Immune-mediated signaling in intestinal goblet cells via PI3-kinase- and AKT-dependent pathways

Mei-Lun Wang,1 Sue A. Keilbaugh,1 Tanesha Cash-Mason,2 Xi C. He,3 Linheng Li,3 and Gary D. Wu1

1Division of Gastroenterology, University of Pennsylvania School of Medicine and 2Division of Gastroenterology, Hepatology, and Nutrition, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania; and 3Stowers Institute for Medical Research, Kansas City, Missouri

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Wang ML, Keilbaugh SA, Cash-Mason T, He XC, Li L, Wu GD. Immune-mediated signaling in intestinal goblet cells via PI3-kinase- and AKT-dependent pathways. Am J Physiol Gastrointest Liver Physiol 295: G1122–G1130, 2008. First published October 2, 2008; doi:10.1152/ajpgi.90430.2008.—In the intestinal epithelium, activation of phosphatidylinositol 3-kinase (PI3-kinase)/AKT pathways, via growth factor-mediated signaling, has been shown to regulate cell proliferation and inhibit apoptosis. An immune-activated receptor critical for Th2 immune responses, IL-4Rα, can also activate PI3-kinase via insulin receptor substrate (IRS)-dependent signaling. Here, using the intestinal goblet cell-specific gene RELMα, we investigated the effect of PI3-kinase activation via Th2 immune responses on the goblet cell phenotype. IL-13 stimulation activated PI3-kinase and AKT signal transduction in LS174T cells. Not only did pharmacological inhibition of PI3-kinase and AKT1/2 inhibit RELMα induction by IL-13, but AKT inhibition also significantly reduced constitutive basal expression of RELMα, a response reproduced by the simultaneous pharmacological inhibition of both epithelial growth factor receptor and IGF-1 receptor signaling. In vivo, the disruption of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), an inhibitor of PI3-kinase activation, led to the activation of RELMα expression in the small intestine. Furthermore, induction of an intestinal Th2 immune response by infection with a small intestinal nematode parasite, Heligmosomoides polygyrus, led to enhanced epithelial cell proliferation, activation of AKT as demonstrated by the loss of Foxo1 nuclear localization, and robust induction of RELMα expression in wild-type, but not IL-4Rα knockout, mice. These results demonstrate that Th2 immune responses can regulate goblet cell responses by activation of PI3-kinase and AKT pathways via IL-4Rα.

phosphatidylinositol 3-kinase; epithelium; intestine

PHOSPHATIDYLINOSITOL 3-KINASE (PI3-kinase) and AKT signaling pathways are regulators of diverse and fundamental cellular processes such as metabolism, proliferation, growth, differentiation, and cell survival. Ubiquitous in eukaryotic cells, PI3-kinase/AKT pathways have been extensively studied in their roles downstream of insulin and insulin-like growth factor (IGF)-1 signaling (45). The intestinal epithelium, through its continuous cycles of renewal, cellular proliferation, differentiation, and senescence, represents an ideal model system to study PI3-kinase/AKT signaling pathways. The small intestinal epithelium is divided into two distinct compartments. Proliferative multipotent stem cells reside within the crypt epithelium, giving rise to daughter cells that differentiate into either absorptive enterocytes or secretory cells including goblet cells, Paneth cells, and enteroendocrine cells. Once differentiated, cells proliferate and migrate toward the villus compartment or, in the case of Paneth cells, to the base of the crypt. The precise mechanisms by which intestinal stem cells are maintained and differentiate into specific cell lineages are complex and involve the interplay of multiple developmental pathways including Notch, Wnt/β-catenin, bone morphogenetic protein (BMP), and PI3-kinase/AKT signaling (37).

In vitro, while some investigators have reported that pharmacological inhibition of PI3-kinase enhances sodium butyrate-induced proliferation of HT-29 and Caco-2 cells (44), others have shown that activation of PI3-kinase signaling is critical for the cell-cell contact-induced enterocyte differentiation of the Caco-2 cell line (26, 27). Furthermore, microarray analysis of Caco-2 cells has also suggested that PI3-kinase pathway-related genes are generally upregulated during progressive stages of differentiation (10). More recent evidence demonstrates that Akt may coordinate with Wnt signals, leading to the enhancement of nuclear β-catenin activity, a process that may be involved in the development of intestinal polyposis (14, 15).

During inflammation, intestinal homeostasis is further challenged by growth factor stimulation, cytokines, and oxidative stress (9, 22, 23, 33, 40). The activation of PI3-kinase/AKT pathways in the setting of intestinal inflammation has been shown to play a critical role in host-protective responses in the intestinal epithelium in vitro. For example, in a cell culture model of Salmonella enteritis, small intestinal epithelial cells are protected from apoptosis through activation of Akt (24). In addition, the morphogenetic protein epi morphin protects intestinal epithelial cells from oxidative stress through phosphorylation of the epidermal growth factor receptor (EGFR) and downstream activation of PI3-kinase/AKT (19).

Of particular interest in the area of intestinal epithelial cell differentiation, growth, and survival is the observation that polarized Th2-type inflammation in the gut, characterized by the production of interleukin (IL)-4 and IL-13, leads to an increase in crypt cell proliferation and goblet cell size and number. The actions of IL-4 and IL-13 are mediated by binding to the common receptor subunit, IL-4Rα, leading to the activation of two independent signal transduction pathways, STAT6 and PI3-kinase/AKT, via insulin receptor substrate (IRS) phosphorylation (32). Although the role of PI3-kinase activation in the biology of the intestinal epithelium has been explored within the context of growth factor receptor-dependen-
dent activation, we hypothesized that immune-mediated stimulation of the intestinal epithelium, via the IL-4Rx receptor, may also regulate intestinal gene expression through this same pathway. Here, using a goblet cell-specific gene known as RELMβ, whose expression is activated by IL-4 and IL-13 in vitro and Th2 immune responses induced by parasitic nematode infection in vivo, we provide evidence for immune-activated gene expression in intestinal goblet cells via PI3-kinase and AKT signaling.

MATERIALS AND METHODS

Cell culture and cytokines. LS174T cells (American Type Culture Collection, Rockville, MD) were maintained in minimum essential medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum and penicillin-streptomycin. Recombinant human (rh)IL-13 was obtained from R&D Systems (Minneapolis, MN).

Animals and pathogens. Mice were maintained under specific pathogen-free (SPF) conditions at the University of Pennsylvania School of Medicine. H. polygyrus was propagated in C57BL/10 mice (Taconic Laboratories), and infective L3 larvae were isolated from stool pellets with previously described methods (3). Primary infection of naive female Balb/c and IL-4Rx−/− mice (Jackson Laboratory, Bar Harbor, ME) was established by oral inoculation of H. polygyrus larvae (300 L3 larvae per mouse) with a ball-tipped oral gavage needle. All experiments were performed under protocols reviewed and approved by the University of Pennsylvania Animal Care and Use Committee.

RNA isolation and quantitative reverse transcription-polymerase chain reaction. RNA was isolated with TRIzol (Invitrogen) per the manufacturer’s instructions. Reverse transcription utilizing the SuperScript II First Strand Synthesis Kit (Invitrogen) was followed by SYBR Green quantitative reverse transcription-polymerase chain reaction (qRT-PCR) performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Primers were designed with Primer Express software (Applied Biosystems). Primer sequences used in this study included hRELM forward: 5′-CACCCAGGAAGCTGATCTAA-3′, hRELM reverse: 5′-ACGGCCCCACCTGTCACA-3′; mouse (m)RELM forward: 5′-ATGGGTGTCACTGGATGTGCTT-3′, mRELM reverse: 5′-AGCAGCTGCCAGTGGCAAGTA-3′; and mRELMα reverse: 5′-TGCTTTTGTGGTGTGAGTTT-3′, mRELMα reverse: 5′-GCAGTGGTCTAGCAGTGAAGT-3′.

5-Bromo-2-deoxyuridine labeling and immunohistochemistry. Mice were injected with 1 ml/100 g body wt 5-bromo-2-deoxyuridine (BrdU) (Sigma, St. Louis, MO) 90 min before euthanasia. Swiss-rolled duodenum sections were fixed in 10% neutral buffered formalin overnight at 4°C. Immunohistochemical analysis for BrdU was performed with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions. Immunohistochemistry for RELMβ with formalin-fixed and paraffin-embedded tissue sections was performed as previously described (13). Hematoxylin was used as the counterstain after development with 3',3'-diaminobenzidine. Foxo1 staining was performed in a similar fashion with an affinity-purified polyclonal antibody (Cell Signaling, Danvers, MA) (12). No counterstain was applied after 3',3'-diaminobenzidine development. For Alcan blue staining, 3% aqueous acetic acid was applied to deparaffinized slides before the application of 1% Alcan blue in 3% acetic acid at pH 2.5. Sections were washed and counterstained with nuclear fast red 0.1%, dehydrated, and mounted.

Cell transfection. LS174T cells were seeded in 12-well plates. The following day, cells were cotransfected with cytomegalovirus (CMV)-β-galactosidase and either the promoterless pGL2 Basic reporter plasmid (Promega, Madison, WI) or the −706 human RELMβ promoter construct (−706 hRELM) (43) and Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Twenty-four hours after transfection, cells were treated with either vehicle or 20 µM Akt1/2i (no. 124017, InSolution Akt Inhibitor VIII, EMD Chemicals, San Diego, CA). Cell lysates were collected 24 h after treatment, and luciferase activity was quantified with the Luciferase Assay System (Promega) according to manufacturer’s recommendations. Transfection efficiency was determined by measurement of β-galactosidase activity with an ortho-nitrophenyl-β-galactoside substrate and a colorimetric assay.

Immunoprecipitation. LS174T cells were seeded in six-well plates and treated with or without rhIL-13 (10 ng/ml, R&D Systems) for the indicated time points. Cells were lysed with lysis buffer (in mM: 50 Tris pH 7.5, 150 NaCl, 1 EDTA, 4 sodium orthovanadate, and 200 µM sodium fluoride, with 1% Triton X-100, 10% glycerol, and Complete protease inhibitor (Roche, Indianapolis, IN)). Protein concentrations of cell lysates were determined by bichinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). Protein lysates (200 µg) were incubated with anti-IRS-2 antibody (2 µg) (Upstate). Cell lysates were collected 24 h after treatment, and luciferase activity was quantified with the Luciferase Assay System (Promega) according to manufacturer’s recommendations. Transfection efficiency was determined by measurement of β-galactosidase activity with an ortho-nitrophenyl-β-galactoside substrate and a colorimetric assay.

Immunoblotting. LS174T cells were treated with or without rhIL-13 (10 ng/ml, R&D Systems) for the indicated time points. Total protein lysate (5 µg per sample) was loaded onto a NuPAGE 4–12% Bis-Tris gel run with MOPS buffer (Invitrogen). The gel was run at 200 V for 1 h and then transferred to PVDF membrane and probed with an anti-phospho-Akt (Ser473) antibody (Cell Signaling Technology, Danvers, MA) at 1:1,000. The blot was then exposed to donkey anti-rabbit conjugated to horseradish peroxidase (HRP) secondary antibody (1:10,000 GE Healthcare). The blot was stripped, reblocked, and exposed to secondary antibody without prior exposure to another primary antibody and then developed to ensure that the original signal had been stripped from the blot. The blot was then rinsed, blocked, and exposed to total Akt antibody (Cell Signaling Technology) 1:1,000 in 1 × Tris-buffered saline –0.1% Tween containing 5% nonfat dry milk overnight at 4°C with shaking. The blot was then exposed to donkey anti-rabbit secondary antibody conjugated to HRP and developed by ECL Plus.

Inhibitor studies. LS174T cells were seeded in six-well plates and treated with rhIL-13 (10 ng/ml, R&D Systems) in the presence of 0, 2, 5, or 10 µM LY-294002 (Sigma). Akti studies were performed with 10 ng/ml rhIL-13 for 72 h with or without concurrent treatment with 20 µM Akt1/2i (InSolution Akt Inhibitor VIII, EMD Chemicals). For tyrosine kinase inhibitor studies, rhIL-13-treated cells were pretreated for 30 min with 10 µM tyrphostin AG-1024 (Sigma) and/or 100 nM tyrphostin AG-1478 (Sigma). After 48 h, RNA was isolated with the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA), including on-column DNase treatment with RNase-free DNase (Qiagen). Statistical analysis. A Student’s t-test, performed with GraphPad Prism software (San Diego, CA), was used to determine statistical significance between two variables. Throughout the analyses P < 0.05 was used as the level of significance, and all data are presented as means ± SE.

RESULTS

Stimulation with Th2-type cytokine IL-13 leads to phosphorylation of IRS-2 and AKT. The colon cancer cell line LS174T is a well-established model used to study goblet cell-specific gene expression (36, 41). We showed previously (2) that the goblet
cell-like intestinal cell line LS174T exhibits constitutive expression of RELMβ that can be enhanced by stimulation with either IL-13 or IL-4. Since these cytokines exert their effects via a common receptor, IL-4R, which is known to activate both STAT6 and PI3-kinase pathways, we performed immunoblots to examine the signal transduction of this receptor specifically in LS174T cells. Immunoblots revealed that IL-13 led to phosphorylation of both IRS-2 and AKT in a time-dependent fashion (Fig. 1).

Activation of RELMβ mRNA expression by IL-13 is prevented by pharmacological inhibition of PI3-kinase and AKT signaling. To determine whether IL-13 activates RELMβ expression via PI3-kinase- and AKT-dependent pathways, LS174T cells were pretreated with pharmacological inhibitors before stimulation with IL-13. The PI3-kinase inhibitor LY-294002 and the AKT1 and -2 inhibitor Akt1/2i reduced IL-13-dependent activation of RELMβ mRNA expression in a dose-dependent fashion (Fig. 2).

Pharmacological inhibition of AKT and growth factor signaling represses constitutive expression of RELMβ. LS174T cells constitutively express a basal level of RELMβ mRNA expression (13) consistent with the goblet cell-like phenotype of this cell line (41) and the observation that RELMβ is constitutively expressed by colonic goblet cells in vivo (13). To determine the mechanism responsible for the constitutive expression of this gene in vitro, we examined the role of AKT in the basal expression of RELMβ in LS174T cells. Pharmacological inhibition of both AKT1 and -2 reduced constitutive expression of RELMβ by ~60% (Fig. 3A). Reporter gene analysis of the RELMβ promoter revealed that the reduction in mRNA induced by AKT1/2i is, at least in part, due to a reduction in transcriptional activation (Fig. 3B).

Since most studies examining the effect of AKT activation on the intestinal epithelium have focused on the role of growth factors such as IGF and EGF that are present in the fetal bovine serum used to supplement cell culture medium, we examined the constitutive expression of RELMβ in LS174T cells on pharmacological inhibition of these two receptors (Fig. 3C). This notion is supported by the recently reported observation that fetal calf serum induces the expression of RELMβ in vitro (25). Although inhibition of the IGF-I receptor (IGFR) significantly reduced RELMβ expression, a slightly more significant reduction was observed when both EGFR and IGFR were

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**Fig. 1.** Activation of phosphatidylinositol 3-kinase (PI3-kinase) and AKT signal transduction pathways in LS174T cells by stimulation with interleukin (IL)-13. Total protein was isolated from LS174T cells stimulated with IL-13 for the times indicated. A: phosphotyrosine immunoprecipitation followed by an immunoblot for insulin receptor substrate (IRS)-2 (top) and an immunoblot of total IRS-2 (bottom). B: immunoblot of AKT phosphorylated at Ser473 (p-AKT; top) and total AKT (bottom). C: densitometric quantification of the immunoblots shown in B.

**Fig. 2.** Pharmacological inhibition of PI3-kinase and AKT inhibits the induction of RELMβ expression by stimulation of LS174T cells with IL-13. A: mRNA expression of RELMβ in LS174T cells stimulated with IL-13 in the presence or absence of the PI3-kinase inhibitor LY-294002 for 48 h. B: mRNA expression of RELMβ in LS174T cells stimulated with IL-13 in the presence or absence of an inhibitor of both AKT1 and AKT2, Akt1/2i, for 72 h. *P ≤ 0.001, **P ≤ 0.03.

**Pharmacological inhibition of AKT and growth factor signaling represses constitutive expression of RELMβ.** LS174T cells constitutively express a basal level of RELMβ mRNA expression (13) consistent with the goblet cell-like phenotype of this cell line (41) and the observation that RELMβ is constitutively expressed by colonic goblet cells in vivo (13). To determine the mechanism responsible for the constitutive expression of this gene in vitro, we examined the role of AKT in the basal expression of RELMβ in LS174T cells. Pharmacological inhibition of both AKT1 and -2 reduced constitutive expression of RELMβ by ~60% (Fig. 3A). Reporter gene analysis of the RELMβ promoter revealed that the reduction in mRNA induced by AKT1/2i is, at least in part, due to a reduction in transcriptional activation (Fig. 3B).

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Constitutive activation of PI3-kinase leads to goblet cell hyperplasia and ectopic small bowel expression of RELMβ.

Inactivation of PTEN leads to constitutive activation of AKT signaling and is associated with the development of Cowden disease and the predisposition to certain neoplastic processes (28, 29). Recently, the conditional inactivation of PTEN in intestinal epithelial stem cells has been shown to alter epithelial proliferation rates, resulting in the recapitulation of an intestinal polyposis phenotype (14).

Quantification of gene expression in the small intestinal polyps of these mice revealed a strong induction of RELMβ, but not RELMα, mRNA (Fig. 4A) expression. Western blot analysis revealed a significant increase in RELMβ protein expression in the PTEN knockout (KO) small intestine (Fig. 4B), with equal protein loading confirmed by silver stain (data not shown). Furthermore, immunohistochemical staining of this tissue for RELMβ confirms the strong induction of RELMβ expression specifically in intestinal goblet cells (Fig. 4, C and D). In total, these results provide in vivo confirmation of our studies in LS174T cells (Figs. 1–3) showing that inhibition of both EGFR and IGFR signaling (Fig. 3C). Thus, although the activation of AKT via tyrosine kinase receptors such as IGFR regulates RELMβ expression, activation of AKT via IL-13 stimulation occurs through the activation of IL-4Rα, a process that is independent of growth factor receptor-mediated activation.

Small intestinal nematode infection with Heligmosomoides polygyrus leads to activation of PI3-kinase/AKT pathways and induction of RELMβ expression in intestinal epithelium of mice via IL-4Rα. Having confirmed the role of PI3-kinase/AKT signaling in the regulation of RELMβ expression in vivo, we then sought to examine the role of immune-mediated expression of this gene in vivo. Intestinal parasitic nematode infections lead to Th2-mediated immune responses resulting in prototypical alterations in the phenotype of the intestinal epithelium, including hyperproliferation of the cells in the crypt compartment and goblet cell hyperplasia (6), changes similar to those observed in the small intestinal polyps of PTEN KO mice (Fig. 4). Infection of wild-type mice with H. polygyrus for 21 days led to the activation of robust RELMβ mRNA expression in the small intestine (Fig. 5A). By contrast, the expression of RELMβ remained undetectable in the small intestine of IL-4Rα KO mice infected with H. polygyrus for the same period of time. Alcian blue staining revealed that H. polygyrus induced goblet cell hyperplasia in the small intestine (Fig. 5B).

Immunohistochemical localization of RELMβ expression in the small intestine of H. polygyrus-infected wild-type mice revealed that this protein was induced in goblet cells located throughout both the crypt and villus (Fig. 5, C and D). To evaluate AKT activation in vivo, tissue sections were stained for the Foxo1 transcription factor. Foxo1 plays a critical role in the regulation of gluconeogenesis when it is localized to the nucleus (1). However, on phosphorylation by AKT, Foxo1 is transported out of the nucleus (4). The cellular localization of

inhibited simultaneously. These results demonstrate that growth factor-induced activation of AKT is largely responsible for the constitutive expression of RELMβ in vitro. Importantly, activation of PI3-kinase and AKT signaling via stimulation by IL-13 (Figs. 1 and 2) still resulted in significant induction of RELMβ mRNA expression even during the simultaneous in-
Fig. 4. Conditional deletion of PTEN induces RELMβ expression in the small intestine. A: mRNA expression of RELMα and RELMβ in the small intestine of wild-type (WT) and PTEN conditional knockout (KO) mice. B: immunoblot of RELMβ using total protein isolated from the small intestine of WT or PTEN conditional KO mice. C and D: immunohistochemistry for RELMβ in the small intestine of WT (C) and PTEN conditional KO (D) mice. Magnification ×200.

Foxo1 can therefore be used as a surrogate indicator of AKT activation in the intestinal epithelium (12). In the small intestine of naive, wild-type mice, Foxo1 was located in the nucleus throughout villus epithelial cells but was absent in BrdU-positive proliferating crypt epithelial cells (Fig. 5E, Fig. 6, A and C). By contrast, Foxo1 was largely excluded from the nucleus throughout both the villus and crypt intestinal epithelium in H. polygyrus-infected mice (Fig. 5F, Fig. 6, B and D), indicating that AKT is activated in the villus epithelium on infection. The relationship between the induction of RELMβ expression and the loss of nuclear staining for Foxo1 in the villus epithelium (Fig. 5D, Fig. 6, B and D) provides in vivo support for our findings in LS174T cells that IL-13 activates RELMβ expression via a PI3-kinase/AKT-dependent pathway. In naive mice, the absence of constitutive RELMβ expression in the crypt epithelium of the small intestine, where Foxo1 is excluded from the nucleus (Fig. 6A), suggests that the activation of AKT is necessary but not sufficient for the induction of RELMβ expression by Th2-mediated immune activation. Indeed, this is consistent with our observation that LS174T cells engineered to express myristolated AKT do not exhibit an increase in RELMβ expression (data not shown).

DISCUSSION

The Th2-type immune response is characterized by an increased production of cytokines IL-4 and IL-13, which exert diverse effects on the intestinal epithelium, including alterations in cell growth, gut barrier function, ion secretion, and goblet cell hypertrophy. Central to IL-4 and IL-13 signaling pathways is the IL-4Rα chain. IL-4Rα, paired with the IL-13Rα chain, binds to both IL-4 and IL-13 (17, 18). Expressed in the intestinal epithelium (35) as well as hematopoietic, endothelial, and muscle cells, the activation of IL-4Rα leads to the activation and recruitment of mediators of gene activation, cell growth, differentiation, and resistance to apoptosis. Two distinct signaling pathways are responsible for the diverse effects of IL-4Rα activation. Well-characterized in immune-regulatory cells (14), IL-4Rα activation of Stat6 signaling pathways leads to the differentiation of naive CD4+ T cells into Th2-type immune cells (31). Alternatively, IL-4/IL-13 signaling through IL-4Rα, via phosphorylation of IRS-1 and IRS-2, leads to activation of downstream PI3-kinase pathways (39, 42, 45).

The importance of immune-mediated PI3-kinase signaling in intestinal epithelial barrier function has been described previously in cell culture model systems. It has been established in several intestinal cell lines that treatment with IL-13 results in an increase in paracellular permeability (34), decreased transepithelial resistance, a decrease in epithelial restitution velocity (16), and inhibition of cytokine-induced apoptosis (46). In these models, PI3-kinase signaling has been implicated as the primary signaling pathway by which IL-13 leads to alterations in barrier function, suggesting that while both Stat6 and PI3-kinase pathways may be simultaneously activated by Th2 immune stimulation, PI3-kinase may play the more prominent role in specific intestinal epithelial responses. Consistent with this notion, the induction of claudin-2 expression in T84 cells by IL-13 is dependent on PI3-kinase/AKT signaling (34).

Although these studies provide support for the relative importance of immune-mediated PI3-kinase signaling pathways in intestinal epithelial cell homeostasis with cell culture models, evidence that Th2 immune responses can alter intestinal goblet cell phenotype through this pathway in vivo is currently lacking. Thus, in the present study, we used an intestinal goblet cell-specific gene as a model to show that PI3-kinase/AKT signaling cascades are activated by Th2-type immune stimulation in an intestinal epithelial cell line in vitro.
Intestinal goblet cells occupy a unique niche in the setting of Th2-type epithelial immune responses, prototypically undergoing both hyperplasia and hypertrophy in response to intestinal nematode infection. Hence, our investigation into the regulation of RELMβ, an intestine-specific goblet cell gene, is an attractive model in which to study Th2-type immune-mediated signaling pathways within the intestinal epithelium. We have shown previously (2) that RELMβ is highly induced in vivo in the setting of Th2-type cytokine stimulation by intestinal nematode infection in mice and that RELMβ is induced by cytokines IL-4 and IL-13 in the goblet cell-like LS174T colon cancer cell line in vitro. The robust induction of RELMβ contrasts with only modest changes in other goblet cell-specific genes such as TFF3 and Muc2 on Th2 immune stimulation (2).

With respect to these latter two gene products, the signaling pathways that regulate their expression remain controversial. Although some have reported that both transcription and translation of the goblet cell-specific gene TFF3 may be dependent on Stat6 signaling in vitro (5), others have demonstrated that PI3-kinase pathways, but not Stat6, play a critical role in the expression of goblet cell proteins TFF3 and Muc2 during goblet cell differentiation in HT29 cell culture subpopulations (8). In addition, it has also been suggested that MAPK pathways, important in epithelial proliferation, are critical for the induction in Muc2 mRNA expression by IL-13 in cell culture models (21, 41). Although we did not interrogate MAPK signaling pathways in our study, the possibility that MAPK may also be involved in the Th2 immune-mediated induction of RELMβ cannot be excluded, and may require future investigation.

In the present study, we show that activation of the PI3-kinase/AKT pathway regulates goblet cell gene expression...
both in vitro and in vivo. Our findings are in keeping with the results described by Duan et al. (7), who observed that intra-tracheal administration of the PI3-kinase inhibitor LY-294002 in mice not only inhibited an ovalbumin (OVA)-induced increase in airway mucus production and goblet cell hyperplasia but also decreased OVA-induced phosphorylation of Akt in whole lung lysates. However, our results contrast with a recent study with a mouse model of allergic asthma reporting that RELMα could be induced in goblet cells in the airway epithelium of mice by the intratracheal administration of IL-4, IL-13, or OVA in a Stat6-dependent fashion (30). It is possible that there are tissue-specific mechanisms by which RELMα expression is regulated. Indeed, in contrast to the intestinal tract, where RELMα is constitutively expressed at high levels in the colon and can be highly induced in the small intestine on nematode infections (2, 13), the induced expression in the lung is very modest (unpublished observations), with absolute levels of RELMα being >1,000-fold lower than those of RELMβ in allergen-challenged mice (38). In part, this may be due to the important role that the intestine-specific transcriptional activator Cdx2 plays in the regulation of this gene (43). Alternatively, these differences might also be explained by other signaling pathways, including the IL-13/Stat6-dependent phosphorylation of p38 MAPK, which has been described in airway epithelial cells (11).

In the absence of immune stimulation, we show in Fig. 4 that constitutive activation of PI3-kinase/AKT signaling in the intestinal epithelium of PTEN KO mice is sufficient for high-level expression of RELMβ in the small intestine. Since the Mx1-Cre transgene, used to delete PTEN in the intestinal epithelium, also results in targeted deletion in hemopoietic progenitors (14), we cannot exclude the possibility that alterations in the immune response may have also contributed to the induction of RELMβ expression in the KO mice. However, since the mRNA expression of either IL-4 or IL-13 was not induced in the intestine of these mice (data not shown), this possibility seems unlikely. Rather, this finding is consistent with the dependence of constitutive RELMβ expression on EGFR and IGFR signaling in LS174T cells via PI3-kinase/AKT activation (Figs. 2 and 3). In part, the activation of RELMβ expression in this manner is regulated at the level of gene transcription. These findings may be particularly relevant to our previous observations (13) that, in the absence of Th2-mediated immune stimulation, bacterial colonization of the gut leads to constitutive expression of RELMβ in colonic goblet cells. Indeed, Yan et al. (47) recently showed that the Lactobacillus rhamnosus GG strain of probiotic bacteria expresses two proteins that activate AKT and inhibit cytokine-induced epithelial apoptosis in vitro. Since it has been reported that goblet cell hyperplasia as well as activation of RELMβ expression occurs with both bacterial colonization of the gut (13, 20) and Th2-mediated immune responses in vivo, our observations suggest that PI3-kinase/AKT signaling may be a common pathway through which both innate and adaptive immune responses are coordinated in intestinal goblet cells.

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Fig. 6. Infection with H. polygyrus prevents nuclear localization of Foxo1 in the small intestinal epithelium. Foxo1 staining in the small intestine of naive (A) and H. polygyrus-infected (B) mice. C and D: expanded images of Foxo1-stained small intestine obtained from naive (C) and H. polygyrus-infected (D) mice with labeling identifying the location of the epithelial nuclei.
IMMUNE-MEDIATED SIGNALING IN GOBLET CELLS VIA AKT

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REFERENCES


