

Anaphylatoxin-induced Histamine Release with Human Leukocytes

STUDIES OF C3a LEUKOCYTE BINDING AND HISTAMINE RELEASE

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ABSTRACT Purified human C3a and synthetic COOH-terminal peptides of C3a, i.e., a pentapeptide, Leu-Gly-Leu-Ala-Arg (5R), and an octapeptide, Ala-Ala-Ala-Leu-Gly-Leu-Ala-Arg (8R) induced histamine release from human basophil granulocytes. On a molar basis, 5R was one-tenth and 8R was one-fifth as active as C3a in causing histamine release.

It was found that ^{125}I -C3a binds to whole leukocytes, interacting with both mononuclear cells and neutrophils and the binding was inhibited by preincubation of cells with unlabeled C3a, but not by C5a. 5R and 8R also inhibited the binding of ^{125}I -C3a to the cells. However, on a molar basis, 2,000 times more 8R or 6,000 times more 5R is required for 50% inhibition of ^{125}I -C3a binding as compared with native C3a. Autoradiography of cells using ^{125}I -C3a and ^{125}I -C5a showed preferential binding of ^{125}I -C3a to eosinophils and basophils, whereas ^{125}I -C5a binds primarily to neutrophils and eosinophils and to a lesser extent to basophils. The preferential binding of C3a and C5a to different cell types may herald significance related to their physiological functions.

INTRODUCTION

Within the last decade, the role of complement-derived anaphylatoxins, i.e., C3a and C5a in causing smooth muscle contraction and histamine release from basophils and mast cells has come into focus. Partial purification of human C3a and C5a from complement-activated serum demonstrated that C3a (1) and C5a (1, 2) could

contract guinea pig ileum and enhance vascular permeability in guinea pig skin. The addition of C3a to guinea pig ileum produced tachyphylaxis to C3a but not to C5a or histamine (2). Likewise C5a induced smooth muscle contraction-produced tachyphylaxis to C5a and not to C3a or histamine, reflecting the likelihood that each of these agents acted on separate receptors (2).

Recently, several studies have emphasized that C5a seemed to be the major anaphylatoxin-producing basophil histamine release after complement activation with immune complexes or zymosan (3-7). In this paper, we have demonstrated histamine release from human basophil granulocytes by purified human C3a and the synthetic COOH-terminal peptides based on the structure of C3a, a pentapeptide, Leu-Gly-Leu-Ala-Arg (5R),¹ and an octapeptide, Ala-Ala-Ala-Leu-Gly-Leu-Ala-Arg (8R). Furthermore, attempts were made to identify the cells to which ^{125}I -C3a or ^{125}I -C5a were bound using autoradiography.

METHODS

Preparation of leukocytes. Venous blood was collected from human donors and leukocytes were separated by dextran sedimentation (8). Mononuclear cells and neutrophil granulocyte fractions were obtained from whole blood by differential centrifugation on a Ficoll-Hypaque layer (9) (Ficoll, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.; Hypaque, Winthrop Laboratories, New York).

Purified anaphylatoxin and peptides. C3a and C5a were purified from fresh human serum by the methods previously described (10, 11). Purified C3a and C5a gave a single band in polyacrylamide gel electrophoresis. Antiserum specific for C3a and C5a were prepared in rabbits as described (10). C3a_{des Arg}¹ and C5a_{des Arg}¹ were prepared as described (11).

¹Abbreviations used in this paper: 5R, Leu-Gly-Leu-Ala-Arg; 8R, Ala-Ala-Ala-Leu-Gly-Leu-Ala-Arg.

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COOH-terminal octapeptide (8R)² and pentapeptide (5R)² of C3a were synthesized by a solid-phase method as described (12).

Both C3a and C5a were labeled with ¹²⁵I by either the solid-phase lactoperoxidase method (13) or the acylating agent technique (14). Specific radioactivity of the preparations varied between 13 and 75 $\mu\text{Ci}/\mu\text{g}$. C3a and C5a concentrations, unless otherwise stated, are expressed as moles per liter.

¹C3a_{des Arg} and C5a_{des Arg} are natural catabolites of C3a and C5a, respectively. The des Arg derivative is rapidly formed in blood by serum carboxypeptidase (EC 3.4.12.7), which cleaves an arginyl residue from the COOH-terminus of the anaphylatoxin. The des Arg forms of either C3a and C5a can also be formed by short-term digestion with the pancreatic carboxypeptidase B (EC 3.4.12.3). C3a_{des Arg} and C5a_{des Arg} are both inactive as spasmogens, (e.g. ability to contract smooth muscle).

Synthetic peptide, 5R,² mimics the COOH terminal sequence of human C3a including residues 73 to 77 of the natural polypeptide. The octapeptide, 8R, is an analogue peptide, composed of three alanine residues followed by C3a residues 73–77, which exhibits C3a-like activity and is approximately fivefold more potent than 5R in eliciting smooth muscle contraction and for enhancing vascular permeability.

Determination of the binding of ¹²⁵I-C3a and ¹²⁵I-C5a to leukocytes. Leukocytes (2.5×10^6) were incubated with 2 ng of ¹²⁵I-C3a or 4 ng of ¹²⁵I-C5a in EDTA-Tris A buffer, pH 7.5 containing 25 mM Tris, 120 mM NaCl, 5 mM KCl, 10 mM EDTA, and 0.5 g human serum albumin/liter, in a final vol of 0.5 to 1 ml. The final concentration of ¹²⁵I-C3a and ¹²⁵I-C5a in the cell suspension were 0.22 and 0.41 nM, respectively. As a control, an aliquot of the same cell suspension was preincubated in a high concentration of C3a (550 nM), C5a (455 nM), or C5a_{des Arg} (91 nM) at 37°C for 15 min before the addition of radiolabeled protein. All tubes were incubated at 37°C for 30 min with constant shaking, and the amount of radiolabeled protein bound to the cells was determined by the method of Kulczycki et al. (15).

Because a part of C3a may be degraded by radioiodination, the proportion of ¹²⁵I-C3a bindable to leukocyte was estimated by adding 10^6 to 4×10^7 leukocytes to 1 ng ¹²⁵I-C3a. The results showed that 15–50% of total radioactivity in the preparations were bindable to leukocytes. In addition, the integrity of the ¹²⁵I-C3a and ¹²⁵I-C5a binding to rabbit anti-C3a and -C5a sera was tested with each labeled preparation and varied from 20 to 60% binding. Preparations with <20% binding to specific antisera were discarded.

Autoradiography. Leukocytes ($5 \times 10^6/\text{ml}$) suspended in EDTA Tris A buffer were incubated with an appropriate concentration of ¹²⁵I-C3a (56 $\mu\text{Ci}/\mu\text{g}$) ¹²⁵I-C5a (60 $\mu\text{Ci}/\mu\text{g}$) at 37°C for 30 min. 1 ml of the cell suspension was layered on 3 ml of heat-inactivated fetal calf serum and centrifuged at 200 g for 10 min. Cells in the pellet were washed with the same buffer and the smears of the cells were stained with Wright stabilized staining solution. Autoradiographs of the smears were performed as described (16). After 14–28 d exposure, the slides were developed. 200–500 cells of each type were examined for the presence of radioactive grains and the number of grains per cell was recorded.

Histamine release. The histamine release from human leukocytes was performed in Tris albumin, calcium, and magnesium (ACM) buffer, pH 7.6, containing 25 mM Tris, 120 mM NaCl, 5 mM KCl, 0.6 mM CaCl₂, 1 mM MgCl₂, and 0.5 g human serum albumin/liter (8). Briefly, 0.1 ml of a leukocyte suspension in Tris ACM buffer was incubated with an equal volume of C3a or anti-immunoglobulin(Ig)E at various concentrations. The reaction volume was adjusted to 0.3 ml with Tris ACM (or other agents designated by the protocol). Unless otherwise specified, the reaction

was allowed to proceed for 45 min at 37°C. Reaction tubes containing only cells and buffer, and cells lysed in 1% perchloric acid were included in each assay. Duplicate determinations were performed for all experiments. After incubation, the volume of each tube was adjusted to 1.0 ml, and centrifuged to obtain the supernate. The histamine content in the supernate was measured by the automated technique of Siraganian (17).

Kinetic studies of histamine release. Multiple reaction tubes containing 0.1 ml of an appropriate concentration of C3a or anti-IgE and 0.1 ml Tris ACM were assembled as described above. The reaction was terminated at various time intervals by the addition of 0.2 ml ice-cold 0.02 M EDTA Tris A buffer, and the reaction tubes were moved to an ice bath. The final volume in the tubes was adjusted to 1.0 ml with cold buffer, and the supernates were obtained for histamine analysis.

RESULTS

Binding of ¹²⁵I-C3a and ¹²⁵I-C5a to human leukocytes.

To demonstrate the binding of C3a and C5a to human leukocytes, 2–4 ng of ¹²⁵I-C3a or 4 ng of ¹²⁵I-C5a was added to a suspension of leukocytes that contained 2.5×10^6 nucleated cells. The final concentration of ¹²⁵I-C3a and ¹²⁵I-C5a in the cell suspension was 0.22–0.41 nM and 0.36 nM, respectively. After incubation at 37°C for 30 min, the cells were centrifuged in a microfuge and radioactivity bound to the cell pellets was determined. The results showed that approximately 2% of the added radioactivity in ¹²⁵I-C3a and 5% of the added ¹²⁵I-C5a were bound to the leukocytes (Table I). When mononuclear cells and neutrophil fractions, (both containing 2.5×10^6 cells), were incubated in higher concentration of ¹²⁵I-C3a (up to 4 nM), ¹²⁵I-C3a bound to the mononuclear cells was similar to that bound to the granulocytes (Fig. 1A). When the same cell fractions were incubated in ¹²⁵I-C5a (up to 1.7 nM), however, counts per minute bound to the neutrophil fraction was 7–10 times greater than counts per minute bound to mononuclear cells (Fig. 1B) suggesting a higher affinity of C5a for neutrophils.

TABLE I
Relative ¹²⁵I-C3a and ¹²⁵I-C5a Binding to
Unfractionated Human Leukocytes

Material added	Amount added	Leukocyte binding \pm SE
$\mu\text{Ci}/\mu\text{g}$	ng	%
¹²⁵ I-C3a, 26.5	4	2.12 ± 0.08 (a)
¹²⁵ I-C5a, 13	4	5.1 ± 0.20 (b)

Results indicate mean of duplicate or triplicate tubes performed with four different leukocyte donors (a) and two different donors (b). Purified mononuclear cell ¹²⁵I-C3a binding was slightly less (20–25% less) than that observed with unfractionated leukocytes in two separate experiments (a).

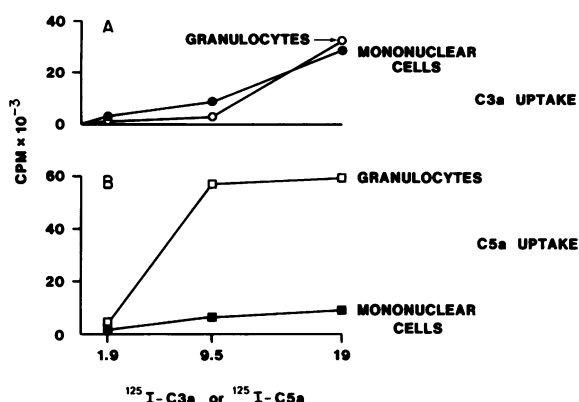


FIGURE 1 Relative binding of ^{125}I -C3a and ^{125}I -C5a to granulocytes and mononuclear cells. Varying amounts of ^{125}I -C3a (56 $\mu\text{Ci}/\mu\text{g}$) and ^{125}I -C5a (60 $\mu\text{Ci}/\mu\text{g}$) were added to 2.5×10^6 cells. Counts per minute bound to 1.0×10^6 cells is shown on the y-axis; i.e., total counts per minute bound would be 2.5 times the given values. Note relatively similar binding of C3a to granulocytes and mononuclear cells, yet markedly increased binding of C5a to granulocytes. Average SD of counts per minute bound in duplicate tubes was $\pm 8.8\%$ of mean. 9 ng/ml C3a and 11 ng/ml C5a = 1 nM/liter.

The specificity of the binding was examined by preincubation of leukocytes with a high concentration of unlabeled C3a, C5a, C3a_{des Arg}, or C5a_{des Arg}, before the radiolabeled peptides. As shown in Fig. 2, preincubation of the cells with unlabeled C3a (550 nM) completely inhibited the binding of ^{125}I -C3a, but failed to inhibit the binding of ^{125}I -C5a. The same concentration of C3a_{des Arg} did not inhibit the binding of ^{125}I -C3a. Preincubation of the cells with 91 nM of unlabeled C5a_{des Arg} however, inhibited the binding of ^{125}I -C5a by 55–75%, whereas the binding of ^{125}I -C3a was not

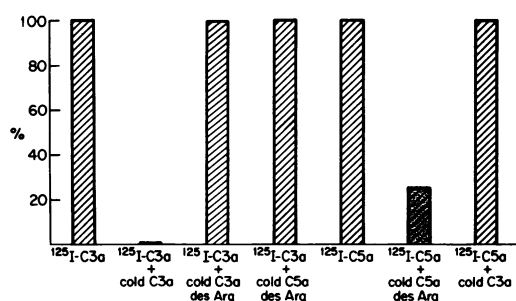


FIGURE 2 The binding of ^{125}I -C3a and ^{125}I -C5a to human leukocytes. 2.5×10^6 leukocytes were incubated with 4 ng of ^{125}I -C3a (26.5 $\mu\text{Ci}/\mu\text{g}$) or 4.5 ng of ^{125}I -C5a (13 $\mu\text{Ci}/\mu\text{g}$) at 37°C for 30 min. The final concentration of ^{125}I -C3a and ^{125}I -C5a were 0.44 and 0.41 nM, respectively. Maximum binding of ^{125}I -C3a and ^{125}I -C5a were 3,383 and 3,452 cpm (100%). Specificity of the binding was examined by preincubating cells in a high concentration of unlabeled C3a (550 nM) C3a_{des Arg} (550 nM) or C5a_{des Arg} (91 nM) before the addition of ^{125}I -C3a or ^{125}I -C5a. Average duplicate tube variability of counts per minute bound $<9\%$ of mean.

affected by C5a_{des Arg}. In separate experiments (not shown), a complete inhibition of ^{125}I -C5a binding to the cells was obtained when the cells were preincubated with 455 nM of unlabeled C5a.

Autoradiography of leukocytes with ^{125}I -C3a and ^{125}I -C5a. To identify the cell types to which C3a or C5a were bound, autoradiography of leukocytes was performed. Leukocytes were incubated at 37°C for 30 min in the presence of 0.55 nM ^{125}I -C3a or 0.36 nM ^{125}I -C5a. Control cells were preincubated in 550 nM of unlabeled C3a or 455 nM of C5a for 15 min at 37°C before the addition of ^{125}I -C3a or ^{125}I -C5a. The results of autoradiography are summarized in Table II. In addition, in separate experiments (Table III), leukocytes from two other normal donors were tested for ^{125}I -C3a and ^{125}I -C5a binding. When the leukocytes were incubated with ^{125}I -C3a, essentially all eosinophils had a significant number of grains ($>10/\text{cell}$) and over 50% had >30 grains/cell. Under the same experimental conditions, only 36–52% of basophils had a significant number of grains and most of them showed <30 grains/cell. Few lymphocytes bound ^{125}I -C3a under these experimental conditions. The relative preferential binding of C3a for eosinophils and C5a for neutrophils and eosinophils was consistent with all three leukocyte preparations. Preincubation of the leukocytes with unlabeled C3a completely inhibited the binding of ^{125}I -C3a with all types of cells. Representative auto-

TABLE II
Binding of ^{125}I -C3a and ^{125}I -C5a with Leukocytes from a Single Donor*

Cell type†	Cells treated with			
	^{125}I -C3a‡		^{125}I -C5a§	
	In buffer	In unlabeled C3a¶	In buffer	In unlabeled C5a¶
	%	%	%	%
Eosinophils	100	0	76	1
Neutrophils	20	1	100	1
Basophils	52	0	32	1
Monocytes	14	1	32	0
Lymphocytes	0	0	2	0

* The binding was evaluated by autoradiography. Numerals in the table represent the percentage of each type of leukocyte giving a significant number of grains ($>10/\text{cell}$). 200–500 of each type of cell was examined.

† Each experiment in Tables II and III involves leukocyte preparations from individual normal donors.

‡ 21 d exposure.

§ 28 d exposure.

¶ Cells were preincubated with 5 $\mu\text{g}/\text{ml}$ (550 nM) of unlabeled C3a or 5 $\mu\text{g}/\text{ml}$ (455 nM) C5a before the addition of ^{125}I -C3a or ^{125}I -C5a.

TABLE III
Binding of ^{125}I -C3a and ^{125}I -C5a with Leukocytes
from Two Additional Donors

Cell type	Cells treated with			
	^{125}I -C3a*		^{125}I -C5a†	
	In buffer	In unlabeled C3a	In buffer	In unlabeled C5a
	%	%	%	%
Eosinophils	100	1	59	1
Neutrophils	31	1	98	1
Basophils	48	1	41	0
Monocytes	22	0	38	1
Lymphocytes	3	0	2	0
Eosinophils	97		64	
Neutrophils	43		98	
Basophils	36	ND [‡]	36	ND [‡]
Monocytes	21		34	
Lymphocytes	2		1	

* ^{125}I -C3a (5.2×10^{-5} cpm/25 μl) was incubated with cells in the presence of 10 μg of cold C3a or in the buffer. Results of autoradiograph were examined after 14-d exposure.

† ^{125}I -C5a (6.5×10^{-5} cpm/25 μl) was incubated with cells in the presence of 2 μg of cold C5a or in the buffer. Results of autoradiograph were examined after 14-d exposure.

‡ No more cold C3a or C5a were available.

radiograph of an eosinophil and a basophil granulocyte treated with ^{125}I -C3a is illustrated in Figs. 3 and 4.

As shown in Tables II and III, the binding of C5a has a different profile from C3a. ^{125}I -C5a bound primarily to neutrophils and eosinophils and to a lesser extent to basophils. Essentially all neutrophils had a significant number of grains (>10 grains/cell) and about 70% showed >30 grains/cell after 14- or 28-d exposure. Specificity of binding was depicted by complete inhibition of ^{125}I -C5a binding when leukocytes were preincubated with unlabeled C5a.

Histamine release with C3a. C3a caused a dose-dependent release of histamine, which began at about 0.2 nM and usually peaked at a 10-fold higher concentration (Fig. 5). Release was usually under 50% of the total available histamine but occasional donors demonstrated over 50% histamine release, and the dose-response curves varied little from donor to donor. Insufficient material was available to ascertain whether higher concentrations of C3a would cause an inhibition of the release.

The kinetics of C3a-induced histamine release were similar to those described for C5a-induced release and quite different from anti-IgE-mediated release (Fig. 6). Release was rapid, being complete at the first time point studied (1 min). In contrast, the response of anti-IgE is sigmoid, with a short lag followed by a release process, which proceeds relatively slowly for

the next 20–30 min. Release was definitely greater at 37°C as opposed to 25°C; there is some conflict on this point with respect to C5a release reported by Siraganian and Hook (7) and Grant et al. (6).

Histamine release with COOH-terminal peptides of C3a, i.e., 5R and 8R. The activity of the synthetic COOH-terminal peptides, 5R and 8R, was studied using a donor whose leukocytes demonstrate $>50\%$ histamine release upon exposure to C3a. In the preliminary experiments, various concentrations of 5R and 8R were incubated with cells in the presence of 0.01 M EDTA Tris-A buffer at 37°C for 45 min, and the supernates of the tubes as well as various concentrations of 5R and 8R in the buffer were analyzed. The results indicated that the peptides were not fluorescent themselves and did not interfere with the fluorometric assay. When the same leukocytes were incubated with 5R or 8R in Tris ACM buffer, both 5R and 8R released a significant amount of histamine from the cells. In this experiment, the concentration of C3a for 50% histamine release was 9 μM , whereas the concentration of 5R and 8R required for 50% histamine release was 80 and 50 μM , respectively (Fig. 7). It appears that on a molar basis 5R was one-tenth, and 8R was one-fifth as active as C3a in inducing histamine release from human leukocytes.

Inhibition of ^{125}I -C3a binding to leukocytes by C3a, 5R, and 8R. Because the results of histamine release indicated that 5R and 8R are capable of reacting with basophils, the ability of the peptides to inhibit the binding of ^{125}I -C3a to leukocytes was examined. Aliquots of a leukocyte suspension, which contained 2.5×10^6 nucleated cells, were mixed with C3a, 8R, or 5R of varying concentrations. After incubation at 37°C for 30 min, 2 ng of ^{125}I -C3a was added to each tube, which was incubated at 37°C for another 30 min with constant shaking. The concentration of "bindable ^{125}I -C3a" in the final cell suspension was 0.066 nM. It is apparent in Fig. 8 that both 5R and 8R inhibited the binding of ^{125}I -C3a. Quantitatively, however, the COOH-terminal peptides were much less active than C3a. Thus, 50% inhibition of the binding was achieved by 14–15 μM of 8R, or 45–50 μM of 5R, whereas as low as 0.7–1 nM of unlabeled C3a was sufficient for 50% inhibition. Similar experiments using leukocytes from a different donor confirmed that molar concentrations of 5R and 8R required for 50% inhibition were $\approx 6,000$ and 2,000 times as high as the concentration of C3a for 50% inhibition.

DISCUSSION

The biological activity of the complement-derived anaphylatoxins has been appreciated for almost 80 yr. Only recently, however, has it been recognized that the components responsible for this activity are split

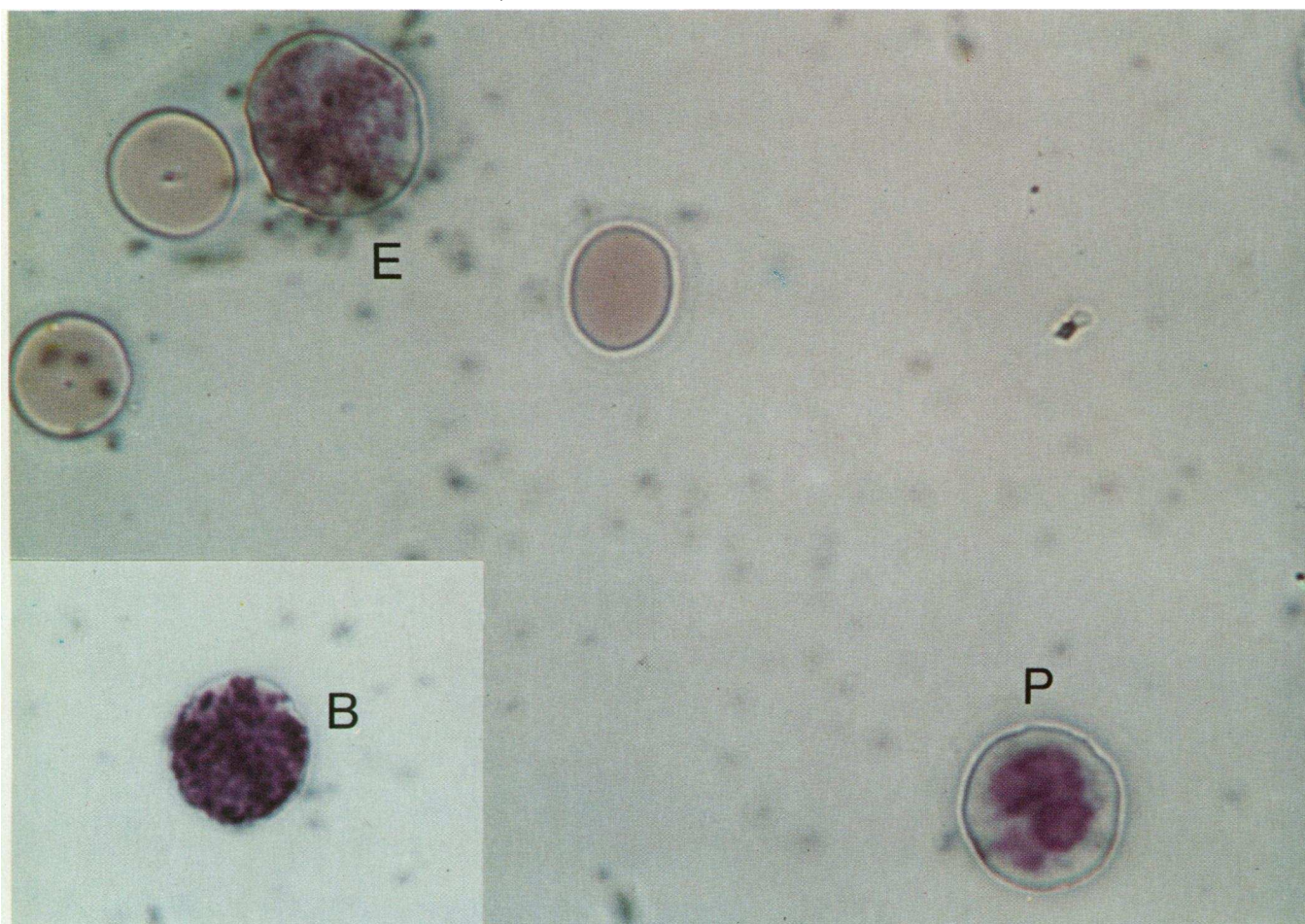


FIGURE 3 Autoradiograph of leukocytes treated with ^{125}I -C3a ($56 \mu\text{Ci}/\mu\text{g}$) or ^{125}I -C5a ($60 \mu\text{Ci}/\mu\text{g}$). Focus was directed on cells for morphology. Basophil was inserted in lower left for photomicrograph clarity; B = basophil, E = eosinophil, P = polymorphonuclear leukocyte.

products of C3 and C5, designated C3a and C5a (1, 2). In this paper we have been concerned with the binding of highly purified C3a or C5a to the cells in human blood particularly and with the ability of C3a to release histamine from human basophils. Histamine release with partially purified C5a has been previously reported (7).

Both C3a and C5a bind specifically to human leukocytes and unlabeled C3a blocks the binding of ^{125}I -C3a but not that of ^{125}I -C5a. These results indicate that the two anaphylatoxins recognize and combine with different leukocyte receptors. An interesting test of specificity was that the removal of the COOH-terminal arginine of C3a led to a totally inactive product that did not inhibit the binding of intact radiolabeled C3a. The des Arg derivative of C5a, on the other hand, retained some ability to block the binding of intact C5a. Thus, the terminal, basic amino acid is

responsible, at least to some degree, for the binding of C5a to leukocytes and is essential for the binding of C3a. This behavior was recently noted (18) for the binding of C5a and C5a_{des Arg} to purified human neutrophils.

Anaphylatoxin binding was further pursued by autoradiography with ^{125}I -labeled C3a and C5a. The two anaphylatoxins were quite different in this respect as well: C3a bound preferentially to eosinophils and to basophils, with lesser binding to neutrophils. On the other hand, C5a bound preferentially greater to neutrophils than to eosinophils and less well to basophils. In neither case was there significant binding to lymphocytes or erythrocytes, although monocytes were variably labeled. This differential binding may well be responsible for the pattern of biological activities that have been described for the two anaphylatoxins. Lack of any C3a uptake by lymphocytes

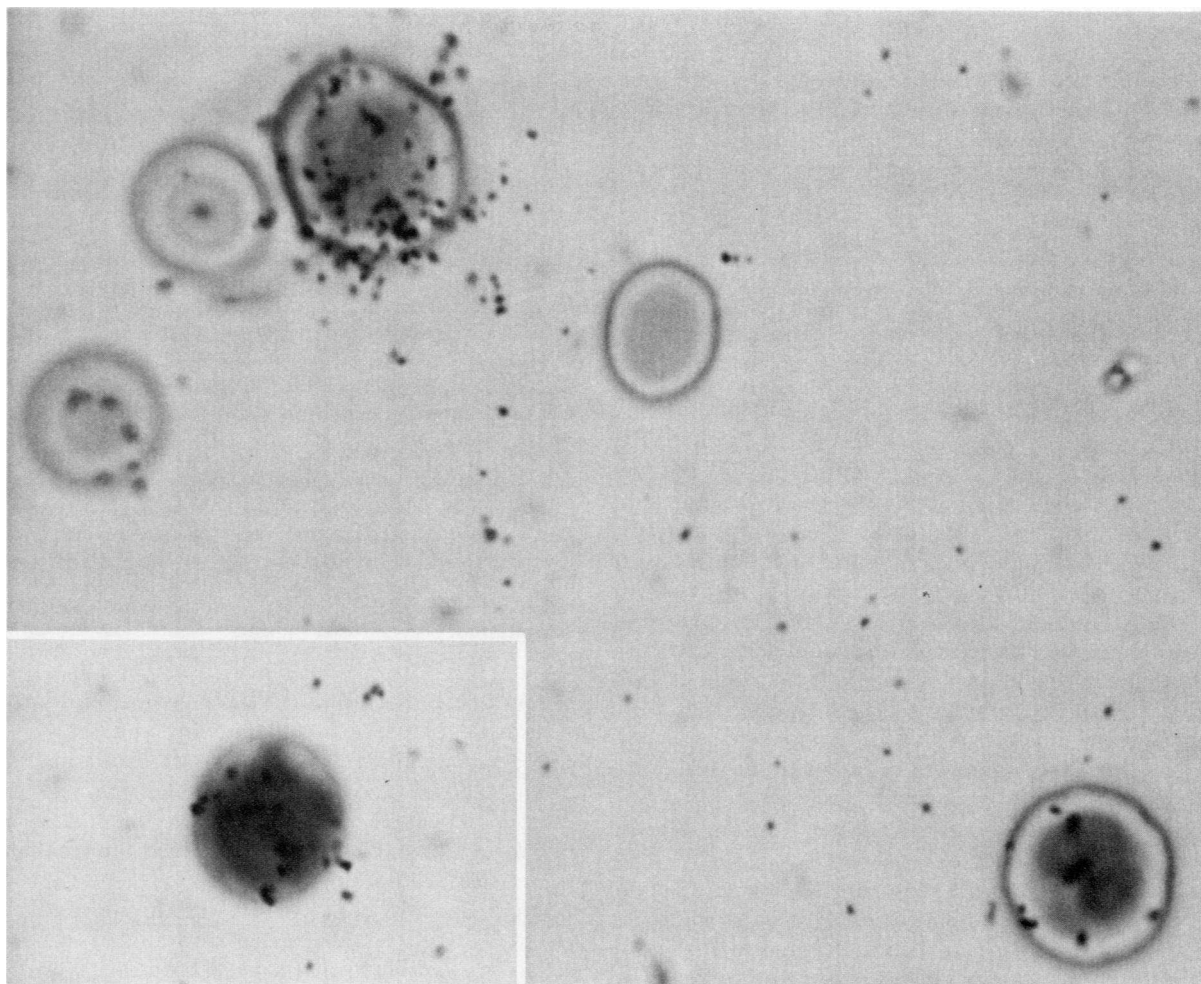


FIGURE 4 Autoradiograph of leukocytes treated with ^{125}I -C3a ($56 \mu\text{Ci}/\mu\text{g}$) or ^{125}I -C5a ($60 \mu\text{Ci}/\mu\text{g}$). Photomicrograph was focused on radioactive grains which can be seen in decreasing quantities above an eosinophil > basophil > polymorphonuclear leukocytes; nonsignificant grain counts are seen on erythrocytes.

suggests that the reported cytolytic effects of C3a may be related to nonspecific influences in the cell cultures studied (19) or to in vitro lymphatic tumor specific reactions.

These experiments constitute the first demonstration that a totally purified anaphylatoxin, C3a, is able to release histamine from human basophils. Previous studies of anaphylatoxin-induced histamine release have used crude activated serum or only partially purified preparations, felt to primarily contain C5a (4, 7). The release is extremely rapid, being over within 1 min. This result is similar to that reported with partially purified C5a but quite different from the release kinetics observed with antigen or anti-IgE-induced histamine release. In most individuals studied, the release induced by C3a was 30% of the total

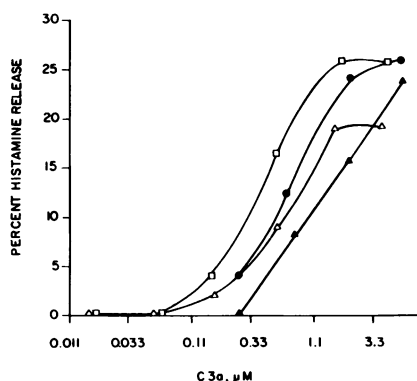


FIGURE 5 Histamine release by C3a from leucocytes obtained from four normal donors. Variation from the mean in duplicate samples tested for histamine release was <5%.

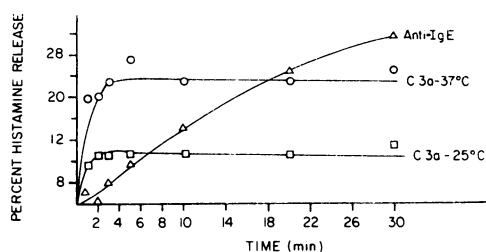


FIGURE 6 Kinetics of C3a-induced histamine release at 25° and 37°C. Histamine release by goat anti-IgE (0.05 μ g/ml) is shown for comparison.

histamine or lower, although occasional individuals demonstrated more than 50% histamine release. The concentrations required for release, 0.3–3.0 μ M, are compatible with those that can be generated from the C3 in serum. Previous studies (using partially purified C5a) of the temperature dependence of anaphylatoxin-induced histamine release have not clarified whether 37° or 25°C is optimal for release (7). In any event, the temperature dependence of this reaction appears to be less marked than that of IgE-mediated release. With the purified C3a, it is clear that 37°C is superior to 25°C, although release occurs at the lower temperature. Moreover, as has been reported for partially purified C5a, cells rapidly lose their ability to respond to C3a when they are allowed to remain at 37°C before challenge.

Of particular interest were the studies carried out with the COOH-terminal peptides of C3a. Both the pentapeptide and the octapeptide caused histamine release; on a molar basis the pentapeptide was one-tenth as active and octapeptide one-fifth as active as native C3a in inducing histamine release. The fact that these low-molecular-weight peptides are capable

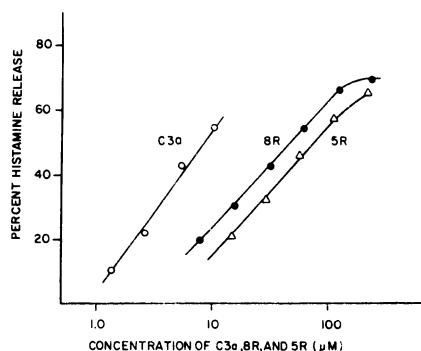


FIGURE 7 Dose-response curves of histamine release with C3a, 8R, and 5R. 2.5×10^6 mononuclear cells were incubated with various concentrations of C3a (○), 8R (●), and 5R (△) at 37°C for 45 min, and histamine released in the supernate was assayed. The experiment was repeated two additional times with similar results. Average SEM in repeat experiments was $\pm 3\%$.

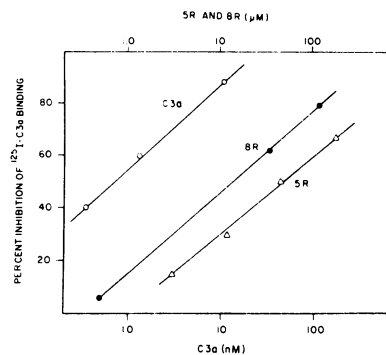


FIGURE 8 Inhibition of 125 I-C3a binding to leukocytes by C3a, 8R, and 5R. Leukocytes (2.5×10^6) were preincubated with various concentrations of C3a (○), 8R (●), and 5R (△) at 37°C, for 30 min before incubation of the cells with a constant amount of 125 I-C3a (60 μ Ci/ng) at 37°C for 30 min. The concentration of 125 I-C3a bindable to cells was 0.066 nM. Radioactivity bound to the cells was measured and the percentage of inhibition was calculated. With leukocytes obtained from three additional normal donors >1,000 times more 8R than C3a on a molar basis was required for inhibition of 50% 125 I-C3a binding.

of inducing histamine release points to another difference between anaphylatoxins and antigen-IgE induced histamine release. In the latter situation, cross-linking is clearly necessary. It seems highly unlikely, however, that a pentapeptide could cause a cross-link between two receptors for C3a. If this is the case, the mechanism of release would not depend upon receptor cross-linking. Because both peptides are highly basic, it is possible that the anaphylatoxin-induced histamine release is similar to that caused by basic peptides in other cell types. The binding studies with the peptides favor this hypothesis. Although both the pentapeptide and the octapeptide are capable of inhibiting the binding of intact C3a, they are much less effective in this than in causing histamine release. It is likely that both the pentapeptide and the octapeptide have a low affinity for the cell receptor. Because the C3a-induced histamine release is such a rapid process, disassociation of the peptide from the cells may not influence the ability to release histamine. In the inhibition assay, however, the peptides once bound to the cells could be easily displaced by the radiolabeled intact C3a. Furthermore, the relatively poor 125 I-C3a-binding inhibition of the octapeptide and the pentapeptide compared to native C3a, strongly implies that optimal binding activity requires C3a fragments greater than eight residues from the COOH-terminus.

The *in vivo* relevance of the anaphylatoxins remains to be established. The ability to work with purified proteins and peptides is, however, a large step forward and in this work we have established that multiple inflammatory cell types have separate receptors for C3a and C5a and that in the case of the basophil,

receptor occupancy leads to histamine release. It seems likely that further studies can define the parameters necessary to assess biological significance.

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