ANAPHYLATOXIN C3a ENHANCES MUCOUS GLYCOPROTEIN RELEASE FROM HUMAN AIRWAYS IN VITRO

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Mucus hypersecretion accompanies such pulmonary diseases as asthma, bronchitis, and pneumonia. While some of the factors capable of influencing mucus secretion have been identified (1–9), no investigations into the possible interaction of activated complement products with mucus secretion have been reported. During the course of complement activation by either the alternative or the classical pathway, anaphylatoxins are generated that are capable of causing mast cell and basophil degranulation, inducing bronchial smooth muscle spasm and attracting polymorphonuclear leukocytes. It is possible that immunologic pulmonary inflammatory reactions are accompanied by anaphylatoxin generation, particularly during the course of fungal or bacterial infections (10), and perhaps in association with other airway diseases as well. Thus, we were interested in determining whether the anaphylatoxin C3a was capable of influencing mucous glycoprotein (MGP)[1] secretion in vitro.

Materials and Methods

Reagents. We used CMRL-1066 medium, penicillin, streptomycin, and amphotericin B (Gibco Laboratories, Grand Island, NY); [^{3}H]glucosamine (20 Ci/mM) (ICN, Irvine, CA); [^{3}H]leukotriene C_4 (LTC_4) (35.7 Ci/mM) (New England Nuclear, Boston, MA); Ultrafluor (National Diagnostics Inc., Somerville, NJ); trichloroacetic acid (J. T. Baker Chemical Co., Phillipsburg, NJ); cycloheximide (Sigma Chemical Co., St. Louis, MO); phosphotungstic acid (Mallinckrodt Inc., St. Louis, MO); and LKGD analytical thin-layer chromatography (TLC) precoated plates (Whatman Laboratory Products Inc., Clifton, NJ). All other materials were obtained as cited in a previous study (6).

Preparation of Human Airways for Culture. Human lungs were obtained at surgery, primarily from tumor resections. Normal-appearing airways, 2–10 mm in diameter, were fragmented into replicates and cultured as previously described (4, 6). The airway explants were maintained in CMRL-1066 medium with penicillin (100 μg/ml), streptomycin (100 μg/ml), and amphotericin B (0.5 μg/ml), in a controlled-atmosphere chamber gassed with 45% O_2, 50% N_2, and 5% CO_2, and incubated at 37°C.

[1] *Abbreviations used in this paper:* ETYA, eicosatetraynoic acid; HETE, hydroxy-eicosatetraenoic acid; HPLC, high-pressure liquid chromatography; LTC_4, leukotriene C_4; MGP, mucous glycoprotein; MMS, macrophage-derived mucus secretagogue; PBS, phosphate-buffered saline; TLC, thin-layer chromatography.

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Radiolabeling of MGP. MGP were radiolabeled by incorporating [^{3}H]glucosamine (1 μCi/ml) from the culture medium. Explants were initially incubated for 16 h in the absence of [^{3}H]glucosamine, washed twice with media, and then incubated with [^{3}H]-glucosamine for a 16-h baseline period (period I). The period I supernatants were harvested; fresh culture medium without glucosamine was added for an additional 4-h period (period II), and the culture supernatants were subsequently harvested. Airway cultures were cycled through 16-h baseline incubations (period I) and 4-h experimental incubations (period II) for up to 8 d.

One-milliliter aliquots of the culture medium and washes from period I and period II were added to 1 ml of 10% trichloroacetic acid and 2% phosphotungstic acid. After 16 h at 4°C, the samples were centrifuged for 5 min at 2,500 *g*, and the supernatant was decanted. The pellet was washed twice with 10% trichloroacetic acid and 1% phosphotungstic acid, and resuspended in 1 ml of 0.2 M NaOH. The suspension was vortexed, incubated at 22°C for 12 h, and vortexed again; 0.5-ml aliquots were then added to 10 ml of Ultrafluor and counted in a Beckman 8100 scintillation counter (Beckman Instruments, Inc., Cedar Grove, NJ).

Effect of Pharmacologic Manipulations on the Release of MGP. The effect of pharmacologic manipulations on the release of [^{3}H]glucosamine-labeled MGP was determined by adding agents to cultures at the beginning of period II. The ratio of cpm of period II to cpm of period I for each sample was termed the secretory index. The effects of the pharmacologic agents were determined by comparing the secretory indices of manipulated samples with matched, unmanipulated control samples. Controls were derived from the same tissue, cultured in parallel, and handled identically to the experimental samples, except that the pharmacologic agents were not added. Thus, each airway culture provided its own baseline period (period I) as well as a stimulated period (period II), and the effects of each pharmacologic manipulation could be compared with matched controls.

Complement Components and Antibody Preparations. C3a was prepared from highly purified human C3 (11), under conditions exactly as previously described (12). The C3a was concentrated with Amicon UM-2 membranes and dialyzed against phosphate-buffered saline (PBS) with the use of spectra-Por 3 tubing. The protein concentrations of the C3a preparations were measured using a microbiuret assay (13) with bovine serum albumin as a standard. At pH 8.3, the C3a produced a typical cationic precipitin arc in immunoelectrophoresis against a burro monospecific antiserum (14). Intact C3 also precipitated with the anti-C3a antiserum, but C3b failed to react. The C3a fragment failed to react with sheep or goat antisera to trypsin-degraded C3b. These antisera were the kind gift of Dr. Carl Hammer, NIH, and have been described elsewhere (14). An anti-C3a immunoadsorbent was prepared by incubating 1 ml of the anti-C3a serum for 2 h at room temperature with 1.5 ml of packed staphylococcal protein A–conjugated Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden) in PBS-carbonate buffer at pH 8.2. The absorbent was washed five times with the same buffer, then 42 μg C3a was added, and incubated for 1 h at room temperature. As a control, an equivalent aliquot of C3a was incubated with another 1.5-ml aliquot of protein A–Sepharose that had not been preincubated with the antiserum. Following incubation, the absorbent and control Sepharose were removed by centrifugation, the pH of the supernatants were adjusted to 7.3 with 2–3 μl of 1N HCl, and the supernatants were filtered through 0.22-μm membranes. $F(ab')_2$ fragments of anti-C3a were prepared as described (15). C3a des Arg was prepared by cleavage of the purified C3a with the use of 1% (wt/wt) diisopropylfluorophosphonate-treated carboxypeptidase B. An anodal shift of the C3a des Arg relative to the uncleaved C3a was the immunoelectrophoretic indicator used to confirm cleavage.

Characterization of LTC_4. Culture medium containing LTC_4 was chromatographed by TLC using a one dimensional, ascending system developed in propanol/ammonia/water (6:3:1) (16). [^{3}H]LTC_4 migrated with an R_f of 0.58. Leukotrienes in tissue culture media from cultured human airways were also analyzed by high-pressure liquid chromatography (HPLC) as described (17). The HPLC analyses were kindly performed by Dr. Jack Vanderhook of George Washington University in Washington DC.

Other Analytical Procedures. The histamine in the culture supernatants was determined

by the automated fluorometric assay (18). Anti-human IgE was prepared as previously described (19).

Statistical Analysis. The data were analyzed by paired sample t tests with $P < 0.05$ considered statistically significant. In experiments analyzing the effects of secretagogues on MGP release, five replicate tissue culture plates were used to generate each experimental point. All results represent data derived from experiments that were repeated on several different human lungs. The results are provided as the mean ±SEM.

Results

To determine whether C3a was capable of causing an increase in mucous glycoprotein release from human airways in culture, four separate airway preparations were exposed to purified C3a in concentrations from 0.5 to 15 μg/ml (Table I). C3a caused a dose-dependent increase in MGP release at concentrations above 0.5 μg/ml, all of which were significantly above control levels.

The ability of C3a des Arg to affect MGP release was compared to that of native C3a (Table II). C3a des Arg failed to cause increased MGP release at 5–20 μg/ml, while active C3a caused a 35% increase in the same experiments.

Effect of Anti-C3a. To determine whether the C3a effect on the airways reflected a specific interaction, the following experiments were performed (Table III). Anti-C3a $F(ab')_2$ were prepared as previously described (15). Antibody concentrations of 5, 10, and 50 μl/plate were added to human airways in culture at the beginning of period II. None of these antibody preparations had any effect

TABLE I
Effect of C3a on MGP Release From Human Airways in Culture

C3a	MGP release (percent increase above control; $n = 4$)
μg/ml	
0.5	7.3 ± 1.0
5	25.0 ± 1.3
10	30.0 ± 3.5
15	40.0 ± 2.0

TABLE II
Effect of C3a vs. C3a des Arg on MGP Release

Treatment	MGP release (percent increase above control; $n = 2$)
	μg/ml
C3a des Arg	
μg/ml	
5.2	11 ± 0.5
7.8	9 ± 0.5
10.4	8 ± 0.4
20.8	3 ± 0.2
C3a	
μg/ml	
10.0	35 ± 0.8

TABLE III
Effect of C3a and Anti-C3a on MGP Release

Airways incubated with:	MGP release (percent increase above control) (n = 2 for each experiment)
Exp. 1*	
C3a, 20 μg/ml	33 ± 0.5
Anti-C3a‡, 5 μl/plate	2 ± 0.8
Anti-C3a plus C3a	9 ± 1.2
Exp. 2§	
C3a, 10 μg/ml	35 ± 1.2
Anti-C3a, 10 μl/plate	11 ± 4.0
Anti-C3a plus C3a	2 ± 1.0
Methacholine, 1 μM	38 ± 2.0
Anti-C3a plus methacholine	35 ± 1.0

* Airways were preincubated with anti-C3a for 20 min, then C3a was added.
‡ Anti-C3a, in concentrations of 5, 10, and 50 μl/plate had no effect on the release MGP.
§ Anti-C3a and C3a were added to airways at the same time.

on MGP release when examined alone. In experiment I, the airways were preincubated for 20 min with 5 μl/plate of antibody, then C3a (20 μg/plate) was added (Table III, experiment 1). C3a in the absence of antisera increased mucus release 33% above the control level, anti-C3a alone had no effect on mucus release, and pretreatment of the airways with the antibody significantly inhibited the stimulatory effect of C3a (73% inhibition). Addition of control rabbit sera to C3a-treated airways had no effect on C3a-induced MGP release.

In a different experiment (Table III, experiment 2), anti-C3a alone again had no effect on MGP release, while C3a alone enhanced mucus release to 35% above control. When C3a was added simultaneously with anti-C3a, it produced no increase in MGP release. Methacholine (1 μM), a known mucus-secretion–stimulator (1–4), enhanced mucus release to 38% above control, and the presence of anti-C3a did not affect the secretion-stimulating activity of methacholine. Thus, while C3a is capable of enhancing mucus release from human airways in vitro, its activity is significantly inhibited by anti-C3a, and this inhibitory action is specific for C3a. In other experiments (not shown), anti-C3a also failed to affect histamine or C5a-induced MGP release.

Specific Immunoabsorption. In order to further investigate the specificity of the anti-C3a and its capacity to inhibit the effect of C3a on human airways in culture, we examined the effects of absorbing and washing C3a from a Sepharose–protein A–antibody immunoabsorption mixture, or from a control Sepharose–protein A mixture. Native C3a, anti-C3a alone, antibody plus C3a, and C3a exposed to a Sepharose–protein A–antibody conjugate or to control Sepharose–protein A were added to human airways in culture to determine whether they influenced MGP release (Table IV). 20, 50, and 100 μl/plate of buffer alone, as well as 5, 10, 20, and 50 μl/plate of antibody alone had no effect on mucus release. C3a alone (Table IV, *A*) increased MGP release in a dose-related manner.

C3a was totally absorbed by the Sepharose–protein A–anti-C3a affinity conjugate and did not elute with buffer washes (Table IV, *B*). C3a that was added to Sepharose–protein A alone was not absorbed, as reflected by its continued secretion-stimulating activity (Table IV, *C*). Thus, it appears that the interaction between C3a and the anti-C3a antibody is specific.

Mechanisms of Action: Mast Cell Degranulation. C3a is capable of causing mast cell degranulation (20) and can induce alveolar macrophages to become "activated" and release enzymes (21). Diverse mast cell mediators (4, 6–9) and a recently recognized macrophage product termed macrophage-derived mucus secretagogue (MMS) (22) are capable of causing mucus secretion. It was, therefore, important to determine whether the C3a effect on MGP release acted directly on mucous glands, or through either mast cell degranulation or macrophage MMS release.

Purified C3a was added to airways in culture in concentrations of 5, 10, and 15 μg/ml, and the supernatants were collected and assayed for both MGP and histamine. As shown in Table V, C3a stimulated mucus release in all concentrations examined, but did not cause an increase in histamine release. Samples from three different lung experiments were assayed for histamine release after exposure to C3a, and in each instance, no histamine release was observed. To determine whether the histamine that might have been released into the culture medium was metabolized during the 4-h incubation, histamine (1,650 ng/ml) was incubated with airways for up to 4 h. The histamine in the medium remained

TABLE IV
Specific Immunoadsorption of C3a

Airways incubated with:	MGP release (percent increase above control)
A. C3a:	
μg/ml	
2.5	15 ± 0.2
5	24 ± 0.3
10	36 ± 0.6
B.* C3a after exposure to Sepharose–protein A–anti-C3a immunoadsorption and washing	
μg/ml	
10	8 ± 0.3
25	6 ± 0.5
50	9 ± 0.8
C.* C3a after exposure to Sepharose–protein A and washing:	
μg/ml	
10	16 ± 0.7
25	21 ± 0.2
50	32 ± 0.3

* Because the concentration of C3a after column washes is 1/10 of the starting concentration, higher concentrations were used in experiments *B* and *C*.

TABLE V
C3a Effects on MGP and Histamine Release

C3a	MGP release (percent increase above control)	Histamine release	
		Control airways	C3a-treated airways
μg/ml		*ng/ml*	
5	21 ± 0.9	6.4 ± 0.2	6.3 ± 0.3
10	33 ± 0.3	6.5 ± 0.4	6.7 ± 0.2
15	43 ± 0.5	6.0 ± 0.2	6.2 ± 0.1

C3a was added to airways, and the supernatants were assayed for MGP release or for histamine. Control airways were treated with tissue culture media alone.

TABLE VI
Comparison of C3a and Reversed Anaphylaxis–induced Histamine Release and MGP Secretion From Human Airways

Experimental condition	Histamine release	MGP release (percent increase above control)
	ng/ml	
Dibutyryl cAMP (1 mM)	8	9.5 ± 2.5
Anti-IgE (1:100)	42	20.0 ± 1.5
Anti-IgE plus dibutyryl cAMP	10	9.5 ± 0.5
C3a (10 μg/ml)	5	28.0 ± 5.5
C3a plus dibutyryl cAMP	8	24.0 ± 3

stable throughout the incubation, indicating that metabolism cannot account for the observed absence of histamine release. Thus, C3a stimulates mucus release apparently without causing detectable histamine release.

In order to further investigate the relationship between C3a-induced MGP release and mast cell activation, the same experiments were used to compare the actions of C3a to reversed anaphylaxis, using rabbit anti–human IgE (19). C3a caused MGP release but no histamine release (Table VI), while anti-IgE caused both histamine and MGP release. It is well-documented that increased cAMP in lung tissue suppresses mast cell activation (23); therefore, the effects of dibutyryl cAMP on C3a and anti-IgE–induced reactions were compared. cAMP alone had minimal effects on MGP and histamine release, while reducing the ability of reversed anaphylaxis to stimulate either response. However, cAMP had no effect at all on C3a-induced MGP release. Thus, these data strongly support the suggestion that C3a is not acting to stimulate MGP release through mast cell degranulation.

Oxidative Products Derived From Arachidonic Acid. To investigate whether the effect of C3a on mucus release might act through generating oxidative products derived from arachidonic acid, airways were incubated with the following: (*a*) 5,8,11,14-eicosatetraynoic acid (ETYA) (35 μg/ml), which, in this concentration, is capable of blocking both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism in the lung (6); (*b*) C3a; (*c*) ETYA plus C3a (added simultaneously); or (*d*) C3a plus ETYA (with the ETYA added 20 min before

the C3a) (Table VII). ETYA alone decreased MGP release (6); C3a (5 μg/ml) increased mucus release; and with either experimental design, C3a in the presence of ETYA increased mucus release. Thus, ETYA did not significantly affect the ability of C3a to stimulate MGP release. These data suggest that the actions of C3a do not involve generation of oxidative derivatives of arachidonic acid.

To further assess the possibility of C3a-induced leukotriene production, pooled supernatants obtained from the ETYA plus C3a–treated airways, were acidified to pH 3.0 and extracted with diethylether, as previously described (6). The organic and aqueous phases were both assayed by HPLC for leukotrienes. No leukotrienes B, C, or D were detected. These experiments thus suggest that the activity of C3a on airways is not mediated through the generation of detectable amounts of leukotrienes.

As it was possible that leukotrienes generated by C3a-stimulated airways might be metabolized under the experimental conditions, it was essential to determine whether the leukotrienes in the culture medium were stable for up to 4 h. Therefore, [^{3}H]LTC_4 was added to the airways in culture, and aliquots of the incubation medium were removed 30–240 min later. After acidification, the medium was extracted into ethyl acetate, and chromatographed on TLC. Authentic LTC_4 migrated with an R_f of 0.58, as did the radioactivity in each aliquot. The authentic LTC_4 migrated with 12,000 cpm/ml at time 0; LTC_4 migrated with 12,240 cpm/ml after 30 min of incubation with lung tissue; 11,460 cpm/ml after 120 min; and 11,790 cpm/ml after 240 min. Therefore, no significant metabolism of LTC_4 takes place during human airway incubations lasting up to 4 h. Moreover, these data strongly support the conclusion that C3a-induced MGP release is independent of leukotriene (or other eicosanoid) generation.

MMS Generation. Next, we investigated the possibility that C3a acts through the generation of MMS by pulmonary macrophages. Initially, the time course of C3a-induced MGP release was determined to see whether it paralleled the time course for the appearance of MMS activity from macrophages in vitro (Table VIII). C3a-induced MGP release is appreciable after a 1-h incubation, and peaks by 6 h. MMS release from macrophages peaks in 2–4 h after stimulation (22). Thus, it seemed possible that C3a interacted with macrophages in culture to stimulate MMS release which, in turn, increased MGP release.

MMS is synthesized by macrophages through a cycloheximide-sensitive pathway (22). Therefore, we reasoned that exposure of airways to cycloheximide for 16 h before adding C3a should suffice to determine whether C3a was acting by

TABLE VII

Effect of Influencing Arachidonic Acid Metabolism on C3a-induced MGP Release

Airways incubated with:	MGP release (percent change from control)
ETYA (35 μg/ml)	−25 ± 1.4
C3a (5 μg/ml)	+27 ± 2.0
ETYA plus C3a:	
Simultaneous addition	+29 ± 0.9
After 20 min ETYA preincubation	+26 ± 1.2

TABLE VIII
Time Course of MGP Release From Human Airways Exposed to C3a

Time of incubation after C3a addition	MGP release (percent increase above control)
h	
0.5	8.4 ± 2.0
1	28.5 ± 0.5
2	30.5 ± 0.5
4	39.5 ± 0.5
6	42.0 ± 1.0
8	25.0 ± 1.0

TABLE IX
Effect of Cycloheximide on C3a-induced MGP Release

Experimental condition	MGP release (percent increase above control)
C3a (10 μg/ml)	27 ± 1.0
Cycloheximide (10 μg/ml)	4 ± 0.2
C3a plus cycloheximide	30 ± 3.0
Methacholine (1 μM)	34 ± 1.0
Methacholine plus cycloheximide	33 ± 2.5

stimulating MMS synthesis. In this experiment, cycloheximide-treated airways, and airways cultured in parallel in the absence of cycloheximide, were challenged with C3a or the muscarinic agonist, methacholine (Table IX). Cycloheximide had no effect on spontaneous MGP release, and also failed to influence either C3a or methacholine-stimulated MGP release. Thus, it is unlikely that C3a acts to stimulate MGP release by activating MMS generation.

Discussion

The anaphylatoxin, C3a, generated during complement activation, can stimulate a variety of biologic responses by interacting with mast cells (20), basophils and eosinophils (24, 25), neutrophils (26, 27), and macrophages (21, 28). It is very likely that anaphylatoxins play a role in host defense mechanisms, particularly those causing inflammation. While there is no current documentation of anaphylatoxin generation during pulmonary infections in humans, it is likely that complement activation occurs, particularly during the course of infections associated with IgG antibody formation. As mucus secretion accompanies many pulmonary infections, it was appropriate to determine whether C3a influences MGP release. C3a was found to be a potent mucus secretagogue, with impressive activity at concentrations as low as 5 μg/ml—amounts generated by activation of <10% of normal serum complement. The activity of C3a was rapid, causing MGP release within 30 min, and specific, as reflected in the ability of C3a antisera to prevent or absorb C3a activity. In addition, it was demonstrated that C3a des Arg did not produce such activity.

Neither the role of mucus nor the mechanisms responsible for its secretion in

different pulmonary diseases is clear, although recent studies have uncovered a number of putative secretagogues, including neurohormones (1–5), mast cell–derived mediators (4, 6–9), and a molecule termed MMS, released from human pulmonary macrophages and peripheral blood monocytes after surface activation (22, 29). Thus, it became important to examine whether the secretion-stimulating activity of C3a is related to the generation of any of these recognized secretagogues.

C3a is capable of activating mast cells and releasing histamine from pulmonary tissue (30); therefore, in several experiments where C3a had stimulated MGP release, histamine levels in the same supernatants were measured. No increase in histamine release was observed in four separate experiments in which C3a-treated airways were compared to control airways. In order to validate this observation, histamine was incubated with airways, and its complete recovery after a 4-h incubation was documented. Thus, this data indicated that C3a did not cause detectable histamine release in these cultured airways. This observation was explored further by comparing the activity of C3a with reversed anaphylaxis. Both C3a and reversed anaphylaxis led to increased MGP release, but the former was not associated with detectable histamine release, while the latter was. Moreover, cAMP inhibited both the MGP and the histamine release induced by reversed anaphylaxis, while having no effect on C3a actions. Therefore, C3a does not act as a mucus secretagogue through mast cell histamine release.

Arachidonic acid, several prostaglandins, several hydroxyeicosatetraenoic acids (HETE) and hydroperoxy-eicosatetraenoic acids, and leukotrienes C_4 and D_4 are potent mucus secretagogues (6, 7, 9). C5a is capable of stimulating leukotriene D_4 formation from pulmonary tissue (31) and causing prostaglandin formation from neutrophils (32), while terminal complement components cleave arachidonic acid from the cell membrane and cause prostaglandin, thromboxane, and HETE formation (33). C3a stimulates macrophages to release thromboxane (34) and causes neutrophils to generate leukotrienes (32). Thus, it was essential to examine the relationship between C3a-induced MGP secretion and eicosanoid formation.

The first approach used was to modulate cyclooxygenase and lipoxygenase pathways of archidonic acid metabolism by pharmacologic inhibitors. ETYA, in concentrations that inhibit both enzymes (6), did not influence C3a stimulation of MGP release. In data not presented, aspirin and indomethacin, which selectively inhibit the cyclooxygenase pathway, also failed to affect C3a-induced MGP secretion. Thus, impairment of both enzymatic pathways involved in the oxidation of arachidonic acid failed to influence C3a actions on MGP release. In another experiment, diethylether extracts of airway supernatants after C3a treatment were analyzed by HPLC, and no leukotrienes were found. This experiment was validated by demonstrating that LTC_4 added to airway in culture was not metabolized significantly during a 4-h incubation. Thus, several lines of evidence indicate that C3a does not cause MGP release from airways through the generation of eicosanoids. Therefore, we conclude that the effect of C3a on MGP release is not through histamine or eicosanoid release from airway tissue.

Since pulmonary macrophages constitute a major cell type found in human airways, and the time course of C3a-induced MGP release approximates the peak

of MMS release from macrophages (22), it was conceivable that C3a acted through the generation of MMS. However, MMS is synthesized by macrophages through a cycloheximide-inhibitable pathway. Therefore, we used cycloheximide to determine whether C3a action paralleled MMS synthesis. Neither C3a nor the muscarinic agonist methacholine was influenced by prior exposure of the airways to cycloheximide. Therefore, it is unlikely that C3a acts by stimulating MMS formation.

In conclusion, C3a is a potent mucus secretagogue, but its mechanism of action is unclear. The data indicate that mast cells are probably not involved, although it is possible that mast cells in close proximity to mucous glands release mediators that stimulate glandular activity without achieving detectable concentrations in the diffusate. It also appears that neither arachidonic acid products nor macrophage generation of MMS is involved in these C3a actions. Therefore, it seems possible that C3a acts directly on mucous glands (and/or globet cells) to directly stimulate MGP release. Regardless of the mechanism, the capacity of C3a to stimulate mucus secretion is an additional, potentially important activity to be included in the spectrum of actions caused by this anaphylatoxin.

Summary

Because C3a may be generated during the course of pulmonary inflammatory reactions, we investigated the ability of C3a to affect mucous glycoprotein (MGP) secretion from cultured human airways. C3a, but not C3a des Arg, caused a dose-related increase in MGP release (maximal after 4–6 h), with as little as 15 μg of C3a per milliliter stimulating a 40% increase.

The experimental evidence suggested that immunologically specific C3a was required for the secretagogue actions, as monospecific anti-C3a inhibited the reaction, as well as specifically absorbing the secretagogue from solution. Moreover, it appeared that C3a does not require mast cell activation, eicosanoid generation, or macrophage-derived mucus secretagogue synthesis for its effect, since (*a*) no evidence of histamine release accompanied C3a-induced MGP release, and dibutyryl cAMP failed to affect C3a-induced MGP release, while reducing the actions of reversed anaphylaxis; (*b*) MGP release caused by C3a was not influenced by eicosatetraynoic acid or specific cyclooxygenase inhibitors, and no leukotrienes were detectable on the supernatants of C3a-stimulated airways; and (*c*) cycloheximide failed to affect C3a secretion-stimulating actions. Thus, C3a is a potent mucus secretagogue, and, possibly, acts directly as a glandular stimulant. It seems likely that C3a generated in the course of pulmonary inflammation might contribute to the mucus secretion associated with pulmonary infections.

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