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IL-11 Receptor α in the Pathogenesis of IL-13-Induced Inflammation and Remodeling[1]

Qingsheng Chen,[2]* Lesley Rabach,[2]* Paul Noble,* Tao Zheng, Chun Geun Lee,* Robert J. Homer,[†‡] and Jack A. Elias[3]*

IL-13 is a major stimulator of inflammation and tissue remodeling at sites of Th2 inflammation. In Th2-dominant inflammatory disorders such as asthma, IL-11 is simultaneously induced. However, the relationship(s) between IL-11 and IL-13 in these responses has not been defined, and the role(s) of IL-11 in the genesis of the tissue effects of IL-13 has not been evaluated. We hypothesized that IL-11, signaling via the IL-11Rα-gp130 receptor complex, plays a key role in IL-13-induced tissue responses. To test this hypothesis we compared the expression of IL-11, IL-11Rα, and gp130 in lungs from wild-type mice and transgenic mice in which IL-13 was overexpressed in a lung-specific fashion. We simultaneously characterized the effects of a null mutation of IL-11Rα on the tissue effects of transgenic IL-13. These studies demonstrate that IL-13 is a potent stimulator of IL-11 and IL-11Rα. They also demonstrate that IL-13 is a potent stimulator of inflammation, fibrosis, hyaluronic acid accumulation, myofibroblast accumulation, alveolar remodeling, mucus metaplasia, and respiratory failure and death in mice with wild-type IL-11Rα loci and that these alterations are ameliorated in the absence of IL-11Rα. Lastly, they provide insight into the mechanisms of these processes by demonstrating that IL-13 stimulates CC chemokines, matrix metalloproteinases, mucin genes, and gob-5 and stimulates and activates TGF-β1 via IL-11Rα-dependent pathways. When viewed in combination, these studies demonstrate that IL-11Rα plays a key role in the pathogenesis of IL-13-induced inflammation and remodeling. ***The Journal of Immunology,*** **2005, 174: 2305–2313.**

Interleukin-13 is a pleiotropic 12-kDa product of a gene on chromosome 5 at q31 that is produced in large quantities by stimulated Th2 cells. It was originally described as an IL-4-like molecule based on shared effector properties, including the ability to stimulate IgE production. Subsequent studies demonstrated that IL-13 and IL-4 often play distinct roles in biology. A prominent aspect of this distinction is the appreciation that IL-4 plays a key role in Th2 cell differentiation and response generation, whereas IL-13 contributes as the major effector of Th2 inflammation and tissue remodeling (1–4). In accord with these observations, IL-13 dysregulation has been documented, and IL-13 has been implicated in the pathogenesis of a variety of diseases characterized by inflammation and tissue remodeling, including asthma, idiopathic pulmonary fibrosis, scleroderma, viral pneumonia, hepatic fibrosis, nodular sclerosing Hodgkin's disease, and chronic obstructive pulmonary disease (COPD)[4] (1–11). Studies from our laboratory and others have demonstrated that IL-13 mediates its tissue effects by activating a broad array of downstream target genes, including chemokines, matrix metalloproteinases (MMPs), TGF-β1, and chitinases (12–16). The importance of IL-6-type cytokines in the generation of the effects of IL-13, however, have not been investigated.

IL-11 is a multifunctional IL-6-type cytokine with diverse biologic properties, including the ability to stimulate hemopoiesis, thrombopoiesis, megakaryocytopoiesis, and bone resorption; regulate macrophage differentiation; and confer mucosal protection after chemotherapy and radiation therapy (17–22). These effects are mediated by a multimeric receptor that contains a ligand-binding α subunit, IL-11Rα, and the ubiquitous β subunit, gp130, that triggers intracellular signaling (18, 23, 24). Previous studies from our laboratory and others demonstrated that, like IL-13, IL-11 is expressed in an exaggerated fashion in the dysregulated Th2 response in the asthmatic airway (25). Although IL-11 can inhibit Th1 responses, inhibit the production of Th1-related cytokines such as IL-12, and shift inflammation in a Th2 direction (22, 26–29), little else is known about the role(s) of IL-11 in the generation and/or expression of Th2 tissue responses. In particular, interactions between IL-11 and IL-13 have not been defined, and a role for IL-11 in the genesis of IL-13-induced pathologies has not been established.

We hypothesized that IL-11 signaling plays a key role in IL-13-induced Th2 inflammation. To test this hypothesis, we characterized the expression of IL-11, IL-11Rα, and gp130 in lungs from wild-type (WT) mice and mice in which IL-13 was overexpressed in a lung-specific fashion. We also characterized the effects of a null mutation of IL-11Rα on the tissue effects of transgenic IL-13. These studies demonstrate that IL-13 is a potent stimulator of IL-11 and IL-11Rα. They also demonstrate that IL-11Rα plays a key role in IL-13-induced inflammation, fibrosis, hyaluronic acid

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[2] Q.C. and L.R. made equal contributions to this work.

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[4] Abbreviations used in this paper: COPD, chronic obstructive pulmonary disease; BAL, bronchoalveolar lavage; HA, hyaluronic acid; MMP, matrix metalloproteinase; Tg, transgenic; Timp, tissue inhibitor of MMP; WT, wild type; TARC, thymus and activation-regulated chemokine.

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(HA) accumulation, myofibroblast accumulation, alveolar remodeling, mucus metaplasia, and respiratory failure and death. Lastly, they provide insights into the mechanisms of these processes by demonstrating that IL-13 stimulates CC chemokines, MMPs, mucin genes, and gob-5 and stimulates and activates TGF-β1 via IL-11Rα-dependent pathways.

Materials and Methods

Overexpression transgenic mice

CC10-IL-13 transgenic mice were generated in our laboratory, bred onto a C57BL/6 background, and used in these studies. These mice use the Clara cell 10-kDa protein (CC10) promoter to target IL-13 to the lung. The methods used to generate and characterize these mice were described previously (30). In this modeling system, IL-13 caused a mononuclear cell- and eosinophil-rich tissue inflammatory response, alveolar enlargement, subepithelial and parenchymal fibrosis, mucus metaplasia, and respiratory failure and death, as previously described (12, 13, 30).

IL-11Rα-null mice (IL-11R$\alpha^{-/-}$) were provided by Drs. L. Robb and C. Glenn Begley (Walter and Eliza Hall Institute, Victoria, Australia) (31, 32). These mice were bred for more than eight generations onto a C57BL/6 genetic background. CC10-IL-13 mice with WT$^{+/+}$ and null$^{-/-}$ IL-11Rα loci were generated by breeding the IL-13 transgenic (Tg^{+}) mice with the IL-11R$\alpha^{-/-}$ animals. Genotyping was accomplished as previously described (30, 32). Littermate control WT mice with (+/+) or without (−/−) IL-11Rα loci were used as controls.

Bronchoalveolar lavage (BAL)

Lung inflammation was assessed by BAL as previously described (13, 33). The BAL samples from each animal were then pooled and centrifuged. The number and types of cells in the cell pellet were determined as previously described (12, 13). The supernatants were stored at −20°C until used.

Lung volume and morphometric assessments

Animals were anesthetized, the trachea was cannulated, and the lungs were removed and inflated with PBS at 25 cm. The size of each lung was evaluated via volume displacement, and alveolar size was estimated from the mean chord length of the airspace, as previously described by our laboratory (13). Chord length increases with alveolar enlargement.

Histologic evaluation

Animals were killed, a median sternotomy was performed, and right heart perfusion was accomplished with calcium- and magnesium-free PBS. The heart and lungs were then removed en bloc, inflated at 25 cm pressure with neutral-buffered 10% formalin, fixed in 10% formalin, embedded in paraffin, sectioned, and stained. H&E, Mallory's Trichrome, and periodic acid-Schiff with diastase stains were performed at Yale University School of Medicine.

mRNA analysis

The levels of mRNA encoding IL-11 and IL-11Rα were evaluated with a commercial RNase protection assay (BD RiboQuant; BD Biosciences) as described by the manufacturer. Other mRNA levels were evaluated by RT-PCR analysis as previously described (13). The primers used have been described previously (12, 13, 15, 16). For each cytokine, the optimal numbers of cycles that will produce a quantity of cytokine product that is directly proportional to the quantity of input mRNA was determined experimentally. β-Actin was used as an internal standard. Amplified PCR products were detected using ethidium bromide gel electrophoresis, quantitated electronically, and confirmed by nucleotide sequencing.

Quantification of IL-13 and chemokines

BAL IL-13 and chemokine levels were quantitated using commercial ELISA kits (R&D Systems) according to the manufacturer's instructions.

Immunohistochemistry

α-Smooth muscle actin and myosin H chain staining cells were evaluated by immunohistochemistry as previously described by our laboratory (15). The primary Abs were obtained from DakoCytomation. Specificity was assessed by comparing the staining of serial sections that were incubated in the presence and the absence of the primary Ab.

Quantification of lung collagen

Collagen content was determined biochemically by quantifying total soluble collagen using the Sircol collagen assay kit (Biocolor) according to the manufacturer's instructions (15). The data are expressed as the collagen content of the entire right lung. Collagen was also assessed morphometrically using picosirius red staining, performed as described previously by our laboratory (15). These data are expressed as the percentage of the histologic section with picosirius red staining.

Quantification of HA

The levels of BAL HA were measured using a competitive ELISA using biotinylated HA-binding protein as described previously (34, 35). Microtiter plates were coated with HA by combining rooster comb HA, carbodiimide HCl, and HCl. Samples were incubated with biotinylated HA-binding protein for 1 h and then added to the wells. The plate was then agitated, washed, developed with HRP-streptavidin, and exposed to peroxidase substrate for 30 min. OD at 405 nm was evaluated. Samples were compared with a simultaneously performed standard curve.

TGF-β bioassay

The levels of total and bioactive TGF-β1 were evaluated by ELISA (R&D Systems) using untreated and acid-treated BAL fluids according to the manufacturer's instructions.

Murine 100% O_2 exposure

Adult 6- to 8-wk-old Tg^{-} and Tg^{+} mice with WT or null mutant IL-11Rα loci were exposed to room air (controls) or continuously to 100% O_2 in a Plexiglas chamber as previously described (19, 36). All protocols were reviewed and approved by the institutional animal care and use committee at Yale University School of Medicine.

Statistics

Normally distributed data are expressed as the mean ± SEM and assessed for significance by Student's t test or ANOVA as appropriate. Data that were not normally distributed were assessed for significance using the Wilcoxon rank-sum test.

Results

Effect of IL-13 on IL-11 and IL-11R expression

Studies were undertaken to define the effects of IL-13 on IL-11 and its receptor components in murine lung. These studies demonstrated that transgenic IL-13 is a potent stimulator of the expression of IL-11 and IL-11Rα. These effects were readily apparent at all time points evaluated (1–4 mo; Fig. 1 and data not shown). The induction of IL-11 was associated with similar increases in the levels of mRNA encoding other IL-6-type cytokines, including IL-6 and LIF (Fig. 1). A modest increase in gp130 expression was also observed (Fig. 1). Similar alterations in M-CSF, GM-CSF, stem cell factor, L32, and GAPDH, however, were not found. The alterations in IL-11Rα were also at least partially specific, because

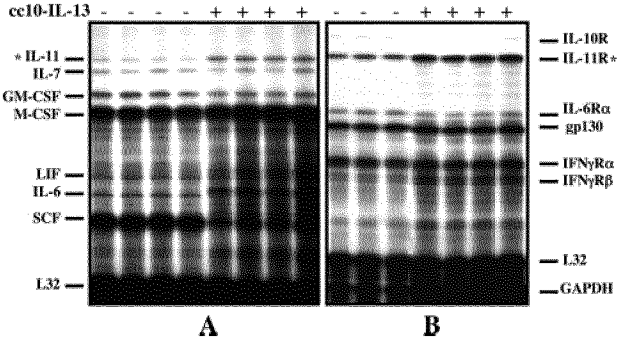

FIGURE 1. IL-13 regulation of IL-11Rα and IL-11. Lungs were obtained from 2-mo-old CC10-IL-13 Tg^{-} and Tg^{+} mice, and the levels of mRNA encoding the noted cytokines, proteins, and receptors were evaluated using RNase protection. Each lane represents an individual animal.

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comparable alterations in the expression of IL-6Rα and IFN-γRα were not observed (Fig. 1). These studies demonstrate that IL-13 is a potent stimulator of IL-11 and the α subunit of its receptor in murine lung.

Role of IL-11R signaling in IL-13-induced inflammation

To address the importance of IL-11 in the pathogenesis of IL-13-induced tissue inflammation, CC10-IL-13 transgenic mice were bred with IL-11Rα$^{-/-}$ mice. The inflammatory responses in IL-13 Tg^{+} mice with WT and null IL-11Rα loci were then compared. As previously reported (12, 30), IL-13 was a potent stimulator of tissue inflammation that caused a progressive increase in the accumulation of macrophages, lymphocytes, and eosinophils in the tissues and BAL fluids of IL-13 Tg^{+} mice with normal IL-11Rα loci. In the absence of IL-11Rα, an impressive decrease in this inflammatory response was noted. In 2- and 4-mo-old mice, impressive decreases in BAL total cell, macrophage, and eosinophil recovery were noted (Fig. 2, *A* and *B*). A similarly, impressive decrease in tissue inflammatory cell accumulation was apparent (Fig. 2*C* and data not shown). In BAL and tissues, compensatory increases in neutrophils were not noted (Fig. 2).

Role of IL-11Rα in IL-13-induced chemokine elaboration

Previous studies from our laboratory demonstrated that IL-13 induces its tissue alterations in part via the induction of a wide array of CC chemokines (12). To investigate the mechanism by which IL-11Rα deficiency diminished IL-13-induced inflammation, we compared the expression of selected chemokines in IL-13 Tg^{+} mice with WT and null IL-11Rα loci. In Tg^{-} mice with WT or null IL-11Rα loci, the levels of mRNA encoding MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MIP-1α/CCL-3, MIP-1β/CCL4, MIP-2/CXCL-2/3, MIP-3α/CCL20, C10/CCL-6, eotaxin/CCL-11, eotaxin-2/CCL21, and thymus and activation-regulated chemokine (TARC)/CCL17 were comparable and in many cases were near or below the limits of detection of our assays (Fig. 3*A*). As previously reported (12, 37), IL-13 increased the levels of mRNA encoding these chemokine moieties in Tg^{+} mice with WT IL-11Rα loci (Fig. 3*A*). In contrast, in the absence of IL-11Rα, the ability of IL-13 to induce MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCL2–3, MIP-3α/CCL20, C10/CCL6, eotaxin/CCL11, eotaxin-2/CCL21, and TARC/CCL17 was markedly diminished (Fig. 3*A*). In accord with these mRNA alterations, comparable alterations in BAL MCP-1/CCL2, MIP-1α/CCL-3, and eotaxin/CCL-11 protein were observed (Fig. 3, *B–D*). Thus, IL-11Rα plays an essential role in the stimulation of selected chemokines by IL-13.

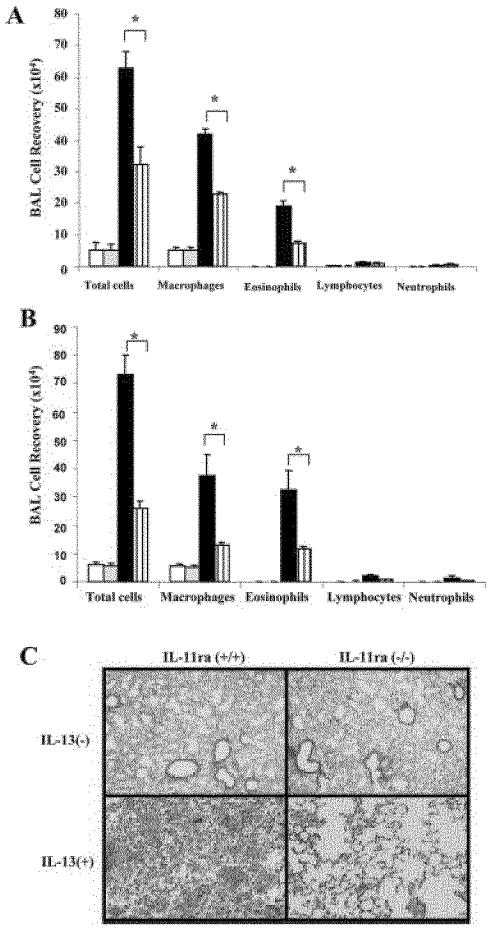

FIGURE 2. Role of IL-11Rα in IL-13-induced inflammation. *A* and *B*, The BAL cell recoveries of Tg^{-}/IL-11Rα$^{+/+}$ mice (□), Tg^{-}/IL-11Rα$^{-/-}$ mice (▨), Tg^{+}/IL-11Rα $^{+/+}$ mice (■), and Tg^{+}/IL-11Rα $^{-/-}$ mice (▥) at 2 (*A*) and 4 (*B*) mo of age are compared. The histologic effects in 4-mo-old mice are illustrated in *C* (original magnification, ×10). *, $p < 0.01$.

Role of IL-11Rα in IL-13-induced fibrosis and HA and myofibroblast accumulation

Quantitative morphometric, biochemical, and immunohistochemical approaches were used to define the role of IL-11Rα in IL-13-induced pulmonary fibrosis and HA and myofibroblast accumulation. In these studies, we compared these collagen, HA, and cellular parameters in IL-13 Tg^{+} mice with WT and null IL-11Rα loci. Similar amounts of collagen and BAL HA and similar numbers of anti-smooth muscle actin-staining parenchymal cells were noted in lungs from WT littermate control mice and IL-11Rα$^{-/-}$ animals (Fig. 4). In WT mice, IL-13 caused an impressive increase in lung collagen content (Fig. 4, *A* and *B*) and BAL HA levels (Fig. 4*C*) that could be easily determined by histochemical and biochemical measurement techniques. In addition, IL-13 increased the accumulation of parenchymal myofibroblast-like cells that contained anti-smooth muscle actin, but did not stain with Abs against smooth muscle myosin (Fig. 4*D* and data not shown). In contrast, the levels of IL-13-induced collagen and HA were significantly reduced in lungs from Tg^{+} mice with null vs WT IL-11Rα loci (Fig. 4, *A–C*). Myofibroblast accumulation was similarly decreased in lungs from IL-13 Tg^{+}/IL-11Rα$^{-/-}$ mice compared with Tg^{+}/IL-11Rα$^{+/+}$ animals (Fig. 4*D*). Interestingly, the anti-smooth muscle actin staining of vascular smooth muscle cells was not altered in the absence of IL-11Rα (Fig. 4*D*). Thus, IL-11 signaling plays a critical role in IL-13-induced tissue fibrosis and HA and myofibroblast accumulation.

Role of IL-11Rα in IL-13-induced production and activation of TGF-β1

Previous studies from our laboratory demonstrated that the fibrotic effects of IL-13 are mediated by its ability to induce and activate TGF-β1 and that this activation is mediated to a great extent by MMP-9 (15). To define the importance of IL-11Rα in these responses, we evaluated the TGF-β1 production of Tg^{+} mice with WT and null IL-11Rα loci. In mice with a WT IL-11Rα locus, IL-13 was a potent stimulator of the levels of mRNA encoding TGF-β1, TGF-β2, and TGF-β3 (Fig. 5*A*). IL-13 also augmented MMP-9 mRNA accumulation (Fig. 5*A*). In accord with these observations, IL-13 increased the levels of spontaneously activated and total TGF-β1 protein in BAL fluids from these animals (Fig. 5, *B* and *C*). In all cases, these inductive effects appeared to be

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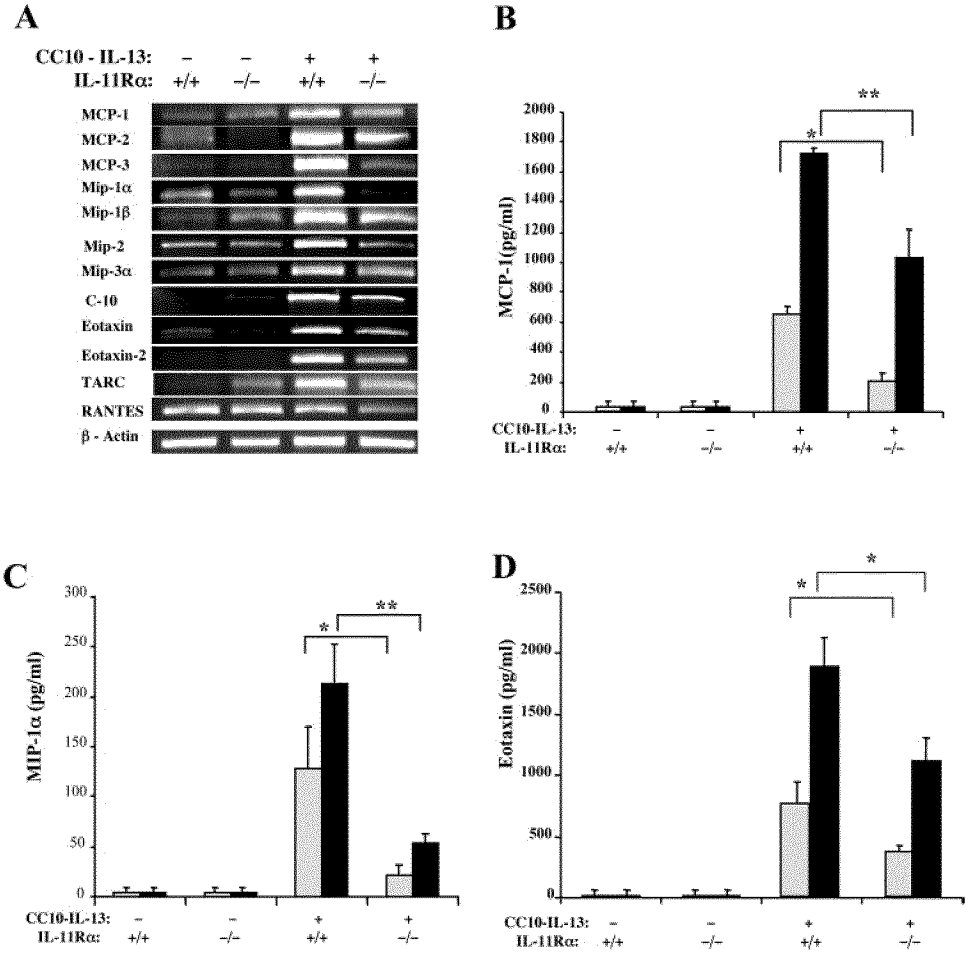

FIGURE 3. Role of IL-11Rα in IL-13-induced chemokine stimulation. *A*, Comparison of the levels of mRNA encoding the noted chemokines in lungs from Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci. *B–D*, Levels of MCP-1, MIP-1α, and eotaxin protein were assessed by ELISA in BAL fluids from 2-mo-old (▨) and 4-mo-old (■) mice. The evaluations in *A* are representative of four similar evaluations. *B–D*, Each value is the mean ± SEM of evaluations in a minimum of five mice. *, $p < 0.05$; **, $p < 0.01$.

IL-11Rα-dependent, because the levels of mRNA encoding TGF-β1, -β2, and -β3 and MMP-9 and the production and activation of TGF-β1 were significantly decreased in IL-11Rα-null mutant mice (Fig. 5). Thus, IL-13 stimulates and activates TGF-β1 and induces production of the TGF-β1 activator, MMP-9, via an IL-11Rα-dependent mechanism.

Role of IL-11Rα in IL-13-induced alveolar remodeling

To define the role(s) of IL-11Rα in the pathogenesis of IL-13-induced alveolar remodeling, we compared the alterations in lung volume and alveolar size in IL-13 Tg^+ mice with WT and null IL-11Rα loci. In accord with previous observations (13), IL-13 caused an impressive increase in these parameters in lungs from mice with WT IL-11Rα loci (Fig. 6, *A* and *B*). In contrast, these effects of IL-13 were significantly diminished in mice with null IL-11Rα loci (Fig. 6, *A* and *B*). Thus, IL-11Rα plays a key role in this remodeling response.

Effects of IL-11Rα deficiency on lung proteases

To determine whether a deficiency of IL-11Rα could modulate the IL-13-induced alveolar phenotype by decreasing the production of respiratory proteases, we compared the levels of mRNA encoding lung-relevant MMPs and cathepsins in WT and IL-11Rα$^{-/-}$ mice. As noted above (Fig. 5*A*), IL-13 is a potent stimulator of MMP-9, and this inductive event was mediated via an IL-11Rα-dependent pathway. As shown in Fig. 6*C*, IL-13 was also a potent stimulator of MMP-2, MMP-12, tissue inhibitor of MMP (Timp)-1, Timp-2, Timp-3, Timp-4, cathepsin K, cathepsin S, cathepsin B, and cathepsin L. Interestingly, the induction of MMP-2, MMP-12, Timp-1 to -4, cathepsin K, and cathepsin B was decreased in the absence of IL-11Rα (Fig. 6*C*). Thus, in the setting of a deficiency of IL-11Rα, IL-13 is unable to optimally stimulate lung proteases.

Role of IL-11Rα in IL-13-induced mucus metaplasia

Studies were next undertaken to determine whether IL-11Rα played an important role in the pathogenesis of IL-13-induced mucus metaplasia. In these studies we compared mucin gene expression in Tg^+ mice with WT and null IL-11Rα loci. The expression of gob-5, a calcium-activated chloride channel involved in the mucus response (38), was also evaluated. In lungs from Tg^- mice with WT or null IL-11Rα loci, the levels of expression of Muc-5ac and gob-5 were at or near the limits of detection in our assay (Fig. 7). In contrast, IL-13 was a potent stimulator of muc-5AC and gob-5 in murine lung (Fig. 7). Interestingly, the stimulation of muc5AC and gob-5 gene expression were diminished in Tg^+ mice with null mutant IL-11Rα loci (Fig. 7). These studies demonstrate that IL-11 plays an important role in the pathogenesis of IL-13 stimulation of mucin and gob-5 gene expression.

Role of IL-11Rα in IL-13-induced respiratory death

In CC10-IL-13 Tg^+ mice, progressive lung pathology is noted. As a result, these mice die prematurely from a fibrodestructive, inflammatory alveolar filling process that abrogates normal respiratory function (12). To define the role of IL-11Rα in this fatal response, we compared the survival of IL-13 Tg^+ mice with WT and null IL-11Rα loci. Tg^+ mice with IL-11Rα$^{+/+}$ loci started to die at ~100 days of age, and 100% of these animals were dead by 4.1 mo of age (Fig. 8). As shown in Fig. 8, a deficiency of IL-11Rα

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