

In vivo detection of a novel macrophage-derived protein involved in the regulation of nasal mucus-like glycoconjugate secretion

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Background: We recently described a novel 68 kd mucus secretagogue (MMS-68) derived from human monocytes, pulmonary macrophages, and a macrophage hybridoma, clone 63. We detected MMS-68 in monocyte culture supernatants from patients with steroid-dependent asthma and in bronchoalveolar lavage fluid from patients with chronic bronchitis by antigen capture ELISA and in normal lung tissue by immunohistochemistry.

Methods: To determine a role for MMS-68 in the regulation of nasal mucus, we labeled human nasal turbinates with tritiated glucosamine and assayed for the ability of the previously purified MMS-68 (stock solution) to induce mucus-like glycoconjugate release (MLGC). We also performed immunohistochemistry stains with an anti-MMS-68 antibody (1-D-10) on frozen sections ($n = 5$) of nasal turbinates from patients with allergic and nonallergic rhinitis who were undergoing rhinoplasty and measured MMS-68 levels in nasal lavages from patients who were undergoing topical nasal histamine or methacholine challenge.

Results: MMS-68 is a potent nasal MLGC secretagogue causing a dose-dependent increase in MLGC release in vitro. Staining revealed a subepithelial distribution for MMS-68. Antigen capture ELISA of nasal lavages demonstrated mean MMS-68 levels from saline control challenge of 0.9 ± 0.5 μg MMS-68 per milligram of protein ($n = 5$), 8.6 ± 1.4 μg MMS-68 per milligram of protein from histamine challenge and 20.7 ± 2.3 μg MMS-68 per milligram of protein ($n = 5$) after methacholine challenge.

Conclusion: Taken together these data suggest that MMS-68 may play a role in the normal regulation of mucus secretion. (*J ALLERGY CLIN IMMUNOL* 1993;92:581-8.)

Key words: Monocyte, nasal mucus, regulatory protein

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Abbreviations used

MLGC: Mucus-like glycoconjugate
MMS-68: 68,000d Macrophage/monocyte-derived mucus secretagogue
SI: Secretory index

Excessive mucus secretion is associated with inflammatory nasal diseases including allergic rhinitis.^{1, 2} Many mediators including leukotrienes and cyclooxygenase products of arachidonic acid,³⁻⁵ as well as histamine⁶ and methacholine,⁷ have been implicated as possible contributors to this process. Because we have associated pulmonary macrophages⁸ and peripheral blood mono-

cytes⁹ with excessive mucus production in the lower airways, we were interested in investigating the potential role of monocyte- and macrophage-derived products in the regulation of nasal mucus secretion. We have recently described a novel 68,000d macrophage-monocyte-derived mucus secretagogue (MMS-68)¹⁰ that can induce mucus-like glycoconjugate (MLGC) release from cultured airways, respiratory epithelial cells, and the Ishikawa adenocarcinoma cell line. Using purified MMS-68 protein, we generated polyclonal and monoclonal antibodies against MMS-68. Using these antibodies in an antigen capture ELISA and in immunohistochemical staining, we were able to detect MMS-68 in human airways, bronchoalveolar lavage fluid of patients with chronic bronchitis and smokers and in culture supernatants of peripheral blood monocytes and pulmonary macrophages of normal subjects, patients with chronic bronchitis, and patients with steroid-dependent asthma.¹¹ To establish a possible role for MMS-68 in the regulation of nasal mucus secretion, we investigated the ability of purified MMS-68 (stock solution) to induce MLGC secretion from normal nasal tissue *in vitro*. We also performed immunohistochemical stains of frozen sections of nasal turbinates with the previously described anti-MMS-68 monoclonal antibody (1-D-10)¹¹ and measured MMS-68 levels in nasal lavages of patients with seasonal allergic rhinitis who were undergoing topical nasal histamine and methacholine challenges. We demonstrated that (1) MMS-68 is a potent nasal MLGC secretagogue *in vitro*, (2) it is present in normal nasal tissue, and (3) it is markedly elevated in nasal lavage after both histamine and methacholine challenges.

METHODS

MMS-68 generation and purification

We have used our previously described isolation and purification method^{10, 11} for MMS-68. In brief, 1 L of conditioned media from clone 63, a macrophage hybridoma, which makes large quantities of MMS-68, was concentrated by passage through a YM-30 ultrafiltration membrane in the cold at 4° C. The YM-30 retentate material (containing proteins with molecular weight > 30 kd) was dialyzed in the cold at 4° C against 2 to 5 L of deionized water, lyophilized, resuspended in 0.05 mol/L of Tris/0.2 mol/L of KCl, pH 7 and then passed through an ion-exchange column, Q-Sepharose (Pharmacia, Piscataway, N.J.) fast-flow anion-exchange gel. Proteins were eluted by gradient elution with 0.1 to 1 mol/L of KCl/0.05 mol/L of Tris buffer, and 0.5 ml fractions were collected. Each fraction was screened by an immunodot-blot assay, and positive peak

fractions (1 to 2 ml) eluted at 0.3 mol/L KCl were pooled, dialyzed at 4° C, and concentrated for additional testing. Protein concentration was determined according to the assay of Lowry. Further isolation was done by applying MMS-68 to an affinity column with the purified 1-D-10 IgG antibody as described.¹¹ This purified MMS-68 served as our stock solution for the dose-response experiments on the nasal tissue (see below) and for the ELISA of the unknown MMS-68 in the nasal lavage (see below).

Nasal provocation challenge and lavage

Histamine challenges were performed on subjects with seasonal allergic rhinitis¹² after approval was obtained from the Institutional Review Board at St. Louis University Medical Center. All subjects were between the ages of 20 and 59 years. They had a clinical history of at least 1-year duration of seasonal allergic rhinitis to grass, tree, and/or ragweed pollen. They had 3+ or 4+ positive epicutaneous skin test results to the appropriate allergen with a positive histamine control and negative saline control (Multi-Test, Lincoln Diagnostics, Decatur, Ill.). All subjects had a positive symptomatic response (paroxysmal sneezing, copious rhinorrhea, or both) to at least one of the histamine doses administered during the nasal challenge performed during a screening visit. All patients were otherwise healthy and free of symptoms and were not receiving medications at the time of study.

Subjects with other local nasal disease, including perennial allergic rhinitis or nonallergic rhinitis, nasal polyps, or deviated septum were excluded from the study. Subjects with an upper respiratory tract infection within 15 days of the study were also excluded.

Challenge procedure

Baseline nasal challenges were performed on all patients who qualified up to this point. One naris (right or left) was selected, and four nasal prewashes with 4 ml of saline solution each were done to reduce cell-free mediator concentrations to baseline. An 8F g catheter was introduced along the nasal floor before the prewashes and all subsequent nasal lavages. This procedure was followed by a control nasal challenge, which consisted of the instillation of 0.3 ml of diluent into each nasal cavity from a calibrated pump-spray device. Ten minutes later, a nasal wash with 4 ml of saline solution was done in only the prewashed naris.

Immediately after the postdiluent saline wash, a dose of 30 µg histamine in 0.3 ml total volume was instilled into each nasal cavity from the pump spray. A 4 ml nasal wash was performed 10 minutes after the challenge. Challenges with histamine doses of 300, 1000, and 3000 µg, in 0.3 ml total volume followed, respectively, in similar fashion at 10-minute intervals. All posthistamine washes were performed on the same naris.

Methacholine challenges were performed on pa-

tients with nonallergic perennial rhinitis after approval was obtained from the Institutional Review Board at St. Louis University Medical Center. A single dose of 0.3 ml (50 mg/ml) of methacholine hydrochloride was introduced. Lavage was performed 10 minutes later and collected as described above. Saline challenges were performed on all subjects.

To perform serial provocations, metered-dose nasal aerosol dispensers were weighed and calibrated. A challenge volume of 0.3 ml was dispensed to each nostril. Ten minutes after challenge, lavage with 4.0 ml of saline solution was performed in the nostril. The lavage fluid was collected through a modified 8F rubber catheter connected to a mucus trap and suction device. Typical recovery was 60% to 80% of instilled volume.

Antigen capture ELISA to detect MMS-68 in nasal lavages

An antigen capture assay was constructed with the 1-D-10 monoclonal antibody as the capture antibody and an alkaline-phosphatase-conjugated polyclonal guinea pig anti-MMS-68 sera as the developing antibody, which has been previously described.¹¹ Ninety-six-well microtiter plates were coated with 5 µg/ml purified 1-D-10 monoclonal antibody for 16 hours at 4° C. The plates were then washed five times with phosphate-buffered saline/0.05% Tween 20 and blocked with phosphate-buffered saline/0.05% Tween 20 and 0.25% bovine serum albumin for 1 hour at 37° C. Dilutions of duplicate test samples were incubated in blocked pre-coated 96-well microtiter plates for 2 hours at 37° C. The plates were extensively washed and incubated for 2 hours at 37° C with the alkaline-phosphatase-conjugated polyclonal guinea pig antibody, followed again by five washes. p-Nitrophenyl phosphate disodium (Substrate 104; Sigma Chemical Co., St. Louis, Mo.) was added for 30 minutes at 25° C, and the plates were read on an ELISA reader at 405 nm. Standard curves of known concentrations of purified MMS-68 (stock solution), obtained from clone 63, were generated for each assay.

Patient population

Five subjects were screened for the study. Subjects ranged in age from 20 to 59 years, with a mean age of 36 years. They had seasonal rhinitis for a mean of 18 years. Skin test results demonstrated that all of the five had positive responses to an extract of mixed tree pollen with an appropriate correlation of rhinitis symptoms by season. Subjects were studied out of their symptomatic season.

Statistical analysis of antigen capture assay of nasal lavage

Nonparametric statistical analysis was performed with the SAS statistical program package (SAS Institute, Cary, N.C.)¹³ and analyzed by previously described methods.¹⁴ Data were compared with the Mann-Whit-

ney test. A *p* value of less than 0.05 was considered positive for statistical significance.

Assay of nasal tissue explant MLGC secretion

Although ELISAs in which monoclonal antibodies were used to determine mucus release have been described,^{15, 16} their superiority as compared with the previously used methods has not been demonstrated. Therefore the MLGC secretion assay was performed with a slight modification of our (and others') previously described methods.^{3-11, 17-20} Nasal turbinates were obtained from allergic and nonallergic patients who were undergoing turbinate resection for cosmetic rhinoplasty and were maintained in MEM medium (Gibco, Grand Island, N.Y.) containing Earle's salts, 100 µg/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B. Two-millimeter tissue explants were placed in 35 × 100 mm plastic dishes and incubated for 24 hours in MEM medium containing antibiotics (as described above) and tritiated glucosamine (1 µCi/ml) (Amersham, Arlington Heights, Ill.), which was incorporated into newly synthesized and secreted glycoconjugate. After the initial 24-hour incubation period, the labeling medium was discarded, the explants were washed in fresh MEM medium without serum, and tritiated glucosamine was added. The plates were then incubated for two 1-hour incubation periods, period I (baseline) and period II (experimental period), in MEM medium containing antibiotics, aprotinin (10 µg/ml), and phenylmethylsulfonyl fluoride, 0.5 mmol/L (Sigma Chemical Co.). These compounds by themselves do not have any modulating effect on MLGC secretion and were added as anti-proteases to prevent possible degradation of the MMS-68 protein. The experiment started with period I (1 hour) to measure baseline release of MLGC from the cells. At the beginning of period II, purified MMS-68 (stock solution) was added to the medium of the experimental plates, whereas controls were treated with medium alone. The cells were incubated for 1 hour, and the medium was harvested. The harvested media from periods I and II were then processed for determination of MLGC content. The MLGC was separated during an overnight precipitation of 1 ml of the collected media mixed with an equal volume of 0.1 mol/L of potassium acetate in absolute ethanol. This was then centrifuged at 3500 rpm for 15 minutes. The supernatant was discarded, and the pellet was suspended in 250 µl of formic acid. This suspension was then added to 4 ml of scintillation fluid (Dimiscit, National Diagnostics, Manville, N.J.) and counted in a scintillation counter. The biologic effect of MMS-68 was quantified by establishing a secretory index (SI) for the control cultures in counts per minute and comparing it with the SI for the experimentally manipulated cultures (period I vs period II). The effect of MMS-68 tested was assessed by relating its SI to the average SI for the corresponding set of controls and expressing the result as percent

TABLE I. MMS-68 causes MLGC release from nasal explants

MMS-68 ($\mu\text{g/ml}$)	MLGC secretion (% increase above control)
1	12 $\pm$ 0.57
5	21 $\pm$ 1.0*
10	27 $\pm$ 1.0*
20	56 $\pm$ 1.0*
40	209 $\pm$ 1.1*
Carbachol (10^{-5} mol/L)†	55 $\pm$ 1.2*

Purified MMS-68 causes MLGC release from tritiated glucosamine-labeled nasal explants in a dose-dependent fashion. Maximum mucus release occurs at 1 hour after incubation with MMS-68. These data represent the pooled values of seven separate experiments with the standard error of the mean.

* $p < 0.001$.

†Carbachol was used as a positive control.

change, namely $100 \times (\text{SI experimental} \div \text{average SI control} - 1)$. Percent changes were then averaged over experiments and tested for significance by a paired-sample t test (t , mean $\pm$ SEM). A p value of less than 0.05 was considered statistically significant. MMS-68 biologic activity was always compared with the known secretagogue, methacholine (positive control), which at concentrations of 10^{-5} mol/L always caused a significant increase of MLGC release above control.

Detection of MMS-68 in nasal tissue sections

Frozen sections of 4 μm in thickness were obtained from patients who were undergoing turbinate resection. Staining was performed with the previously described 1-D-10 (anti-MMS-68) monoclonal antibody and W6/32 (anti-class I) by means of an avidin/biotin/peroxidase sandwich technique (Vectastain, Burlingame, Calif.).^{11,21} In each experiment isotype-matched controls were used to control for nonspecific Fc receptor binding (murine IgG1 and IgG2a, anti-dinitrophenol). Sections were counterstained with hematoxylin and examined by means of light microscopy by two observers.

RESULTS

MMS-68 is a potent mucus secretagogue in nasal tissue

Purified MMS-68 was biologically active and caused a dose-dependent release of MLGC in the nasal turbinates (Table I). The values, including the positive methacholine control in Table I, are pooled from seven separate experiments. MMS-68 at a concentration of 40 μg per milligram of protein stimulated an increase in MLGC release of $209\% \pm 1.1\%$ above control, which was seven times greater than that of carbachol. It is quite

apparent from the table that this dose response is narrow. The reason for this narrow dose-response range is unclear (see Discussion section). However, as determined by lactate dehydrogenase release experiments, MMS-68 was not toxic for the turbinates. In a series of time course experiments (data not shown), maximum MLGC release in all of these studies occurred 1 hour after addition of MMS-68. Carbachol (10^{-5} mol/L) served as the positive control and induced a significant enhancement of MLGC release above control values. In data not shown, the 1-D-10 monoclonal antibody inhibits MMS-68-induced MLGC release but not carbachol-induced MLGC release, demonstrating the specificity of the antibody. Other methods were also used to demonstrate that MLGC was released in response to MMS-68. In data not shown, immunodot-blot assays from supernatants of MMS-68 stimulated explants, in which an anti-mucus IgG monoclonal antibody was utilized, demonstrated an increase in MLGC release in response to MMS-68.

MMS-68 can be detected in normal nasal tissue

Frozen sections of human nasal turbinates were obtained from a patient with allergic rhinitis out of season and from a nonallergic control subject and stained with 1-D-10. In the patient with allergic rhinitis 1-D-10 demonstrated a subepithelial distribution of MMS-68 by immunohistochemistry in sections of nasal turbinate (Fig. 1, B), which was similar to the subepithelial distribution in the bronchus demonstrated in human lung.¹¹ This staining is diminished (Fig. 1, C) when excessive MMS-68 is added. A similar distribution of staining is seen with the anti-CD-14 monocyte specific antibody (Fig. 1, D). IgG2a anti-DNP (Fig. 1, A) is the negative control. Similar staining for MMS-68 was observed in the specimen from the nonallergic control subject (data not shown).

MMS-68 is present in nasal lavage after histamine and methacholine challenge

As previously described,¹¹ an antigen capture ELISA was constructed to measure MMS-68 in biologic fluids. The range of the ELISA for MMS-68 was from a concentration of 10 ng/mg protein to 1000 ng/mg protein. The lavage fluid was used without any further processing. The specificity of the ELISA was determined by adding known quantities of MMS-68 to the isotonic saline washes. The patients were sequentially challenged with isotonic saline solution, histamine, or

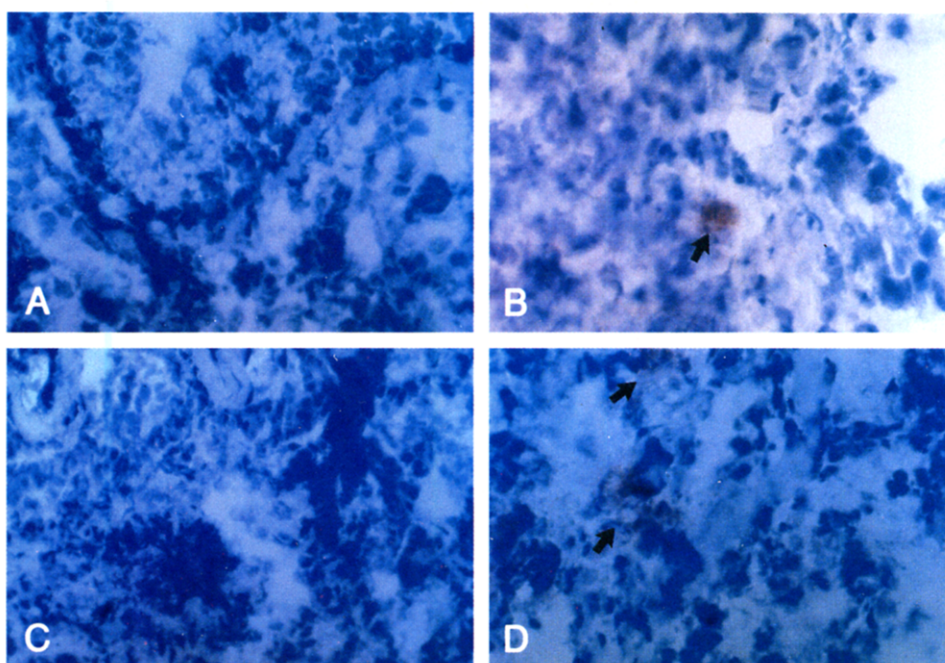


FIG. 1. Demonstration of MMS-68 by immunohistochemistry in sections of nasal turbinate (hematoxylin counterstain) from patients with allergic rhinitis. This is representative of multiple sections. MMS-68 has a subepithelial distribution (arrow) as is noted in **panel B**. **Panel A** (IgG2a anti-dinitrophenol) serves as the negative control, and **panel C** demonstrates that excess MMS-68 can diminish staining for MMS-68. **Panel D** shows that MMS-68 has a distribution that is similar to the macrophage-specific anti-CD-14 antibody (arrow). ($\times 40$.)

methacholine as described in the Methods section. The protein content of the nasal lavage was determined by absorbance at 280 nm. The specificity of the ELISA was determined by adding known quantities of MMS-68 to the isotonic saline washings (data not shown). As can be seen in Fig. 2, A, challenge with methacholine resulted in the highest levels ($20.7 \pm 2.3 \mu\text{g/ml}$ of washing) of MMS-68 recovered from nasal lavage. Histamine challenge increased levels of MMS-68 ($8.6 \pm 1.4 \mu\text{g/ml}$ of washing) significantly above challenge levels with normal saline solution ($0.9 \pm 0.5 \mu\text{g/ml}$ of washing) but less than half of the value obtained with methacholine. According to a nonparametric analysis, there was a statistical difference between MMS-68 after isotonic saline lavage compared with both histamine ($p < 0.01$) and methacholine ($p < 0.001$). The protein concentration in the nasal lavage was also higher after the histamine ($p < 0.01$) and methacholine challenges ($p < 0.001$) compared with the isotonic saline challenge (Fig. 2, B). Baseline MMS-68 concentrations in the allergic patients were higher compared with those in the nonallergic control subjects; $4.1 \pm 1.02 \mu\text{g/ml}$ of washing compared with $0.9 \pm 0.5 \mu\text{g/ml}$ of washing ($p < 0.01$). Thus patients with allergic

rhinitis had elevated baseline MMS-68 levels and secreted more MMS-68 after methacholine and histamine challenges than after isotonic saline challenge.

DISCUSSION

To study the role of inflammation in nasal mucus secretion, we investigated mediators secreted by monocytic cells to determine whether these products might be involved in the regulation of mucus secretion. Our laboratory has generated a series of human macrophage hybridomas,²² which were used to study the regulation of a previously described 2000d monocyte/macrophage-derived mucus secretagogue.^{8, 9, 17} The same clone that produces the 2000d secretagogue (clone 63) also produces large amounts of a novel 68,000d protein (MMS-68), which has been previously described to cause mucus release from cultured airways, respiratory epithelial cells, and the Ishikawa adenocarcinoma cell line.¹⁰ To better characterize this high molecular weight mucus secretagogue, we generated a panel of monoclonal antibodies and detected MMS-68 in peripheral blood monocytes, pulmonary macrophages, and airways in bronchoalveolar lavage fluid

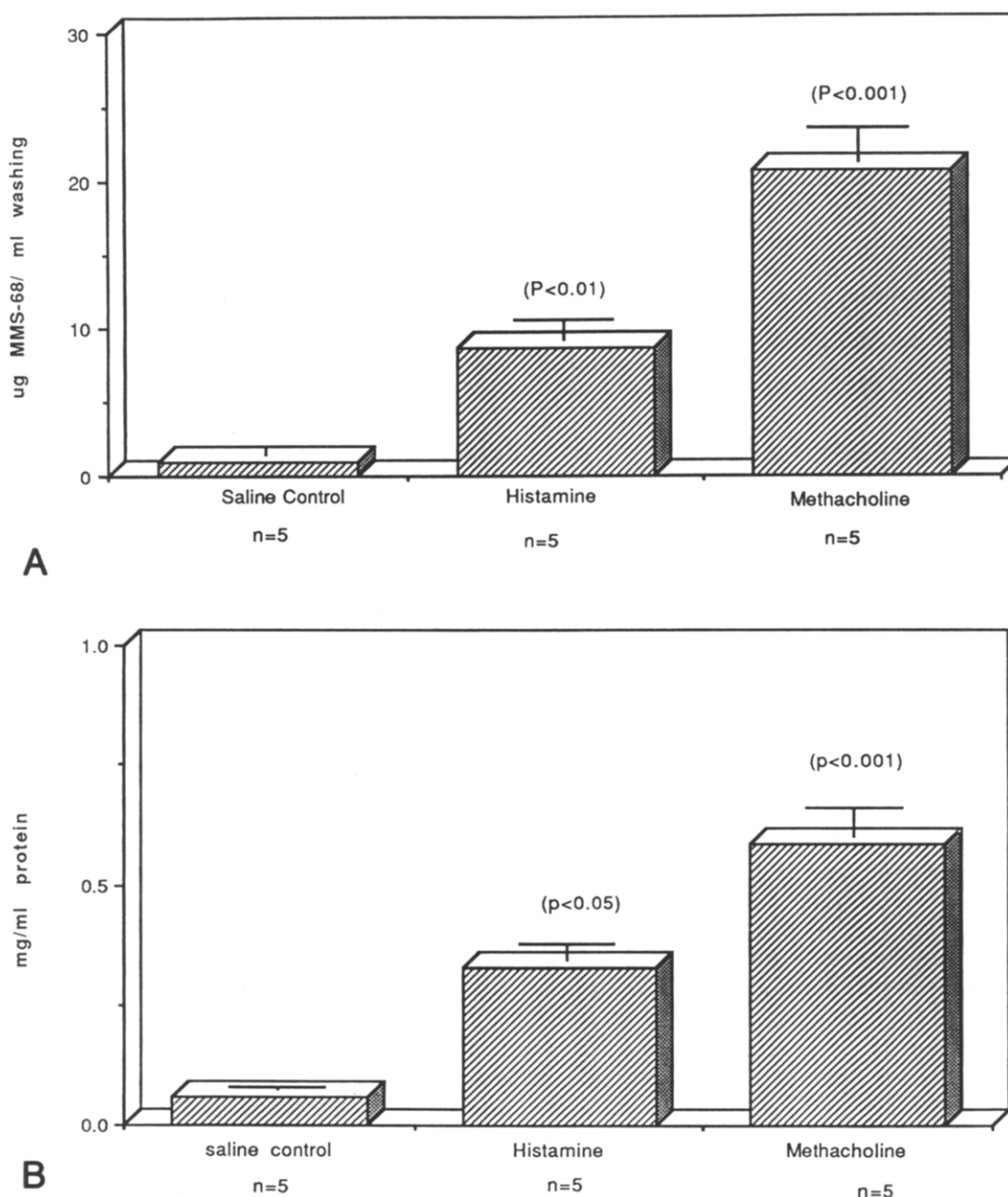


FIG. 2. A, Demonstration of MMS-68 in nasal lavage fluid. With the use of the antigen capture ELISA, MMS-68 was measured in nasal lavage. The mean levels in the saline control challenge were 0.9 ± 0.5 μg MMS-68 per milligram of protein compared with 8.6 ± 1.4 μg MMS-68 per milligram of protein after histamine challenge and 20.7 ± 2.3 μg MMS-68 per milligram of protein after methacholine challenge. Statistically significant differences were observed between isotonic saline and histamine challenges ($p < 0.01$) and between isotonic saline and methacholine challenges ($p < 0.001$). **B,** Protein content of the nasal lavage fluid. The protein content of the nasal lavage fluid was determined by absorbance at 280 nm. Statistically significant differences were observed between isotonic saline and histamine challenges ($p < 0.05$) and between isotonic and methacholine challenges ($p < 0.01$).

and in monocyte culture supernatant from normal subjects, patients with chronic bronchitis, and patients with steroid-dependent asthma.¹¹ We have extended these studies to investigate a po-

tential role for MMS-68 in nasal mucus secretion.

MMS-68 is a potent nasal secretagogue, which acted in a dose-dependent fashion (Table I) and at maximum concentrations (40 $\mu\text{g}/\text{ml}$) caused an

increase in MLGC release seven times greater than that induced by 10^{-5} mol/L methacholine (positive control). The dose-response curve is narrow, but it still appears to be going up (i.e., it has not reached a plateau). Thus it is possible that the occupancy of a putative receptor has not reached saturation at these concentration ranges. MMS-68 was present as determined by immunohistochemical staining in nasal tissue from surgical specimens of patients with allergic and nonallergic rhinitis who were undergoing cosmetic rhinoplasty (Fig. 1, C). The subepithelial staining pattern for MMS-68 in the normal nasal tissue is similar to its subbronchial distribution in the lower airways.¹¹ MMS-68 also had in vivo mucus stimulatory properties, and in an experimental rat model system it was demonstrated to be a potent nasal mucus secretagogue (personal observation).

MMS-68 levels were measured in nasal lavage fluid of allergic patients after normal saline, methacholine, and histamine challenges with an antigen capture assay as previously described.¹¹ Markedly elevated levels of MMS-68 were observed after methacholine and histamine challenges compared those after isotonic saline challenge. Increased secretion of MMS-68 may contribute to the increased nasal secretions that have been previously described in atopic patients after parasympathetic stimulation, which were attributed to abnormal autonomic function.²³⁻²⁵ Baseline MMS-68 concentrations were elevated in patients with allergic rhinitis compared with nonallergic control subjects, suggesting that the allergic state may be associated with increasing levels of MMS-68 either because of preexisting inflammation or the recruitment of other cells and mediators. The finding of increased levels of MMS-68 after histamine challenge suggests a potential role for MMS-68 as a second mediator for histamine-induced mucus hypersecretion. Many of the clinical symptoms associated with histamine response including nasal irritation, sneezing, and hypersecretion of mucus, which are induced by allergens, are due to direct stimulation of sensory nerves in the nasal mucosa.^{26, 27} There is evidence that histamine is involved in these processes, since they are blocked by H_1 antagonists.¹² Histamine has been variably reported to have direct MLGC-releasing capacity in vitro⁶ and may induce MLGC release through either an axon response or through the induction of other mediators,²⁸ including MMS-68.

Taken together, these data strongly suggest that MMS-68 plays an important role in the regulation of nasal mucus secretion. We have demonstrated its presence in normal nasal tissue and found

increased levels of MMS-68 in nasal lavage after both histamine and methacholine challenges. Overproduction of MMS-68 may contribute to the hypersecretion of mucus that is observed in patients with allergic rhinitis. Further studies are required to delineate the role of MMS-68 in nasal mucus production.

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REFERENCES

1. Lundgren JD, Shelhamer JH. Pathogenesis of airway mucus hypersecretion. *J ALLERGY CLIN IMMUNOL* 1990;85:399-419.
2. Kaliner MA, Marom Z, Patow C, Shelhamer J. Human respiratory mucus. *J ALLERGY CLIN IMMUNOL* 1984;73:318-23.
3. Marom Z, Shelhamer J, Sun F, Kaliner M. Effects of arachidonic acid, monohydroxyeicosatetraenoic acid, and prostaglandin on the release of mucous glycoproteins from human airways in vitro. *J Clin Invest* 1981;67:1695-1702.
4. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances (leukotrienes C_4 and D_4) increase the release of mucus from human airways in vitro. *Am Rev Respir Dis* 1982;126:449-551.
5. Marom Z, Shelhamer J, Sun F, Kaliner M. Human airway monohydroxyeicosatetraenoic acid generation and mucus release. *J Clin Invest* 1983;72:122-7.
6. Shelhamer JH, Marom Z, Kaliner M. Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. *J Clin Invest* 1980;66:1400-8.
7. Borson DB, Chinn RA, Davis B, Nadel JA. Adrenergic and cholinergic nerves mediate fluid secretion from tracheal glands of ferrets. *J Appl Physiol* 1980;49:1027-31.
8. Marom Z, Shelhamer JH, Kaliner M. Human pulmonary macrophage-derived mucus secretagogue. *J Exp Med* 1984;159:844-60.
9. Marom Z, Shelhamer JH, Kaliner M. Human monocyte derived mucus secretagogue. *J Clin Invest* 1985;75:191-8.
10. Gollub E, Goswami S, Sperber K, Marom Z. Isolation and characterization of a macrophage-derived high molecular weight protein involved in the regulation of mucus-like glycoconjugate secretion. *J ALLERGY CLIN IMMUNOL* 1992;89:656-62.
11. Sperber K, Gollub E, Goswami S, Kalb T, Mayer L, Marom Z. In vivo detection of a novel macrophage derived protein involved in the regulation of mucus-like glycoconjugate secretion. *Am Rev Respir Dis* 1992;146:1589-97.
12. Druce HM. The effects of an H_1 -receptor antagonist, terfenadine, on histamine-induced microcirculatory changes and vasopermeability in nasal mucosa. *J ALLERGY CLIN IMMUNOL* 1990;86:344-52.
13. Zar JH. Biostatistical analysis. 2nd ed. Englewood Cliffs, New Jersey: Prentice Hall Inc., 1984:176-9.
14. SAS Institute Inc. User's guide. Cary, North Carolina: SAS Institute, 1985.
15. Lin H, Carlson DM, St. George JA, Plopper CG, Wu R. An ELISA method for quantitation of tracheal mucins from human and non-human primates. *Am J Respir Cell Mol Biol* 1989;1:41-8.
16. Logun C, Mullol J, Rieves D, et al. Use of monoclonal antibody enzyme-linked immunosorbent assay to measure

- human respiratory glycoprotein in vitro. *Am J Respir Mol Biol* 1991;5:71-9.
17. Sperber K, Goswami S, Gollub E, Mayer L, Marom Z. Mucus secretagogue production by a human macrophage hybridoma. *J ALLERGY CLIN IMMUNOL* 1990;87:490-8.
 18. Amin DN, Goswami SK, Klein T, Maayami S, Marom Z. Functional antagonism between hormone receptor systems: modulation of glycoprotein secretion in secretory epithelial cells. *Am J Respir Cell Mol Biol* 1991;4:135-9.
 19. Goswami SK, Kivity S, Marom Z. Erythromycin inhibits respiratory glycoprotein secretion from human airways in vitro. *Am Rev Respir Dis* 1990;141:72-9.
 20. Fahy JV, Schuster AJ, Ueki I, Boushey HA, Nadel J. Mucus hypersecretion in bronchiectasis. *Am Rev Respir Dis* 1992;146:1430-3.
 21. Kalb T, Chuang M, Marom Z, Mayer L. Accessory cell function by airway epithelial cells. *Am J Respir Cell Mol Biol* 1991;4:319-329.
 22. Sperber K, Bauer J, Pizzimenti A, Najfeld V, Mayer L. Identification of subpopulations of human macrophages through the generation of human macrophage hybridomas. *J Immunol Methods* 1990;129:31-40.
 23. Mullol J, Raphael GD, Lundgren JD, et al. Comparison of human nasal mucosal secretion in vivo and in vitro. *J ALLERGY CLIN IMMUNOL* 1992;89:584-92.
 24. Raphael GD, Druce HM, Baraniuk JN, Banks SM, Kaliner M. The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine induced nasal secretion. *Am Rev Respir Dis* 1989;139:791-800.
 25. Druce HM, Wright RH, Kossoff D, Kaliner M. Cholinergic nasal hyperreactivity in atopic subjects. *J ALLERGY CLIN IMMUNOL* 1985;76:445-52.
 26. Raphael GD, Jeney EV, Baraniuk JN, Kim I, Meredith SD, Kaliner M. Pathophysiology of rhinitis: lactoferrin and lysozyme in nasal secretion. *J Clin Invest* 1989;84:1528-35.
 27. Raphael GD, Druce HM, Baraniuk JN, Kaliner M. The pathophysiology of rhinitis. I. Assessment of the sources of protein in methacholine-induced nasal secretion. *Am Rev Respir Dis* 1988;138:413-20.
 28. Platshon LF, Kaliner M. The effect of immunologic release of histamine upon human cyclic nucleotide levels and prostaglandin generation. *J Clin Invest* 1978;62:1113-21.