

IL-11: Insights in asthma from overexpression transgenic modeling

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The evolution of our understanding of IL-11 mirrors, in many ways, the problems that are faced by investigators in the post-genome era and the types of techniques that might need to be used to deal with these issues. IL-11 was discovered as a soluble factor in fibroblast supernatants that stimulated the proliferation of "IL-6-dependent" plasmacytoma cells. It was subsequently demonstrated to be an important stimulator of platelet reconstitution and a pleiotropic regulator of nonrespiratory tissues. In the lung, IL-11 is produced by a variety of structural cells and eosinophils in response to a variety of stimuli, including TGF- β , major basic proteins, and viruses. IL-11 is also detected in exaggerated quantities at sites of virus infection. Its potential effector functions at these sites were defined with constitutive and inducible overexpression transgenic modeling systems which demonstrated that IL-11 causes nodular mononuclear infiltrates, airway remodeling with subepithelial fibrosis, airways obstruction, and airways hyperresponsiveness and can block alveolar development when expressed during development. In accord with these murine findings, IL-11 is selectively expressed in eosinophils and epithelial cells in patients with moderate and severe asthma where expression correlates directly with disease severity and inversely with FEV₁. Studies using transgenic mice also demonstrated that IL-11 inhibits antigen-induced tissue inflammation. Thus IL-11 might be an important regulator of inflammatory and remodeling responses in the asthmatic airway. (*J Allergy Clin Immunol* 2001;108:489-96.)

Key words: Interleukin 11, asthma, transgenic modeling, airway remodeling, fibrosis

Present day students of lung biology are living through the transition from the pre-genome scientific era to the post-genome scientific era. As a result, we have access to

Abbreviations used

IL-11R α : IL-11 receptor α
rtTA: Reverse tetracycline transactivator
STAT: Signal transducer and activator of transcription
TGF- β : Transforming growth factor β
tet-O: Tetracycline operator
tet-R: Tetracycline repressor

an impressive amount of information regarding the primary structure of the genes in the genome. In the majority of cases, however, we are totally or partially lacking in our understanding of the effector functions of their protein products and of how they interact to generate complex physiologic and pathologic responses. IL-11 was discovered in 1990 as a soluble factor distinct from IL-6 in supernatants from transformed fibroblasts that stimulated the proliferation of "IL-6-dependent" plasmacytoma cells.¹ In many ways, the approaches that were used to characterize the IL-11 system and define its role(s) in biology reflect this scientific transition. They also illustrate the types of studies that will need to be undertaken with other proteins to define their role(s) in health and disease. In this article, we review the biology of IL-11 in the context of the approaches that were used to obtain this information. Particular attention will be directed toward the insights obtained with overexpression transgenic modeling systems, which contributed impressively to our understanding of the effector functions of IL-11 in the lung and provided guideposts for studies of IL-11 in human asthmatic tissues.

THE IL-11 CYTOKINE AND RECEPTOR SYSTEM

After the discovery of human IL-11 and the description of murine IL-11 in 1992, subsequent studies provided knowledge of the structure of the genes that encode these moieties and the proteins that they produce. The human and murine IL-11 genes both contain 5 exons and 4 introns, are 80% homologous at the nucleotide level, and have been mapped to chromosome 19 (q13.3-q13.4) and to the centromeric region of chromosome 7, respectively.^{2,3} The 5' flanking region of the human IL-11 gene con-

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tains a "TATA" boxlike sequence 180 nucleotides upstream from the translation initiation codon ATG.³ In addition, our studies demonstrated 2 activator protein 1 sites between -100 and -82 in the IL-11 promoter that interact in a complex fashion with activator protein 1 transcription factor proteins, conferring transforming growth factor β (TGF- β) inducibility on the human cytokine moiety.⁴ There are 2 polyadenylation sites located in the 3' untranslated region of IL-11, at nucleotides 6762 and 6591. These sites give rise to the 1.5 and 2.5 Kb IL-11 mRNA transcripts that have been noted to be expressed in a variety of cells and tissues.^{1,3} The IL-11 precursor protein contains 199 amino acids, is rich in proline residues, lacks cysteine residues, is highly helical, and is thermally stable.⁵ It is also impressively cationic, with an isoelectric point that is more basic than that of eosinophil major basic protein.*

IL-11 is classified with IL-6, ciliary neurotropic factor, leukemia inhibitory factor, oncostatin M, and cardiotropin 1 as an IL-6-type cytokine. Members of this cytokine family share a 4-helix bundle fold motif.⁶ More importantly, they have overlapping effector profiles and multimeric receptor complexes in which the promiscuous gp130 molecule serves as a signaling β subunit.⁷⁻¹⁰

IL-11 initiates signaling via binding to a unique IL-11 receptor α chain (IL-11R α). Isoforms of IL-11R α have been described that contain (IL-11R α_1) and do not contain (IL-11R α_2) a cytoplasmic domain.¹¹ The binding of IL-11 to IL-11R α forms a complex that binds to and induces gp130 molecule homodimerization, resulting in the assembly of the active IL-11 receptor trimer. This activates Janus kinases,^{12,13} which phosphorylate tyrosine residues within the cytoplasmic region of gp130 that are specific docking sites for signal transducer and activator of transcription (STAT)-1 and STAT-3 proteins.^{14,15} The activated Janus kinases also phosphorylate tyrosine residues within the bound STAT proteins, causing the STATs to dissociate from gp130, dimerize, and enter the nucleus to act as transcriptional activators of target genes.^{16,17} STAT dimers might be additionally phosphorylated by mitogen-activated protein kinases^{18,19} that are also activated in response to cytokine binding to the receptor.^{20,21}

EXTRAPULMONARY EFFECTOR FUNCTIONS OF IL-11

IL-11 is a pleiotropic cytokine (Fig 1) with hematopoietic, osseous, and mucosal protective effects that have received significant investigative attention and lesser understood effects on immune cells and other tissues. In the hematopoietic system, IL-11 interacts with other early and late growth factors to stimulate various stages and lineages of hematopoiesis and regulate macrophage proliferation and differentiation. Its effects on platelets are particularly prominent, inasmuch as IL-11 synergizes with IL-3, thrombopoietin, or stem cell factor to stimulate megakaryocytopoiesis and thrombopoiesis.²² The

latter property underlies the recent US Food and Drug Administration approval of recombinant human IL-11 as a treatment that fosters platelet reconstitution after bone marrow ablative chemotherapy.

In bones, IL-11 is produced by cytokine and parathyroid hormone-stimulated osteoblasts.²³ After its release, it activates osteoclasts that increase bone absorption and decrease new bone formation. The effects of IL-11 on osteoclasts might be mediated by its ability to inhibit the production of osteoprotegerin, a naturally occurring protein with potent osteoclastogenesis inhibitory activity, and stimulate the production of RANK ligand, a transmembrane receptor on hematopoietic osteoclast precursor cells.^{24,25} IL-11 also stimulates chondrocyte and synovio-cyte production of tissue inhibitor of metalloproteinase 1, which regulates local matrix metabolism.²⁶

One of the intriguing properties of IL-11 is its ability to provide protective effects in the setting of mucosal injury. These effects have been investigated most thoroughly in the gastrointestinal tract, where IL-11 protects small intestinal cells after combined radiation therapy and chemotherapy, reduces experimental mucositis, and ameliorates injury in models of inflammatory bowel disease.²⁷ IL-11 has also been shown to improve survival after thoracic irradiation.²⁸ The mechanism of IL-11-induced protection is not known; it might, however, relate to the ability of IL-11 to inhibit macrophage production of TNF, IL-1, IL-12, and/or nitric oxide and to prevent nuclear factor κ B activation.²⁹ IL-11 also inhibits oxidant-induced cell death responses,³⁰ prevents epithelial apoptosis, regulates epithelial cell proliferation and retinoblastoma protein phosphorylation,^{31,32} and protects clonogenic stem cells. Recent *in vitro* studies also demonstrated that IL-11 activates human endothelial cells to resist oxidant-mediated cellular injury,³³ inhibits T-cell IL-2 and IFN- γ production, and augments T-cell IL-10 elaboration.³⁴

In addition to its hematopoietic, mucosal, and osseous effects, IL-11 stimulates the proliferation of plasmacytoma cells, inhibits adipogenesis, regulates neuronal differentiation, regulates T-cell function, and stimulates hepatocyte production of acute phase proteins. The T-cell effects of IL-11 include the ability to stimulate B-cell immunoglobulin production via a T cell-dependent mechanism³⁵ and the ability to enhance T_H2 and inhibit T_H1 cytokine production in murine modeling systems.^{34,36} In contrast to IL-6, IL-11 is not a major autocrine growth factor in plasma cell dyscrasis, such as multiple myeloma.³⁷ The role of IL-11 in the *in vivo* acute phase response has also been questioned.³⁸

IL-11 IN THE LUNG

To define the role(s) of IL-11 in pulmonary homeostatic responses, early studies from our laboratory and others used *in vitro* approaches to determine which lung cells have the ability to produce this cytokine. At baseline, IL-11 mRNA was not detected or was barely detected in normal lung tissue and a variety of primary lung cells and

*The isoelectric point of IL-11 is 11.7.

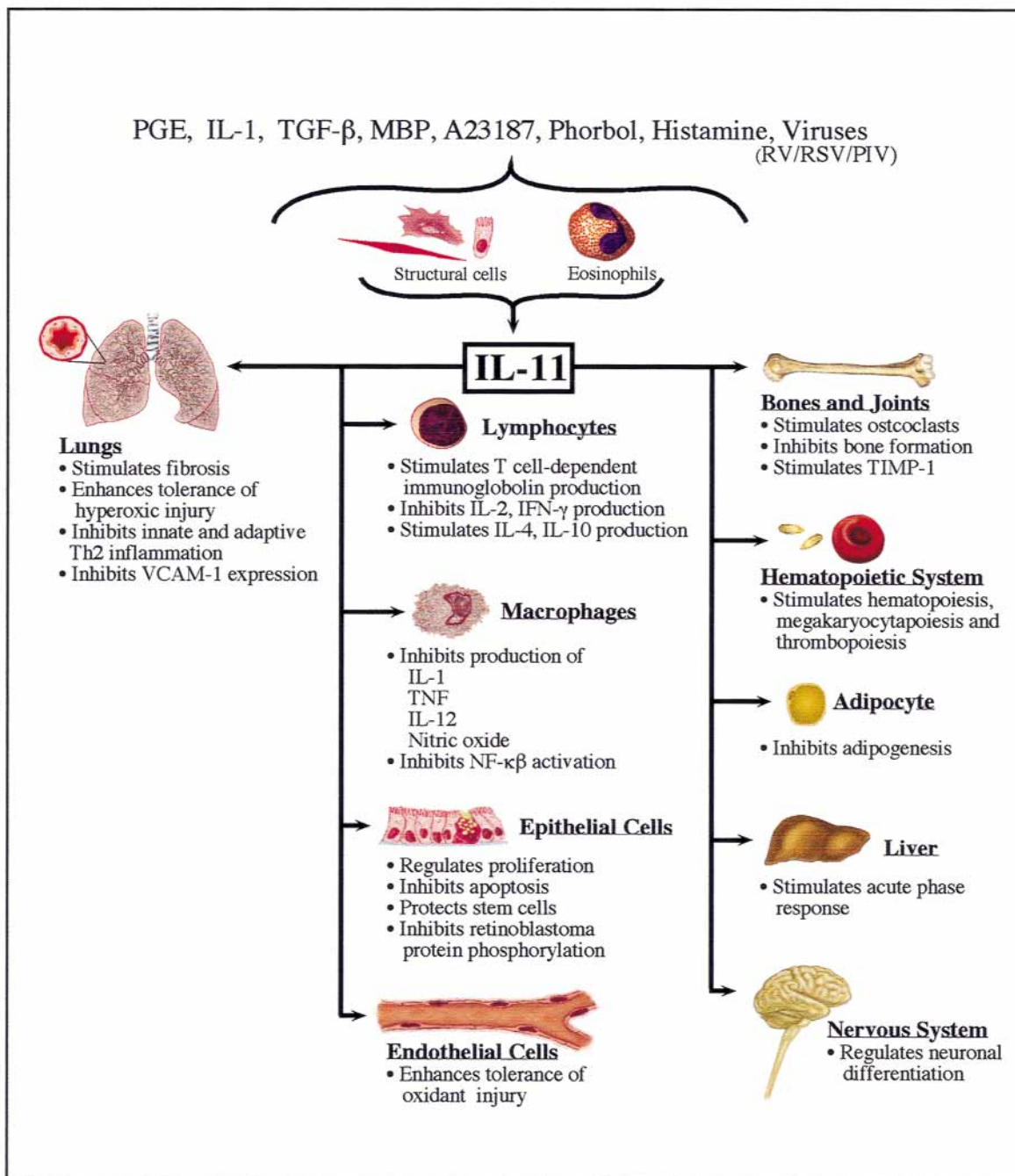


FIG 1. Schematic illustration shows the stimuli that induce IL-11 production, the cells that produce IL-11 in the lung, and the major effector functions of IL-11 in pulmonary and extrapulmonary tissues.

transformed lung cell lines. In contrast, appropriately stimulated fibroblasts, epithelial cells, human airway smooth muscle cells, and eosinophils were noted to produce and/or contain impressive quantities of this moiety.³⁹⁻⁴² A variety of stimuli were shown to induce IL-11 elaboration by these cells, including TGF- β_1 , TGF- β_2 , TGF- β_3 , IL-1, TNF, calcium ionophore, phorbol esters, and prostaglandin E₂.^{39-41,43} Histamine and eosinophil major basic proteins were also demonstrated to augment

cytokine-induced IL-11 elaboration.^{44,45} Surprisingly, the asthma-associated viruses, rhinovirus, respiratory syncytial virus, and parainfluenza virus type 3 also stimulated IL-11 elaboration.^{41,46} In accord with these findings, exaggerated levels of IL-11 were found in nasal secretions from children with viral upper respiratory tract infections in which the presence of IL-11 correlated with upper respiratory tract infection symptomatology and the presence of clinically detectable bronchospasm.⁴⁶

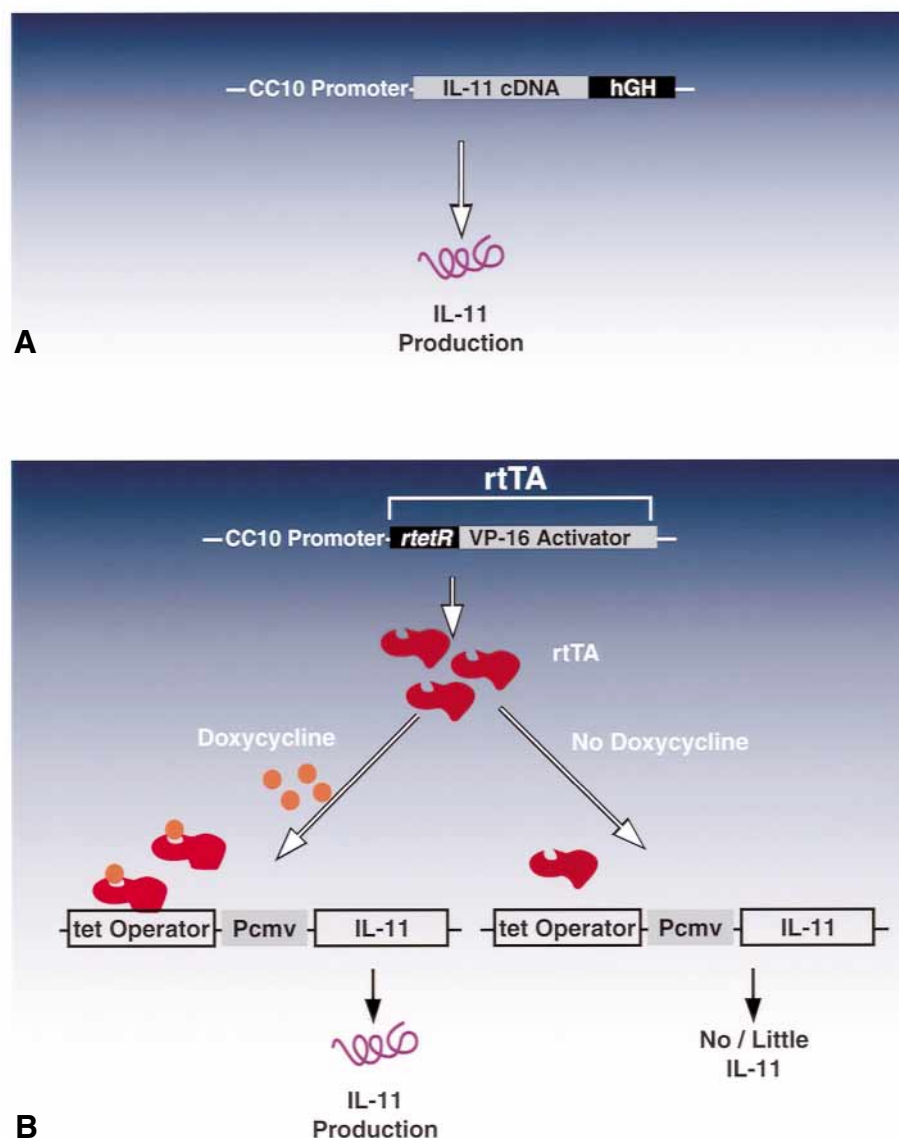


FIG 2. Components of the lung-targeted overexpression transgenic systems used in the investigation of IL-11. The single-construct constitutive system is presented in **A**; the dual-construct inducible system is presented in **B**.

OVEREXPRESSION TRANSGENIC MODELING OF IL-11

At the conclusion of the experiments noted above, experimentalists were faced with a dilemma that is characteristic of the post-genome era. They knew that the protein product of the gene in question (IL-11) was produced in large quantities by lung cells and was present in exaggerated amounts in secretions from patients with lung disease. However, the effector functions of the protein in the lung were completely undefined. To begin to define the effects of IL-11 in the lung, overexpression transgenic modeling was used. Both constitutive and externally regulatable (inducible) modeling systems were part of this effort. The advantages and disadvantages of these systems and the insights that they provided are outlined below.

Constitutive modeling

In these early studies, the Clara cell 10-kDa protein (CC10) promoter was used to target IL-11 to the lung. The CC10 promoter was chosen on the basis of its known propensity to express transgenes in airway Clara cells (and to a lesser extent alveolar type II cells) and the knowledge that Clara cells make up approximately 40% of the cells in the murine airway epithelium (versus 3% in human beings). In these systems, the CC10 promoter initiates gene transcription in utero and drives transgene expression in a constitutive fashion thereafter. To generate these (and the other mice described in this report), the requisite genetic constructs (Fig 2) were prepared, linearized, purified, and microinjected into the pronuclei of fertilized zygotes. The eggs were then placed in the uteri of pseudopregnant

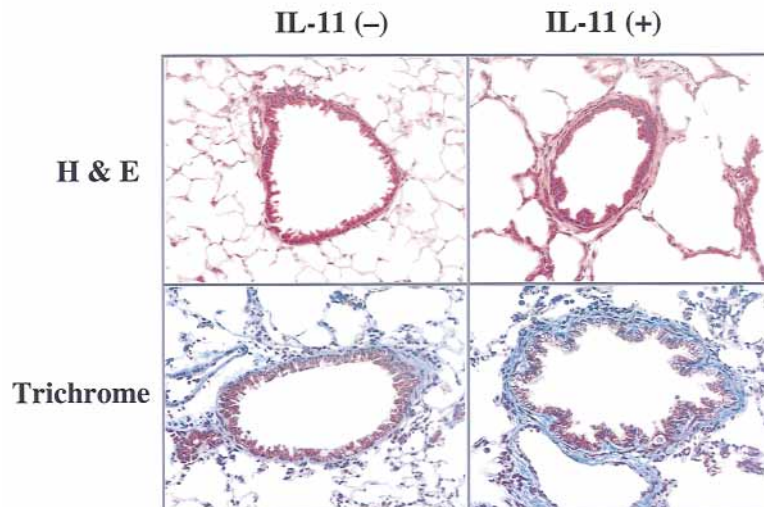


FIG 3. Histologic features of CC10-IL-11 transgenic mice. The photomicrographs compare transgene-negative and transgene-positive mice through use of hematoxylin-eosin and Trichrome evaluations. Airway fibrosis and enlarged alveoli are readily apparent.

female mice that were allowed to carry to term. Transgene-positive founder animals and transgene-negative littermate controls were identified through use of Southern and/or PCR analysis of DNA derived from tail biopsies, and the phenotypes of the animals were defined. When lungs from the transgene-positive CC10-IL-11 mice were compared with lungs from the transgene-negative littermate control animals, an interesting phenotype was appreciated in the former.⁴⁷ This phenotype included (1) nodular collections of peribronchiolar mononuclear cells, (2) subepithelial airway fibrosis, and (3) enlarged alveoli (Fig 3; some data not shown). The mononuclear nodules contained large numbers of B220-positive and MHC Class II-positive B cells and lesser numbers of CD3⁺, CD4⁺, and/or CD8⁺ T cells. They might represent exaggerated accumulations of bronchus-associated lymphoid tissue. The fibrotic response contained increased amounts of type III and type I collagen, increased numbers of α -smooth muscle actin- and desmin-containing cells, and a spectrum of structural elements, including fibroblasts, myofibroblasts, and smooth muscle cells. Physiologic abnormalities were also prominent in these animals; IL-11 transgene-positive animals manifested airways obstruction at baseline and airways hyperresponsiveness on methacholine challenge.⁴⁷ The airway remodeling response and the physiologic abnormalities that were noted were similar in many ways to the findings in human asthma.

Inducible overexpression transgenic modeling

The constitutive overexpression transgenic system described above has a number of limitations. Specifically, it is unable to accurately model waxing and waning disease processes such as asthma. In addition, it does not allow development-dependent and adult-onset responses to be differentiated. The latter may be particularly relevant to the large alveoli seen in the CC10-IL-11 trans-

gene-positive mice. It is well known that the normal mouse is born without true alveoli. Instead, it has large, sacklike alveolar structures and forms true alveoli from these sacks during the first month of life as a result of a developmental process that includes alveolar septation. Thus alveolar enlargement in a murine model can be caused by processes that block alveolar development (which is presumed to occur in bronchopulmonary dysplasia) or by processes that destroy otherwise normally formed alveoli (as in human emphysema). Because emphysema is not a routine consequence of chronic asthma, we reasoned that it was important to determine which processes were responsible for the enlarged alveoli in our CC10-IL-11 transgenic animals. To allow waxing and waning responses to be emulated and to allow the influences of lung development to be adequately assessed, we developed an inducible overexpression transgenic system that allowed us to control the timing of transgene expression.⁴⁸ This system is based on the generation of dual transgenic mice. As illustrated in Fig 2, the first construct in this system contains the CC10 promoter driving the expression of the reverse tetracycline transactivator (rtTA). The rtTA is a fusion protein made up of a mutated tetracycline repressor (tet-R) protein and the herpes virus VP-16 transactivator. tet-R proteins bind with high avidity to tetracycline operator (tet-O) sites. However, the tet-R in these constructs has been mutated in a fashion that allows it to bind to tet-O sites only in the presence of doxycycline. VP-16 is a powerful activator of gene transcription.

The second construct contains multimers of the tet-O, a minimal promoter, and the transgene of interest (IL-11). Under normal circumstances, the CC10 promoter targets rtTA to the lung. If the animal is receiving doxycycline (either in its drinking water or as a subcutaneous pellet), the rtet-R binds to the tet-O sequences, allowing VP-16 to activate the transgene. In the absence of doxy-

cycline, rTA is produced but does not bind or binds weakly to the tet-O. As a result, little or no transcription of the transgene is noted. Thus this transgenic system allows the investigator to induce high-level transgene expression by adding doxycycline to the animal's drinking water and to decrease transgene expression by withdrawing doxycycline at any point in time. In addition, because doxycycline readily crosses the placenta and is present in large quantities in breast milk, the system can also be used to activate transgene expression in utero or in neonatal (lactating) animals, respectively.

To investigate the processes that generated the enlarged alveoli in the CC10-IL-11 transgene-positive mice, we used the CC10-rTA system described above and compared the pulmonary effects of IL-11 when the IL-11 gene was activated in utero and when it was activated in the adult lung. When gene activation was initiated in utero and continued into adulthood, subepithelial airway fibrosis, peribronchiolar mononuclear nodules, and alveolar enlargement were noted. When IL-11 gene expression was delayed until the animal was >1 month old, subepithelial fibrosis and inflammatory nodules were noted; alveolar enlargement, however, was not appreciated. In contrast, induction in utero and for the first 14 days of life caused alveolar enlargement without airway remodeling or peribronchiolar nodules.⁴⁸ Thus subepithelial fibrosis and mononuclear nodules were seen whenever IL-11 was induced for an adequate period. In contrast, alveolar enlargement was seen only when IL-11 was induced during crucial stages of early lung development. When viewed in combination, these studies demonstrate that IL-11 causes development-dependent and development-independent phenotypes in the murine lung and that the alveolar enlargement in the CC10-IL-11 transgene-positive mice is not caused by an alveolar destructive process analogous to that seen in emphysema. They also demonstrate that IL-11 can generate airway remodeling in the absence of major alveolar abnormalities, which is analogous to the clinical picture that is seen in most patients with chronic asthma.

IL-11 IN HUMAN ASTHMA

The utility of an animal modeling system ultimately depends on the degree to which the paradigms that are operative in the model reflect events in human tissues. As a result of the studies noted above, we hypothesized that IL-11 plays an important role in the pathogenesis of the airway events in human asthma. To begin to test this hypothesis, we collaborated with Minshall et al⁴² in studies in which *in situ* hybridization and immunohistochemistry were used to evaluate the expression of IL-11 mRNA and the accumulation of IL-11 protein in biopsies from patients with mild, moderate, and severe asthma and normal controls. IL-11 protein and mRNA were not prominent features of the normal airway. In addition, IL-11 protein and mRNA were not found in exaggerated quantities in biopsies from patients with mild asthma. In contrast, heightened expression of IL-11 mRNA and

exaggerated accumulation of IL-11 protein were noted in epithelial cells and subepithelial eosinophils in tissues from patients with moderate and severe disease. When the patients with asthma in this study were looked at as a group, an impressive correlation was seen between the expression of IL-11 and disease severity. In addition, an impressive inverse correlation was noted between the number of cells expressing IL-11 and the FEV₁ in these patients.⁴² These studies demonstrate that IL-11 is selectively expressed in airways from patients with moderate and severe asthma with airway remodeling. They also highlight the impressive similarities between the airway phenotype of the CC10-IL-11 transgene-positive mice and the pathologic features in these human airway biopsies. Last, these studies illustrate how knowledge of the phenotype of the CC10-IL-11 transgene-positive mice helped us to decide which subpopulation of patients with asthma to study. Had our studies of human asthma been performed without knowledge of the remodeling phenotype induced by IL-11 in these animals, patients with mild disease would have been the first to be studied, a negative result would have been obtained, and we would have falsely concluded that IL-11 was not expressed in an abnormal fashion in asthmatic tissues.

EFFECTS OF IL-11 ON AIRWAY INFLAMMATION AND INJURY

The studies noted above demonstrate that IL-11 induces tissue fibrosis and is found in exaggerated quantities in airway biopsies from patients with asthma. Studies were next undertaken to determine whether this IL-11 was produced as a manifestation of disease pathogenesis and/or a manifestation of airway healing and repair. We reasoned that cytokines that are involved in healing and repair would simultaneously induce tissue fibrosis and the resolution of tissue inflammation (as seen with TGF- β). In contrast, we reasoned that cytokines involved in inducing a fibrotic response that represents an attempt to preserve organ integrity in the face of ongoing injury would induce tissue fibrosis without necessarily inhibiting ongoing inflammation. To gain insight into the role(s) of the IL-11 that is produced in the asthmatic airway, we characterized the effects of IL-11 in a standard murine asthma model that involves systemic sensitization and aerosol challenge with ovalbumin. This was done by comparing the ovalbumin-induced responses in IL-11 transgene-positive mice and wild-type littermate transgene-negative control animals. Lung-targeted IL-11 did not alter the ability of transgene-positive and -negative mice to be sensitized after ovalbumin exposure. IL-11 did, however, have a profound inhibitory effect on antigen-induced inflammatory responses in the lung. This inhibitory response was associated with marked diminution in eosinophil recruitment, T_H2 cell accumulation, T_H2 cytokine production, and antigen-induced endothelial cell VCAM-1 expression.⁴⁹ Interestingly, IL-11 did not alter ovalbumin-induced mucus metaplasia and did not augment T_H1 cytokine elaboration. These studies

demonstrate that IL-11 inhibits antigen-induced inflammation while inducing local tissue fibrosis. This suggests that IL-11 acts as a healing cytokine in the asthmatic airway and in other tissues. In accord with these observations, studies from our laboratory have also demonstrated that IL-11 has mucosal protective effects in hyperoxic acute lung injury³⁰ and thoracic irradiation.²⁸

SUMMARY

Our knowledge of IL-11 has progressed impressively. We started with a bioactivity and then used traditional cloning and protein expression approaches to characterize the structure of the IL-11 genes and the proteins that they encode. A variety of *in vivo* and *in vitro* approaches were subsequently used to investigate the effector profiles of these proteins and their role(s) in biology. Overexpression transgenic modeling was particularly important, because it demonstrated that IL-11 induces an airway remodeling response characterized by tissue fibrosis, deposition of types I and III collagen, and myocyte and myofibroblast hyperplasia. The studies also guided investigations using human tissues which validated the predictive nature of the overexpression transgenic models. Experimentation using the overexpression transgenic mice also provided insights into the role of IL-11 in the asthmatic airway by demonstrating that IL-11 inhibits inflammation while simultaneously generating tissue fibrosis. As a result of these studies, it is reasonable to hypothesize that IL-11 is an important mediator of the remodeling response in the asthmatic airway and that its elaboration reflects, at least in part, an attempt at healing and repair in this setting.

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REFERENCES

- Paul SR, Bennett F, Calvetti JA, Kelleher K, Wood CR, O'Hara RM, et al. Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc Natl Acad Sci U S A* 1990;87:7512-6.
- Morris JC, Neben S, Bennett F, Finnerty H, Long A, Beier DR, et al. Molecular cloning and characterization of murine interleukin-11. *Exp Hematol* 1996;24:1369-76.
- McKinley D, Wu Q, Yang-Feng T, Yang YC. Genomic sequence and chromosomal location of human interleukin-11 gene (IL11). *Genomics* 1992;13:814-9.
- Tang W, Yang L, Yang YC, Leng SX, Elias JA. Transforming growth factor-beta stimulates interleukin-11 transcription via complex activating protein-1-dependent pathways. *J Biol Chem* 1998;273:5506-13.
- Czupryn M, Bennett F, Dube J, Grant K, Scoble H, Sookdeo H, et al. Alanine-scanning mutagenesis of human interleukin-11: identification of regions important for biological activity. *Ann N Y Acad Sci* 1995;762:152-64.
- Bazan JF. Hemopoietic receptors and helical cytokines. *Immunol Today* 1990;11:350-4.
- Grotzinger J, Kernebeck T, Kallen KJ, Rose-John S. IL-6 type cytokine receptor complexes: hexamer, tetramer or both? *Biol Chem* 1999;380:803-13.
- Taga T. Gp130, a shared signal transducing receptor component for hematopoietic and neuropoietic cytokines. *J Neurochem* 1996;67:1-10.
- Zhang XG, Gu JJ, Lu ZY, Yasukawa K, Yancopoulos GD, Turner K, et al. Ciliary neurotrophic factor, interleukin 11, leukemia inhibitory factor, and oncostatin M are growth factors for human myeloma cell lines using the interleukin 6 signal transducer gp130. *J Exp Med* 1994;179:1337-42.
- Yang YC, Yin T. Interleukin (IL)-11-mediated signal transduction. *Ann N Y Acad Sci* 1995;762:31-40.
- Cherel M, Sorel M, Lebeau B, Dubois S, Moreau JF, Bataille R, et al. Molecular cloning of two isoforms of a receptor for the human hematopoietic cytokine interleukin-11. *Blood* 1995;86:2534-40.
- Yin T, Yasukawa K, Taga T, Kishimoto T, Yang YC. Identification of a 130-kilodalton tyrosine-phosphorylated protein induced by interleukin-11 as JAK2 tyrosine kinase, which associates with gp130 signal transducer. *Exp Hematol* 1994;22:467-72.
- Wang XY, Fuhrer DK, Marshall MS, Yang YC. Interleukin-11 induces complex formation of Grb2, Fyn, and JAK2 in 3T3L1 cells. *J Biol Chem* 1995;270:27999-8002.
- Luticken C, Wegenka UM, Yuan J, Buschmann J, Schindler C, Ziemiecki A, et al. Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science* 1994;263:89-92.
- Hemmman U, Gerhartz C, Heesel B, Sasse J, Kurapatk G, Grotzinger J, et al. Differential activation of acute phase response factor/Stat3 and Stat1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. II. Src homology SH2 domains define the specificity of stat factor activation. *J Biol Chem* 1996;271:12999-3007.
- Ihle JN. STATs: signal transducers and activators of transcription. *Cell* 1996;84:331-4.
- Akira S. IL-6-regulated transcription factors. *Int J Biochem Cell Biol* 1997;29:1401-18.
- Zhang X, Blenis J, Li HC, Schindler C, Chen-Kiang S. Requirement of serine phosphorylation for formation of STAT-promoter complexes. *Science* 1995;267:1990-4.
- Boulton TG, Zhong Z, Wen Z, Darnell JE, Stahl N, Yancopoulos GD. STAT3 activation by cytokines utilizing gp130 and related transducers involves a secondary modification requiring an H7-sensitive kinase. *Proc Natl Acad Sci U S A* 1995;92:6915-9.
- Adunyah SE, Spencer GC, Cooper RS, Rivero JA, Ceesay K. Interleukin-11 induces tyrosine phosphorylation, and c-jun and c-fos mRNA expression in human K562 and U937 cells. *Ann N Y Acad Sci* 1995;766:296-9.
- Yin T, Yang YC. Mitogen-activated protein kinases and ribosomal S6 protein kinases are involved in signaling pathways shared by interleukin-11, interleukin-6, leukemia inhibitory factor, and oncostatin M in mouse 3T3-L1 cells. *J Biol Chem* 1994;269:3731-8.
- Du X, Williams DA. Interleukin-11: review of molecular, cell biology, and clinical use. *Blood* 1997;89:3897-908.
- Elias JA, Tang W, Horowitz MC. Cytokine and hormonal stimulation of human osteosarcoma interleukin-11 production. *Endocrinology* 1995;136:489-98.
- Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J Bone Miner Res* 2000;15:2-12.
- Nakashima T, Kobayashi Y, Yamasaki S, Kawakami A, Eguchi K, Sasaki H, et al. Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. *Biochem Biophys Res Commun* 2000;275:768-75.
- Maier R, Ganu V, Lotz M. Interleukin-11, an inducible cytokine in human articular chondrocytes and synoviocytes, stimulates the production of the tissue inhibitor of metalloproteinases. *J Biol Chem* 1993;268:21527-32.
- Keith JC, Albert L, Sonis ST, Pfeiffer CJ, Schaub RG. IL-11, a pleiotropic cytokine: exciting new effects of IL-11 on gastrointestinal mucosal biology. *Stem Cells* 1994;12 Suppl 1:79-89; discussion 89-90.
- Redlich CA, Gao X, Rockwell S, Kelley M, Elias JA. IL-11 enhances survival and decreases TNF production after radiation-induced thoracic injury. *J Immunol* 1996;157:1705-10.
- Trepicchio WL, Bozza M, Pedneault G, Dornier AJ. Recombinant human IL-11 attenuates the inflammatory response through down-regulation of proinflammatory cytokine release and nitric oxide production. *J Immunol* 1996;157:3627-34.
- Waxman AB, Einarsson O, Seres T, Knickelbein RG, Warshaw JB, Johnston R, et al. Targeted lung expression of interleukin-11 enhances murine tolerance of 100% oxygen and diminishes hyperoxia-induced DNA fragmentation. *J Clin Invest* 1998;101:1970-82.
- Orazi A, Du X, Yang Z, Kashai M, Williams DA. Interleukin-11 prevents apoptosis and accelerates recovery of small intestinal mucosa in mice treated with combined chemotherapy and radiation. *Lab Invest* 1996;75:33-42.
- Peterson RL, Bozza MM, Dornier AJ. Interleukin-11 induces intestinal

- epithelial cell growth arrest through effects on retinoblastoma protein phosphorylation. *Am J Pathol* 1996;149:895-902.
33. Mahboubi K, Biedermann BC, Carroll JM, Pober JS. IL-11 activates human endothelial cells to resist immune-mediated injury. *J Immunol* 2000;164:3837-46.
 34. Bozza M, Bliss JL, Dorner AJ, Trepicchio WL. Interleukin-11 modulates th1/th2 cytokine production from activated cd4+ T cells. *J Interferon Cytokine Res* 2001;21:21-30.
 35. Yin TG, Schendel P, Yang YC. Enhancement of in vitro and in vivo antigen-specific antibody responses by interleukin 11. *J Exp Med* 1992;175:211-6.
 36. Hill GR, Cooke KR, Teshima T, Crawford JM, Keith JC, Brinson YS, et al. Interleukin-11 promotes T cell polarization and prevents acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Clin Invest* 1998;102:115-23.
 37. Paul SR, Barut BA, Bennett F, Cochran MA, Anderson KC. Lack of a role of interleukin 11 in the growth of multiple myeloma. *Leuk Res* 1992;16:247-52.
 38. Gabay C, Singwe M, Genin B, Meyer O, Mentha G, LeCoultré C, et al. Circulating levels of IL-11 and leukaemia inhibitory factor (LIF) do not significantly participate in the production of acute-phase proteins by the liver. *Clin Exp Immunol* 1996;105:260-5.
 39. Elias JA, Wu Y, Zheng T, Panettieri R. Cytokine- and virus-stimulated airway smooth muscle cells produce IL-11 and other IL-6-type cytokines. *Am J Physiol* 1997;273(3 Pt 1):L648-L655.
 40. Elias JA, Zheng T, Whiting NL, Trow TK, Merrill WW, Zitnik R, et al. IL-1 and transforming growth factor-beta regulation of fibroblast-derived IL-11. *J Immunol* 1994;152:2421-9.
 41. Elias JA, Zheng T, Einarsson O, Landry M, Trow T, Rebert N, et al. Epithelial interleukin-11. Regulation by cytokines, respiratory syncytial virus, and retinoic acid. *J Biol Chem* 1994;269:22261-8.
 42. Minshall E, Chakir J, Laviolette M, Molet S, Zhu Z, Olivenstein R, et al. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. *J Allergy Clin Immunol* 2000;105(2 Pt 1):232-8.
 43. Matsui S, Yamashita N, Mino T, Taki H, Sugiyama E, Hayashi R, et al. Role of the endogenous prostaglandin E2 in human lung fibroblast interleukin-11 production. *Respir Med* 1999;93:637-42.
 44. Zheng T, Nathanson MH, Elias JA. Histamine augments cytokine-stimulated IL-11 production by human lung fibroblasts. *J Immunol* 1994;153:4742-52.
 45. Rochester CL, Ackerman SJ, Zheng T, Elias JA. Eosinophil-fibroblast interactions. Granule major basic protein interacts with IL-1 and transforming growth factor-beta in the stimulation of lung fibroblast IL-6-type cytokine production. *J Immunol* 1996;156:4449-56.
 46. Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: stimulation in vivo and in vitro by respiratory viruses and induction of airways hyperresponsiveness. *J Clin Invest* 1996;97:915-24.
 47. Tang W, Geba GP, Zheng T, Ray P, Homer RJ, Kuhn C, et al. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. *J Clin Invest* 1996;98:2845-53.
 48. Ray P, Tang W, Wang P, Homer R, Kuhn C, Flavell RA, et al. Regulated overexpression of interleukin 11 in the lung. Use to dissociate development-dependent and -independent phenotypes. *J Clin Invest* 1997;100:2501-11.
 49. Wang J, Homer RJ, Hong L, Cohn L, Lee CG, Jung S, et al. IL-11 selectively inhibits aeroallergen-induced pulmonary eosinophilia and Th2 cytokine production. *J Immunol* 2000;165:2222-31.