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Functional Importance of Regional Differences in Localized Gene Expression of Receptors for IL-13 in Murine Gut1

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Abstract

IL-13 induces a STAT6-dependent hypercontractility of intestinal smooth muscle that is mediated by binding to the IL-13Rα1 component of the type 2 IL-4R that is linked to STAT6. IL-13 also binds to the IL-13Rα2 that is not linked to STAT6 and functions to limit the effects of IL-13 in vivo. In this study we assessed the contributions of regional and cellular differences in the distribution of the IL-13R components to the physiological regulation of smooth muscle function in wild-type mice and mice deficient in STAT6 or IL-13Rα2. The expression of IL-13 and IL-13Rα2 was higher in colon than in small intestine. Laser capture microdissection of specific cell types revealed that the expression of IL-13Rα2 was higher in the smooth muscle layer compared with levels in the epithelial cells of the mucosa. In contrast, there was a uniform distribution of IL-13α1 in smooth muscle, epithelia, and myenteric neurons. The significant

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Disclosures

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hypercontractility of smooth muscle in mice deficient in IL-13Rα2, but not in STAT6, shows the physiological importance of IL-13 binding to IL-13Rα2. The pronounced differences in the expression of IL-13Rα2 suggest that the gut has developed sophisticated mechanisms for controlling the physiological and pathophysiological activities of IL-13.

Much of our knowledge of the Th2 cytokines, IL-4 and IL-13, is derived from studies of enteric nematode infection. These cytokines have a number of overlapping effects that contribute to worm expulsion, including stimulation of IgE, mast cells, eosinophils, and mucus production, as well as induction of specific changes in epithelial cell and smooth muscle function. An emergent therapeutic potential of Th2 cytokines is their ability to protect against Th1-driven pathologies, such as inflammatory bowel disease (1–4). Because of their critical role in host resistance against parasite infection and their ability to modulate Th1 cytokines, there is considerable interest in the contributions of Th2 cytokines and their individual receptors to both Th1- and Th2-predominant pathologies.

IL-4 and IL-13 share immunologic and biologic functions that can be explained in part by their sharing a receptor component. It is reported that there are at least three different receptors that bind IL-4 and/or IL-13. The type 1 receptor is composed of the common IL-2R γ-chain and the IL-4R α1-chain, whereas the type 2 receptor is composed of the IL-4R α1- and IL-13R α1-chains. Binding of IL-4 to the type 1 or type 2 receptor or of IL-13 to the type 2 receptor leads to phosphorylation of the IL-4R α-chain and activation of the transcription factor STAT6. Th2 cytokines must bind to their receptors to induce biological responses; therefore, the location and level of expression of these receptors are of interest.

A second IL-13R chain, IL-13Rα2, reportedly binds IL-13 with high affinity, but is not linked with STAT6 signaling (5). The function of IL-13Rα2 remains unclear, but previous studies demonstrated that soluble IL-13Rα2 in serum acts as a decoy receptor to limit the actions of IL-13 in vivo (6, 7). Despite the number of studies demonstrating the potent effects of Th2 cytokines on the gastrointestinal tract, there is little information on the locations of these receptors, particularly on nonhemopoietic effector cells, such as epithelial cells, smooth muscle cells, and enteric neurons. The aim of the present study was to determine in the intestine and colon 1) the regional differences in the constitutive expression of the cytokine receptors for IL-4 and IL-13, 2) the expression of cytokine receptors in specific cell types harvested by laser capture microdissection, and 3) the functional importance of IL-13Rα2. The results of the present study are the first to demonstrate regional as well as cell-specific differences in cytokine receptor distribution that potentially impact Th2 effects on gut function. In addition, they support a phenotype for IL-13Rα2 in the physiological regulation of endogenous IL-13 on gut motility.

Materials and Methods

Animals

Tissues were taken from BALB/c mice, 8–12 wk old (National Cancer Institute), mice deficient in the expression of STAT6 (STAT6$^{-/-}$)), IL-4 (IL-4$^{-/-}$; both from F. Finkelman),

IL-13Rα2 (IL-13Rα2$^{-/-}$ from T. Wynn, or IL-13$^{-/-}$ from W. Paul), to assess the contribution of IL-13 acting though STAT6-dependent and -independent receptors. Serum levels of IL-13Rα2 were determined as described previously (8).

Preparation of tissue, frozen blocks, and sectioning

Segments of tissue were taken from the mid small intestine (jejunum) and colon, and one piece was homogenized in TRIzol (Invitrogen Life Technologies) and stored at −80°C until needed. A second section was slit longitudinally, laid flat with the mucosal surface up, rolled around a wood stick (Swiss roll), and embedded in Tissue-Tek OCT compound (Sakura Finetek) in cryomold. The tissues were frozen on dry ice-acetone, removed from the cryomold while still frozen, and placed at −80°C in an airtight container until sectioned (HM505E cryostat; Richard-Allan Scientific). For laser capture microdissection (LCM[4], 4-μm tissue sections were made from frozen blocks and placed immediately on plain uncoated slides on dry ice, then stored at −80°C until needed. Sections of frozen tissue were stained with H&E to assess changes in tissue morphology (ECLIPSE TE2000E; Nikon Instruments). For each mouse, the thickness of the muscle layer was assessed in four different architecturally normal sites in each region and then averaged to obtain one value (micrometers) per region.

Laser capture microdissection

LCM was performed as described previously (9, 10). Briefly, cryosectioned tissue was stained with H&E and dehydrated. LCM was performed on a PicCell II (Arcturus Engineering), and target cells were transferred to Cap-Sure LCM Caps (Arcturus Engineering). Cells were captured from the region of the epithelium, lamina propria, muscles, and myenteric plexus in small intestine and colon.

Gene expression analysis (RNA extraction, RT, and real-time PCR)

Total RNA extraction from whole tissue was performed according to the manufacturer's instructions. RNA was extracted from LCM samples, and RT was performed as described previously (9, 10). Primers and probes for IL-13, IL-4, STAT6, IL-4Rα1, IL-13Rα1, and IL-13Rα2 were designed using Primer Express software (Applied Biosystems) with sequences obtained from GenBank as follows: IL-4, 5′-CGGAGATGGATGTGC CAAAC-3′ (sense), 5′-GCACCTTGGAAGCCCTACAG-3′ (antisense), and 5′-TCCTCACAGCAACGAAGAACACCACA-3′ (probe); IL-13, 5′-GACCAGACTCCCCTGTGCAA-3′ (sense), 5′-TGGGTCCTGTA GATGGCATTG-3′ (antisense), and 5′-CGGGTTCTGTGTAGCCC TGGATTCC-3′ (probe); IL-4Rα1, 5′-CCCACCTGCTTCTCTGA CTACAT-3′ (sense), 5′-GTTTTCAGAGAACTCGAAGAACATCA-3′ (antisense), and 5′-ACTGCAGTTCTCAGCTCTGCCTACACTA CAGG-3′ (probe); IL-13Rα1, 5′-CATGGAGGGTACAAGTTGTTTCC-3′ (sense), 5′-GTTTTGACTCTTACTCTGACTGTGTA GACA-3′ (antisense), and 5′-CTCCCTGGTGTTCTTGCCGACGC-3′ (probe); IL-13Rα2, 5′-

[4]Abbreviations used in this paper: LCM, laser capture microdissection; WT, wild type.

TGTCTTTTCTTTATATTCCTTTTGTTA CTTCT-3′ (sense), 5′-ACACACTTCTTTGTTCAGATCCACAT-3′ (antisense), and 5′-AAGAACCTGAACCCACATTGAGCCTCCA-3′ (probe); and STAT6, 5′-GACTATGGTAGAGGACAGTTGCCTAA-3′ (sense) and 5′-GGTTCTCCAGGAGAAGCTTGGT-3′ (antisense).

Real-time PCR was performed for STAT6 using the SYBR Green Supermix (Bio-Rad) with iCycler (Bio-Rad). For all other probes, real-time PCR was performed using the Brilliant Quantitative PCR Core Reagent kit (Stratagene) on an ABI PRISM 7700 Sequence Detector System (Applied Biosystems). Amplification conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. Fluorescence signals measured during amplification were processed after amplification. Data for whole tissue were normalized to 18S rRNA and then expressed as the fold change in mRNA expression compared with wild type (WT) (Applied Biosystems). For LCM samples, each experiment was repeated three times ($n \geq 3$ each time).

Smooth muscle contractility

Segments of jejunum or mid-colon (1 cm) were flushed of their intestinal contents, suspended longitudinally in individual 8-ml organ baths, and maintained in oxygenated Krebs solution at 37°C. One end of the tissue was attached to an isometric tension transducer (model FT03; Grass Medical Instruments), and the other to the bottom of the bath. Tissues were stretched to a load of 9.9 mN ($2 \times g$), because preliminary experiments showed that this load stretched tissues to their optimal length for active contraction (11). Tissues were allowed to equilibrate for at least 30 min in Krebs buffer solution before concentration-response curves to acetylcholine, a major neurotransmitter in the gut (1 nM to 100 μM), were constructed. The bath solution was replaced every 10 min throughout each study. Tension was recorded using a Grass model 79 polygraph and was expressed as force per cross-sectional area (12).

Statistics

Concentration-dependent responses to acetylcholine were compared using MANOVA (Systat 5.2) with post-hoc analysis for multiple comparisons. A value of $p < 0.05$ was considered significant. Differences in mRNA expression among the different treatment groups were determined using one-way ANOVA, followed by Tukey's test. Appropriate vehicle and time- and age-matched controls were performed for each group ($n = 3$–5/group). There were no significant differences among WT controls; therefore, data were pooled.

Results

Regional differences in whole tissue gene expression of cytokine and cytokine receptors

To investigate differences in the expression of IL-4 and IL-13 between small intestine and colon, sections of whole tissue were taken from WT mice, and RNA was extracted and analyzed using real-time PCR. IL-4 expression in the small intestine and colon was low; however, IL-13 expression was significantly higher in the colon than in the small intestine (Fig. 1*A*). In addition, STAT6 expression was ~3-fold higher in the small intestine than in

the colon (small intestine, 1.00 ± 0.12; colon, 0.33 ± 0.03). A comparison of cytokine receptor expression in whole tissue showed little difference in the expression of IL-4Rα or IL-13Rα1 between the small intestine and colon, but a striking difference in IL-13α2 expression between the two areas, with a much higher expression in the colon (Fig. 1*B*). This high expression of IL-13Rα2 in colon was unexpected and was investigated further.

Regulation of IL-13Rα2 gene expression

To determine the physiological regulation of IL-13Rα2 gene expression, full-thickness sections of intestine and colon were taken from WT, IL-4$^{-/-}$, and IL-13$^{-/-}$ mice. Because the expression of IL-13Rα2 was constitutively low in the intestine (Fig. 1*B*), we did not perform additional analyses in deficient mice. In contrast, the high constitutive expression of the decoy receptor in the colon was inhibited significantly in IL-4$^{-/-}$ mice and to an even greater extent in IL-13$^{-/-}$ mice (Fig. 2).

Localization of cytokine receptors using LCM

There are limits to the interpretation of observations in whole tissue, and given the heterogeneity of cell types along the gut, expression is not likely to be uniform in all cell types on specific nonimmune cells. To identify microenvironments where the receptors are expressed, we used LCM to rapidly isolate specific cell populations or regions from tissue sections on a microscope slide. Cells were captured from epithelium, lamina propria, smooth muscle, and myenteric plexus in small intestine and colon. Total RNA was isolated from LCM samples, converted to cDNA, and IL-4Rα, IL-13Rα1, IL-13Rα2, and ribosomal RNA were assessed by real-time PCR. All values were normalized to constitutive ribosomal RNA values.

We showed previously that, with the exception of Peyer's patches, IL-13 and IL-4 gene expression in a number of cell types was very low in uninfected mice (10). Constitutive expression of IL-4Rα in normal mice was low in epithelial cells of the intestine and colon (Table I). Smooth muscle expression was higher than that in the epithelia, but was similar in both regions, whereas expression in myenteric plexus was much greater in the colon relative to the small intestine. IL-13Rα1 expression levels in the epithelium were greater in the colon than in the small intestine, but smooth muscle and myenteric plexus were similar in both regions (Table I). Finally, the expression of IL-13Rα2 in small intestine was very low, with detectable levels only in smooth muscle, correlating with the low levels observed in whole tissue (Fig. 1*B*). In contrast, IL-13Rα2 expression was dramatically higher in colonic smooth muscle (Fig. 3). These levels in the colon were cell specific, because there was no IL-13Rα2 expressed in the epithelial cells or myenteric plexus, demonstrating the value of LCM for probing selective cell types.

In vitro contractility

Enteric helminth infection induced a STAT6-dependent hypercontractility of smooth muscle, demonstrating a role for Th2 cytokines (11). In normal mice, however, there were no differences in response to acetylcholine between WT and STAT6$^{-/-}$ mice in either the intestine (Fig. 4*A*) or colon (Fig. 4*B*) despite regional differences in the expression of STAT6. IL-13 has a greater effect on smooth muscle than IL-4 (11), suggesting a greater

contribution of IL-13 to helminth-induced hypercontractility. To investigate the contribution of the IL-13Rα2, which is not linked to STAT6, to the physiological control of gut function, smooth muscle responses to acetylcholine were evaluated in IL-13Rα2$^{-/-}$ mice. In both the small intestine and colon, responses to acetylcholine were elevated significantly in IL-13Rα2$^{-/-}$ mice compared with WT (Fig. 4) in the absence of any change in smooth muscle thickness (small intestine, 41.0 ± 6.2 vs 34.8 ± 2.2 μm; colon, 107.6 ± 14.1 vs 134.8 ± 10.2 μm). This is the first demonstration of a change in constitutive gut function in the absence of a specific cytokine receptor, IL-13Rα2$^{-/-}$.

Gene expression of cytokines and cytokine gene receptors in IL-13Rα2$^{-/-}$ mice

Serum concentrations of IL-13Rα2 were undetectable in IL-13Rα2$^{-/-}$ mice compared with WT mice (7.2 ± 4.9 ng/ml). To determine whether the enhanced response to acetylcholine in IL-13Rα2$^{-/-}$ mice could be attributed to changes in cytokine gene or other cytokine receptor components, the expressions of IL-4, IL-13 gene, as well as IL-4Rα and IL-13Rα1 in intestine and colon were determined by real-time PCR. There were no differences in the expression of either IL-4 or IL-13 in the intestine of IL-13Rα2$^{-/-}$ mice (Table II). In contrast, in the colon, there was a 3-fold greater expression of IL-4 and a 20-fold greater expression of IL-13 in IL-13Rα2$^{-/-}$ mice compared with WT mice. The expression of the receptor components, IL-4Rα and IL-13Rα1, was similar in WT and IL-13Rα2$^{-/-}$ mice (Table II).

Discussion

There is considerable interest in the relative contributions of individual Th2 cytokines to the development of Th2-driven pathologies. Although Th2 cytokines are instrumental for protective immunity against enteric helminth infection, persistent and/or robust polarization of the Th2 cytokine profile can have deleterious effects on the host. Previous reports showed that the dominant profile of cytokines is a major determinant of gut function in parasite infection (11, 13–15). There is evidence for both direct actions on effector cells, such as smooth muscle cells, epithelial cells, and enteric neurons, as well as indirect effects mediated by other immune or nonhemopoietic cells (11, 13–17). The interaction of Th2 cytokines and the receptor components, however, is not well understood and the mechanisms by which cytokines exert their functional effects in the gut remain unclear. The present study demonstrates that nonimmune cells exhibit a constitutive expression of receptors for IL-4 and IL-13 with striking region- and cell-specific differences in the cytokine gene expression as well as the distribution of receptor components, especially IL-13Rα2. In the absence of IL-13Rα2, there is a smooth muscle hypercontractility and an elevation in IL-13 gene expression in both regions in the absence of significant alterations in smooth muscle morphology. In addition, IL-13Rα2 expression in uninfected mice is regulated in part by IL-13 and IL-4. These data are the first to demonstrate roles for endogenous IL-13 and IL-13Rα2 in the physiological regulation of smooth muscle function in the gut.

IL-4 and IL-13 share a receptor component as well as some immunological functions, but they also exhibit different biological effects. Helminth-induced alterations in epithelial cell function and smooth muscle contractility in the small intestine are STAT6-dependent (11,

14, 15) and are mimicked by exogenous IL-4 and IL-13, indicating that these effects are due in part to activity at the type 2 receptor. Studies of the exogenous administration of IL-4 or IL-13 reveal that these cytokines have remarkably similar effects on sodium-linked glucose absorption and tissue permeability, but that there are also several differences, such as the prosecretory effects of IL-4 in the small intestine, which are mast cell-dependent and are not shared by IL-13 (16). In addition, IL-13 has a greater effect than IL-4 on intestinal smooth muscle contractility (11). Some of these differences between IL-4 and IL-13 on gut function can be explained by differences in the structure and expression of their receptors. Although both receptors are linked to STAT6 signaling through the IL-4R α-chain, the type 1 receptor is expressed predominantly by bone marrow-derived cells and binds IL-4, but not IL-13. The tissue distribution of type 1 and type 2 receptors, therefore, will influence responses to IL-4 vs IL-13.

In the present study there were striking regional variations in cytokine and cytokine receptor gene expression. STAT6 expression was higher in the small intestine than in the colon, yet there were no differences between WT and STAT6$^{-/-}$ mice in the contractile response to acetylcholine. This is consistent with previous reports showing the lack of a constitutive effect of STAT6 signaling on smooth muscle (11) or epithelial cell responses (16) and suggests that other factors influence the activities of IL-13 and IL-4. IL-13 gene expression was markedly higher in the colon compared with the small intestine. Although the expression of IL-4Rα and IL-13Rα1 was similar in both areas, there was a predominant expression of IL-13Rα2 RNA in the colon. The contribution of IL-13Rα2 to cytokine effects in the gut is unknown, but evidence suggests that soluble IL-13Rα2 in the serum functions as a decoy receptor to limit the effects of IL-13 in vivo. In this paradigm, responses to IL-13 should be enhanced in the absence of IL-13Rα2. There are convincing data showing an elevated responsiveness to IL-13 in IL-13Rα2$^{-/-}$ mice marked by elevated production of IgE and increased macrophage progenitors (7) as well as an exaggerated liver fibrosis in *Schistosoma mansoni*-infected mice (6). This was attributed to an apparent movement of IL-13 from serum to tissue (6). This is consistent with the results of the present study showing that IL-13Rα2$^{-/-}$ mice have undetectable levels of the receptor in serum and exhibited a significant hypercontractility of smooth muscle in both the intestine and colon. This could not be attributed to changes in the tissue expression of other receptor components, IL-4Rα or IL-13Rα1. It is important to note that the effects of IL-13 on smooth muscle contractility as well as on epithelial cell function are dependent largely on STAT6. In the intestine and colon, therefore, the functional effects of IL-13 are mediated by binding to IL-13Rα1, which is part of the type 2 receptor containing IL-4Rα that is linked to STAT6, whereas the constitutive levels of IL-13Rα2 act to limit the amount of IL-13 available to bind to IL-13Rα1. The greater movement of IL-13 to the tissue in IL-13Rα2$^{-/-}$ mice elicited a comparable hypercontractility in the small intestine and colon because of the similar expression of IL-13Rα1.

If the level of IL-13Rα1 limits the functional impact of IL-13 in the gut, what is the role for IL-13Rα2? The gut exhibited pronounced regional differences in IL-13Rα2 RNA expression, with a markedly higher expression in the colon. LCM analysis of specific cell types revealed that RNA expression of IL-13Rα2 was low in mucosal epithelial cells in both regions, but was constitutively expressed in smooth muscle cells, particularly in the colon.

This regional and cellular distribution of IL-13Rα2 suggests that there may be a greater need for the decoy receptor in the colon, because constitutive IL-13 gene expression is higher in this area. This is supported by the observation that in the absence of IL-13Rα2$^{-/-}$, the expression of IL-13 was elevated markedly above that in WT mice, but only in the colon. The immune regulation of this receptor is demonstrated further by the finding that the constitutive expression of IL-13Rα2 in the colon is regulated by IL-13 and, to a lesser extent, by IL-4. This cytokine- and region-specific effects on gene expression imply that in the colon, IL-13 positively regulates IL-13Rα2 expression, which, in turn, negatively regulates IL-13 gene expression, and that there is a physiological reason for limiting effects of IL-13 in this region. In response to certain enteric helminth infections, IL-13 can functionally replace IL-4 and initiate parasite expulsion (18). IL-13 has potent effects on mucosal permeability and smooth muscle contractility in the small intestine that are part of the host response to increase intraluminal fluid accumulation and promote motility to facilitate helminth expulsion. Comparable permeability changes in the colon would be detrimental given the bacterial load in this region and the need to maintain the high mucosal resistance required in this area for ion and fluid absorption. Although the source of soluble IL-13Rα2 is unclear, these data also suggest that the colon may serve as a constitutive source of this receptor that can be amplified during helminth infection. The restriction of IL-13Rα2 expression to smooth muscle may serve to limit the effects of IL-13 on the adjacent mucosa.

In summary, these data are the first to show the functional importance of the regional- and cell-specific differences in the distribution of cytokine gene and receptor expression in the gastrointestinal tract. Despite the importance of IL-13 in protective immunity, however, the prominent differences in the expression of IL-13Rα2, but not IL-13Rα1, suggest that the gut has developed sophisticated mechanisms for controlling or focusing the physiological and pathophysiological activities of IL-13. Disruption of this balance may have both physiological and clinical relevance.

References

1. Elliott DE, Urban JF Jr, Argo CK, Weinstock JV. Does the failure to acquire helminthic parasites predispose to Crohn's disease? FASEB J. 2000; 14:1848–1855. [PubMed: 10973934]
2. Elliott DE, Li J, Blum A, Metwali A, Qadir K, Urban JF Jr, Weinstock JV. Exposure to schistosome eggs protects mice from TNBS-induced colitis. Am. J. Physiol. 2003; 284:G385–G391.
3. Khan WI, Blennerhasset PA, Varghese AK, Chowdhury SK, Omsted P, Deng Y, Collins SM. Intestinal nematode infection ameliorates experimental colitis in mice. Infect. Immun. 2002; 70:5931–5937. [PubMed: 12379667]
4. Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am. J. Gastroenterol. 2003; 98:2034–2041. [PubMed: 14499784]
5. Donaldson DD, Whitters MJ, Fitz LJ, Neben TY, Finnerty H, Henderson SL, O'Hara RM Jr, Beier DR, Turner KJ, Wood CR, et al. The murine IL-13 receptor α2: molecular cloning, characterization, and comparison with murine IL-13 receptor α1. J. Immunol. 1998; 161:2317–2324. [PubMed: 9725226]
6. Chiaramonte MG, Mentink-Kane M, Jacobson BA, Cheever AW, Whitters MJ, Goad ME, Wong A, Collins M, Donaldson DD, Grusby MJ, et al. Regulation and function of the interleukin 13 receptor α2 during a T helper cell type 2-dominant immune response. J. Exp. Med. 2003; 197:687–701. [PubMed: 12642601]

7. Wood N, Whitters MJ, Jacobson BA, Witek J, Sypek JP, Kasaian M, Eppihimer MJ, Unger M, Tanaka T, Goldman SJ, et al. Enhanced interleukin (IL)-13 responses in mice lacking IL-13 receptor α2. J. Exp. Med. 2003; 197:703–709. [PubMed: 12642602]
8. Mentink-Kane MM, Cheever AW, Thompson RW, Hari DM, Kabatereine NB, Vennervald BJ, Ouma JH, Mwatha JK, Jones FM, Donaldson DD, et al. IL-13 receptor α2 down-modulates granulomatous inflammation and prolongs host survival in schistosomiasis. Proc. Natl. Acad. Sci. USA. 2004; 101:586–590. [PubMed: 14699044]
9. Morimoto, M.; Morimoto, M.; Whitmire, J.; Star, RA.; Urban, JF., Jr; Gause, WC. In situ localization of gene expression using laser capture microdissection. In: Dieffenbach, CW.; Dveksler, GS., editors. PCR Primer. 2nd. Plainview: Cold Spring Harbor Laboratory Press; 2003. p. 135-148.
10. Morimoto M, Morimoto M, Whitmire J, Xiao S, Anthony RM, Mirakami H, Star RA, Urban JF Jr, Gause WC. Peripheral CD4 T cells rapidly accumulate at the host: parasite interface during an inflammatory Th2 memory response. J. Immunol. 2004; 172:2424–2430. [PubMed: 14764713]
11. Zhao A, McDermott J, Urban JF Jr, Gause W, Madden KB, Yeung KA, Morris SC, Finkelman FD, Shea-Donohue T. Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. J. Immunol. 2003; 171:948–954. [PubMed: 12847266]
12. Zhao A, Bossone C, Pineiro-Carrero V, Shea-Donohue T. Colitis-induced alterations in adrenergic control of circular smooth muscle in vitro in rats. J. Pharmacol. Exp. Ther. 2001; 299:768–774. [PubMed: 11602693]
13. Dawson HD, Beshah E, Nishi S, Solano-Aguilar G, Morimoto M, Zhao A, Madden KB, Ledbetter TK, Dubey JP, Shea-Donohue T, et al. Localized multigene expression patterns support an evolving Th1/Th2-like paradigm in response to infections with *Toxoplasma gondii* and *Ascaris suum*. Infect. Immun. 2005; 73:1116–1128. [PubMed: 15664955]
14. Madden KB, Yeung KA, Zhao A, Gause WC, Finkelman FD, Katona IM, Urban JF Jr, Shea-Donohue T. Enteric nematodes induce stereotypic STAT6-dependent alterations in intestinal epithelial cell function. J. Immunol. 2004; 172:5616–5621. [PubMed: 15100305]
15. Akiho H, Blennerhassett P, Deng Y, Collins SM. Role of IL-4, IL-13, and STAT6 in inflammation-induced hypercontractility of murine smooth muscle cells. Am. J. Physiol. 2002; 282:G226–G232.
16. Madden KB, Whitman L, Sullivan C, Gause WC, Urban JF Jr, Katona IM, Finkelman FD, Shea-Donohue T. Role of STAT6 and mast cells in IL-4- and IL-13-induced alterations in murine intestinal epithelial cell function. J. Immunol. 2002; 169:4417–4422. [PubMed: 12370375]
17. Shea-Donohue T, Sullivan C, Finkelman FD, Madden KB, Morris SC, Goldhill J, Pineiro-Carrero V, Urban JF Jr. The role of IL-4 in *Heligmosomoides polygyrus*-induced alterations in murine intestinal epithelial cell function. J. Immunol. 2001; 167:2234–2239. [PubMed: 11490010]
18. Urban JF Jr, Noben-Trauth N, Donaldson DD, Madden KB, Morris SC, Collins M, Finkelman FD. IL-13, IL-4Rα, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. Immunity. 1998; 8:255–264. [PubMed: 9492006]

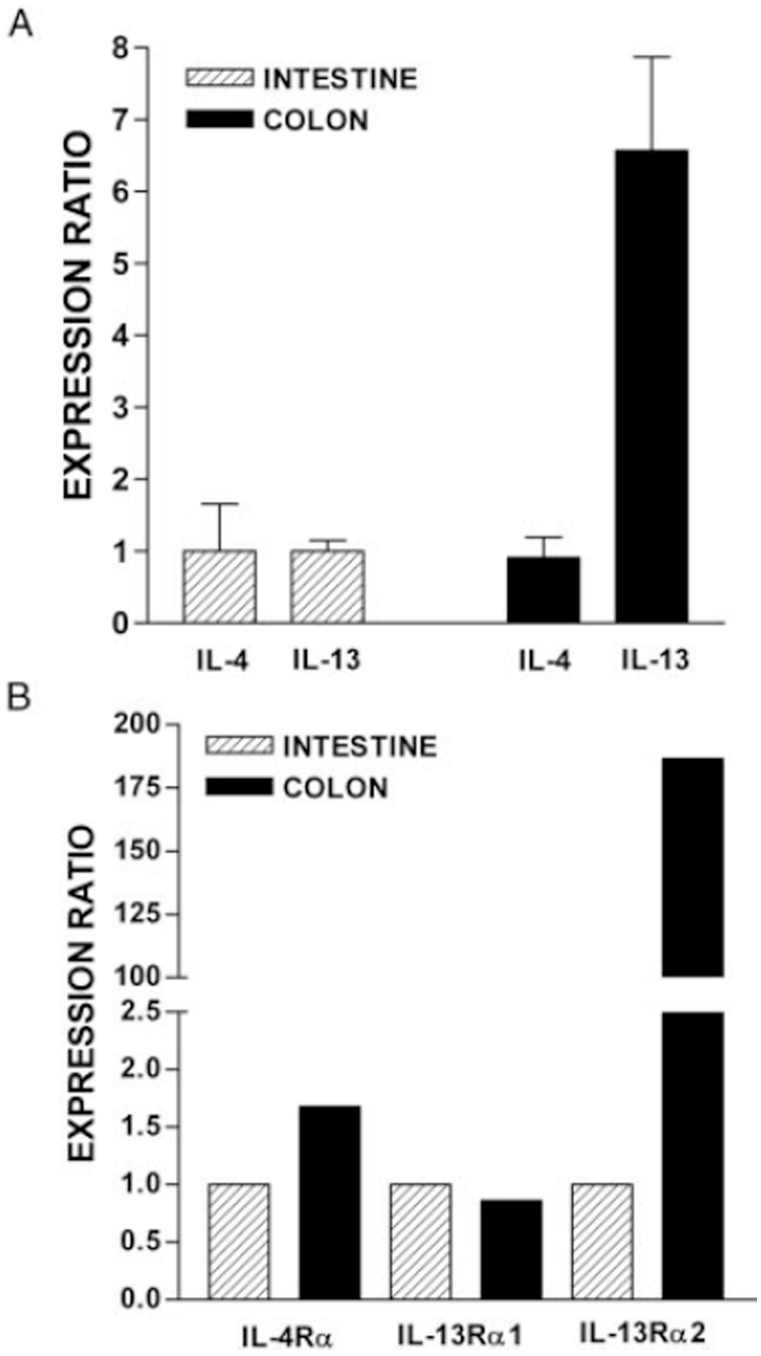

FIGURE 1.

Constitutive gene expression of type 2 cytokines (*A*) and cytokine receptors (*B*) in whole intestine and colon from WT mice measured by real-time PCR. *A*, Gene expression of IL-4 was similar in the two regions, but expression of IL-13 was significantly greater in the colon compared with the small intestine. *B*, The expression of IL-4Rα1 and IL-13Rα1 was similar in small intestine and colon. In contrast, the expression of IL-13α2 was markedly higher in the colon.

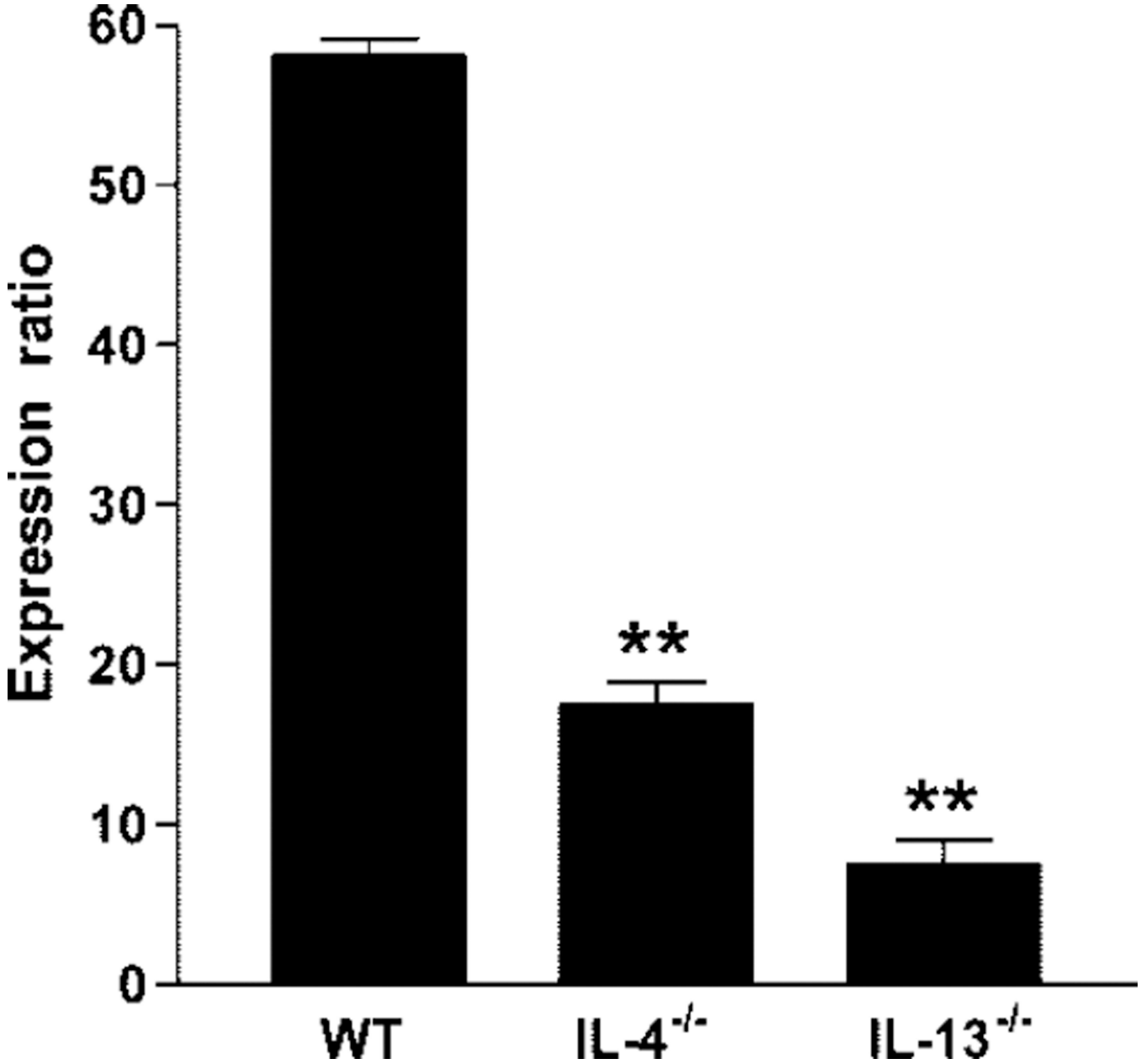

FIGURE 2.

IL-13Rα2 gene expression in colon in IL-4$^{-/-}$ and IL-13$^{-/-}$ mice measured by real-time PCR. Data were normalized to 18S rRNA and then expressed as the fold change in mRNA expression compared with WT intestine. The high constitutive expression of the decoy receptor in the colon was inhibited significantly in IL-4$^{-/-}$ mice and to a greater extent in IL-13$^{-/-}$ mice.

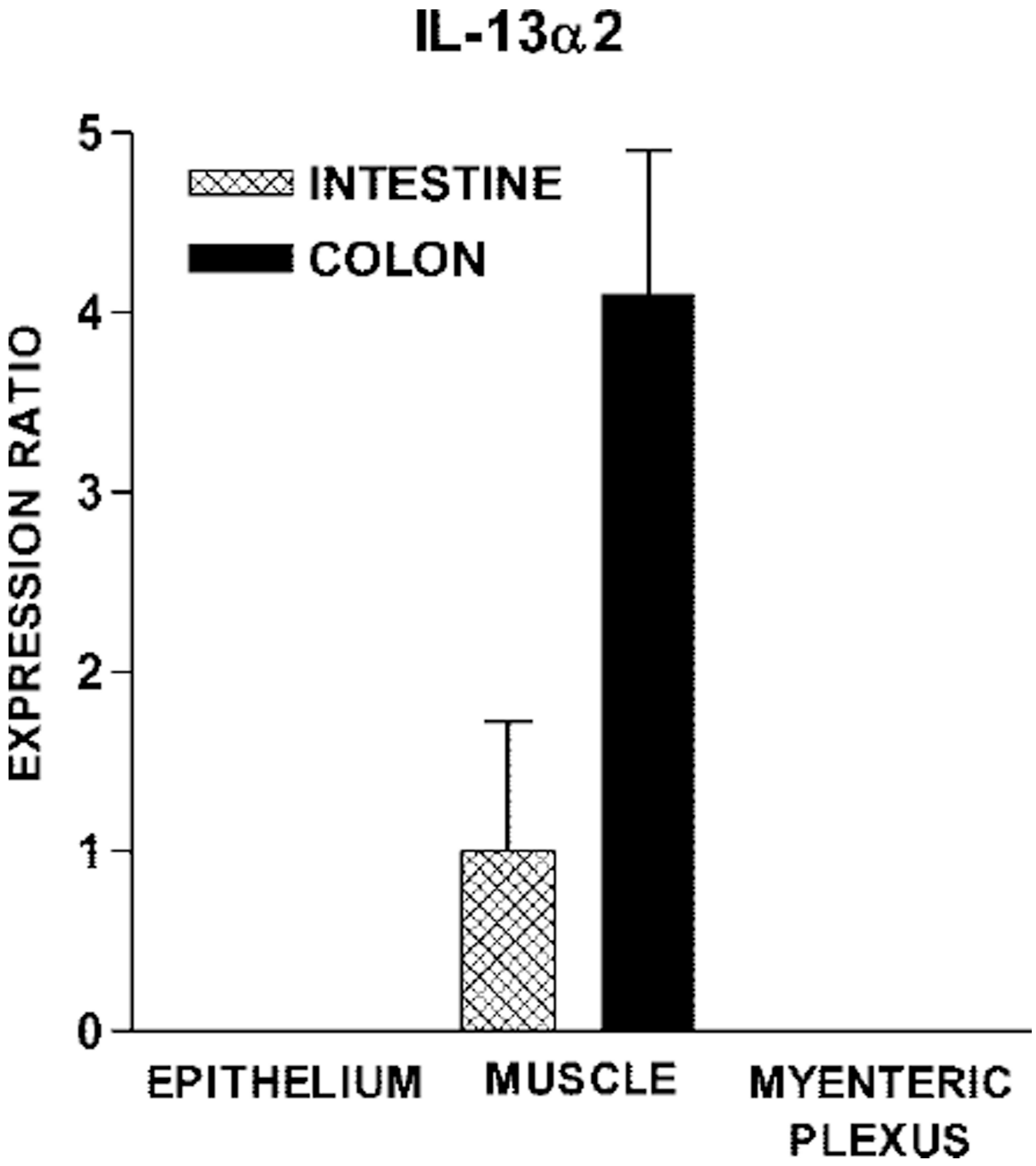

FIGURE 3.

IL-13Rα2 gene expression in specific cells in small intestine and colon. Using LCM, followed by real-time PCR, the IL-13Rα2 gene was undetectable in small intestine, except in smooth muscle; therefore, in small intestine, all data were expressed relative to muscle. IL-13Rα2 expression was much greater in smooth muscle in the colon compared with the small intestine.

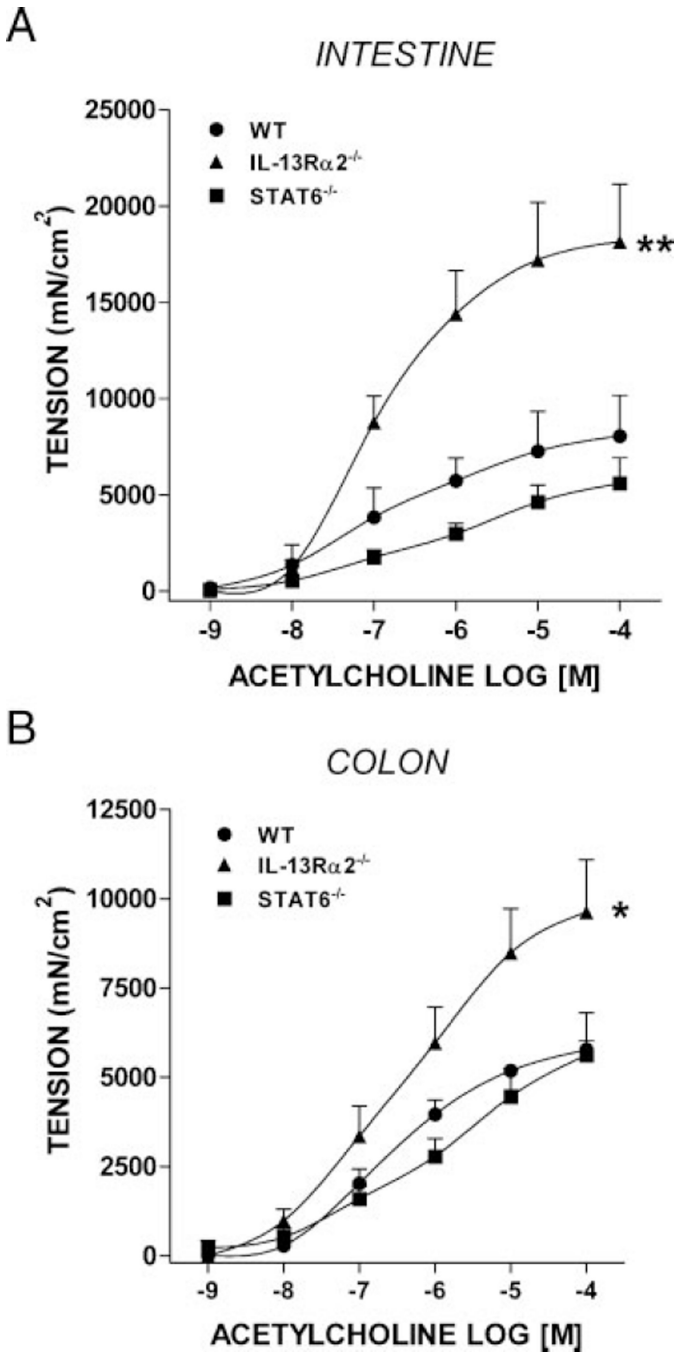

FIGURE 4.

In vitro smooth muscle contractile responses to acetylcholine. Muscle strips (1 cm) taken from the intestine or colon were suspended in organ baths and stretched to optimum length (Lo). Concentration-dependent responses to acetylcholine were constructed in small intestine (*A*) and colon (*B*) taken from STAT6$^{-/-}$ and IL-13Rα2$^{-/-}$ mice. Reponses to acetylcholine were enhanced significantly in the small intestine and colon of IL-13Rα2$^{-/-}$ mice, but not in STAT6$^{-/-}$ mice. *, $p < 0.05$; **, $p < 0.01$ vs WT.

Table I

Cell specific expression of IL-4Rα and IL-13Rα1 in the small intestine and colon in specific cells captured by LCM, followed by real-time PCR[a]

	Epithelial Cells		Myenteric Plexus		Smooth Muscle	
	Intestine	**Colon**	**Intestine**	**Colon**	**Intestine**	**Colon**
IL-4Rα	0.6 ± 0.2	0.2 ± 0.1	1.5 ± 0.4	5.9 ± 0.7*	3.3 ± 1.0	3.4 ± 0.9
IL-13Rα1	3.4 ± 0.6	5.9 ± 0.8*	2.6 ± 0.8	2.0 ± 0.2	3.3 ± 0.2	2.4 ± 0.2

[a]Values are expressed as fold differences from the corresponding receptor in WT intestine ($n \geq 3$).

*$p < 0.05$ vs intestine. All data are expressed relative to expression in the lamina propria in small intestine.

Table II

Expression of IL-4, IL-13, IL-4Rα, and IL-13Rα1 in whole tissue of small intestine and colon obtained from WT and IL-13Rα2$^{-/-}$ mice measured by real-time PCR

	WT		IL-13Rα2$^{-/-}$	
	Intestine	**Colon**	**Intestine**	**Colon**
IL-4	1.0 ± 0.7	0.9 ± 0.3	1.2 ± 0.2	3.5 ± 2.1
IL-13	1.0 ± 0.8	7.6 ± 3.1*	0.95 ± 0.4	19.1 ± 5.4**
IL-4Rα	1.0 ± 0.1	1.7 ± 0.1	1.0 ± 0.1	1.1 ± 0.3
IL-13Rα1	1.0 ± 0.1	1.6 ± 0.3	1.2 ± 0.2	2.0 ± 0.4

Values are expressed as fold differences from the corresponding cytokine or receptor in WT; $n \geq 3$;

* $p < 0.05$;

** $p < 0.01$ vs intestine.