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Humanized Mouse Model Used to Monitor MUC Gene Expression in Nasal Polyps and to Preclinically Evaluate the Efficacy of Montelukast in Reducing Mucus Production

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Abstract

Objectives—To determine whether MUC gene expression could be down-regulated in nasal polyps by the leukotriene receptor antagonist montelukast, we developed a system in which nondisrupted human nasal polyps could be successfully implanted into severely immunocompromised mice, and in which the histopathology of the original nasal polyp tissue could be preserved for long periods. In addition, the histopathologic changes in the human nasal polyps were carefully examined to determine the origin of the submucosal glands (SMGs) that develop in true nasal polyps found in the anterior third of the nose.

Methods—Small, nondisrupted pieces of human nasal polyp tissues were subcutaneously implanted into NOD-*scid*IL-2 γ^{null} mice. Xenograft-bearing mice were treated with either montelukast or saline solution. Xenografts at 8 to 12 weeks after implantation were examined histologically, and expression of MUC genes 4, 5AC, and 7 was studied in the polyps before implantation and in the 8-week xenograft. Alzet pumps were inserted into the mice, and montelukast (Singulair) was continuously delivered to determine its effect on goblet cell hyperplasia, mucus production, and the enlargement of nasal polyps over an 8-week period.

Results—The xenografts were maintained in a viable and functional state for up to 3 months and retained a histopathology similar to that of the original tissue, but with a noticeable increase in goblet cell hyperplasia and marked mucus accumulation in the SMGs. MUC4 and MUC5AC were significantly increased in the xenograft 8 weeks after implantation, but MUC7 was significantly decreased compared to the preimplantation polyps. Inasmuch as MUC7 is found exclusively in serous glands, the findings suggest that serous glands are not found in polyps in the anterior third of the nose. The histopathologic findings confirm the original findings of Tos et al suggesting that the SMGs are derived from pinching-off of the epithelium of the enlarging polyp following inflammatory changes. These SMGs have the same epithelium as surface epithelium and consist of multiple goblet cells that secrete periodic acid Schiff stain–positive mucin into the interior of the SMGs. A progressive increase in the volume of the xenografts was observed, with little or no evidence of mouse cell infiltration into the human leukocyte antigen–positive human tissue. An average twofold increase in polyp volume was found 2 months after engraftment. Montelukast did

not decrease the growth of the xenograft in the 8-week NOD-*scid* mice, nor did it affect MUC gene expression.

Conclusions—The use of innate and adaptive immunodeficient NOD-*scid* mice homozygous for targeted mutations in the IL-2 gamma-chain locus NOD-*scid* IL-2 γ^{null} for establishing engraftment of nondisrupted pieces of human nasal polyp tissues represents a significant advancement in studying chronic inflammation over a long period of time. In the present study, we utilized this humanized mouse model to confirm our prediction that MUC genes 4 and 5AC are highly expressed and significantly increased over those of preimplanted polyps. The overexpression of these 2 MUC genes correlates with both the goblet cell hyperplasia and the excessive mucus production that are found in nasal polyp xenografts. MUC7, which is primarily associated with the submucosa, as opposed to MUC4 and MUC5AC, which are primarily expressed in the epithelium, was significantly decreased in the nasal polyp xenografts. Montelukast had no significant effect on MUC gene expression in the xenografts. In addition to the MUC gene expression patterns, the histology of the xenografts supports the concept that mucinous glands that are characteristic of true nasal polyps are significantly different from those in the mucosa found in the lateral wall of the nose in patients with chronic sinusitis without nasal polyps. The mucinous glands seen in nasal polyps (which appear to be derived from an invagination of hyperplastic epithelial mucosa containing large numbers of goblet cells) are histologically distinct from the seromucinous glands found in the submucosa of hyperplastic middle turbinates. The data presented here establish a humanized mouse model as a viable approach to study nasal polyp growth, to assess the therapeutic efficacy of various drugs in this chronic inflammatory disease, and to contribute to our understanding of the pathogenesis of this disease.

Keywords

montelukast; mucous gland; nasal polyp; SCID mouse

INTRODUCTION

The exact origin, pathogenesis, and development and growth of nasal polyposis in humans have not been adequately defined. However, it is now clear that chronic hyperplastic sinusitis with nasal polyposis is a chronic inflammatory disease in which proinflammatory cytokines play a major role.¹ In an effort to understand the mechanisms of this disorder and to develop new therapeutic strategies, specific biochemical mediators and cytokines involved in chronic hyperplastic sinusitis with nasal polyposis, as well as their genetic control, have been targeted,² but progress in these areas of study has been limited by the lack of a suitable animal model of human nasal polyposis. A promising new model for studying many different human diseases is the humanized mouse. Most notably, mouse strains, such as the NOD-*scid* IL-2 γ^{null} mice that lack the interleukin (IL)-2 receptor common gamma chain, have been utilized to study many areas of immunology, including autoimmunity, transplantation, cancer, and infectious diseases.³ We previously demonstrated that human nasal polyp xenografts can be maintained in a viable and functional state for up to 3 months and retain a histopathology similar to that of the original tissue, with a noticeable increase in goblet cell hyperplasia and marked mucus accumulation in the submucosal glands (SMGs) compared to the glands of the original polyp tissue.⁴

Because the major histopathologic finding made with the humanized mouse model demonstrated significant growth in nasal polyps to 2 to 3 times their original implanted size associated with a massive increase in mucus secretion and goblet cell hyperplasia, the present study was designed to qualitatively and quantitatively monitor MUC gene expression and to assess the ability of this model to evaluate the efficacy of montelukast to

suppress MUC gene expression and to alter the progression and histopathology of human nasal polyps.

Several studies have indicated that a large set of mucin genes is expressed in nasal polyps. For example, Ali et al⁵ studied the expression of 8 mucin genes (MUC1-4, MUC5AC and 5B, MUC6, and MUC7) in nasal polyps and normal mucosa (sphenoid sinus) by in situ hybridization. The MUC6 and MUC7 genes were not expressed in normal mucosa, but all 8 mucin genes were expressed in nasal polyps, and the expression patterns varied widely between individual polyps. The predominantly expressed genes were MUC4 and MUC5AC, followed by MUC3, MUC5B, and MUC7. The predominant epithelial mucins were MUC4 and MUC5AC, whereas MUC5B and MUC7 were mainly of SMG origin. The major alterations in gene expression in nasal polyps were found in the SMGs. Another study⁶ evaluated the expression of the same set of mucins, and in addition, MUC8, in nasal polyps, mainly by immunohistochemistry. MUC1, MUC4, and MUC5AC were highly expressed in the epithelium, with MUC1 and MUC4 being increased and MUC5AC decreased compared to the expression in normal mucosa. MUC5B was mainly detected in SMGs, and the expression was higher in nasal polyps than in normal nasal mucosa. MUC8 was highly expressed in both epithelium and SMGs in both normal tissue and nasal polyps. MUC7 was detected in 50% of normal tissue samples (6 of 11), but was not detected in nasal polyps, and was restricted to SMGs. Note that the latter study finding of MUC7 is contrary to the finding of Ali et al,⁵ in which MUC7 was not expressed in normal mucosa but was expressed in nasal polyps.

On the basis of the above findings and our interest in the significant increase in mucus production in the xenografts previously reported, we elected to study the expression of 3 mucin genes, MUC4 and MUC5AC (mainly expressed in the epithelium) and MUC7 (expressed in SMGs). Both MUC4 (membrane-tethered mucin) and MUC5AC (secreted mucin) are high-molecular weight mucins, highly expressed in the respiratory tract, whereas MUC7 is a low-molecular weight secreted mucin, expressed primarily in the salivary glands but also in low amounts in the respiratory tract. We have previously described the function and expression of MUC7.⁷

It has also been demonstrated that leukotriene receptors are present in nasal polyps.⁸ Cysteinyl leukotrienes have been demonstrated to be present in both polyps that contain abundant eosinophils and those that contain few eosinophils.⁹ Leukotrienes are derivatives of arachidonic acid through the 5-lipoxygenase pathway.¹⁰ These compounds are potent inflammatory mediators and are strong promoters of eosinophilic infiltration. Furthermore, mucin gene expression and mucin production can be markedly increased as a result of leukotriene receptor stimulation.¹¹ It has also been demonstrated that some of these receptors can be inhibited by leukotriene receptor antagonists such as montelukast (Singulair).¹²

In summary, the purpose of the present investigation was to monitor the type and amount of MUC gene expression in human nasal polyp xenografts over time and to determine whether a leukotriene receptor antagonist (montelukast) can inhibit mucus production, down-regulate MUC gene expression, and alter the histopathology of nasal polyps.

MATERIALS AND METHODS

Patient Characteristics

Nasal polyp samples were taken from 5 different patients who were undergoing surgery for chronic hyperplastic sinusitis with nasal polyposis. Three patients were male, and 2 were female. Three of the patients had a diagnosis of both allergic rhinitis and asthma, and 2

patients had asthma but not allergic rhinitis. One patient had a diagnosis of aspirin intolerance. None of the patients had cystic fibrosis (primary ciliary dyskinesia). Surgical specimens from all 5 patients were sent for bacterial and fungal culture. All 5 mucous secretion cultures from the lateral wall of the nose adjacent to the nasal polyps grew out various microorganisms, including *Staphylococcus aureus* in 2, *Streptococcus viridans* in 2, and *Klebsiella* species in 1. Fungal species were not detected in any patient.

Tissue Samples

Nasal polyp tissue was obtained from the surgical suites at the DeGraff Memorial Hospital, North Tonawanda, New York. All specimens were obtained under sterile conditions and according to an Institutional Review Board–approved protocol. The tissue was transported in Dulbecco's modified Eagle's medium (DMEM)–F12 medium for preservation until implantation.

Implantation of nondisrupted nasal polyp tissue into NOD Cg-*Prkdc*^{scid} *IL-2rg*^{tmWjl}/Sz mice, abbreviated as NOD-*scid* *IL-2r*^γnull mice, was performed according to an approved Institutional Animal Care and Use protocol as previously described.¹³ The surgical specimens of nasal polyps were bathed for 90 minutes at room temperature in DMEM/F12 culture medium containing penicillin G (800 μg/mL), streptomycin sulfate (800 μg/mL), and amphotericin B (2 μg/mL; all from Gibco, Grand Island, New York).

Xenograft Implantation

The specimens were cut into cubic pieces, 0.5 cm on a side as measured with a ruler. Twenty-one immune-deficient NOD-*scid* *IL-2r*^γnull mice were obtained from a research colony at The Jackson Laboratory, Bar Harbor, Maine. The mice were anesthetized with Avertin, 0.5 mg/g body weight (Sigma-Aldrich, St Louis, Missouri). A small midline incision was made on the abdomen and extended with blunt forceps to form a pocket. A single nondisrupted fragment of nasal polyp was inserted into the pocket, which was then closed with the surgical glue Nexaband liquid topical tissue adhesive (Burns Veterinary Supply, Guilford Center, New York). There were no deaths associated with the surgery. An Alzet Pumps model 2006 pump (Durect Corp, Cupertino, California) was installed under anesthesia at the same time the polyps were implanted in 11 mice. A spot was shaved in the shoulder blade area of the mice, and the skin was prepared for incision by an approved procedure. A 5-mm cutaneous incision was made, and a pocket was opened under the skin with a blunt instrument. The pump was inserted into the pocket, and the opening was sealed with surgical glue. There were no deaths associated with this surgery. The animals were painlessly sacrificed at various times after surgery, and the polyp xenografts and other tissues were removed.

The model 2006 Alzet pumps were loaded under sterile conditions with 1 mL of sterile Singulair solution obtained from Merck Laboratories, Newark, New Jersey, at a concentration of 6 mg/mL. The drug was calculated to be delivered at the rate of 5 mg/kg body weight per day for 60 days. Control animals were implanted with a model 2006 Alzet pump filled with 1 mL of sterile saline solution. There were 10 nondisrupted nasal xenografts as controls, and 11 nondisrupted nasal xenografts treated with Singulair (montelukast).

Histopathology

The original nasal polyps and polyp xenografts were prepared for histologic examination. Tissues were fixed in neutral buffered formalin and embedded in paraffin, and 8-μm sections were cut and mounted according to standard procedures by the State University of New York at Buffalo Histology Service Laboratory. Sections were stained with hematoxylin

and eosin (H & E) for histologic evaluation. Periodic acid–Schiff reagent (PAS) staining, which detects carbohydrate moieties such as those found in mucin, was also performed. To estimate the relative percentages of tissue taken up by SMGs and stroma, a pathologist (A.S.) examined the H & E– and PAS-stained slides under low-power and high-power magnifications.

Semiquantitative evaluation of original polyps, implanted control polyps, and implanted polyps treated with montelukast were analyzed for the percentage of stroma, including extravasation exclusive of mucus; the percentage of SMGs filled with mucus; and semiquantitation of macrophages, lymphocytes, plasma cells, eosinophils, PMNs, and goblet cells.

Quantitation of Mucin Gene Expression in Nasal Polyps by Quantitative Real-Time PCR

To determine the expression of mucin genes in the original nasal polyps and in polyp xenografts that were either untreated or treated with montelukast, we used quantitative real-time reverse-transcriptase polymerase chain reaction (RT-qPCR). Reverse transcription (RT) combined with polymerase chain reaction (PCR) has proven to be a powerful method to quantify gene expression.¹⁴ MUC4, MUC5AC, and MUC7 were studied. The expression of these genes was determined from 5 sets of polyp samples, as mentioned above. The RT-qPCR involves 3 steps: RNA preparation, RT (first strand complementary DNA synthesis), and real-time PCR.

RNA Preparation

Total RNA from polyp tissues was prepared with TRIzol reagent (Invitrogen, Carlsbad, California) according to the manufacturer's specifications. The quality and quantity of RNA were determined spectrophotometrically.

RT

The RT was carried out with SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Reactions contained total RNA (2 µg), random hexamer primers, dNTPs rRNasin, reverse transcriptase, and 5× first-strand buffers. The reactions were incubated at 65°C for 5 minutes, followed by 1 minute of incubation on ice, 10 minutes at 25°C, and 50 minutes at 50°C. The reactions were inactivated at 70°C for 15 minutes.

Real-Time PCR

Quantitative real-time PCR was performed with Applied Biosystems 7500 Real Time PCR System (Foster City, California) according to the TaqMan Gene Expression Assay protocol (Applied Biosystems). TaqMan Gene Expression Assay mix, containing primers and an FAM dye–labeled TaqMan probe for the selected human MUC genes (MUC4, MUC5AC, and MUC7), the internal control housekeeping gene GAPDH, and TaqMan universal PCR master mix were purchased from Applied Biosystems. The data were analyzed with Relative Quantification Study Document. The relative quantity of mucin messenger RNA was normalized to human GAPDH expression. The $\Delta\Delta C_t$ method was used to analyze relative quantitative gene expression (not absolute quantification, which determines the absolute copy number). The relative change in mucin gene expression compared to the nonimplanted polyp was reported as x-fold change.

Statistics

For the analysis of the change in the percentage of stroma versus the glandular percentage in original polyps versus xenograft polyps after 8-week implantation, a random-effects model

was utilized. The use of the model allows for accommodation of the potential dependence that may exist between observations based on the same sample.

The statistical evaluation of MUC gene expression in xenografts treated with montelukast versus untreated controls compared to original polyps required a mixed linear model. For MUC4, MUC5AC, and MUC7, the relative quantity value was modeled as a function of treatment (control versus montelukast) and a random effect—presenting polyp. The inclusion of the random effect was for the purposes of accommodating the potential dependence that may exist between observations based on the same sample. A log transformation was applied in the case of MUC4 and MUC5AC in order to meet model assumptions. A nominal significance level of 0.05 was used throughout, and SAS (version 9.2) was used for all analyses.

RESULTS

Long-Term Engraftment and Growth of Nasal Polyp Tissue in NOD-scid IL-2ry^{null} Mice

The mice implanted with intact fragments of nasal polyp tissues obtained surgically were monitored over an 8-week period for evidence of a palpable nodule, and the nodules were measured externally at 5 weeks and 8 weeks after engraftment. This design was already described in our previous publication.⁴ However, the results in this study, with a different set of 21 polyp fragments, confirmed our original finding that there was a statistically significant increase in the size of the nodules for all engrafted mice 8 weeks after implantation, as shown in Fig 1. The average size of the original polyp fragments was 25 mm³, and the average size of the 8-week xenografts was 150 mm³ ($p = 0.01$).

MUC Gene Expression in Control Xenografts

Expression levels of all 3 MUC genes in the original polyps (before implantation) were set at a relative quantity value of 1. The levels of MUC gene expression in the implanted polyp xenografts (untreated control or montelukast-treated) are graphically depicted in Fig 2 as the log fold change (increase or decrease) in the original nonimplanted polyps. The messenger RNA fold changes for all 3 MUC genes are depicted in the Table.

The expression of MUC4 and MUC5AC genes in the 8-week untreated xenografts increased more than 5-fold and more than 8-fold, respectively, compared to the original polyp before implantation. Statistically, these are highly significant increases ($p < 0.001$). In contrast, MUC7 was significantly decreased in the xenografts in comparison to the original nasal polyp. The average decrease was 0.68-fold; this decrease is also highly significant ($p < 0.001$).

MUC Gene Expression in Xenografts Treated With Montelukast

Expression levels of MUC4 and MUC5AC were significantly increased in the xenograft treated with montelukast over an 8-week period. MUC4 increased 9.5-fold, and MUC5AC increased 11.65-fold compared to the original nonimplanted control. Montelukast had no significant effect on the size or rate of growth of the xenograft at 8 weeks.

Having established the utility of the humanized model to monitor the possible therapeutic effects of a drug by quantifying MUC gene expression patterns, we chose to take a closer look at the histopathology of the nasal polyp xenografts in these mice. We anticipated that further histopathologic details could be used, in addition to gene expression patterns, to assess drug effects.

Effect of Montelukast on Goblet Cell Hyperplasia and Mucus Production

The presence of continuous montelukast infiltration did not have any effect on goblet cell hyperplasia in this mouse model.

Presence and Expression of Mucinous Glands in Nasal Polyps

The xenograft tissue in both the control xenografts and the xenografts treated with montelukast demonstrated goblet cell hyperplasia, more pronounced than that of the original nasal polyp, and also demonstrated a marked accumulation of PAS-positive mucus (Fig 3). In the original nasal polyps, SMGs accounted for 5% to 20% of the tissue volume, whereas 8 weeks after implantation in both the controls and montelukast-treated mice, the glandular tissue expanded to 75% to 90% of the tissue volume. This difference was significant ($p = 0.001$).

Figure 4 demonstrates inflammatory nasal mucosa found in the lateral wall of the nose of a patient with chronic sinusitis with nasal polyposis. The histopathology demonstrates classic seromucinous glands in which both serous tubules and mucous glands are present side by side with inflammatory infiltrative lymphocytes. It is emphasized that this is not characteristic of true nasal polyps located in the front of the nose, medial to the middle turbinate, which never possess serous glands.¹⁵ The pathogenesis of the SMG-like structures in the lamina propria of nasal polyps in the present study follows closely the theory of the pathogenesis of mucous glands proposed by Tos et al^{16–18} more than 35 years ago.

Figure 5 shows a classic SMG in the nasal polyp. The histopathology demonstrates a circular cyst surrounded by goblet cells secreting PAS-positive substances, and the entire cyst is filled with mucus.

Figure 6 demonstrates the similarity between the surface epithelium and the epithelium surrounding the SMG. These findings confirm the original concept of the pathogenesis of nasal polyps by Tos and Mogensen,¹⁸ in which there is a prolongation of the epithelial cords of a growing nasal polyp with a pinching-off of the surface epithelium that becomes invaginated into the lamina propria; the resulting surface epithelium possessing multiple goblet cells continues to secrete mucus into these large SMGs. Figure 7 demonstrates the pinching-off of the surface epithelium with the production of an SMG. In summary, our results establish a humanized model that provides an opportunity to test the therapeutic efficacy of drugs for nasal polyposis and has the potential to explore cellular and molecular factors that contribute to the progression of this chronic inflammatory disease. Furthermore, the type and amount of MUC gene expression and the histopathologic findings in the nasal polyp xenografts are consistent with the notion that the mucus associated with nasal polyps is derived primarily from epithelial mucins MUC4 and MUC5AC and that the SMGs found in the lamina propria of nasal polyps are likely formed by invagination of the hyperplastic epithelium, which contains a plethora of mucin-producing goblet cells.³

DISCUSSION

Mucus hypersecretion is one of the main symptoms of nasal polyposis and occurs as a result of the increase in the quantity of mucus present in both goblet cells and the enlarged SMGs in the lamina propria of nasal polyps.¹⁹

Perhaps the most elegant studies on the origin of mucous glands in nasal polyps and goblet cell density, as mentioned above, have been those performed by Tos et al^{16–18} over the past 30 years. The quantitative study of glands suggests that these SMGs are tubular, of different shapes and sizes, and differ widely from those in the normal nasal mucosa. These true nasal polyp glands are formed from the surface epithelium after the polyp has attained a certain

size. They do not arise from the normal nasal mucosa and cannot be compared to the true seromucinous glands found in the nasal septal mucosa and the mucosa of the lateral wall of the nose or of the nasal turbinates in chronic sinusitis. Many theories have been established for the development of glands in nasal polyposis. The present study confirms those of Tos et al,^{16–18} in that these SMGs appear to arise from invagination of the surface epithelium resulting from increased inflammation in the true nasal polyp.

The present study confirms that in true nasal polyps, the SMGs are totally different from the seromucinous glands of normal nasal mucosa and chronic sinusitis without nasal polyposis. The size of these newly formed glands increased significantly in the xenograft polyps. It is therefore likely that the massive accumulation of mucus in the xenograft polyps was the result of a marked increase in goblet cell numbers. The present mouse model allows the investigator to follow the histopathologic progression of nasal polyps over time, unlike any model that has previously been reported. The present study demonstrates the quantitative increase in MUC gene expression in nasal polyp xenografts. MUC4 and MUC5AC are increased significantly in the polyp tissue after 8 weeks in the xenografts. Previous studies have demonstrated that MUC4 and MUC5AC are present in both the epithelial cells and the SMGs of nasal polyps.²⁰ Our study differs significantly from that of Ali et al,⁵ who demonstrated MUC7 to be expressed in, and to be of glandular origin in, nasal polyps. It may be, as mentioned above, that a polypoid middle turbinate would contain seromucinous glands, which are never found in true nasal polyps. Thus, the presence of MUC7 in the study of Ali et al⁵ might be explained by the location of the tissue that was analyzed. The present study demonstrates, for the first time, the actual time course for the development of goblet cell hyperplasia and MUC gene expression over time. The increase in MUC gene expression may be due to an actual increase in synthesis of the genes or to an increase in the genes secondary to goblet cell hyperplasia.

The concept that MUC7 is exclusively found in serous cells of SMGs is well illustrated by the study of Sharma et al,²¹ who demonstrated that there is a marked difference between MUC5B and MUC7, wherein the former is expressed in mucous cells and MUC7 is exclusively found in serous cells of SMGs of the trachea. The original studies of Tos et al,^{16–18} in which the polyp tissue was taken only from the anterior third of the nasal cavity, demonstrate the absence of serous glands. MUC7 expression is seen in a subset of lysozyme-expressing serous tubules, but not in mucous glands.

Expression of MUC5AC in human nasal polyps and inferior turbinate epithelium was studied by Lü et al.²² These investigators noted that the number of MUC5AC-positive cells in nasal polyps was significantly greater than that of the normal inferior turbinate and that the MUC5AC-positive cells were mainly concentrated in the goblet cells, as suggested by the present study.

Because MUC5AC is a genetic marker for goblet cells in human airways,²⁰ it seems reasonable to conclude that the increased numbers of goblet cells present in our xenograft polyps are associated with an increased expression of MUC5AC. The increased expression of MUC5AC may be responsible for the mucus overproduction in the chronic inflammatory state of nasal polyposis.

Previous studies from our laboratory have demonstrated the presence of tumor necrosis factor α in the epithelial cells of nasal polyps.²³ Other studies have demonstrated the up-regulation of epithelial growth factor receptor in nasal polyps, as well.²⁴ A potential mechanism for the development of increased mucin expression in goblet cells in nasal polyps, as shown in the present study, may exist as follows. Tumor necrosis factor α up-regulates epithelial growth factor receptor expression. Epithelial growth factor receptor and

its ligands (epithelial growth factor, transforming growth factor α) increase tyrosine phosphorylation in the epithelial cell with the ultimate conversion into a goblet cell.²⁴ MUC4 is also predominantly expressed in polyp epithelium, as well as in SMGs.²⁵

Although we could not demonstrate the effect of a leukotriene receptor antagonist (montelukast) in either decreasing the size of the implanted xenograft or down-regulating the production of mucus or MUC gene expression, our findings do not eliminate the possibility that cysteinyl leukotriene receptor antagonists have the ability to modulate nasal polyps in humans. However, at least in this model, we could not demonstrate either a decrease in the rate of growth of the xenograft or a down-regulation of the expression of these 2 MUC genes. We can only state that in the present mouse model, montelukast secreted by Alzet pumps in the subcutaneous tissue did not down-regulate MUC gene expression, decrease the size of the mucous glands, or have any effect on the rate of growth of the xenograft nasal polyp.

CONCLUSIONS

The present investigation demonstrates a marked increase in mucus production in human nasal polyp xenografts implanted in NOD-*scid*IL-2 γ^{null} mice. The marked increase in the size of the SMGs appears to be the result of hyperplasia of goblet cells in the xenograft compared to the original polyp.

This increase in mucus production caused by goblet cell hyperplasia may be explained by the up-regulation of MUC4 and MUC5AC, which were statistically significantly elevated in polyp tissue compared to the original polyp. The significant decrease in expression of MUC7, however, may only indicate that this gene is found primarily in serous cells. It is our belief that serous glands are not present in true nasal polyps that appear in the anterior part of the nose, and that the expression of MUC7 found by other investigators indicates that tissues expressing MUC7 are probably related to hyperplastic middle turbinate mucosa or even sinus mucosa, which may contain true seromucinous glands, as suggested in the present study. This study establishes the viability of the humanized mouse model to evaluate therapeutic efficacy studies on nasal polyps and supports the original claim of Tos et al¹⁶⁻¹⁸ that mucus-producing glands in nasal polyps are formed by the infolding and pinching-off of hyperplastic epithelium, and that serous glands do not exist in true nasal polyps.

The lack of inhibition by a leukotriene receptor antagonist (montelukast) does not indicate that this drug may not be effective in chronic sinusitis with nasal polyposis. It only demonstrates that in this particular mouse model, the abundant production of mucus is not inhibited by this drug. Therefore, the effect of montelukast on the clinical improvement of nasal polyposis may be due to some mechanism other than a decrease in MUC gene expression and subsequent mucus production.

Finally, one of the most important ideas expressed in this article is that “humanized” mice are a promising model for studying human diseases and immunity. The NOD-*scid*IL-2 γ^{null} mouse that lacks the IL-2-receptor gamma chain represents a new stock of immunodeficient hosts.³ These mice lack adaptive immune function, display multiple defects, and, most importantly, support heightened levels of human engraftment. Humanized mice can support studies in many areas of immunology, including infectious diseases and chronic inflammation.³ This model is particularly valuable in following the course of chronic inflammation in a disease such as nasal polyposis. This model also provides a method for evaluating various drugs that may be effective in the treatment of chronic hyperplastic sinusitis with nasal polyposis.

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Fig 1. Change in xenograft volume 8 weeks after implantation in NOD-*scid*IL-2 γ^{null} mice. Mean postimplantation volume was found to be 150 mm³. Mean size of original polyp at time of implantation was 25 mm³. This increase in polyp volume of 125 mm³ was statistically significant ($p = 0.01$).

Fig 2. MUC4, MUC5AC, and MUC7 gene expression in polyp xenografts for 10 untreated (U) xenografts and 11 montelukast-treated (M) xenografts. Messenger RNA levels were determined by quantitative real-time reverse-transcriptase polymerase chain reaction, and are depicted as log fold change (increase or decrease) relative to original nonimplanted polyp (n = 5). See Table for numerical fold changes.

Fig 3.

Periodic acid-Schiff (PAS) staining of **A**) original nasal polyp tissue versus **B**) nasal polyp xenograft at 8 weeks after implantation. **A**) Arrows in pre-implanted polyp point to small glands, which represent 10% to 20% of polyp (PAS, original $\times 100$). Stroma (STR) area is 80% to 90% of pre-implanted polyp. **B**) At 8 weeks after implantation in NOD mouse, xenograft tissue shows extensive mucus production in large gland (PAS, original $\times 400$). Average glandular occupation of polyp is 66% greater than that of pre-implanted polyp and constitutes 80% to 90% of volume of xenograft polyp ($p = 0.001$).

Fig 4.

Hematoxylin and eosin stain of sinus mucosa taken from *lateral wall of nose* in patient with chronic sinusitis with nasal polyposis (original $\times 40$). Histopathology demonstrates classic seromucinous glands in which both serous glands (Se) and mucous glands (Mu) are present side by side with inflammatory infiltrative lymphocytes (Ly). This tissue was derived from proximal portion of polyp arising from anterior ostiomeatal complex.

Fig 5. PAS stain of typical submucosal gland in nasal polyp (original $\times 400$). Gland has appearance of large, cystically dilated accumulation of mucus (M) resulting from secretion of mucus from surrounding goblet cells (Go).

Fig 6.

High-power photomicrographs of submucosal glands (PAS, original $\times 400$). **A)** Classic submucosal gland found in nasal polyp from anterior third of nose. This submucosal gland arises from invagination of surface epithelium (Su Ep) adjacent to sub-mucosal gland. Epithelium of surface of polyp and epithelium of surface of submucosal gland (Gl Ep) are identical and consist mainly of goblet cells filled with mucin. M — mucus. **B)** Polyp submucosal gland demonstrates secretion of mucus from goblet cells (Go) directly into lumen of submucosal gland (M).

Fig 7. Submucosa of nasal polyp demonstrates pinching-off of epithelium (Ep) in development of submucosal gland (PAS, original $\times 400$). Interior of gland is filled with mucus (M) secreted by surrounding epithelial cells that have been transformed into goblet cells.

Table 1**THREE MUCIN GENES IN POLYP XENOGRAFTS**

Gene	Untreated Xenografts	Xenografts With Montelukast
MUC4	5.36 ± 3.28	9.50 ± 6.84
MUC5AC	8.15 ± 8.84	11.65 ± 13.91
MUC7	0.68 ± 1.30	0.19 ± 0.55

Mean ± SD of messenger RNA levels (relative quantity) of 3 mucin genes in polyp xenografts that were untreated and xenografts treated with montelukast (n = 10 to 11 for each experimental group for each MUC gene). Values are shown as fold changes (increase or decrease) relative to levels in original polyps before implantation (n = 5; relative quantity value of 1). For all 3 MUC genes, differences between untreated and montelukast-treated xenograft groups were not statistically significant. In contrast, differences in MUC gene expression in untreated xenografts and original polyps and between montelukast-treated xenografts and original polyps were highly statistically significant (p < 0.001 in test of mean relative quantity equal to 1). See Fig 2 for graphical depiction of changes in MUC gene expression.