I-C5a was completely inhibited compared to control experiments in which cells were simply incubated with buffer. This is in keeping with the slow rate of dissociation of previously bound C5a. When PMN were exposed to $C5a_{des\ Arg}$ (10 μg/ml, 1.2 μM) before incubation with ^{125}I-C5a, ligand binding was also inhibited although the magnitude of this inhibition was less

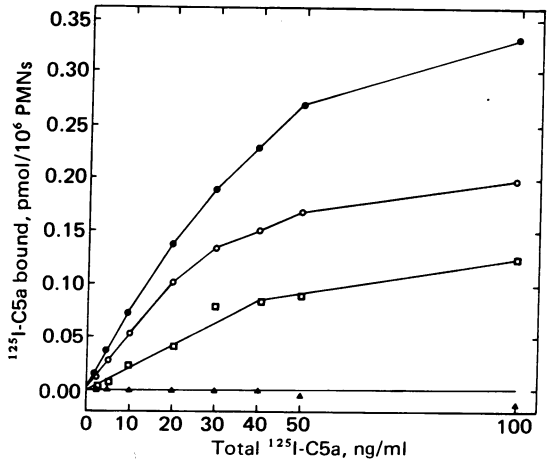

FIG. 4. Inhibition of ^{125}I-C5a uptake by human PMNs with unlabeled C5a or unlabeled structural analogs of C5a. Purified cells were incubated with buffer as a control (●), unlabeled C5a (100 ng/ml; ▲), $C5a_{des\ Arg}$ (10 μg/ml; □), or C5-(1-69) (7.7 μg/ml; ○) for 15 min at 24° before addition of varying amounts of ^{125}I-C5a. After ^{125}I-C5a addition, the mixtures were incubated for 30 min at 24°, and the amount of cell-bound ligand was determined.

than that produced by the native glycoprotein. The shorter analog C5a-(1-69) (7.7 μg/ml, 1 μM) also inhibited ^{125}I-C5a binding under these conditions but less effectively than either C5a or $C5a_{des\ Arg}$. At lower concentrations of C5a, $C5a_{des\ Arg}$, or C5a-(1-69), correspondingly less inhibition of ligand binding was found. In addition to being dependent on the inhibitor concentration, the magnitude of the inhibitory effect was also dependent on the duration of incubation of ^{125}I-C5a with the cell/inhibitor mixture—i.e., prolonging exposure to ^{125}I-C5a increased the uptake of radiolabeled ligand.

Although not shown in Fig. 4, neither C3a anaphylatoxin nor its des Arg derivative significantly inhibited ^{125}I-C5a binding even when their concentrations were increased to 50 μg/ml (5.5 μM).

Mixing the various unlabeled factors with ^{125}I-C5a before instead of after the cells were added reduced the effectiveness of inhibiting radiolabeled ligand binding. In the case of unlabeled C5a, cells that were exposed simultaneously to the labeled and native glycoprotein bound ^{125}I-C5a in proportion to the respective concentrations of each factor. This finding provided a convenient means of quantitating the radiolabeled glycoprotein and suggested that native and ^{125}I-C5a could be considered identical with regard to cellular binding.

Comparison of PMN Binding of ^{125}I-C5a and Biological Activity. One of the most important criteria used to establish the presence of specific cellular receptors is that the affinity of the ligand must be consistent with its biological activity (22). Therefore, we first assessed both the unlabeled and radiolabeled ligands' abilities to promote chemotaxis of purified human PMNs. As shown in Fig. 5, directed cellular migration was enhanced by extremely low concentrations of C5a. The maximal effect was evident at 50–100 ng/ml with a calculated ED_{50} of 2 nM. At concentrations greater than 100 ng/ml, progressively lower chemotactic index values were noted. This phenomenon, termed "desensitization," has been consistently noted with C5a used as a chemoattractant (6, 7). Within the limits of this experimental method there was no significant difference in the results produced by radiolabeled and unlabeled C5a throughout the concentration range tested (1–1000 ng/ml).

The ability to promote lysosomal enzyme release from cytochalasin B-treated PMN was examined. Cytochalasin B-treated (5 μg/ml) purified human PMNs were incubated with various concentrations of unlabeled C5a or ^{125}I-C5a for 30 min at 24°, and the amount of extracellular β-glucuronidase was subsequently determined. The native glycoprotein and the radiolabeled ligand were equally and completely capable of inducing the release of this lysosomal enzyme (Fig. 5), with a calculated ED_{50} of 3 nM.

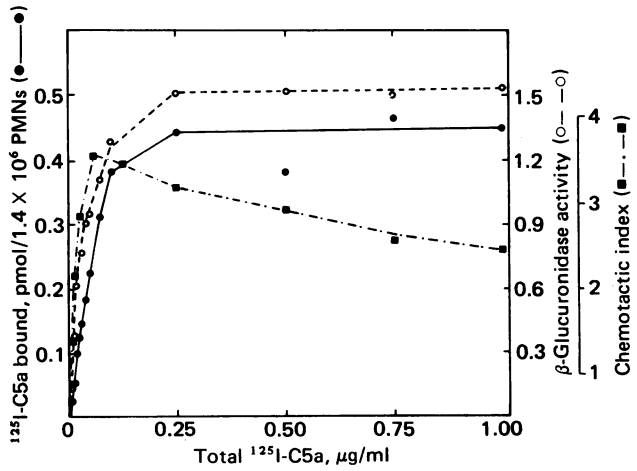

FIG. 5. Comparison of C5a-induced lysosomal enzyme release from cytochalasin-B treated PMNs, chemotaxis mediated by C5a, and ^{125}I-C5a uptake on purified human PMNs. Extracellular levels of β-glucuronidase activity are shown as μg of phenolphthalein/hr per 10^6 PMNs.

DISCUSSION

With homogeneous preparations of ^{125}I-C5a, we have delineated characteristics of ligand–cell interaction that are consistent with the presence of a specific receptor for C5a on PMNs. Human PMNs bind ^{125}I-C5a within 5 min, suggestive of a receptor–ligand interaction. Although the degree of ligand binding was temperature-dependent, the initial rate of ^{125}I-C5a uptake was essentially independent of temperature and equivalent, whether measured at 4°, 24°, or 37°. Equilibrium between the ligand and the cells was established and maintained for up to 90 min. Unlike the chemotactic *N*-formylmethionyl peptides (23, 24), C5a did not degrade or spontaneously dissociate once bound to human PMNs. In addition, at equilibrium, ^{125}I-C5a uptake ceased, suggesting that no additional binding sites were available during the test.

Once ^{125}I-C5a became cell-associated, at least at low concentrations, it was only slowly dissociated. When an attempt was made to determine the rate of dissociation of the radiolabeled ligand by the usual competition method (25), only 5–10% of the bound ^{125}I-C5a was dissociated from the cells after 1 hr. Longer incubation periods, which would be required for the determination of k_{-1}, were technically precluded because of limited PMN viability. Therefore, the usual kinetic parameters (25) were not directly measurable. Although the precise explanation for the limited dissociation of ^{125}I-C5a remains to be determined, ligand concentration-dependent receptor–receptor interactions, receptor redistribution, internalization, and simply the very high affinity of the ligand for the receptor all should be considered as possible explanations.

Examination of the concentration-dependent uptake of ^{125}I-C5a showed a typical saturation curve with no evidence of statistically significant nonspecific binding. As noted, for slowly dissociating ligands, the Scatchard plot (20) analysis was inappropriate for calculating receptor affinity (K_a) or total number of receptors per cell (q). For this reason, we used a computer-assisted probit analysis of the concentration-dependent uptake data to estimate these parameters. Such calculations gave an ED_{50} value on the order of 3–7 nM, thus providing an approximation of K_a. From the uptake data at saturation (ED_{99}), a total of 1.6–2.2 × 10^5 binding sites per cell was estimated, although this value varied somewhat with increasing cell density (Fig. 1). Variation in ligand uptake with cell density probably reflects a decreased concentration of the ligand in the supernatant or a masking of binding sites caused by cellular aggregation. The aggregation of leukocytes that is promoted by addition of C5a (26) would concurrently increase the steric masking of receptors.

Other findings support the concept that ^{125}I-C5a uptake by human PMNs results from a specific ligand–cell interaction. Failure of either erythrocytes or cultured lymphoblastoid cells (WI-L2) to bind the radiolabeled polypeptide over the same concentration range at which PMN uptake is maximal confirms the cellular specificity of ^{125}I-C5a binding.

Our current studies, in which structural analogs of C5a interfered with cellular uptake of ^{125}I-C5a, suggest that several specific features of the C5a structure play important roles in governing ligand–receptor interaction. As shown in Fig. 4, intact unlabeled C5a completely prevented ^{125}I-C5a uptake by human PMNs under appropriate conditions. $C5a_{des\ Arg}$ and

C5a-(1-69), which differ from C5a only with regard to their COOH-terminal amino acid sequences, inhibited ^{125}I-C5a cellular binding under similar conditions, with $C5a_{des\ Arg}$ being about twice as effective an inhibitor as C5a-(1-69). These factors apparently act as inhibitors because of their interaction with the C5a receptor. Inhibition depends on both the concentration of the inhibitor and the time of exposure of the cell/inhibitor mixture to ^{125}I-C5a. Thus, it appears that the measured ^{125}I-C5a binding to PMNs in the presence of these structural analogs reflects the relative receptor affinities as well as the rate of dissociation for each factor. It is also possible that the inhibitory effects of C5a and its structural analogs are in part related to their induction of neutrophil aggregation (26). However, *N*-formyl-L-methionyl-L-phenylalanine, which also induces aggregation, does not demonstrate significant inhibition of ^{125}I-C5a uptake (27). Consequently, cellular aggregation alone would not account for the inhibitory effects of the complement-related factors. Nevertheless, our findings clearly show that $C5a_{des\ Arg}$, which is a chemotaxin (6, 7), inhibits ^{125}I-C5a binding to PMNs. The analog C5a-(1-69) lacks only the COOH-terminal five amino acid residues of intact C5a and is inactive as a chemotactic factor (7) but still inhibits ^{125}I-C5a uptake by PMNs. On the other hand, the chemically similar C3a anaphylatoxin, which is not a chemotaxin (6, 7), does not inhibit ^{125}I-C5a binding to PMNs.

Evidently, the PMN C5a receptor recognizes the bulk of the C5a molecule, as represented by C5a-(1-69), but the COOH-terminal five residues of the molecule seem to play a critical role in governing both receptor–ligand interactions and in eliciting the cellular response. The concept that several highly specific structural features are required for maximal C5a-receptor interaction is further supported by the finding that C3a fails to inhibit ^{125}I-C5a binding.

Comparison of the concentration-dependent uptake of ^{125}I-C5a with the biological activity of either labeled or unlabeled C5a (Fig. 5) revealed that nearly identical concentrations of ligand are required for binding and for eliciting cellular function. In addition to confirming that ligand affinity and biological activity correlate, these findings strongly suggest that ligand–receptor interaction is necessary to promote leukocyte chemotaxis and lysosomal enzyme release from cytochalasin B-treated cells. This is reinforced by the remarkably similar ED_{50} values obtained for each parameter studied—i.e., ^{125}I-C5a binding (3–7 nM), chemotaxis (2 nM), and enzyme release (3 nM). In addition, these studies show that at C5a concentrations of about 100 ng/ml (10 nM) or less, increasing quantities of C5a become cell-associated and leukocyte chemotaxis increases correspondingly to a maximum. With the saturation of binding sites at higher concentrations of C5a ($\geq$ 250 ng/ml; 30 nM), PMN migration becomes progressively impaired. Although the exact mechanism is unknown, this response seems to provide a delicate control mechanism that could effectively regulate the extent of cellular influx at sites where C5a is actively being generated (6).

To summarize, this report provides experimental evidence to substantiate the presence and characterize the nature of the C5a receptor on intact human PMNs. That ^{125}I-C5a is cell-bound via a specific receptor is demonstrated by the facts that cellular uptake of the ligand was rapid, although the rate of dissociation was slow. Concentration-dependent uptake of the ligand was specific and saturable. Demonstration that the affinity of ligand binding was consistent with two distinct biological activities fulfilled the final criterion to establish the presence of a specific cellular receptor. With this information, we can now begin an accurate investigation of the cellular localization of the C5a receptor and the mechanisms whereby C5a–receptor interactions promote PMN chemotaxis and related cellular functions.

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