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Human Nasal Polyp Microenvironments Maintained in a Viable and Functional State as Xenografts in NOD-*scid* *IL2 γ ^{null}* Mice

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Abstract

Objectives—The objective was to develop a model with which to study the cellular and molecular events associated with nasal polyp progression. To accomplish this, we undertook to develop a system in which nondisrupted human nasal polyp tissue could be successfully implanted into severely immunocompromised mice, in which the histopathology of the original nasal polyp tissue, including inflammatory lymphocytes, epithelial and goblet cell hyperplasia, and subepithelial fibrosis, could be preserved for prolonged periods.

Methods—Small, non-disrupted pieces of human nasal polyp tissues were subcutaneously implanted into NOD-*scid* *IL2 γ ^{null}* mice. Xenografts at 8 to 12 weeks after implantation were examined histologically and immunohistochemically to identify human inflammatory leukocytes and to determine whether the characteristic histopathologic characteristics of the nasal polyps were maintained for a prolonged period. The xenografts, spleen, lung, liver, and kidneys were examined histologically and immunohistochemically and were evaluated for changes in volume. The sera of these mice were assayed for human cytokines and immunoglobulin.

Results—Xenografts of human nasal polyp tissues were established after their subcutaneous implantation into NOD-*scid* *IL2 γ ^{null}* mice. The xenografts were maintained in a viable and functional state for up to 3 months, and retained a histopathologic appearance similar to that of the original tissue, with a noticeable increase in goblet cell hyperplasia and marked mucus accumulation in the submucosal glands compared to the original nasal polyp tissue. Inflammatory lymphocytes present in the polyp microenvironment were predominantly human CD8+ T cells with an effector memory phenotype. Human CD4+ T cells, CD138+ plasma cells, and CD68+ macrophages were also observed in the xenografts. Human immunoglobulin and interferon- γ were detected in the sera of xenograft-bearing mice. The polyp-associated lymphocytes proliferated and were found to migrate from the xenografts to the spleens of the recipient mice, resulting in a significant splenomegaly. 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 at 3 months after engraftment.

Conclusions—The use of innate and adaptive immunodeficient NOD-*scid* mice homozygous for targeted mutations in the interleukin-2 receptor γ -chain locus NOD-*scid* *IL2 γ ^{null}* for establishing xenografts of nondisrupted pieces of human nasal polyp tissues represents a significant improvement over the previously reported xenograft model that used partially

immunoincompetent CB17-*scid* mice as tissue recipients. The absence of the interleukin-2 receptor γ -chain results in complete elimination of natural killer cell development, as well as severe impairments in T and B cell development. These mice, lacking both innate and adaptive immune responses, significantly improve upon the long-term engraftment of human nasal polyp tissues and provide a model with which to study how nasal polyp-associated lymphocytes and their secreted biologically active products contribute to the histopathology and progression of this chronic inflammatory disease.

Keywords

mucin; nasal polyp; splenomegaly; xenograft

INTRODUCTION

Chronic hyperplastic sinusitis with nasal polyposis, the ultimate manifestation of chronic inflammation in the lateral wall of the nose, possesses many of the histopathologic features of asthma and allergic rhinitis. The nasal polyp arises as a *de novo* tissue growth from the anterior and posterior ostiomeatal complexes, and demonstrates a characteristic histologic appearance that differs dramatically from that of normal nasal mucosa. The histopathologic features of a nasal polyp include hyperplasia of surface epithelium and goblet cells, eosinophilia, lymphocytosis, marked edema, and the generation of cystically dilated and distorted submucosal glands.¹ The cell biology and pathogenesis of nasal polyposis have been studied extensively. Characteristic cytokines, chemokines, adhesion molecules on vascular endothelial cells, and integrins on the surface of inflammatory cells such as lymphocytes and eosinophils and neutrophils have been identified in nasal polyps.² However, the functional significance of these inflammatory cells and the biologically active factors they produce with respect to the generation and progression of the underlying disorder has not been well defined or causally linked to this chronic inflammatory disease. A better understanding of the immunology of nasal polyposis could be achieved by selectively blocking active factors with function-blocking antibodies and immunodepleting polyp-associated lymphocytes and monitoring the effect of each blocking or depletion protocol upon changes in the histopathologic characteristics and progression of the polyp. For obvious ethical reasons, this sort of controlled study is not feasible in patients. Therefore, we set out to design and test animal models in which human nasal polyp tissues could be engrafted into immunodeficient mice, the resultant xenografts could be manipulated (by factor blocking and cell depletion studies), and the effects of the manipulation on the histopathologic characteristics and progression of the polyps could be monitored and quantified.

The development of animal models to study human cells, tissues, and organs *in vivo* without putting individuals at risk has given us new and useful research tools. One of the most widely used of these tools is the mouse-human chimera in which human cells or tissues are implanted into severe combined immunodeficient CB17 mice (abbreviated *scid*). The first use of CB17-*scid* mice to develop these chimeras was reported over 20 years ago.³ Since this initial report, there have been several thousand reports on the successful engraftment into *scid* mice of a variety of different normal and neoplastic human cells and tissues. These studies have led to advances and insights into human cancer, autoimmunity, and infectious diseases.^{4,5} Several limitations have been recognized with the *scid* model, including high levels of host natural killer (NK) cells and other innate immune activity that prevents the long-term engraftment of human cells and tissues.⁴ Two significant breakthroughs have led to improvements in the engraftment and survival of human tumor xenografts. The first was the crossing of the *scid* mutation onto the nonobese diabetic (NOD) mouse, which has defects in innate immune activity, including NK cells. But the use of the humanized NOD-

scid mouse as a model remained limited by its relatively short life span and the residual activity of NK cells and other components of innate immunity that impeded the engraftment of human cells and tissues. The second major breakthrough came with the development of the NOD-*scid* immunodeficient mouse homozygous for the targeted mutation at the interleukin (IL)-2 receptor γ -chain (IL-2R γ chain) locus (abbreviated NOD-*scid* IL2r^{null} mice).^{5,6} The IL-2R γ chain is part of the high-affinity receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 and is required for signaling via these cytokine receptors.⁷ In the absence of these receptors, NK cells fail to develop and T and B cell development and function is impaired.⁸ These mice have greatly increased the success of engrafting both normal human cells and tissues⁸ and neoplastic cells and tissues.⁹

In the first attempt to establish a xenograft model of nasal polyposis, small fragments of human nasal polyp tissue were implanted into subcutaneous pockets in CB17-*scid* mice.¹⁰ Xenografts were established that retained some of the anatomic features of the original nasal polyp microenvironment for up to 6 months. However, these xenografts did not expand in volume, and the evidence of a host-versus-graft response (murine granulocytes and NK cells infiltrating the xenografts) and a coinciding elimination of human inflammatory leukocytes was observed just 2 weeks after implantation of the nasal polyp tissue. These factors severely limited the utility of the *scid* mouse xenograft model. Many, if not all, of the limitations of this model were most likely due to the presence of functionally active NK cells and contributions from macrophages and granulocytes present in CB17-*scid* mice that attack and destroy lymphocytes in the xenografts.⁴

Using the NOD-*scid* IL2r^{null} mice as the recipient of intact human nasal polyp tissue, we report here the generation of an optimal model with which to study human nasal polyps as xenografts in vivo. The advantages of this new model, which include the presence of the characteristic histopathologic characteristics, the persistence and expansion of a variety of different nasal polyp-associated inflammatory lymphocytes, and the continued growth of the polyp tissue in the xenograft, are presented and discussed.

MATERIALS AND METHODS

Patient Characteristics

Nasal polyp samples were taken from 6 different patients who were undergoing surgery for chronic hyperplastic sinusitis with nasal polyposis. Three patients were female, and 3 were male. Four of the 6 patients had a diagnosis of both allergic rhinitis and asthma, 1 patient had asthma but no allergic rhinitis, and 1 patient had allergic rhinitis but no evidence of asthma. None of the 6 patients had a diagnosis of aspirin sensitivity. None of the patients had cystic fibrosis, primary ciliary dyskinesia, or fungal rhinosinusitis. Surgical specimens from all 6 patients were sent for bacterial and fungal culture. Two specimens were positive for *Staphylococcus aureus*, and 2 were positive for coagulase-negative *Staphylococcus* species and *Streptococcus viridans*. The remaining 2 specimens were positive for *Streptococcus viridans* and *Klebsiella* species. Fungal cultures were negative in all 6 patients.

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 tissue into NOD. Cg-*Prkdc^{scid} IL2rg^{tmWjl}/Sz*, abbreviated as NOD-*scid IL2rg^{null}* mice, was performed according to an approved Institutional Animal Care and Use protocol as previously described.⁹ 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, 0.4 cm on a side, measured with a ruler. This was an estimated implantation size, but the exact size of each implant was not recorded. Thirty immunodeficient NOD-*scid IL2rg^{null}* 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 mid-line incision was made on the abdomen and extended with blunt forceps to form a pocket. A single non-disrupted 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, Guilderland Center, New York). There were no deaths associated with the surgery. Twenty-five animals were painlessly sacrificed at 8 weeks after surgery, and the polyp xenografts and other tissues were removed. Another 5 animals were painlessly sacrificed at 12 weeks after implantation, and the xenografts, spleens, and other organs were removed.

Polyp Xenograft Measurement

The size of the polyp xenografts after removal from the mouse hosts was measured with a caliper. The volume of the xenograft was calculated by the formula $\text{volume (mm}^3\text{)} = a^2 \times b / 2$, where a is the short axis of the polyp and b is the long axis of the polyp.

Histology and Immunohistochemistry

The original nasal polyp, polyp xenograft, lung, liver, spleen, intestine, and lymph nodes were prepared for histologic and immunohistochemical 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 submucosal glands and stroma, a pathologist (A.S.) examined H & E- and PAS-stained slides under low-power magnification. As a probe for human leukocyte subsets, human-specific antibodies to the following markers were used for immunohistochemical analysis: CD3, CD19, CD138, CD68, CD44, and CD45RO (BD Pharmingen, San Diego, California). The Dako Labs (San Diego) peroxidase kit was used for detection.

Phenotyping

Spleens from the polyp-implanted NOD-*scid IL2rg^{null}* mice were pressed through a screen to harvest leukocytes. Cells were separated on a 1077 Histopaque gradient (Sigma-Aldrich) to remove the red blood cells. The cell suspensions were stained with primary conjugated human-specific antibodies anti-CD3, anti-CD4, anti-CD8, anti-human leukocyte antigen (HLA)–DR, anti-CD19, and anti-CD45RO (BD Pharmingen). Data were collected on a FACS Calibur flow cytometer (BD Biosciences, San Jose, California; State University of New York at Buffalo; and Roswell Park Cancer Institute, Buffalo, New York) and were analyzed in our laboratory with WinList software.

Enzyme-Linked Immunosorbent Assays for Human Interferon γ and Immunoglobulin G

Assays of mouse serum to detect human interferon γ (IFN γ) and immunoglobulin G were conducted as previously described.¹¹ Both assays are standard sandwich enzyme-linked immunosorbent assays that use commercially available reagents (Endogen, St Louis; Sigma-Aldrich; and Kirkegaard & Perry Laboratories, Gaithersburg, Maryland).

Statistical Analysis

In order to assess the growth in nasal polyp volume after implantation into immunodeficient mice, we used simple descriptive statistics to summarize the postimplantation volume, as well as the change in volume, defined as the measured postimplantation volume minus the implantation volume of 64 mm³. Statistical significance of the change was established with the sign test in conjunction with a nominal significance level of 0.05. All analyses were performed with SPSS version 9.1.3.

RESULTS

Long-Term Engraftment and Growth of Nasal Polyp Tissue in NOD-scid IL2 γ ^{null} Mice

Twenty small (estimated 0.4 cm on a side) pieces of human nasal polyp tissue from 4 different patients were surgically implanted into a subcutaneous pocket on the ventral midline of 20 NOD-scid IL2 γ ^{null} mice. The mice 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. A single palpable nodule was present in all 20 mice and was significantly increased in size by 15 days after engraftment (Fig 1A,B). The increase in volume for all engrafted mice 8 weeks after implantation is shown in Fig 2. There is an approximate twofold increase over the volume of the tissue that was initially engrafted in the mice. To determine whether this increase in volume was due to an expansion and growth of the human tissue, edematous swelling, infiltration of the human xenografts with mouse cells, or a combination of these factors, we fixed and stained the xenografts with H & E and immunohistochemically stained them to distinguish between human and mouse tissues and to phenotypically identify human lymphocyte subsets.

Histopathology and Immunohistochemistry of Nasal Polyp Xenografts 8 to 12 Weeks After Engraftment

Thirty mice were implanted with nasal polyp tissue from 6 different patients. Twenty-five xenografts were removed at 8 weeks after engraftment, and 5 xenografts were removed at 12 weeks after engraftment. The histologic architecture of the xenografts at both 8 weeks (data not shown) and 12 weeks (Fig 1C–E) after the nasal polyp tissue implantation was very similar to that of the original polyp tissue. Both the xenografts and the original polyp tissues showed evidence of a thickened basement membrane, hyperplasia of the ciliated epithelial cells, subepithelial fibrosis, and a florid accumulation of mononuclear leukocytes and plasma cells.

The xenograft tissue also appeared to develop goblet cell hyperplasia even more pronounced than that of the original nasal polyp tissue, and demonstrated a marked accumulation of PAS-positive mucus (Fig 3). Glandular tissue was examined microscopically in preimplantation nasal polyp tissue from 5 patients. In these original nasal polyps, submucosal glands accounted for 5% to 20% of the tissue volume, whereas 8 weeks after implantation into 25 NOD-scid IL2 γ ^{null} mice, glandular tissue expanded to take up 75% to 90% of the tissue volume. This expansion of glandular tissue was also observed 12 weeks after implantation.

Xenografts were also examined histologically for composition of inflammatory cell infiltrates in comparison to original nasal polyps. Of the 6 original nasal polyp samples, 3 showed a predominantly eosinophilic infiltrate, 2 demonstrated marked lymphocytic infiltrates with fewer eosinophils, and the remaining polyp showed roughly equal numbers of eosinophils and lymphocytes per high-power field. After engraftment, both 8- and 12-week xenografts showed preservation of lymphocytic infiltrates but a complete absence of eosinophils. We have noted that these terminally differentiated shorter-lived granulocytes are found in xenografts in the first few weeks after engraftment, but are typically absent in the implants beyond 3 weeks after engraftment (data not shown).

An immunohistochemical analysis of the xenograft tissue 8 weeks after implantation was performed on 25 xenografts from 5 different patients. This analysis revealed that the majority of the cells stained positive for HLA-DR, indicating that the bulk of the xenografts was human tissue (Fig 4A). No evidence of mouse cell infiltration into the xenografts was seen. Pockets of mononuclear cells stained positively for human CD45 (Fig 4B), and the majority of these human leukocytes were CD3+ T lymphocytes (Fig 4C,D). The nasal polyp-associated T cells were positive for CD45RO (Fig 4E) and negative for CD45RA (Fig 4F), as is consistent with either activated effector T cells or effector memory T cells. A positive stain for CD44 suggests that a significant portion of the CD45RO+ T cells are memory T cells (Fig 4G). A small but distinct population of CD68+ macrophages was observed throughout the xenografts (Fig 4H). Terminally differentiated shorter-lived eosinophils, mast cells, and basophils present in the original nasal polyp tissue were not observed in the xenografts 8 weeks after implantation. These granulocytes were found in the xenografts in the first few weeks after engraftment, but were typically absent beyond 3 weeks after engraftment (data not shown).

Lymphocytes Present in Nasal Polyp Xenograft Expand and Migrate to Other Host Tissues

Five mice were implanted with a 0.4-cm piece of nasal polyp tissue from a single patient. Twelve weeks after nasal polyp engraftment, the xenograft-bearing mice were painlessly sacrificed and their organs were examined for gross evidence of changes. A significant increase was observed in the size of the spleens of these mice (Fig 5A). To investigate the possible cause of the splenomegaly, we fixed and stained the spleens. The normal architecture of the spleens was completely effaced, with a diffuse accumulation of lymphocytes (Fig 5B,C). An immunohistochemical stain revealed that these cells were HLA-DR+ human lymphocytes (Fig 5D) and that the majority of the cells were CD3+ T cells (Fig 5E). Fewer CD20+ B cells were seen interspersed among the larger number of T cells (Fig 5F). We conclude that the pronounced splenomegaly was the result of the migration and expansion of human lymphocytes from the nasal polyp xenografts. HLA+, CD45+, CD3+ human T cells were also observed in the lung (Fig 5G,H), liver, and gut (not shown) of the polyp-bearing mice, but were far fewer in number than those observed in the spleen.

Human Nasal Polyp Xenograft–Associated T Cells Migrating to Spleen Have Activated or Memory Phenotype and Inverted CD4:CD8 Ratio

Single cell suspensions of the 5 enlarged spleens from nasal polyp-bearing mice sacrificed at 12 weeks were analyzed by flow cytometry. The CD3+ T cells were CD45RO+ and CD45RA– (Fig 6), so these cells were either effector or effector memory T cells, similar to the phenotype of the T cells that were stained in situ in the nasal polyp xenografts (Fig 4). The majority of the splenocytes (more than 68%) were human CD3+, CD8+ T cells, and fewer than 6% were human CD3+, CD4+ T cells. It is of interest to note that this atypical CD4:CD8 ratio of 1:10, and the effector memory phenotype, is similar to what has been observed previously in human nasal polyp tissues.¹² The expansion and migration of these

cells into the spleen provides a valuable source of a large number of cells for studying the specificity of nasal polyp-associated T cells and further exploring their potential to be activated via the T cell receptor, as well as their role in the histopathologic mechanism of nasal polyps.

Interferon- γ in Sera of Mice Bearing Nasal Polyp Xenografts

Having established the long-term presence of human T lymphocytes in the nasal polyp xenografts, we were interested in determining whether these cells retained their functional properties after engraftment into NOD-*scid* *IL2 γ ^{null}* mice. To address this issue, we obtained blood samples from 20 xenograft-bearing mice 8 weeks after the engraftment of nondisrupted pieces of 2 different nasal polyp specimens and assayed the sera for human IFN- γ . The average IFN- γ serum level was 750 ± 648 pg/mL. The serum levels of human IFN- γ were variable, but were well above those in normal non-xenograft-bearing NOD-*scid* *IL2 γ ^{null}* mice (less than 5 pg/mL). We conclude that the nasal polyp-associated T lymphocytes present within the xenografts remain viable for at least 8 weeks after the engraftment of nasal polyp tissue into NOD-*scid* *IL2 γ ^{null}* mice.

Human Serum Immunoglobulin Detected in Xenograft-Bearing Mice

Plasma cells were found in the nasal polyp xenografts, and up to 9% of the cells in the enlarged spleens of xenograft-bearing mice were CD138+ plasma cells (data not shown). Since NOD-*scid* *IL2 γ ^{null}* mice lack murine plasma cells, we assumed that the plasma cells we observed were of human origin. Consistent with this assumption, human immunoglobulin was detected in the sera of 9 nasal polyp-bearing mice at 3 to 5 weeks after engraftment. The average human immunoglobulin serum level was $1,311 \pm 152$ ng/mL. Thus, in addition to the T cells, nasal polyp-associated antibody producing plasma cells are retained in the xenografts, migrate to the spleen of recipient mice, and remain functional, as evidenced by their continued production and secretion of human immunoglobulin.

DISCUSSION

NOD-*scid* mice homozygous for a targeted mutation at the IL-2 receptor common gamma chain locus were shown here to greatly increase the long-term engraftment of human nasal polyp tissues in comparison to that of a previous study that used CB17-*scid* mice.¹⁰ Two of the most significant improvements of the new xenograft model presented here were the sustained presence and expansion of the nasal polyp-associated lymphocytes and the observed growth of the nasal polyp tissues in the NOD-*scid* *IL2 γ ^{null}* mice.

We have demonstrated that the human nasal polyp tissues engraft well in the NOD-*scid* *IL2 γ ^{null}* mice and that the microenvironment and histopathologic features characteristic of nasal polyps are maintained or expanded in the xenografts. Of particular interest is that the majority of the tissue in the xenografts was HLA-DR+ human tissue, and that the increase in size of the xenografts was largely a reflection of an increase in the nasal polyp tissue, with some contribution from enhanced mucus production and edema.

We have proposed and will test the possibility that the growth and expansion of the nasal polyp tissues in these mice may be dependent upon one or more of the lymphocyte populations (ie, T cell, B cell, plasma cell, and macrophage) and the cytokines and antibodies produced by these polyp-associated lymphocytes. A test of this hypothesis will be carried out by selectively immunodepleting lymphocytes and biologically active factors produced by the lymphocytes. Presently, the major factor related to the growth of the nasal polyp xenograft is the extraordinary increase in the size of the mucus-secreting submucosal glands.

The survival and expansion of effector or effector memory T cells and antibody-producing plasma cells provide a valuable resource with which to explore the specificity of these cells and their functional capacity to contribute to the pathology seen in nasal polyps. By determining the specificity of the T cells and the antibody produced by the polyp-associated plasma cells, one may be able to gain some insight with respect to the cause and pathogenesis of nasal polyps.

Current theories of the etiologic factors behind the development of nasal polyposis are varied. Allergies,¹³ bacterial infection,^{14,15} fungal infection,^{16,17} and viral infection^{18,19} have all been implicated, as well as more mechanical factors such as altered sodium absorption, alteration in aerodynamics of the nasal passages with trapping of pollutants, defects in the CFTR (cystic fibrosis transmembrane regulation) protein, and epithelial disruption.^{2,20}

Our xenograft model has the potential to provide valuable insights with respect to the cause of nasal polyps. Our initial work has shown that although nasal polyp tissue continues to expand after implantation into NOD-*scid* *IL2 γ* ^{null} mice, there is no evidence of bacterial or fungal growth in these severely immunodeficient mice after implantation. This finding suggests that although bacteria or fungi may contribute to the initiation of nasal polyps, it is less likely that they are responsible for the continuation and expansion of polyp tissue and pathology.

In addition, this model of nasal polyposis makes it possible to evaluate novel drug and immunotherapeutic strategies for their efficacy in reducing or even eradicating the histopathology that is characteristic of this chronic inflammatory disease.

The robust and reproducible engraftment of human nasal polyp tissues in the NOD-*scid* *IL2 γ* ^{null} mice occurs without host preconditioning, and although the success of this model represents a significant advancement over the previous *scid* xenograft model, at least a few limitations remain. Eosinophils, a characteristic inflammatory cell type present in approximately 80% of nasal polyps,²⁰ are conspicuously absent in the xenografts 3 weeks after engraftment. Therefore, it will not be possible with this model to assess the role of eosinophils or other short-lived inflammatory cells in sustaining and advancing the nasal polyp histopathology. Nor, in the model's present configuration, will it be possible to monitor and assess the contribution of chemokines in the recruitment of inflammatory cells to the nasal polyp microenvironment. These limitations may be resolved in future models by the adoptive transfer of eosinophils and other inflammatory cells to mice with existing nasal polyp xenografts or by co-engrafting mice with mobilized stem cells from the polyp donors.

We conclude that the engraftment of nondisrupted nasal polyp tissues into severely immunocompromised mice can serve as an important tool for better understanding the cellular and molecular events that are causally linked to maintaining nasal polyp pathology, and that it represents a viable model for evaluating single and multiple therapeutic methods.

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References

- Bernstein JM, Gorfien J, Noble B, Yankaskas JR. Nasal polyposis: immunohistochemistry and bioelectrical findings (a hypothesis for the development of nasal polyps). *J Allergy Clin Immunol* 1997;99:165–75. [PubMed: 9042040]
- Bernstein JM. The molecular biology of nasal polyposis. *Curr Allergy Asthma Rep* 2001;1:262–7. [PubMed: 11892044]
- Reddy S, Piccione D, Takita H, Bankert RB. Human lung tumor growth established in the lung and subcutaneous tissue of mice with severe combined immunodeficiency. *Cancer Res* 1987;47:2456–60. [PubMed: 3567931]
- Bankert RB, Egilmez NK, Hess SD. Human-SCID mouse chimeric models for the evaluation of anti-cancer therapies. *Trends Immunol* 2001;22:386–93. [PubMed: 11429323]
- Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol* 2007;7:118–30. [PubMed: 17259968]
- Ito M, Hiramatsu H, Kobayashi K, et al. NOD/SCID/ γ c^{null} mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 2002;100:3175–82. [PubMed: 12384415]
- Sugamura K, Asao H, Kondo M, et al. The interleukin-2 receptor γ -chain: its role in the multiple cytokine receptor complexes and T cell development in XSCID. *Annu Rev Immunol* 1996;14:179–205. [PubMed: 8717512]
- Shultz LD, Lyons BL, Burzenski LM, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid *IL2rg*^{null} mice engrafted with mobilized human hemopoietic stem cells. *J Immunol* 2005;174:6477–89. [PubMed: 15879151]
- Simpson-Abelson MR, Sonnenberg GF, Takita H, et al. Long-term engraftment and expansion of tumor-derived memory T cells following implantation of non-disrupted pieces of human lung tumor into NOD/scid *IL2R* γ ^{null} mice. *J Immunol* 2008;180:7009–18. [PubMed: 18453623]
- Bernstein JM, Broderick L, Parsons RR, Bankert RB. Human nasal polyp microenvironment maintained in viable and functional states as xenografts in SCID mice. *Ann Otol Rhinol Laryngol* 2006;115:65–73. [PubMed: 16466102]
- Williams SS, Umemoto T, Kida H, Rapsky EA, Bankert RB. Engraftment of human peripheral blood leukocytes into severe combined immunodeficient mice results in the long-term and dynamic production of human xenoreactive antibodies. *J Immunol* 1992;149:2830–6. [PubMed: 1401915]
- Bernstein JM, Ballow M, Rich G, Allen C, Swanson M, Dmochowski J. Lymphocyte subpopulations and cytokines in nasal polyps: is there a local immune system in the nasal polyp? *Otolaryngol Head Neck Surg* 2004;130:526–35. [PubMed: 15138416]
- Settipane GA, Chafee FH. Nasal polyps in asthma and rhinitis. A review of 6,037 patients. *J Allergy Clin Immunol* 1977;59:17–21. [PubMed: 833373]
- Gevaert P, Holtappels G, Johansson SG, Cuvelier C, Cauwenberge P, Bachert C. Organization of secondary lymphoid tissue and local IgE formation to *Staphylococcus aureus* enterotoxins in nasal polyp tissue. *Allergy* 2005;60:71–9. [PubMed: 15575934]
- Bernstein JM, Ballow M, Schlievert PM, Rich G, Allen C, Dryja D. A superantigen hypothesis for the pathogenesis of chronic hyperplastic sinusitis with massive nasal polyposis. *Am J Rhinol* 2003;17:321–6. [Erratum in *Am J Rhinol* 2004;18:62.]. [PubMed: 14750606]
- Ponikau JU, Sherris DA, Kern EB, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999;74:877–84. [PubMed: 10488788]
- Ponikau JU, Sherris DA, Kita H, Kern EB. Intranasal antifungal treatment in 51 patients with chronic rhinosinusitis. *J Allergy Clin Immunol* 2002;110:862–6. [PubMed: 12464951]
- Kozak FK, Mahony JB, Chernesky MA, et al. Nasal polyposis: in search of a viral etiology using DNA hybridization. *J Otolaryngol* 1991;20:404–7. [PubMed: 1774797]
- Hoffmann M, Kahn T, Goeroegh T, et al. Tracing human papillomavirus DNA in nasal polyps by polymerase chain reaction. *Acta Otolaryngol* 2000;120:872–5. [PubMed: 11132723]

20. Mygind N. Nasal polyposis. *J Allergy Clin Immunol* 1990;86:827–9. [PubMed: 2262640]

Fig 1.

Histology of nasal polyp xenograft tissue at 12 weeks after implantation. Expansion of polyp is observed in short time interval from **A)** implantation to **B)** 15 days after implantation into mice. Characteristic histologic features include preserved ciliated epithelial cells (white arrows), mucin-producing goblet cells (white arrowheads) and thickened basement membrane (black arrows). **C,D)** Before implantation. **C)** Original $\times 100$. **D)** Original $\times 400$. **E,F)** At 12 weeks after implantation. **E)** Original $\times 100$. **F)** Original $\times 400$.

Fig 2.

Change in xenograft volume 8 weeks after implantation in NOD-*scid* *IL2r^{null}* mice. Median postimplantation volume was found to be 124.3 mm³, with lower and upper quartiles being estimated at 63.2 and 152.3 mm³, respectively. Median change in polyp volume of 60.3 mm³ was statistically significant ($p = 0.0414$).

Fig 3.

Periodic acid-Schiff (PAS) staining of original nasal polyp tissue versus nasal polyp xenografts at 8 weeks after implantation. **A,B)** Original nasal polyp tissue shows areas of positive PAS staining in dilated submucosal glands and in goblet cells interspersed throughout epithelium (original $\times 50$). **C,D)** At 8 weeks after implantation in immunodeficient mice, xenograft tissue shows numerous PAS-staining goblet cells in epithelium (**C**; original $\times 200$) and pronounced dilatation of sub-mucosal glands with accumulation of PAS-staining mucus (**D**; original $\times 50$).

Fig 4.

Immunohistochemistry of nasal polyp xenografts in NOD-*scid* $IL2\gamma^{null}$ mice, 8 weeks after engraftment. Nasal polyp xenograft is human in origin, positive for human leukocyte antigen (HLA) (A; original $\times 100$), with significant population of human CD45-positive (huCD45+) leukocytes (B; original $\times 100$). C,D) Lymphocytes in xenograft are mostly CD3+ T cells. C) Original $\times 100$. D) Original $\times 400$. E,F) Infiltrating lymphocytes are CD45RO+ (E; original $\times 100$) and CD45RA- (F; original $\times 100$) activated phenotype consistent with lymphocytes from original nasal polyp tissue. G) There is also population of CD44+ lymphocytes (original $\times 100$), suggesting effector memory cell phenotype. H) huCD68+ macrophages are also found in xenograft (original $\times 400$).

Fig 5.

Enlarged spleens from nasal polyp–engrafted mice show infiltration with human lymphocytes. **A)** Infiltration of human lymphocytes into spleen results in splenomegaly. Top — xenograft spleen; bottom — control spleen. **B,C)** Spleens from 12-week xenografts are populated with human lymphocytes. **B)** Original $\times 100$. **C)** Original $\times 400$. **D)** These cells are human in origin, positive for HLA staining by immunohistochemistry (original $\times 100$). **E,F)** Immunohistochemistry of spleen demonstrates CD3+ T cells (**E**; original $\times 400$) and CD20+ B cells (**F**; original $\times 100$). **G,H)** HLA+ human lymphocytes are also present in other xenograft tissues, including mouse lung. **G)** Original $\times 100$. **H)** Original $\times 400$.

Fig 6.

Spleens from polyp-engrafted mice are infiltrated with T lymphocytes with effector memory phenotype. Flow cytometry of enlarged spleens from 8-week xenografts shows small population of CD4⁺ cells in CD3⁺ lymphocyte gate (less than 6%). Majority of lymphocytes were CD3⁺, CD8⁺ (more than 68%). Ninety-eight percent of CD3⁺ lymphocytes were positive for human-specific activation marker CD45RO.