

Airway glycoprotein secretion parallels production and predicts airway obstruction in pulmonary allergy

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Background: Airway obstruction, perhaps the most relevant clinical feature of asthma, is typically assessed in allergic asthma models as airway hyperresponsiveness. Excess secretion of airway glycoproteins also contributes to airway obstruction in asthma but is not measured as part of most experimental models. **Objective:** The purposes of this study were to develop a reliable, quantitative assay for detecting secreted airway glycoproteins and to assess the secretion of airway glycoproteins in comparison with other markers of airway obstruction resulting from allergic lung inflammation.

Methods: Two microtiter plate-based glycoprotein-detecting methods were developed, one using an antiglycoprotein antibody and the other using the glycoprotein-binding plant lectin, jacalin. Both methods were used to assess airway glycoprotein secretion in response to 2 defined agonists given intranasally, IL-13 and an allergen derived from *Aspergillus fumigatus*. Glycoprotein secretion was assessed concomitant with another measure of airway obstruction, airway hyperresponsiveness provoked by acetylcholine challenge, and a histologic method for quantitating glycoprotein production.

Results: Both assays were sufficient for quantitating airway glycoproteins over the full range of values encountered from murine bronchoalveolar lavage and yielded highly reproducible data. Secretion of airway glycoproteins increased commensurate with the detection of both airway hyperresponsiveness and airway glycoprotein production induced by IL-13 and allergen.

Conclusion: Airway glycoprotein secretion is a consistent feature of the allergic lung phenotype and likely contributes to airway obstruction induced by allergen in both humans and rodents. (*J Allergy Clin Immunol* 2004;113:72-8.)

Key words: Asthma, glycoprotein, jacalin, IL-13, allergen, airway hyperresponsiveness

The utility of animal disease models correlates with the accuracy with which the human condition is reproduced and the validity of the experimental data. One of the most widely used models of human disease is that of allergic airway inflammation, a model of allergic asthma. Airway obstruction, perhaps the most relevant clinical feature of

Abbreviations used

BAL: Bronchoalveolar lavage
PAS: Periodic acid-Schiff
RAG: Recombinase activating gene

asthma, is determined in most models by assessing airway hyperresponsiveness, a physiologic alteration of the airway that results in exaggerated bronchoconstriction.¹ A second component of airway obstruction in the allergic setting is glycoprotein hypersecretion. In severe disease, secreted airway glycoproteins accumulate massively, producing contiguous plugs that might result in fatal airflow limitation.²⁻⁵ Indeed, airway glycoprotein impaction is likely the major cause of death as a result of asphyxiation in asthma.⁶ Detection of secreted airway glycoproteins is therefore fundamental to understanding asthma from both clinical and research perspectives.

Despite the importance of secreted glycoproteins to airway obstruction in asthma, glycoprotein secretion in experimental asthma models is, in general, not determined directly but is estimated by using a variety of histologic grading scores.⁷⁻¹² These methods are usually based on the periodic acid-Schiff (PAS) reaction, which shows glycoprotein-containing goblet cells lining the airways. Although glycoprotein production has thus been shown to increase under conditions of allergic airway inflammation, it is less clear whether production is linked under the same conditions to secretion. Furthermore, PAS-based histologic methods have major shortcomings including their laborious, time-consuming, and subjective nature and sampling bias resulting from the heterogeneous distribution of glycoprotein-secreting cells. Rapid, fully quantitative assays of secreted airway glycoproteins are likely to provide more objective, physiologically relevant data.

Immune-based assays potentially offer a highly quantitative means for directly determining secreted glycoproteins from biologic fluids, especially of the airway. Whole lung bronchoalveolar lavage (BAL) offers an unbiased sampling of total secreted airway glycoproteins, is easily obtained, and is readily applied to standard immunoassays. However, most commercially available antiglycoprotein antibodies, although specific for the protein core, do not detect native glycoproteins, presumably because the extensive glycosaminoglycan side chains interfere with antibody binding (unpublished observations). In addition, controversy exists regarding

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the feasibility of detecting secreted airway mucin, one of the principal secreted airway glycoproteins.¹³ For both of these reasons, airway glycoprotein secretion is not routinely evaluated, and consequently the contribution of glycoproteins to airway obstruction is underinvestigated.

To understand whether airway glycoproteins are secreted under conditions known to induce glycoprotein production and airway obstruction, we developed an affinity assay based on the plant lectin jacalin^{14,15} and compared it with a similar ELISA developed by using a mucin-specific antibody. Jacalin binds to many glycoproteins possessing O-linked oligosaccharides^{16,17} and has previously been shown to bind IgA, a property that proved useful in devising immunoassays for detection of this glycosylated Ig from bodily fluids.¹⁸ Our data indicate that the assays provide comparable measurements of total airway glycoproteins that correlate with both glycoprotein production and airway hyperresponsiveness, commonly used indexes of airway obstruction. These assays should prove useful in investigating the biology of airway glycoprotein secretion further under diverse conditions.

METHODS

Animals

Four- to 6-week-old recombinase activating gene 2 (RAG-2)-deficient mice (BALB/c, H-2^d)¹⁹ and wild-type BALB/c control mice were purchased from Taconic (Germantown, NY) and maintained in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited transgenic animal facility at Baylor College of Medicine. All procedures on mice were conducted according to approved Baylor College of Medicine protocols and National Institutes of Health guidelines.

Antibodies and reagents

Raw mouse ascites fluid containing mAb 17Q2 was purchased from Covance (Princeton, NJ). IgA capture mAb C10-3 and secondary biotinylated antibody C10-1 were purchased from BD Biosciences-Pharmingen (San Diego, Calif). Biotinylated jacalin was purchased from Vector Laboratories (Burlingame, Calif). Horseradish peroxidase conjugated streptavidin was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Alkaline phosphatase conjugated streptavidin was purchased from BD Pharmingen. Recombinant IL-13 was obtained from Genetics Institute (Cambridge, Mass). *Aspergillus fumigatus* allergen was prepared free of hyphae and spores and mixed with chicken egg ovalbumin (Sigma, St Louis, Mo) immediately before administration to mice as described.²⁰ I-Block was purchased from Applied Biosystems (Foster City, Calif). P-nitrophenyl phosphate, alkaline buffer, and glycoprotein standard (derived from porcine stomach) were purchased from Sigma.

Purification and biotinylation of antiglycoprotein antibody

mAb 17Q2 was purified from raw ascites fluid by using the NAb Protein G Spin Chromatography Kit and biotinylated by using 2-mercaptoethylamine-HCl and EZ-link PEO-Maleimide activated biotin (all from Pierce, Rockford, Ill) according to the manufacturer's instructions.

Western blot

Equal volumes of BAL fluid were electrophoresed into 8% SDS polyacrylamide gels. The separated proteins were transferred to

nitrocellulose membranes (BioRad, Hercules, Calif) and analyzed by Western blotting with either biotinylated jacalin (1 µg/mL) or biotinylated antiglycoprotein mAb (5 µg/mL) as primary reagents and streptavidin-horseradish peroxidase (10 ng/mL) as the secondary reagent. Blots were then developed by using SuperSignal West Dura Extended Duration Substrate (Pierce).

Enzyme-linked affinity assay for glycoprotein quantitation

The glycoprotein quantitation assay was developed to take advantage of standard reagents and equipment used for conventional ELISAs. Briefly, glycoprotein standard dissolved in PBS was diluted serially 2-fold beginning at 1 µg/mL and continued out to 7.8 ng/mL. BAL samples were diluted in PBS serially 2-fold beginning at a 1:100 dilution and continued out to 1:12,800 by using polypropylene microtiter plates. Forty microliters from each well was then transferred to flat bottom polystyrene microtiter plates treated for high protein binding (Immulon 4HBX; Fisher Scientific Company, Pittsburgh, Pa) and incubated at 37°C for 2 hours or equivalently at 4°C overnight. After washing with PBS containing 0.05% Tween 20 (BioRad), plates were blocked by adding 200 µL of 0.2% I-block and incubating at 37°C for 2 hours or equivalently at 4°C overnight. After washing, 40 µL of chilled biotinylated jacalin diluted in PBS/Tween with 0.1% BSA to 5 µg/mL was added, and plates were kept at 4°C overnight. Plates were washed again, and 40 µL of alkaline phosphatase conjugated streptavidin diluted in PBS/Tween/BSA (1:1000 dilution from original stock) was added, and plates were incubated at room temperature for 30 minutes. After a final wash, nitrophenyl phosphate substrate (5 mmol/L in 0.1 mol/L alkaline buffer) was added, and plates were allowed to develop until the standard curve was readily apparent. The colorimetric reaction was terminated by adding 40 µL of 0.5 N sodium hydroxide, and optical density of the individual wells was determined at 405 nm by using a standard microplate spectrophotometer.

ELISAs

IgA was measured from BAL by standard sandwich ELISA with antibodies C10-3 and C10-1 and developed with streptavidin conjugated alkaline phosphatase followed by nitrophenyl phosphate. Antibody-based glycoprotein ELISA was performed identically to the jacalin assay but substituting jacalin with 0.2 µg/mL biotinylated antibody 17Q2 and using pooled BAL from IL-13-challenged mice as the standard.

Glycoprotein production score

We determined glycoprotein production according to our previous methods.¹¹ Briefly, PAS-stained sections of mouse lung were prepared for viewing by light microscopy at 250× magnification. At least 50 consecutive airways from non-antigen-challenged mice or 20 consecutive medium-sized airways demonstrating mural inflammation from antigen-challenged mice were categorized according to the abundance of PAS+ goblet cells and assigned numeric scores (0, less than 5% goblet cells; 1, 5% to 25%; 2, 25% to 50%; 3, 50% to 75%; 4, greater than 75%). Airway scores from each lung were totaled, and the sum was divided by the number of airways examined for the histologic goblet cell score (arbitrary units; U).

Induction of the asthma phenotype and airway glycoprotein secretion

Enhanced airway glycoprotein secretion was induced in mice according to previously published protocols involving intranasal challenge with either allergen²⁰ or recombinant IL-13.¹¹ Briefly, mice were anesthetized with an isoflurane vapor/oxygen mixture, held upright, and allowed to inspire through the nares 50 µL of

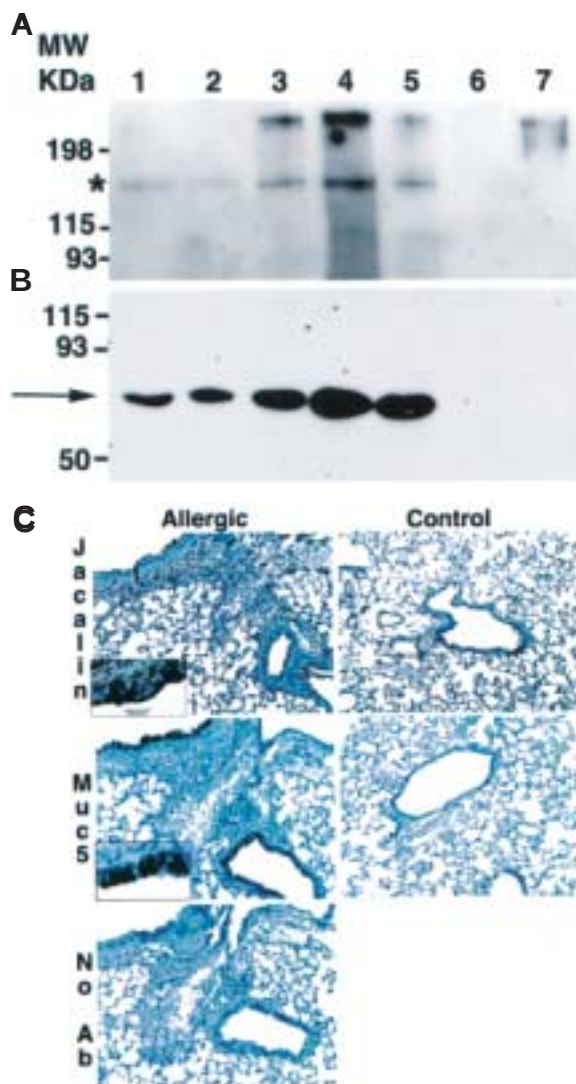


FIG 1. Specificity of jacalin for lung glycoproteins. PAGE gels were developed by using jacalin under nonreducing (**A**) or antiglycoprotein mAb under reducing conditions (**B**). BAL was obtained from wild-type and RAG-2^{-/-} mice challenged with saline (lanes 1-2), wild-type and RAG-2^{-/-} mice challenged with IL-13 (lanes 3-4), wild-type mice challenged with *A fumigatus* allergen (lane 5) and compared with porcine stomach glycoprotein (150 ng; lane 6) and murine IgA (3.7 μ g; lane 7). **C**, Immunohistochemistry was performed on mouse lungs of saline (Control) and allergen-challenged (Allergic) mice. The same regions of lung were stained with jacalin and anti-MUC5AC antibody and compared with no antibody (No Ab) control sections.

either allergen or IL-13. Allergen challenges were continued on a schedule of every 4 days for 5 challenges, and IL-13 was administered twice daily for 3 consecutive days. Twenty-four hours after the final allergen or IL-13 challenge, mice were given a lethal injection of pentobarbital, and the trachea was surgically exposed, cannulated with a 20-gauge angiocatheter, and securely tied. One milliliter of chilled PBS was instilled and withdrawn through the catheter and repeated once, pooling the BAL fluid (typically, 1.5-mL total volume was retrieved). Goblet cell metaplasia was scored and airway hyperresponsiveness was determined according to previously published methods.^{11,21}

Statistics

Data are expressed as means $\pm$ SEM with significant differences ($P \leq .05$) assessed with the nonparametric Kruskal-Wallis test.

RESULTS

Characterization of jacalin and antimucin antibody

Jacalin is plant lectin that has previously been shown to specifically bind O-linked glycoproteins,¹⁶ a variety of which are potentially present in mammalian airways, including mucins, IgA, and likely others. We conducted an initial series of studies to understand whether, and under what conditions, jacalin is able to bind glycoproteins present in mouse BAL. PAGE and Western blot analyses were performed on BAL samples obtained from naive mice and mice treated with allergen and IL-13, treatments that have both been shown to increase airway glycoprotein production (Fig 1, A and B).^{7,9,11,12,15,20,22-27} Wild-type and RAG-2^{-/-} mice, the latter unable to produce Ig, were compared under the same nonreducing conditions to understand whether secreted IgA could potentially contribute to the wild-type signal. IgA, run as a positive control, bound to jacalin (Fig 1, A, lane 7), but jacalin also bound to high and low molecular weight substances in all BAL samples, with the bands, regardless of molecular size, clearly enhancing after treatment with either IL-13 or allergen (Fig 1, A, lanes 3 to 5). The degree of enhancement of these bands was approximately equivalent regardless of mouse genotype or stimulus, indicating that, although it was of the same molecular size as IgA, any contribution of this Ig was likely to be minimal. Therefore, jacalin is binding to the same non-IgA substance in lanes 1 to 6, which likely exists in several multimeric forms under these nonreducing conditions. Under these conditions, jacalin did not react with the porcine glycoprotein standard.

The same BAL samples were probed with an antibody specific for mucins secreted by cultured tracheal epithelial cells and that reacts only with the glycoprotein side chains, not the protein core²⁸ (Fig 1, B). Under reducing conditions, the antibody readily detected an apparently single protein of between 50 and 93 kd in all BAL samples, which appeared to enhance with IL-13 and allergen challenge, as did the jacalin-binding substance in Fig 1, A. The antibody detected neither IgA nor the glycoprotein standard (Fig 1, B). These studies therefore show that both jacalin and the antiglycoprotein antibody bind glycoproteins present in murine BAL whose production is enhanced during allergic lung inflammation and by IL-13 challenge of the airways.

The cellular sources of airway glycoproteins are potentially many and include airway epithelial goblet cells, alveolar epithelial cells, and recruited inflammatory cells. To be used in a broad spectrum assay of secreted glycoproteins, we reasoned that the jacalin reagent should bind to the intracytoplasmic granules of glycoprotein-secreting cells. These granules, which are readily visualized with routine glycoprotein stains such as PAS, are most readily appreciated in goblet cells of larger airways. We used jacalin and another antibody specific for MUC5AC (antibody 17Q2 was unsuitable for immunohistochemistry) to detect glycoprotein-containing cells in situ (Fig 1, C). Both jacalin and anti-MUC5AC antibody readily stained airway goblet cells, especially intracytoplasmic granules, in a pattern indistinguishable from that seen with PAS (Fig 2, A to C). Furthermore, whereas anti-MUC5AC antibody was highly specific for only airway epithelial goblet cells after antigen challenge, jacalin stained airway and alveolar epithelial cells as well as inflammatory cells (Fig 1, C). Thus, the specificities of jacalin and anti-MUC5AC antibody overlap, but jacalin binds to a much broader spectrum of lung glycoproteins.

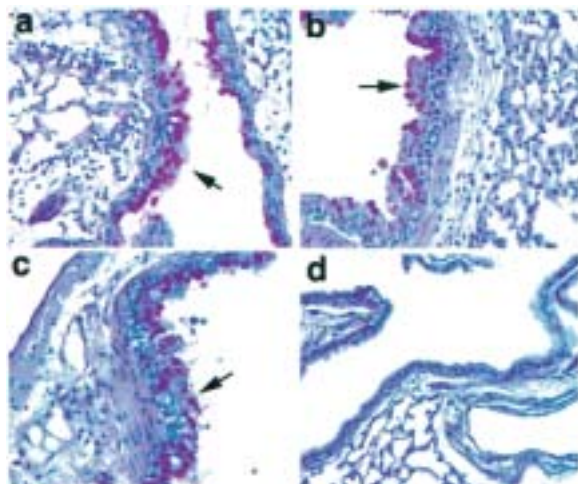


FIG 2. Goblet cell metaplasia induced by IL-13 and allergen. Wild-type mice challenged with either *A fumigatus* allergen (a) or recombinant IL-13 (b) were compared with RAG-2^{-/-} mice challenged with recombinant IL-13 (c) or saline (d). PAS was then used to show the presence of goblet cells (arrows) within airways.

Affinity assays for mouse airway glycoproteins

The above results encouraged us to develop microtiter plate-based assays for quantitation of the glycoproteins detected in murine BAL, the details of which are described in Methods. Fig 3 shows standard curves from the jacalin-based and antiglycoprotein antibody-based assays. To be used comparably with the biotinylated jacalin, the antibody required purification from ascites and biotinylation (data not shown). We used pooled BAL fluid from allergen-challenged mice as a standard for the mAb-based assay because the antibody does not recognize porcine glycoproteins, which are used as the standard for the jacalin-based assay (data not shown). Both standards yielded acceptable titration curves that are linear over an approximately 20-fold concentration difference in standard. In our experience, this range is sufficient to cover the entire physiologic range of secreted airway glycoproteins.

To compare the ability of the antibody- and jacalin-based assays to detect differences in airway glycoprotein secretion, both were used to quantitate BAL glycoproteins from sham (saline) and IL-13-challenged mice (Fig 4, A and B). Wild-type mice were compared with RAG-2^{-/-} mice to detect any possible contribution of IgA to wild-type glycoprotein levels. As shown, regardless of the detection method, baseline levels of BAL glycoproteins (saline challenge) were not significantly different between wild-type and RAG-2^{-/-} mice. Furthermore, IL-13 induced significant increases (approximately 2- to 3-fold) in BAL glycoproteins regardless of genotype. The absolute magnitude of difference in change in BAL glycoprotein levels between the different groups did not differ, indicating that any contribution of IgA to the signal observed in wild-type mice was insignificant compared with the “pure” signal obtained from RAG-2-deficient animals.

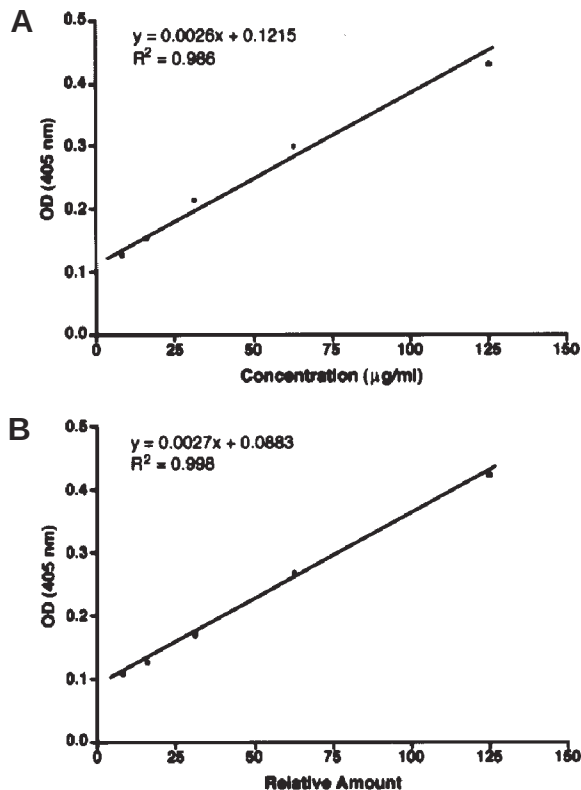


FIG 3. Standard curves generated by using jacalin (A) and antiglycoprotein mAb (B). Porcine glycoprotein starting at 1 μ g/mL and diluted serially 2-fold was used as a standard for jacalin. Pooled BAL from antigen-challenged mice diluted 1:50 was diluted serially 2-fold as the standard in the antiglycoprotein mAb assay, giving arbitrary units.

We also assessed glycoprotein secretion from allergen-challenged animals by using a fungal-derived preparation that we have previously shown induces strong allergic lung disease and airway obstruction²⁰ (Fig 4, C and D). As shown, BAL glycoprotein levels were increased 1.5- to 2-fold regardless of which assay was used. Therefore, both glycoprotein-binding reagents are capable of quantifying secreted airway glycoproteins when applied to simple microtiter plate-based assays. Furthermore, because the magnitude of increase of secreted glycoproteins did not differ between a reagent detecting a specific glycoprotein (mucin, antibody 17Q2) and a less specific reagent (jacalin), we conclude that mucin is a significant component of secreted airway glycoproteins under the conditions examined.

We also measured IgA in the BAL samples analyzed above by using a specific ELISA. As expected, the highest amount of IgA was found in BAL of allergen-challenged mice (maximum, 20 ng/mL). A modified ELISA was then performed in which IgA was bound to the wells of a microtiter plate in a standard curve covering a concentration range of 0.5 to 1000 ng/mL and developed with biotinylated jacalin. The lowest detectable concentration of IgA by using jacalin was 125 ng/mL, demonstrating that maximum expected levels of IgA in mouse BAL are well below the detection limit of jacalin. These findings

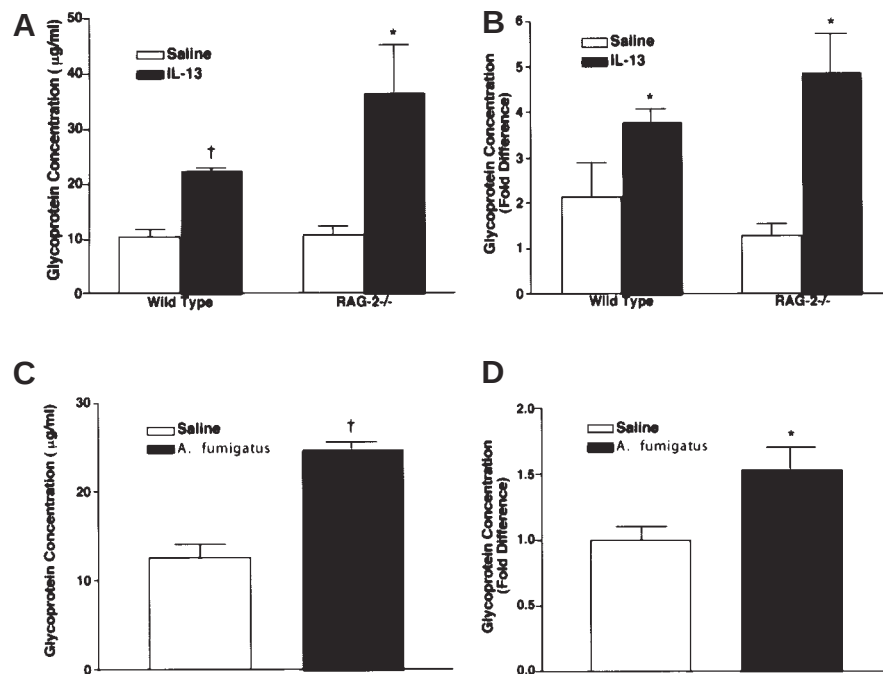


FIG 4. Glycoprotein detection after IL-13 and allergen challenge. BAL fluid samples from wild-type and RAG-2-deficient mice challenged either with saline or recombinant IL-13 were examined for secreted glycoproteins by using jacalin (A) or antiglycoprotein mAb (B) as the detecting reagents. Both assays were also used to quantitate BAL glycoproteins from wild-type mice challenged with saline or *A. fumigatus* allergen (C, D). † $P < .001$ compared with saline challenge; * $P < .03$ compared with saline.

confirm that the jacalin signal derived from mouse BAL, whether from naïve or stimulated animals, is indicative of the presence of glycoproteins other than IgA.

Glycoprotein secretion in relation to glycoprotein production and airway hyperresponsiveness

Although it is clear that glycoprotein secretion must follow its production, it is less obvious that these processes occur simultaneously under diverse agonist conditions. We therefore assessed glycoprotein production under the same conditions used in the prior studies by using a histologic grading method from PAS-stained lung sections.¹¹ Relative to saline-challenged animals, IL-13 challenge of wild-type and RAG-2^{-/-} mice induced robust goblet cell metaplasia of the airway epithelium, comparable with that seen in allergen-challenged wild-type mice (Fig 2). The goblet cell lesion, which is typically assessed as a measure of glycoprotein production, was clearly present under the same conditions in which we observed glycoprotein secretion, regardless of whether the inciting agent was chronic (allergen, Fig 2, A) or acute (IL-13, Fig 2, B). These findings indicate that glycoprotein secretion rapidly follows glycoprotein production.

Airway obstruction has been shown anatomically to be due in part to accumulation of excess secreted airway glycoproteins,²⁻⁵ but real-time measurements of glycoprotein-induced airway obstruction have not been described. Thus, to understand the association between

airway glycoprotein secretion and airway obstruction, we assessed mice that exhibited glycoprotein hypersecretion for airway hyperresponsiveness, another index of airway obstruction that is more readily obtained from living animals. Consistent with our prior studies, both IL-13 and allergen induced robust airway hyperresponsiveness as determined, relative to sham-challenged mice, by a significantly decreased concentration of acetylcholine inducing a 200% increase in respiratory system resistance (Fig 5, A and C). In addition, the same mice showed a marked increase in airway glycoprotein production as determined from histologic lung sections (Fig 5, B and D). Therefore, agonists that induce airway hyperresponsiveness and glycoprotein production also elicit glycoprotein secretion, suggesting that excess airway glycoproteins might contribute to airway obstruction induced during experimental allergic lung inflammation.

DISCUSSION

During the past decade much has been learned regarding the immune basis of airway hyperresponsiveness in experimental allergic lung disease.¹ However, airway hyperresponsiveness represents only one of several pathophysiologic processes that contribute to airway obstruction. In its most exaggerated form, glycoprotein hypersecretion causes asphyxiation-related asthma death and is thus an important additional factor underlying allergic airway obstruction. Aside from this dramatic and fortunately uncommon clinical picture, the contribution of secret-

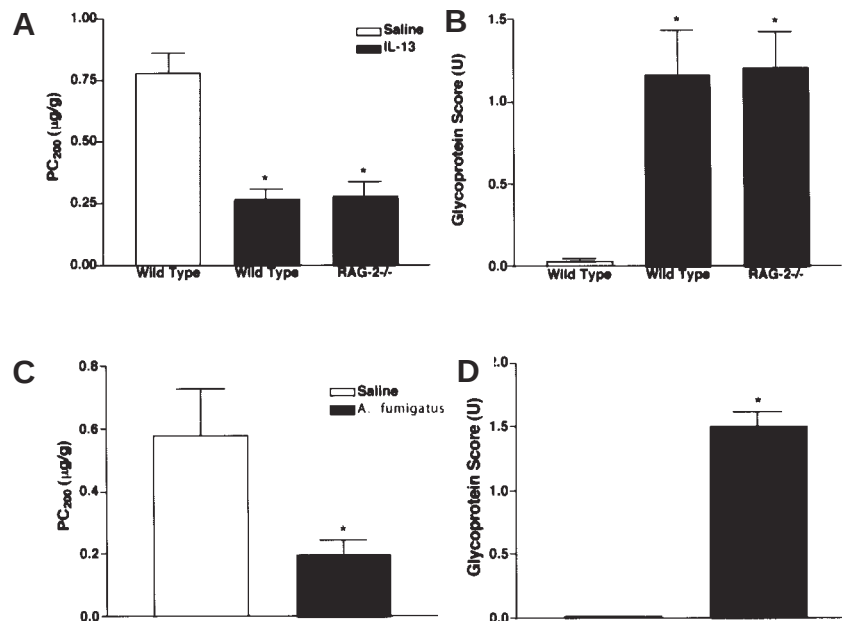


FIG 5. Glycoprotein production in comparison with airway hyperresponsiveness. Mice challenged with IL-13 (A, B) or *A. fumigatus* allergen (C, D) as in Fig 4 were assessed for the presence of airway hyperresponsiveness to acetylcholine challenge (A, C). Lungs were then resected and scored histologically for the presence of goblet cells (B, D; see Methods). PC₂₀₀, Provocative concentration of acetylcholine chloride to induce a 200% increase in respiratory system resistance from baseline. * $P < .03$ compared with saline challenge.

ed glycoproteins to less severe forms of airway obstruction is poorly defined, largely because assays readily capable of measuring airway glycoproteins have not been available. In this study we have developed and used 2 independent assays to demonstrate that glycoprotein secretion closely parallels glycoprotein production and is strongly associated with airway hyperresponsiveness induced by IL-13 and allergen. Glycoprotein secretion is therefore a consistent feature of experimental allergic lung disease and, with the described assays, is a phenomenon that might be directly addressed experimentally.

A variety of immune molecules have been implicated in the pathogenesis of airway hyperresponsiveness, including IgE, histamine, and other mast cell products and IL-4, IL-5, IL-9, and other cytokines. Although these molecules all contribute to airway obstruction induced by inhaled allergens in some way, airway hyperresponsiveness and mucus overproduction have been shown to depend on a final common signaling pathway initiated by the cytokines IL-4 and IL-13.¹¹ The studies here indicate that glycoprotein secretion induced by allergen is controlled through a similar cytokine signaling mechanism, although other potentially important secretagogues such as neutrophil elastase should not be discounted.²⁶ Nonetheless, little is known of the IL-4/IL-13-dependent elements of the glycoprotein secretory apparatus, an area with much potential for future inquiry.

Controversy exists regarding the precise composition of respiratory tract mucus, and, similarly, questions remain regarding the nature of the molecules that are measured by the assays described here and their relative importance to airway obstruction. Analysis of human respiratory tract mucus showed that a maximum of 25% represented spe-

cific mucins, whereas the remainder was largely unaccounted for.²⁹ Additional studies have noted extreme heterogeneity in human airway mucus in terms of mucin genes represented and their degree of glycosylation.¹³ In addition, the jacalin reagent detects at least one other glycosylated molecule present in respiratory tract secretions, IgA, although it might not contribute to the overall signal. Thus, despite the specificity of the antimucin antibody, which appears to detect a relatively homogeneous molecular species, the above considerations argue strongly that respiratory tract mucus should be considered a heterogeneous molecular complex. Therefore, although alternate methods and reagents for detecting specific mucin gene products have been described,^{30,31} these findings strongly support the use of nonspecific reagents such as jacalin for the detection of generic glycoproteins implicated in airway obstruction in the setting of allergic inflammation.

We realized during assay development that the jacalin method is less tolerant to protocol variations than standard immunoassays, and the described method should be rigorously applied to avoid uninterpretable or spurious data. Several methodologic points deserve particular attention. Serum is enriched with jacalin-binding substances, and reagents containing serum of any kind (especially FCS) must be avoided entirely. For this reason, it is recommended that BAL be collected by using PBS only. Similarly, conditions that induce significant hemorrhage or extravasation into the airway are likely to produce spurious results by using jacalin. We determined that contaminating blood does not measurably affect BAL glycoprotein detection (artificially increasing the BAL value) except at dilutions less than 1:50,000. On the

basis of an average hematocrit of mice being 50% to 60%, BAL samples contaminated with red blood cells in excess of 1 to $2 \times 10^5/\text{mL}$ are likely to be significantly contaminated with serum and the jacalin-based assay commensurately inaccurate for detecting BAL glycoproteins alone. This value for red blood cell contamination, readily detected from BAL by using a hemacytometer, can be used as a cutoff mark to include or exclude samples for glycoprotein analysis, depending on the intent of the investigator. In addition, background readings tend to be higher with jacalin compared to most antibody-based ELISAs, and the I-block is strongly recommended for plate blocking to minimize this issue. BSA, which is often used for blocking in immunoassays, is variably contaminated with jacalin-binding substances and is not recommended as a diluent. Finally, although acceptable results might be obtained if incubation times are kept to 1 hour, the overnight incubation of jacalin at 4°C is recommended to maximize detection efficiency.

Jacalin is clearly capable of binding glycoproteins from different species and organs, suggesting that the described assay might be adapted to other situations requiring quantitation of glycoproteins from bodily fluids. Although such modification is encouraged, the limitations of the reagent should be kept in mind, and protocols that deviate from the described method should be validated carefully to ensure specificity of results. A modified assay capable of quantitating glycoproteins from human BAL or induced sputum might prove particularly useful in establishing the severity or monitoring the progression of a variety of airway inflammatory disorders.

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