American Journal of Pathology, Vol. 131, No. 2, May 1988

Monoclonal Antibodies Directed Against Human Airway Secretions

Localization and Characterization of Antigens

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Cellular mechanisms of normal airway mucus secretion and their alterations in chronic obstructive lung disease are poorly understood. To aid in their study, the authors have produced a panel of monoclonal antibodies directed against various constituents of human airway secretions. Two fusions yielded 401 hybridoma-containing cultures. Supernatants from 150 of these cultures stained human tracheal secretory cells by immunofluorescence. Twenty-nine hybridomas were selected for expansion because they selectively stained a single cell type or displayed another interesting distribution. Antigens were further characterized by their localization in glycol methacrylate sections of human trachea, sensitivity to periodate oxidations, selective affinity for fraction peaks obtained by Sepharose 4B chromatography, and reactivity with molecules of various sizes, as estimated by SDS-PAGE. These antibodies will be useful for 1) quantitative detection of antigens in sputum or lavage samples by immunoassay and 2) purification and biochemical characterization of molecular constituents of airway secretions in health and disease. (Am J Pathol 1988, 131:290–297)

CHRONIC OBSTRUCTIVE lung diseases are disabling and result in considerable mortality. These diseases are often characterized by excessive mucus secretion, but the cellular mechanisms responsible are poorly understood. Because several cell types contribute to the secretion (goblet, serous, and mucous,[1] abnormalities in one or more of these cells might be at fault. Such abnormalities might occur in either the regulatory or the biosynthetic pathway of a secretory cell. Experiments performed on human tracheobronchial explants have provided general information about neurotransmitters[2] and inflammatory mediators[3] that influence secretion from the explants as a whole. However, the biochemical role of each cell type in mucus production and its regulation by various secretogogues are unknown.

In order to 1) define the biochemistry and 2) provide a marker for secretory activity of each cell type, we have produced cell-specific monoclonal antibodies against components of human tracheobronchial secretions.[4] In this report, we provide partial characterization of 29 antibodies. Whereas some of the determinants for these antibodies show strict cellular specificity, others are common to two or more cell types. Those that are restricted to a single cell type will be useful not only as affinity purification tools permitting isolation and characterization of their respective antigens[5] but also will serve as biochemical probes for secretory activity of particular cell types.[6]

When used to detect antigen in bronchial lavage samples by immunoassay, appropriate antibodies should help define the biochemistry and regulation of serous, mucous, and goblet cells in human airways, as well as detect cellular abnormalities associated with disease.

Materials and Methods

Preparation of Monoclonal Antibodies

Sputum from a patient with cystic fibrosis, blood group B, Le (a^-b^+), was dialyzed against distilled wa-

Supported by USPHS Grants HL-24136 and HL-01387 and a National Cystic Fibrosis Foundation Research Development Grant.

Accepted for publication December 22, 1987.

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ter (Spectro MW cutoff 12,000–14,000) and concentrated by centrifugal evaporation (Speed Vac). BALB/c mice, 5 weeks of age, were given intraperitoneal injections of 100 μg of the evaporated sample dissolved in 100 μl of sterile phosphate-buffered saline (PBS) emulsified with an equal volume of complete Freund's adjuvant. The intraperitoneal injections were separated by 3 weeks. We stimulated the immune response 2 weeks after the second injection with an intravenous injection of the same material but without the Freund's adjuvant. Three days later, the spleens were removed from the immunized mice and the spleen cells fused with SP 2/0 murine myeloma cells as previously described.[7]

Screening of Antibody

Tissue sections for screening hybridomas were obtained by fixing pieces of normal human trachea in 0.1 M PO_4 buffer (pH 7.4) containing 4% paraformaldehyde (2 hours, 4 C). Tissue pieces were cryoprotected by an 18-hour incubation in 30% sucrose/0.1 M PO_4 buffer (pH 7.4). Tissue pieces were frozen in OCT compound (Miles), and sections (5-μ) were made with a cryostat (Bright). The sections were placed on glass slides and prepared for immunofluorescence. All incubations were at room temperature. Slides were rinsed in PBS for removal of OCT. Hybridoma supernatant was diluted 1:1 with PBS containing 2% normal goat serum/0.6% Triton X-100. The diluted supernatant was applied to the sections for 2 hours. Sections were rinsed in PBS containing 1% normal goat serum/0.3% Triton X-100. Next, the slides were incubated with goat anti-mouse IgG-fluorescein isothiocyanate for 30 minutes. Then they were rinsed in PBS and covered with Dabco (diazabicyclooctane) solution and glass coverslips. The sections were examined in a Zeiss fluorescence microscope.

Localization of Antigens in Plastic-Embedded Tissue

Normal human tracheal tissues were processed by the method of Beckstead.[8] The tissues were embedded in a mixture of glycol methacrylate monomer, benzol peroxide, and polyethylene glycol (Polysciences, Inc.). Sections were cut at 2 μ with glass knives with a Sorvall JB-4 microtome, and the sections were transferred to glass coverslips and air-dried. Immunocytochemical staining was performed by means of a modification of a biotin–avidin procedure.[8]

Determination of Immunoglobulin Subclasses

An enzyme linked immunoassay (Behring Diagnostics) was used to identify antibody subclass. Goat anti-mouse immunoglobulins were immobilized on microtiter plates. Plates were incubated for 1 hour with hybridoma supernatants, diluted 1:2–1:10 in PBS. Negative controls were incubated with nutrient medium alone. Plates were rinsed three times with PBS, then incubated (1 hour) with rabbit anti-mouse typing sera (IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA). Following three PBS rinses, the plates were incubated with peroxidase conjugate (1 hour), and after three more PBS rinses, with 1% urea peroxide. Positive wells developed an amber color within 20 minutes, whereas control wells became pale yellow.

Periodate Sensitivity of Antigens

Sodium periodate in 50 mM sodium acetate buffer (pH 4.5) was applied to tracheal frozen sections, and the slides were incubated in the dark. Three conditions of periodate oxidation were used: mild (10 mM, 10 minutes, 4 C), moderate, (50 mM, 1 hour, 4 C) and harsh (100 mM, 12 hours, room temperature). Sections were rinsed in 10 mM sodium borohydride in PBS for 30 minutes at room temperature for reduction of peroxide groups generated during periodate oxidation and prevention of nonspecific cross-linking of antibody to antigen through Schiff base formation. Next, sections were rinsed five times in PBS containing 1% normal goat serum/0.3% Triton X-100, and monoclonal antibodies were applied to the sections as described above. Specific fluorescence of the periodate-treated sections was compared with control sections incubated in buffer alone.

Gel Filtration Chromatography

Endotracheal secretions were collected from a cystic fibrosis patient other than the one from whom the immunizing secretions were obtained. These secretions were dialyzed against 8 volumes of sodium acetate (0.01 M, pH 5.5) containing 1 mM EDTA, 0.02% sodium azide, and 0.5 mM phenylmethylsulfonyl fluoride for 24 hours at 4 C. The dialyzed material was centrifuged (6000*g*) for 15 minutes. The supernatant was passed through a column (5 × 80 cm) of Sepharose 4B equilibrated with sodium acetate (0.1 M, pH 5.5) containing 0.02% sodium azide. Fractions (12 ml) were collected at room temperature, monitored for protein, and pooled. Pooled fractions were dialyzed against distilled water (30 hours, 4 C) and lyophilized before use in radioimmunoassays or sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Solid-Phase Radioimmunoassay

The solid-phase sandwich radioimmunoassay developed by Podolsky and co-workers[9] was used. Seph-

arose 4B fraction peaks were solubilized in bicarbonate buffer (pH 9.2) at a concentration of 5 μg/ml. Polystyrene beads (6.35 mm, Precision Ball) were coated with material from individual fractions (40 beads/10 ml) by overnight incubation at room temperature with gentle agitation. Next, nonspecific binding sites were blocked by incubating beads for 1 hour in buffer containing supernatant from non-antibody-producing myeloma cells (SP 2/0). Then beads were incubated with monoclonal antibodies (hybridoma supernatant diluted 1:10) for 1 hour at 37 C. After three rinses in distilled water, beads were incubated for 1 hour at 60 C with ^{125}I-labeled sheep anti-mouse Ig Fab fragments (200,000 cpm per assay mixture; specific activity 6.5 μCi/mg; New England Nuclear) in 150 μl of buffer containing 0.01 M Tris-HCl (pH 7.5), EDTA at 2.0 μg/ml, and thimerisol. Finally, beads were washed five times in distilled water before bound radioactivity was measured with a gamma counter (Hewlett Packard). Antibodies were assayed in duplicate, and each antibody was assayed in at least three separate experiments. Activity was compared with a negative control consisting of SP 2/0 supernatant or supernatant obtained from unrelated hybridoma cultures by the Scheffé F test.[10]

SDS-PAGE and Immunoblots

Fractions recovered from Sepharose 4B chromatography were solubilized in Tris buffer (0.0625 M) containing SDS (2%) and mercaptoethanol (5%). SDS-PAGE was performed according to Laemmli.[11] For Fraction I material we used 3–10% acrylamide gradient gels, and for Fractions II and III, 4% stacking gels and 7.5% running gels. Components were stained with Coomassie brilliant blue and compared with high-molecular-weight protein standards (Sigma). Samples were transferred from the gels to nitrocellulose using the method of Towbin and co-workers[12] by means of an electroblotting system (Bio-Rad) operating at 20 V for 17 hours. Successful transfers were confirmed by the absence of staining in gels and the presence of staining (amido black) in nitrocellulose.

The nitrocellulose was cut into strips and incubated in PBS containing 3% bovine serum albumin/0.05% Tween 20 (1 hour, 37 C). The strips were transferred to PBS/Tween 20 containing monoclonal antibody (hybridoma supernatant diluted 1:10) for 2 hours at room temperature. Next the strips were rinsed six times in PBS/0.05% Tween 20 and incubated for 2 hours at room temperature in *Staphylococcus* Protein A horseradish peroxidase conjugate (Sigma) diluted 1:3000 in PBS/0.05% Tween 20. After further rinsing, the peroxidase reaction product was developed by incubating the strips for 2–5 minutes in 0.1% imidazole containing 0.1% diaminobenzidine and 0.03% H_2O_2. The reaction was stopped by transferring the strips to distilled water.

Results

Result of Fusions

Two fusions yielded 401 wells containing hybridoma clones (35% growth). Supernatant from these wells was screened on paraformaldehyde-fixed frozen sections of normal human trachea. One hundred and fifty supernatants stained human tracheal submucosal gland cells alone or in combination with the surface goblet cells. Twenty-nine hybridomas were selected on the basis of their staining pattern for expansion and further characterization.

To better localize the cellular antigens, we performed immunocytochemistry on plastic-embedded tracheal tissues. Mild aldehyde fixation and cold processing of the tissues preserved antigens, and thinly sectioning the tissues allowed their precise localization (Figure 1). All antibodies selected by immunofluorescence on frozen sections also stained the plastic-embedded tissue. Four antibodies stained epithelial goblet cells and submucosal serous and mucous cells, twelve antibodies stained goblet and mucous cells, four stained goblet and serous cells, four stained only mucous cells, and five only serous cells. In some cases, antibodies recognized only subpopulations of a given cell type. Localization of antibody staining and immunoglobulin classification are summarized in Table 1.

Periodate Sensitivity of Antigens

Fluorescence was abolished or considerably reduced for three antibodies after mild periodate treatment, three antibodies after moderate treatment, and nine antibodies after harsh treatment. Fourteen antibodies were unaffected by periodate treatment. For antibodies whose reactivity was reduced by mild treatment, more severe treatment resulted in complete loss of reactivity.

Gel Filtration Chromatography

Fractionation of endotracheal secretions from a cystic fibrosis patient on Sepharose 4B yielded three

Figure 1—Types of staining of human trachea by antibodies directed against secretory cell antigens. **A**—Antibody A1D3. Goblet, serous, and mucous cells are stained. **B**—Antibody A2F3. Goblet and mucous cells are stained. **C**—Antibody B8C3. Goblet and serous cells are stained. **D**—Antibody B8C4. Only mucous cells are stained. **E**—Antibody B7E5. Only serous cells are stained. Bar = 50 μ.

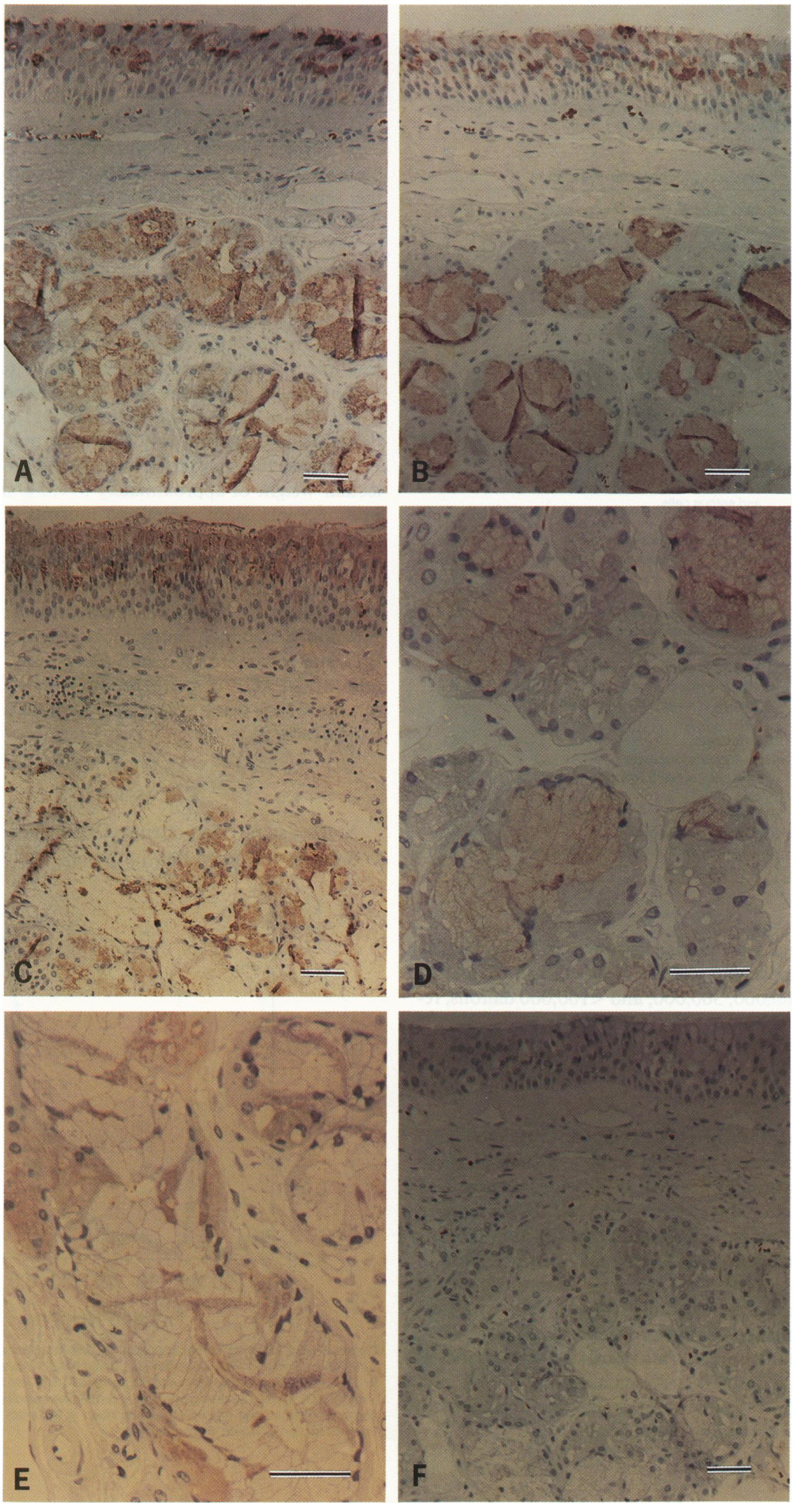
A
B
C
D
E
F

Table 1—Immunocytochemical Localization and Immunoglobulin Classification of Monoclonal Antibodies

Antibody	Immunocytochemical localization	Ig subclass
A1D3	Goblet, mucous, serous cells	M
A1D7	Mucous cells	G1
A1E11	Goblet and mucous cells	G1
A1F8	Mucous	G1
A1F11	Goblet cells, subpopulations of mucous and serous cells	G1
A2E7	Serous cells	G1
A2F3	Goblet and mucous	G1
A3B7	Serous cells	G2b
A3G11	Goblet cells, subpopulation of mucous cells	M
A6D8	Goblet cells, subpopulation of mucous cells	G3
A8E4	Mucous cells	G1
A10F5	Serous cells	G1
A10G5	Goblet cells, subpopulations of mucous and serous cells	G1
B1D8	Serous cells	G1
B2B8	Goblet, mucous and serous cells	G1
B3D11	Goblet cells, subpopulation of mucous cells	G1
B3E8	Goblet and mucous cells	M
B3F2	Goblet and mucous cells	M
B3F10	Serous cells, subpopulation of goblet cells	G1
B4C11	Goblet and mucous cells	M
B4F7	Goblet, mucus and serous cells	M
B5D5	Goblet and mucous cells	M
B5D7	Goblet cells, subpopulation of mucous cells	G1
B5E9	Mucous cells	G1
B6E8	Goblet and mucous cells	M
B6G6	Goblet cells, subpopulation of mucous cells	G1
B7E5	Serous cells	M
B8C3	Goblet and serous cells	G1
B8E10	Goblet cells, serous cells, subpopulation of mucous cells	G1

peaks, which were pooled as shown in Figure 2. The apparent molecular weights of Fractions I, II, and III were >1,000,000, 300,000, and <100,000 daltons, respectively.

Radioimmunoassay (RIA)

The reactivity of each antibody with molecular weight fractions is shown in Table 2. Sixteen antibodies had highest or exclusive affinity for material eluting in Fraction I (void volume), one for material in Fraction II, and one for material in Fraction III. One antibody showed equal affinity for Fractions I and II. Ten antibodies that reacted immunocytochemically with tracheal tissue sections failed to react with fractionated airway secretions in the solid-phase RIA. This phenomenon was also observed for a panel of monoclonal antibodies directed against colonic mucin[13] and may reflect unfavorable conformations of antigens after binding to the beads.

SDS-PAGE and Immunoblots

By immunoblotting, we observed that 19 of 29 antibodies reacted with components of Sepharose 4B further separated by SDS-PAGE. Representative immunoblots are shown in Figure 3 and approximate molecular weights of the antigens are shown in Table 3. Whereas some antibodies reacted with single discrete bands (eg, A1D3, B3D11, A2F3 and A1E11, lanes A, B, C, and G, respectively, Figure 3), others (eg, B6G6, lane E, Figure 3) reacted with single broad zones, or multiple discrete bands and/or zones (eg, B6E8, B4C11, B1D8 and B7E5, lanes E, F, H and I, respectively, Figure 3). Two antibodies, B3D11 and A1E11 (lanes B and G, respectively, Figure 3), may be identical, because they both react with a band having a molecular weight of approximately 377,000 daltons on immunoblots, stain goblet and mucous cells, recognize an antigen that is insensitive to periodate oxidation, and behave similarly in the solid-phase RIA. Another pair, B1D8 and B7E5 (lanes H and I, respectively, Figure 3) show similar immunoblot staining of Fraction II material, immunocytochemical localization, and behavior in the solid-phase assay. However, after SDS-PAGE of the Fraction 3 material, A1D8, but not B7E5, reacts with a 14,000 molecular weight band (Table 3).

Discussion

At least some components of tracheobronchial secretions appear to be very immunogenic on the basis

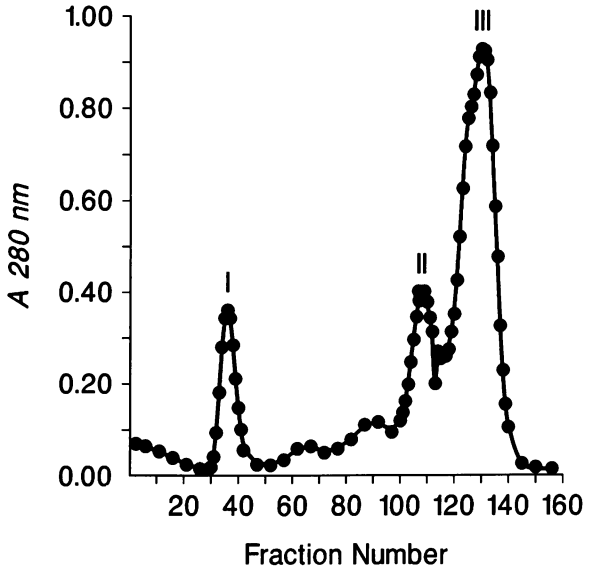

Figure 2—Gel filtration chromatography of endotracheal secretions collected from a patient with cystic fibrosis. Dialyzed secretions were applied to a column of Sepharose 4B in sodium acetate (0.1 M, pH 5.5) containing 1 mM EDTA, 0.02% sodium azide, and 0.5 mM phenylmethylsulfonyl fluoride and collected and monitored as described in Materials and Methods. Pooled Fractions I, II, and III are indicated.

Table 2—Reactivity of Antibodies With Pooled Sepharose 4B Fractions

Antibody	Fraction I	Fraction II	Fraction III
A1D3	306 ± 2*	118 ± 9	119 ± 18
A1D7	137 ± 5	145 ± 5	123 ± 6
A1E11	300 ± 11*	132 ± 2	149 ± 28
A1F8	241 ± 8	318 ± 43*	166 ± 38
A1F11	133 ± 6	121 ± 5	143 ± 17
A2E7	319 ± 22*	125 ± 14	116 ± 22
A2F3	392 ± 42*	130 ± 21	148 ± 30
A3B7	192 ± 6	250 ± 17	282 ± 15*
A3G11	279 ± 29	150 ± 18	160 ± 15
A6D8	363 ± 19*	305 ± 22*	273 ± 16
A8E4	321 ± 12*	203 ± 12	184 ± 4
A10F5	150 ± 20	166 ± 21	131 ± 26
A10G5	455 ± 18*	194 ± 12	161 ± 2
B1D8	126 ± 13	196 ± 32	212 ± 40
B2B8	168 ± 1	154 ± 13	170 ± 18
B3D11	336 ± 26*	114 ± 20	122 ± 19
B38	331 ± 2*	182 ± 12	177 ± 22
B3F2	182 ± 18	151 ± 18	164 ± 12
B3F10	309 ± 15*	104 ± 18	115 ± 15
B4C11	311 ± 14*	162 ± 19	164 ± 21
B4F7	322 ± 8*	206 ± 23	177 ± 27
B5D5	290 ± 11	149 ± 3	127 ± 9
B5D7	415 ± 7*	200 ± 3	177 ± 2
B5E9	294 ± 4*	115 ± 6	143 ± 8
B6E8	311 ± 30*	149 ± 16	158 ± 20
B6G6	493 ± 18*	165 ± 15	165 ± 13
B7E5	139 ± 19	184 ± 15	238 ± 14
B8C3	401 ± 17*	165 ± 15	133 ± 17
B8E10	184 ± 12	152 ± 19	184 ± 23

* Significant at 95%.

Table 3—Molecular Weights of Antigens: Immunoblot Analysis

Antibody	Molecular weights (kd)
A1D3	394
A1E11	377
A2E7	119–131
A2F3	401
A3G11	222–435
A6D8	422; 313–414; 263–305; 116–145; 71; 61; 42; 31; 17–21
B1D8	86–114; 77; 66; 14
B3D11	377
B3E8	353–435
B3F10	323–350
B4C11	367–435; 310–342; 174–200
B4F7	435; 92; 64–74; 39–45; 17–32
B5D5	385–435; 303–350; 222–282; 178–211; 128–151; 93–103
B5D7	376–435; 296–342; 222–269; 67–81
B6E8	362–414; 308–355; 129–146
B6G6	372–414
B7E5	86–114; 77; 66
B8C3	356

of the high proportion of clones secreting antibodies directed against tracheobronchial cells. Table 4 summarizes the characteristics of these monoclonal antibodies. Because the secretory cells of the trachea derive embryologically from common precursor cells, it is not surprising that many antibodies stain more than one cell type.[14,15] Although we detected antigens specific to serous and mucous cells, we did not detect antigens specific to goblet cells. Monoclonal antibodies recognizing goblet cells also recognized mucous cells, serous cells, or both. These results agree with those of St. George et al[16] but differ from those of an earlier study in which monoclonal antibodies directed against sheep tracheal secretion were found to include goblet cell-specific antibodies.[7]

Although some antibodies described in this study are strictly cell-specific, they do not necessarily stain all individual cells of a given type. In agreement with St. George et al,[16] and as expected from histochemical results from Spicer et al,[17] some antibodies identify subpopulations of airway secretory cell types. That is, an antibody that stains serous cells may not stain all serous cells. The significance of subpopulations is unknown at this time. Although staining differences may reflect true phenotypic differences, they might also

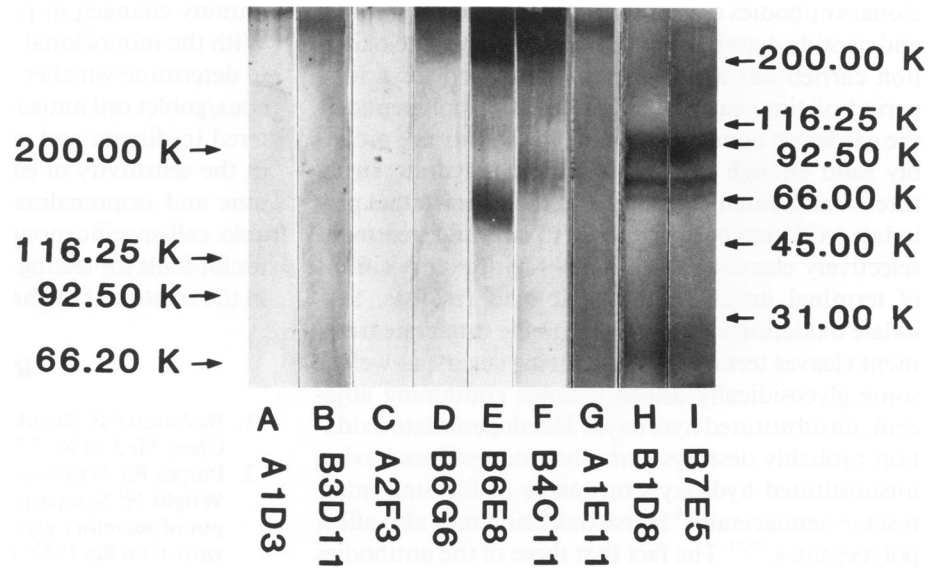

Figure 3—Representative SDS-PAGE and immunoblot analysis of Sepharose 4B material. Lanes A–G, Fraction I material. Lanes H–I, Fraction II material. Left, molecular weight standards lanes A–G; right, molecular standards lanes H–I (myosin, 200,000; β-galactosidase, 116,000; phosphorylase B, 92,500; bovine serum albumin, 66,200; ovalbumin, 45,000).

Table 4—Summary of Monoclonal Antibodies: Localization, Sensitivity, and Apparent Molecular Weight

Antibody	Localization*	Periodate sensitivity†	Column fraction‡
A1D3	G,M, S	NS	I
A1F11	G,M,S	NS	—
A10G5	G,M,S	Harsh	I, II
B2B8	G,M,S	Harsh	—
B4F7	G,M,S	NS	I, II, III
B8E10	G,M,S	NS	—
A1E11	G,M	NS	I
A2F3	G,M	Moderate	I
A3G11	G,M	Mild	I
A6D8	G,M	Harsh	I, II
B3D11	G,M	NS	I
B3E8	G,M	Moderate	I
B3F2	G,M	Mild	—
B4C11	G,M	Moderate	I
B5D5	G,M	NS	I
B5D7	G,M	NS	I
B6E8	G,M	NS	I
B6G6	G,M	Mild	I
B3F10	G,S	Harsh	I
B8C3	G,S	Harsh	I
A1F8	M	NS	II
A8E4	M	Harsh	I
B1D7	M	Harsh	—
B5E9	M	NS	I
A2E7	S	Harsh	I
A3B7	S	Harsh	III
A10F5	S	NS	—
B1D8	S	NS	II, III
D7E5	S	NS	II

* G, goblet cells; M, mucous cells; S, serous cells.
† Mild, 10 mM $NaIO_4$, 10 minutes, 4 C; moderate, 50 mM $NaIO_4$, 1 hour, 4 C; harsh, 100 mM $NaIO_4$, 12 hour, room temperature. NS, not sensitive.
‡ Column fraction results from RIA and SDS-PAGE immunoblots.

simply reflect metabolic or maturational differences among members of a single cell type.

On the basis of the results of periodate oxidation experiments, the epitopes recognized by these monoclonal antibodies appear to include both carbohydrate and peptide determinants. Although periodate oxidation carried out at high temperature and for a long period of time can potentially oxidize polypeptides, the oxidation conditions used in this study are probably mild enough to affect only carbohydrate structures. Van Lenten and Ashwell[18] have shown that periodate oxidation corresponding to our mild treatment selectively cleaves between 7-8-9-hydroxyl positions of terminal unsubstituted sialic acid residues. Periodate oxidation corresponding to the moderate treatment cleaves terminal, nonreducing sugars, as well as some glycosidically linked hexoses containing adjacent, unsubstituted hydroxyls. Harsh periodate oxidation probably destroys carbohydrate residues having unsubstituted hydroxyls incapable of forming interresidue hemiacetals.[19] Harsh oxidation may also affect polypeptides.[20,21] The fact that three of the antibodies (A3G11, B3F2, and B6G6) were sensitive to mild periodate oxidation suggests the involvement of carbohydrate epitopes containing terminal sialic acid residues.

Solid-phase RIA and immunoblot analysis demonstrated that a majority (65%) of antibodies recognized determinants in Fraction I (Table 4). We believe molecules carrying these epitopes are of relatively high molecular weight. Although elution in Fraction I under nondissociating conditions does not in itself constitute evidence for the large size of the antigenic molecules, the limited migration of these molecules on SDS gels strongly indicates they are of large size. High-molecular-weight antigens were identified in all three cell types. However, "low"-molecular-weight antigens (Fractions II and III) were most frequently associated with serous cells, either exclusively or in combination with goblet and mucous cells. These "low"-molecular-weight antigens were resistant to mild and moderate periodate treatments. Additional studies involving enzymatic digestion are needed to determine whether these antigens are associated with protein or carbohydrate moieties.

These monoclonal antibodies showing cellular specificity can serve as probes for the detection of antigen in sputum or lavage samples. Comparisons can be made between healthy and diseased individuals. Previously, the release of glyconjugates from human cells has been detected by incorporated radiolabeled precursors such as ^{35}S- and ^{14}C- or ^{3}H-glucosamine.[2,22,23] Results obtained from these studies, while shedding considerable light on the general control of airway secretion, are unsatisfactory for determining cell-specific differences in secretion. The most promising application of the antibodies described here may lie in the development of immunoassays to monitor cell activity *in vivo* in patients. Recent evidence suggests that the biochemistry of respiratory secretions is profoundly changed in patients with airway disease.[24,25] With the monoclonal antibodies it should be possible to determine whether the normal ratio of serous/mucous/goblet cell antigen in bronchial lavage fluid is altered in disease and whether there are abnormalities in the sensitivity of each cell type to drugs like atropine and isoprenaline. As new drugs become available, cell-specific monoclonal antibodies may provide useful tools for testing their effects at the cellular level in the treatment of chronic obstructive lung disease.

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Acknowledgments

We thank Suzanne Carrier, Marianne Gallup, and Jennifer Mann for technical assistance.