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Research Article

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Differential Effects of the Complement Peptides, C5a and C5a des Arg on Human Basophil and Lung Mast Cell Histamine Release

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Abstract

The ability of purified anaphylatoxins to induce human lung mast cell mediator release was investigated. In eight anti-IgE responsive (histamine release = 22±5%, mean±SEM) mast cell preparations of 1–96% purity, C5a and C5a des Arg (0.55 pg/ml to 55 µg/ml), failed to elicit or potentiate histamine release; lung fragments were similarly unresponsive. The related peptide C3a was also inactive. All anaphylatoxins failed to induce mast cell leukotriene C_4 (LTC_4) and prostaglandin D_2 (PGD_2) release. LTC_4 release was also negligible from basophils where C5a was a potent histamine release stimulus. Supernatants from C5a-challenged mast cells remained fully active on basophils, excluding carboxypeptidase inactivation of C5a as an explanation for the lung mast cell results. In contrast to lung, skin mast cells were C5a-responsive (histamine release = 8±1%, at 55 µg/ml, $n = 2$). We conclude that C5a, though devoid of activity on the human lung mast cell, is a human basophil and skin mast cell secretogogue. These findings demonstrate significant organ-specific heterogeneity in mast cell responsiveness.

Introduction

The biologically active fragment of the fifth complement component, C5a, is capable of triggering release reactions from diverse inflammatory cell types. These reactions result in increased vascular permeability, smooth muscle contraction, and an influx of inflammatory cells, especially neutrophils, that may cause end organ damage (1). A major target organ for these protean effects is the lung (2). Pathologically, dose-dependent lung destruction is characterized by the closure of arterioles and small airways, and disruption of the alveolar network (2). In rodents and rabbits, direct intrabronchial instillation of anaphylatoxins causes severe lung injury and acute respiratory distress (3, 4). Removal of the COOH-terminal arginine of human C5a by serum carboxypeptidase-N results in formation of C5a des Arg, nullifying its anaphylatoxin properties and diminishing its inflammatory effects (5, 6).

Anaphylatoxins have long been suspected of playing a significant role in allergic disease. The effects of C5a as an inducer of histamine release from human basophils (7–9) and rat mast cells (10) have been well characterized. Studies to date characterizing the effects of anaphylatoxins in allergic lung reactions have been performed using animal models (11–14), and significant interspecies differences have been noted (11). This study was undertaken to examine the ability of purified human C5a and C5a des Arg to directly induce histamine release from purified human lung mast cells, the central cell type of human asthma (15, 16). We utilized highly purified, well characterized lipopolysaccharide-free (< 10 ng/ml) preparations of C5a and C5a des Arg to avoid misleading results from minor contaminants (17).

We report that purified C5a, though a potent human basophil secretagogue and a weak human skin mast cell secretagogue, is devoid of direct anaphylatoxin activity for the human lung mast cell.

Methods

Materials. The following were purchased: elastase Type I, chymopapain (Sigma Chemical Co., St. Louis, MO); collagenase (Worthington Biochemicals, Freehold, NJ); DNase, pronase (Calbiochem-Behring Corp., San Diego, CA); gelatin (Difco Laboratories, Detroit, MI). Rabbit anti-human IgE antibody was generously provided by Dr. Terumo Ishizaka, (Johns Hopkins University, Baltimore, MD). Leukotriene C_4 (LTC_4)[1] standard and the antisera for the leukotriene C_4 radioimmunoassay were provided by Dr. Anthony Ford-Hutchinson, (Merck-Frosst Inc., Montreal, Canada). The prostaglandin D_2 standard was purchased from Cayman Chemicals (Denver, CO); the antisera for the radioimmunoassay was provided by Dr. Shuh Naramiya (Kyoto University, Kyoto, Japan). Tritiated standards were obtained from New England Nuclear (Boston, MA).

Buffers. Lung fragment washes were performed in Tyrode's buffer that contained (g/liter): NaCl, 8.0; KCl, 0.2; NaH_2PO_4, 0.05, and glucose, 1.0. The buffer was titrated to pH 7.2 by the addition of $NaHCO_3$. Mast cell isolation and elutriation were performed in TGMD: Tyrode's buffer with gelatin (1.0 g/liter), magnesium (0.25 g/liter; 1 mM), and DNase (0.01 g/liter) added. Basophil washes were performed in a Pipes-albumin (PA) buffer that contained: Pipes 7.6 g/liter; NaCl, 6.4 g/liter; KCl, 0.37 g/liter; NaOH 10 N, 4.2 ml/liter; and 3× recrystallized human serum albumin, 0.03 g/liter. TGMDCM or PAGCM were the Tyrode's or PA buffers containing: $CaCl_2 \cdot 2H_2O$, 0.14 g/liter (1 mM); $MgCl_2 \cdot 6 H_2O$, 0.2 g/liter (1 mM); and glucose, 1.0 g/liter.

Leukocytes. After obtaining informed consent, venous blood was drawn from patients undergoing lung resection, or from atopic and nonatopic normal volunteers, then sedimented over Dextran 75-

1. *Abbreviations used in this paper:* C5a, the fragment of the fifth component of complement with anaphylatoxin activity; C5a des Arg, the fragment formed by removal of the COOH-terminal arginine from C5a; LTC_4, leukotriene C_4; PA, Pipes-albumin; PAF, platelet-activating factor; PAGCM, Pipes-albumin buffer containing gelatin, magnesium, and DNase; TGMD, Tyrode's buffer containing gelatin, magnesium, and DNase.

EDTA (2.5 mM) for 1 h (18). Mixed leukocytes, usually containing ≤ 1.0% basophils, were washed thrice at 22°C in PA buffer.

Human lung mast cells. Mast cells were dispersed from human lung by methods previously reported (19, 20). Briefly, lung specimens obtained at thorocotomy for bronchogenic carcinoma were finely minced and extensively washed in divalent cation-free Tyrode's buffer. Fragments were briefly incubated twice in a mixture of pronase (2 mg/ml) and chymopapain (0.5 mg/ml). Freed cells were harvested through Nytex nylon cloth (150 μm pore size). Residual fragments were further exposed to a mixture of collagenase (1 mg/ml) and porcine elastase (10 U/ml). All incubations and washes were performed at 22°C; all recovered cells were immediately washed three times in large volumes of TGMD. Mast cell purities in these human lung cell suspensions ranged from 1 to 8% as determined by alcian blue staining (21). In experiments requiring purified lung mast cells or designed to examine mast cell subpopulations separated on the basis of diameter, cell suspensions were subjected to counter-current elutriation, using previously reported methods (20). Mast cells were purified (80–96%) by flotation of enriched elutriation fractions through a discontinuous Percoll gradient (22).

Chopped human lung. The methods used to prepare and challenge human lung fragments have been reported in detail previously (23, 24). Briefly, subpleural parenchymal strips well separated from tumor were carefully dissected free of visible blood vessels and pleura. Cut fragments of 10–20 mg each were washed six times in large volumes of Tyrode's buffer at 22°C; fragments were challenged within 1–2 h of lung resection.

Human skin mast cells. Mast cells were dispersed from human foreskin by the methods of Benyon et al. (25). Briefly, skin fragments were finely minced and extensively washed in divalent cation-free Tyrode's buffer. Fragments were exposed to collagenase (1.5 mg/ml) and hyaluronidase (0.5 mg/ml). Dispersed cells were harvested through Nytex cloth and washed with RPMI/1.5% fetal calf serum. Mast cell purities ranged from 1 to 3%.

C5a, C3a, and C5a des Arg. C5a and C3a were purified from human serum as previously described (17). The C5a preparation used for these experiments contained 55 μg protein/ml and had 1×10^6 U/ml of myeloperoxidase-releasing activity when tested with cytochalasin B–treated human neutrophils (26). Both the C3a and C5a were positive when tested for their ability to cause increased vascular permeability in guinea pig skin (5). C5a des Arg was prepared from C5a by treatment with insolubilized carboxypeptidase B in PBS at pH 7.5 at 22°C for 20 h. After treatment with carboxypeptidase, a shift in the R_f from 0.49 to 0.40 was observed on acid 6% polyacrylamide gel electrophoresis, indicating conversion to the C5a des Arg form. All preparations were stored at −20°C until thawed for use. Dilutions were prepared in either PAGCM or TGMDCM.

Histamine release assay. Washed leukocytes (40×10^3 basophils/tube) or mast cells ($10–50 \times 10^3$/tube) were challenged in duplicate with buffer, anti-IgE, C5a or C5a des Arg, or C3a at either 22 or 37°C in PAGCM or TGMDCM. The concentrations of C5a and C3a used covered a physiologic range based on the maximum amounts of each that could be generated from activation of serum complement: C3 1 mg/ml, C3a from 100% C3 conversion 60 mg/ml; C5 50–100 g/ml, C5a from 100% conversion 3–6 g/ml. In chopped lung studies, six 10–20 mg fragments in 0.5–1.0 ml were similarly challenged in TGMDCM. After 45 min for basophil incubations (7, 8, 18) or 20 min for mast cell incubations (19), cells were rapidly pelleted and supernatants removed for analysis of mediator release. In chopped lung studies, one-half of the supernatant was removed for histamine, LTC$_4$ and PGD$_2$ analysis and the volume was replaced with 4% perchloric acid. In the experiments indicated, basophils, mast cells, or chopped lung preparations were pretreated with cytochalasin B (1 μg/ml) for 5 min before addition of C5a or C5a des Arg. Histamine released into the supernatant was expressed as the net histamine released divided by the total histamine content × 100%. The total cellular histamine content was determined by cell lysis in 2% perchloric acid. The total histamine content of lung fragments was determined by boiling the perchloric

acid-treated fragments for 10 min. Spontaneous histamine release was always < 5% of cellular histamine. Histamine measurements were performed by the automated spectrofluorometric method of Technicon Co. (Tarrytown, NY). Variation between replicates was consistently < 5%. All experiments were performed on at least three individual basophil and mast cell donors.

LTC$_4$. LTC$_4$ measurements were performed on unextracted mast cell or lung fragment supernatants using the radioimmunoassay previously described (27). Though the antibody shows 50% cross-reactivity with leukotriene D$_4$ (LTD$_4$), the LTC$_4$ generated by isolated mast cells under these experimental conditions is not further metabolized (28). The assay is sensitive to 0.1 ng/ml of LTC$_4$.

Prostaglandin D$_2$ (PGD$_2$). PGD$_2$ measurements were similarly performed on unextracted supernatants. The antibody has < 0.5% cross-reactivity with PGF$_{2\alpha}$ PGE, TxB$_2$ and 6-keto-PGF$_{1\alpha}$ (29). This assay is sensitive to 0.1 ng/ml of PGD$_2$.

Statistics. Data were expressed as the mean±SEM in the text and figures. Multiple comparisons were performed using an analysis of variance (ANOVA) and a Newman-Keul test. Significance in all tests was taken at $P < 0.05$.

Results

Anaphylatoxin challenge of human lung mast cells. Mast cells from eight different lungs were challenged with release stimuli (Fig. 1). In the first four experiments, mast cells at 80, 92, 93, and 96% purity were challenged for 20 min at 37°C with varying doses of anti-IgE (0.1–30 μg/ml) and C5a and C5a des Arg anaphylatoxins (0.55 pg/ml to 55 μg/ml). Anaphylatoxin, but not anti-IgE, challenges were performed in the presence of cytochalasin B (1 μg/ml). In all four experiments, purified mast cells responded to anti-IgE (peak release of 23±5%, mean±SEM) but failed to respond to anaphylatoxins at any dose. In four additional human lung experiments we examined the possibility that mast cells needed "helper" cell types to respond. Lung cell suspensions containing 1, 4, 6, and 8% mast cells were similarly challenged. Again, anaphylatoxin failed to elicit histamine release, while anti-IgE proved an effective stimulus (peak release = 21±7%).

Because of a prior report showing a loss of basophil responsiveness to complement if cells were exposed to 37°C before challenge (7), lung cells were constantly kept at 25°C until
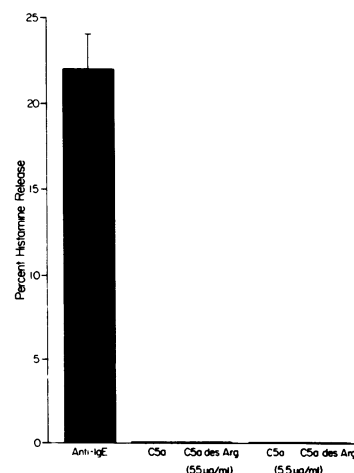

Figure 1. Effects of anti-IgE, C5a and C5a des Arg on mast cells derived from eight human lungs. Mast cells of 1–96% purity were incubated with stimuli for 20 min at 37°C then supernatants were analyzed for histamine release. Mast cells challenged with C5a and C5a des Arg but not with anti-IgE were preincubated with cytochalasin B (1 μg/ml) for 5 min before challenge. Because the complement results from the four lung suspension and four purified mast cell experiments were identical they were combined above. Anti-IgE results represent the peak histamine release response (mean±SEM) from dose-response curves.

challenge in these latter four experiments. The same lung cell suspensions challenged with anaphylatoxins at 25°C instead of 37°C also failed to respond.

Despite the failure to exert a direct mast cell release effect, we investigated the potential for anaphylatoxins to potentiate an anti-IgE challenge in the absence and presence of cytochalasin B (1 µg/ml). As shown in Fig. 2, no enhancement of anti-IgE release was observed. C5a and C5a des Arg also failed to induce mast cell histamine release in buffer containing 44% deuterium oxide (D_2O), an enhancer of IgE-mediated histamine release (results not shown).

Observations by Lepow et al. (30) and Wuepper et al. (31) suggested that the anaphylatoxin C3a functioned as a human mast cell secretagogue. Consistent with this were studies of rat peritoneal mast cells demonstrating that C3a was of equal potency to C5a as a histamine liberator (10). We, therefore, challenged three purified human lung mast cell preparations (76, 92, and 93% purity) with dilutions of C3a and C3a des Arg (55 to 0.55 µg/ml). Similar to C5a and C5a des Arg, these anaphylatoxins failed to elicit lung mast cell histamine release (anti-IgE induced release = 20.8±11.6%).

Challenge of mast cell subsets. Though receptors for C5a have been identified on the surface of almost all human neutrophils, only 50–60% of monocytes and < 10% of lymphocytes bear such receptors (32). Furthermore, the presence in leukocyte subpopulations of C5a receptors may have func-
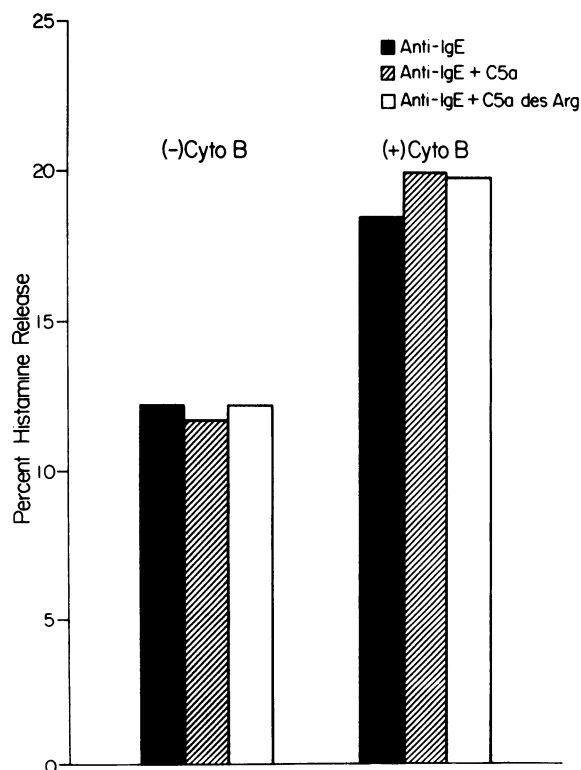
tional correlates (32). We therefore examined functionally distinct human lung mast cell subpopulations, separated on the basis of their diameters by elutriation (20), for selective responses to release stimuli (Fig. 3). As we previously reported, most mast cells eluted in fractions representing cells of 12–15 µm in diameter (20); also, with increases in mast cell diameter, there was an increase in responsiveness to maximal anti-IgE (defined by dose-response curves). However, none of these distinct mast cell subpopulations were stimulated by anaphylatoxins at any concentration.

Anaphylatoxin challenge of fresh lung fragments. During the course of our experiments we examined whether the conditions of our mast cell isolation procedure were responsible for the observed failure of C5a and C5a des Arg to evoke human lung mast cell histamine release. We also questioned if the basophils of our lung donors were likewise unresponsive. In three patients undergoing lung resection, we examined the effects of anti-IgE, C5a, and C5a des Arg on lung fragments challenged within 1 to 2 h of resection, mast cells derived from their lung fragments, and their peripheral blood basophils obtained within 18 h of surgery (Table I). Anti-IgE challenge of lung fragments, mast cells and basophils elicited histamine release responses that were not significantly different (ANOVA) from each other. C5a challenge of each patient's basophils resulted in release responses that were significantly greater ($P < 0.05$) than the responses of their isolated mast cells and lung fragments. The donor basophil responses to C5a were not significantly different than their responses to anti-IgE. Challenge of lung fragments from subject 3 with C3a (final concentration 55 µg/ml) also failed to evoke histamine release.

Examination of mast cells for carboxypeptidase activity. To further examine the differential effects of C5a on basophils and lung mast cells, we considered the possibility that lung mast cells rapidly inactivated C5a by carboxypeptidase-like or oxidative activity. In three experiments, mast cells were challenged with buffer, C5a, or C5a des Arg for 20 min at 37°C (Fig. 4). Part of the mast cell supernatant was withdrawn for histamine release analysis and part was transferred to fresh basophils preincubated at 37°C. Control basophils were chal-
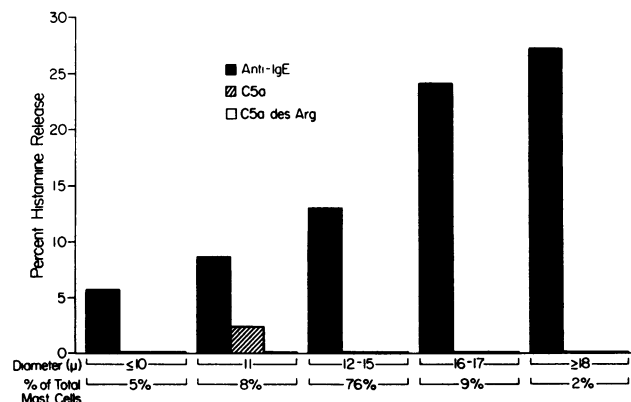

Figure 2. Effects of anaphylatoxins on anti-IgE-induced histamine release. Anaphylatoxins were added to suspension mast cells (80% purity) preincubated for 5 min with cytochalasin B (1 µg/ml) or buffer. After 15 min, anti-IgE (10 µg/ml) was added and the reaction allowed to proceed for an additional 20 min at 37°C. No release enhancement was attributable to anaphylatoxins in the absence or presence of cytochalasin B. The experiment shown is one of three with similar results.

Figure 3. Failure of anaphylatoxins to stimulate diameter-separated human lung mast cell subpopulations. Shown are mast cell responses within subpopulations to maximal anti-IgE challenge, followed by C5a (55 µg/ml) and C5a des Arg (55 µg/ml). The minor release seen in response to C5a in the 11-µm subset was not observed in two additional heterogeneity studies. Figures for mast cell diameters and percent of total mast cells are derived from reference 20.

Table I. Basophil, Mast Cell, and Lung Fragment Responsiveness to Release Stimuli*‡ (Percent Histamine Release)

Subject No.	Basophils			Mast cells			Lung fragments		
	Anti-IgE	C5a	C5a des Arg	Anti-IgE	C5a	C5a des Arg	Anti-IgE	C5a	C5a des Arg
1	8	11	2	10	1	0	7	0	0
2	22	35	1	38	1	0	29	0	0
3	5	14	3	15	0	0	16	1	1

* Dose-response curves were performed for all stimuli, though only peak responses are shown. Peak basophil responses were achieved at anti-IgE concentrations of 0.3–0.45 μg/ml; anaphylatoxins at 0.55–22.5 μg/ml. Peak mast cell anti-IgE responses were obtained at 3–10 μg/ml. The anaphylatoxin concentrations shown for mast cells challenges are 55 μg/ml, but were no different for any concentration of C5a or C5a des Arg. Mast cell purities in the three subjects were 92, 80, and 93%, respectively.

‡ Dispersed (n = 2) and minced (n = 1) foreskin fragments challenged with C5a at 55 μg/ml released 4–9% of cellular histamine. These same preparations challenged with C5a des Arg at 55 μg/ml were unresponsive (see Results).

lenged with equivalent final concentrations of C5a and C5a des Arg not exposed to mast cells. As expected, mast cells were unresponsive to anaphylatoxins. Basophils directly challenged with C5a (22.5 μg/ml) demonstrated 27.2±16.1% histamine release; C5a that was first exposed to mast cells stimulated 27±14.3% histamine release. In addition, C5a mixed in a test tube with supernatant removed from buffer-challenged mast cells resulted in 26.1±16.4% histamine release. These histamine release values were not significantly different from each other.

LTC₄ and PGD₂. In two studies of purified mast cells, release of the non-preformed mast cell mediators, LTC₄ and PGD₂ were examined. Measurements of both mediators after buffer, C5a, C5a des Arg, and C3a challenges were below the levels of detection for the assays. Maximal anti-IgE challenges (histamine release = 20±6%) induced LTC₄ release of 32.7±2.3 fg/mast cell and PGD₂ release of 34.7±19.2 fg/mast cell. In three lung fragment studies, anaphylatoxin challenges

Figure 4. Effects of mast cells on activity of anaphylatoxins. Mast cells were incubated in buffer, anti-IgE (3 μg/ml), C5a (55 μg/ml) or C5a des Arg (55 μg/ml), in a final volume of 200 μl. After a 20-min incubation at 37°C, cells were rapidly sedimented; 50 μl was then removed for histamine analysis and 100 μl was transferred to basophils preincubated at 37°C in 100 μl of buffer. Final anaphylatoxin concentration in all basophil challenges was 22.5 μg/ml. Final anti-IgE concentration in basophil challenges was 0.3 μg/ml. Supernatant A (buffer and mast cells × 20 min, 37°C) was removed and incubated with C5a in a test tube at 37°C for 20 min then added to basophils. Activity was fully evident. The experiment is one of three with similar results.

failed to induce LTC₄ or PGD₂ release. Anti-IgE challenge (histamine release = 17±5%) resulted in LTC₄ release of 6.1±3.3 ng/g wet wt and PGD₂ release of 135±3.5 ng/g wet wt of lung. Interestingly, even though the anaphylatoxins are potent stimulators of basophil histamine release, they failed to activate pathways of LTC₄ generation. After challenges of three highly responsive basophil donors with C5a (maximum histamine release = 48±4% at 5.5 μg/ml, EC₅₀ = 0.9 μg/ml) and with C5a des Arg (maximum histamine release = 26±4% at 55 μg/ml, EC₅₀ = 10.7 μg/ml) LTC₄ was undetectable in the supernatants. This contrasts with anti-IgE–induced (0.3 μg/ml) release: histamine release of 57±6.0% was coupled with appreciable LTC₄ release of 2.4±0.7 fg/basophil.

Human skin mast cells. The impotence of C5a as a lung mast cell secretagogue contrasted strikingly with prior in vivo studies where injection of C5a into human skin produced wheal and flare responses and resulted in mast cell degranulation as visualized both at the light and electron microscopic level (33, 34). To evaluate potential human organ-specific differences in reactivity, mast cells dispersed from human foreskin were challenged with C5a, C5a des Arg, and ionophore A23187 (0.3 μg/ml). In two experiments, C5a induced 8±1% histamine release at 55 μg/ml, 6±1% at 5.5 μg/ml and 0% at 0.55 μg/ml. At the same concentrations, C5a des Arg failed to elicit release. Ionophore-induced release was 28±4%. In a third experiment, minced skin fragments were challenged; C5a induced 4% histamine release, C5a des Arg 0% release and ionophore 6% histamine release. The limitations in specimen size did not allow for arachidonate metabolite measurements in the skin samples.

Discussion

A critical role for the anaphylatoxins C5a and C5a des Arg has been suggested in both immunologic and nonimmunologic lung disease (1–4, 11–14). In fact, the lung may serve as the major target organ for the effects of these mediators (11). Studies undertaken to identify the nature of the mediators released during anaphylatoxin-induced lung injury have found products derived from degranulated mast cells, the central cell type of allergic asthma (11, 12). These studies also emphasized the significant species differences among the specific activities of the anaphylatoxins themselves. For this reason, a study was undertaken to define the actions of purified human C5a and its
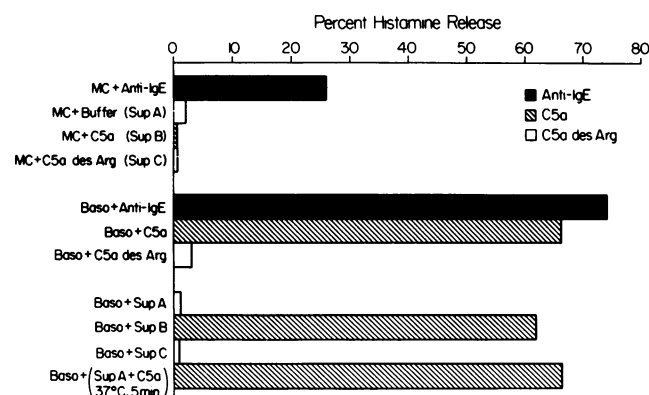
putative physiologic form C5a des Arg, on human lung mast cells.

We conclude that, in contrast to animal mast cells and lung tissues, human lung mast cells and tissues fail to respond to purified human C5a and C5a des Arg, either directly or synergistically with an IgE-mediated stimulus. Furthermore, there is no evidence for inactivation of C5a by any activity derived from mast cells: C5a exposed to mast cells is as active in inducing basophil histamine release as is our fresh C5a preparation. Conditions of the mast cell isolation cannot account for our results. Mast cells in suspension retain their capacity to respond to anti-IgE, a trigger whose release mechanism, like C5a, is also dependent on cell surface receptors. Human lung fragments, challenged within 1 to 2 h of resection and never exposed to mast cell isolation conditions, likewise are entirely unresponsive to anaphylatoxins. Basophils from the same lung donors are quite responsive to anti-IgE and even more responsive to C5a, indicating that our mast cell "donors" are anaphylatoxin "responders."

Since prior studies implicated that small functional subsets of leukocytes can be defined based on C5a receptors (32), we examined functionally distinct subsets of human lung mast cells defined morphologically on the basis of their diameters (20). We felt that direct C5a challenge of quantitatively small subsets might magnify and define effects that are lost in whole lung fragments or in whole lung suspensions. Again, under these conditions, we failed to find a C5a responder subset.

The failure of human lung mast cells to respond to C5a obviously contrasts with older human skin studies (33, 34). In those studies, injection of anaphylatoxins produced wheal and flare responses that were ascribed to cutaneous mast cell degranulation; reactions were partially inhibitable if skin sites were pretreated with an antihistamine (33, 34). One obvious explanation for these differences is that of organ-specific differences in mast cell type and function (lung vs. skin). Studies in rodents have clearly demonstrated marked differences in structure and function between mast cells in the peritonium (connective tissue mast cells) and gastrointestinal mucosa (mucosal mast cells) (35). Even within organs, (e.g., rat skin), there is histochemical evidence of two mast cell subtypes (36).

To test the hypothesis that C5a responsiveness was organ specific, we challenged both skin mast cell suspensions (two experiments) and minced fragments (one experiment) with C5a and C5a des Arg. In each instance, low level (4–9%) histamine release resulted. This in vitro data is consistent with the intradermal injection studies. Since human skin reactivity to C5a (and C3a) are only partially inhibitable with antihistamines, it is possible that the observed wheal and flare responses are heavily dependent on the direct stimulation of cells other than mast cells. In fact, this has been shown in rabbits where the skin reactivity of intradermally administered C5a is independent of histamine release (37): the observed plasma exudation and edema appears to involve a synergism between complement fragments and vasodilator prostaglandins (e.g., PGE_2) derived from non-mast cell sources (37). Another alternative explanation is that target cells (e.g., neutrophils), directly stimulated by C5a, may release their own vasoactive mediators and/or secondarily stimulate secretion of mast cell/basophil mediators. For example, C5a can stimulate neutrophil generation and release of the labile arachidonate intermediate leukotriene A_4 (LTA$_4$) (38); released LTA$_4$ may then be "remodelled" by mast cells and other cells to generate bioactive arachi-

donate products (38). Though this hypothesis is attractive, our results cast doubt that the C5a response is dependent on neutrophils. C5a challenges of whole lung suspension (5% neutrophils) and of mixed leukocyte preparations (50–70% neutrophils) failed to stimulate LTC$_4$ release. This result was particularly surprising in the mixed leukocyte preparation, where C5a proved as effective a histamine liberator as anti-IgE, but was an insufficient signal to activate basophil arachidonate metabolism. Platelet-activating factor (PAF), a mediator capable of contributing to wheal and flare reactions, is directly released from neutrophils following anaphylatoxin challenge (39, 40), and may prove an important contributor to the skin responses.

In conclusion, purified C5a is a potent stimulant of the circulating basophil, a much weaker stimulant of the human cutaneous mast cell, and is devoid of activity on the human lung mast cell. These results point to a major difference in the biology of these related cell types (16). Our studies also suggest that significant differences exist in responsiveness to complement fragments among lung mast cells of human and animal origins.

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