

CD4 T-helper cells engineered to produce IL-10 prevent allergen-induced airway hyperreactivity and inflammation

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Background: T_H2 cells play a critical role in the pathogenesis of asthma, but the precise immunologic mechanisms that inhibit T_H2 cell function *in vivo* are not well understood. **Objective:** The purpose of our studies was to determine whether T cells producing IL-10 regulate the development of asthma.

Methods: We used gene therapy to generate ovalbumin-specific CD4 T-helper cells to express IL-10, and we examined their capacity to regulate allergen-induced airway hyperreactivity. **Results:** We demonstrated that the CD4 T-helper cells engineered to express IL-10 abolished airway hyperreactivity and airway eosinophilia in BALB/c mice sensitized and challenged with ovalbumin and in SCID mice reconstituted with ovalbumin-specific T_H2 effector cells. The inhibitory effect of the IL-10-secreting T-helper cells was accompanied by the presence of increased quantities of IL-10 in the bronchoalveolar lavage fluid, was antigen-specific, and was reversed by neutralization of IL-10. Moreover, neutralization of IL-10 by administration of anti-IL-10 mAb in mice sensitized and challenged with ovalbumin seriously exacerbated airway hyperreactivity and airway inflammation.

Conclusion: Our results demonstrate that T cells secreting IL-10 in the respiratory mucosa can indeed regulate T_H2-induced airway hyperreactivity and inflammation, and they strongly suggest that IL-10 plays an important inhibitory role in allergic asthma. (*J Allergy Clin Immunol* 2002;110:460-8.)

Key words: IL-10, T_H2 cells, airway hyperreactivity, asthma, regulatory cells

Asthma is characterized by airway hyperreactivity (AHR) to a variety of specific and nonspecific stimuli, by chronic airway inflammation with pulmonary eosinophilia, by mucus hypersecretion, and by increased serum IgE levels. The pathology in asthma occurs as a consequence

Abbreviations used

AHR: Airway hyperreactivity
BAL: Bronchoalveolar lavage
KLH: Keyhole limpet hemocyanin
OVA: Ovalbumin
Penh: Enhanced pause
TCR: T-cell receptor
YFP: Yellow fluorescent protein

of excessive production of IL-4, IL-5, and IL-13 by CD4 T_H2 cells.^{1,2} Although the immunologic mechanisms that induce asthma and allergy are relatively well characterized, the specific mechanisms that transpire *in vivo* to downmodulate T_H2 cell-mediated allergic inflammatory responses are not yet clear. T_H1 cells, which secrete IFN- γ , have been proposed to protect against asthma and allergic disease by dampening the activity of T_H2 effector cells³; however, the evidence for this is problematic and is based on data generated in infectious disease models.^{4,5} In a system accentuating pulmonary inflammation and AHR, we recently demonstrated that whereas adoptively transferred, antigen-specific T_H1 cells reduced airway mucus production and eosinophilia, T_H1 cells failed to inhibit T_H2 cell-induced AHR and inflammation, even when the T_H1 cells were given in great excess.⁶ We therefore hypothesized that other cell types and cytokines must be involved in downregulating T_H2-driven immune responses in allergic asthma.

Because IL-10, a pleiotropic cytokine with significant anti-inflammatory and immunomodulatory properties, is a key regulator in the maintenance of immunologic homeostasis, we asked whether IL-10 might play an important role in regulating pulmonary inflammation and asthma. IL-10 inhibits MHC class II expression and macrophage activation and might induce T-cell anergy by enhancing the suppressive effects of TGF- β .⁷ Mice deficient in IL-10 develop chronic enterocolitis with aberrant expression of MHC class II molecules on epithelia,⁸ and administration of IL-10 inhibits the development of inflammatory diseases such as experimental allergic encephalomyelopathy.^{9,10} However, the role of IL-10 in regulating T_H2-mediated diseases such as asthma is controversial, particularly given that IL-10 can enhance and is associated with T_H2-biased immune responses.¹¹

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To study the downmodulatory role of IL-10 at the effector phase of allergen-induced AHR and asthma, we evaluated the effects of IL-10 under conditions in which robust T_H2 responses were already established. We generated CD4 T cells secreting high levels of IL-10 by transducing ovalbumin (OVA)–specific T cells with a retrovirus vector containing the cDNA for IL-10; we also assessed the capacity of these IL-10–secreting T cells to downregulate established allergic inflammation and AHR in mouse models of asthma.

METHODS

Animals

BALB/cBy mice were obtained from the Jackson Laboratory (Bar Harbor, Me), and histocompatible C.B-17Icr^{scid/scid} mice were obtained from the Stanford Medical Center Division of Laboratory Animal Medicine (Stanford, Calif). DO11.10 mice, which are transgenic for T-cell receptor (TCR)–recognizing OVA peptide 323–339 (pOVA 323–339) and backcrossed to BALB/c, were kindly provided by Dennis Loh and were bred in our facilities. The Stanford University Committee on Animal Welfare approved all animal protocols.

Monoclonal antibodies

We used the following mAbs, produced from the hybridomas: R46A2 (anti-IFN- γ mAb), obtained from American Type Culture Collection (Rockville, Md); 11B11 (anti-IL-4 mAb), generously provided by William Paul (National Institutes of Health, Bethesda, Md); XMG1.2 (anti-IFN- γ mAb) and TRFK-4 and TRFK-5 (anti-IL-5 mAbs), generously provided by Tim Mosmann (University of Rochester, Rochester, NY); BVD4-1D11 and BVD6-24G2 (anti-IL-4 mAbs), generously provided by M. Howard (Corixa, Redwood City, Calif.); C17.8 (anti-IL-12 mAb), generously provided by G. Trinchieri (Schering-Plough, Kenilworth, NJ); anti-clonotypic antibody KJ1-26.1, generously provided by Philippa Marrack (National Jewish Medical Center, Denver, Colo); and JES5-2A5 (anti-IL10 mAb), generously provided by Dr John Abrams (DNAX Research Institute, Palo Alto, Calif). Anti-38C13 Id mAb 4G10 (rat IgG2a) was generously provided by S. Levy (Stanford University) and was used as an isotype control.

Preparation of OVA-specific T_H2 cells

OVA-specific T_H2 lines were generated from spleen and lymph node cells from DO11.10 (OVA TCR Tg) mice, as described previously.⁶ By direct examination of supernatants generated from the T_H2 cell lines, the cytokine profiles of these lines were confirmed through use of ELISA for IL-4, IL-5, and IFN- γ , as described previously.^{12,13}

Establishment of an OVA-specific CD4 T-cell line producing IL-10 through use of gene transfer: Generation of retrovirus and CD4 T-cell transduction

The retroviral vector, termed *pGCIREs*, was constructed as previously described.¹⁴ This is a constitutive Moloney murine leukemia virus–based MFG vector. For these studies, the open reading frame of the murine IL-10 gene, consisting of 536 bp (Genbank accession number: M37897) was subcloned upstream of encephalomyocarditis virus internal ribosome entry site (EMCVIRES) and yellow fluorescent protein (YFP; Clontech Laboratories, Palo Alto, Calif). The empty vector contains the multiple cloning site, EMCVIRES, and YFP. Virus supernatant was generated and CD4 T cells were transduced as previously described.¹⁴

Flow cytometry

Murine cells transduced with recombinant retrovirus cells were stained with rat antimouse CD4-PE (GK1.5, BD Pharmingen, San Jose, Calif). Dead cells were excluded through use of propidium iodide staining (Sigma, St Louis, Mo), and live cells were analyzed for CD4-PE (PE channel) and YFP reporter expression (FITC channel). Analysis was performed on a FAC-Scan cytometer (Becton Dickinson, Mountain View, Calif). For T-cell sorting, cells were stained with CD4-PE and sterile-sorted into CD4 populations through use of a modified FACStar flow cytometer (BD, Stanford FACS Facility, Stanford University). Sorted sample populations were reanalyzed after the initial collection to confirm sample fraction purity and CD4 expression (>95%). All data were analyzed through use of FlowJo software (Tree Star, San Carlos, Calif).

Adoptive transfer of T cells into SCID mice

T_H2 cell lines (2.5×10^6 cells per mouse) were transferred intravenously into histocompatible SCID mice, as described previously.⁶ To facilitate homing of the T-cell lines to the lungs, mice were given OVA, 50 μ g in 50 μ L of PBS administered intranasally, 1 day before intravenous transfer of cells. To facilitate pulmonary aspiration of OVA, mice were anesthetized intraperitoneally with 0.25 to 0.35 mL of ketamine (0.44 mg/mL)/xylazine (6.3 mg/mL) in normal saline solution. Seventy-five percent of the intranasally administered antigen could be detected subsequently in the lung.¹⁵

Adoptive transfer of T cells into immunocompetent, allergen-sensitized mice

BALB/c mice were sensitized by intraperitoneal immunization with OVA or keyhole limpet hemocyanin (KLH, CalBiochem, San Diego, Calif) (50 μ g) complexed with aluminum potassium sulfate (alum) on day 0. Mice then received either IL-10 cDNA–transduced cells or vector-only–transduced control cells intravenously (2.5×10^6 cells/mouse) on day 7. To induce AHR, the mice were challenged intranasally on days 7, 8, and 9 with OVA or KLH (50 μ g in 50 μ L of PBS) after administration of light anesthesia. Control mice received intraperitoneal injections of alum alone and also received PBS intranasally. AHR to inhaled methacholine was measured 24 hours after the last intranasal dose of OVA (on day 10). Bronchoalveolar lavage (BAL) and lung fixation were performed on the following day (day 11). In some experiments, mice received anti-IL-10 mAb (JES5-2A5, 500 μ g/mouse, administered intraperitoneally) or control mAb 3 or 4 days after OVA sensitization and again 1 day before challenge with intranasal OVA (day 6).

Measurement of airway responsiveness

Airway responsiveness was assessed by methacholine-induced airflow obstruction from conscious mice placed in a whole-body plethysmograph (model PLY 3211; Buxco Electronics, Troy, NY), as described previously.⁶ Results were expressed for each methacholine concentration as the percentages of baseline enhanced pause (Penh) values after NaCl exposure.

Collection of BAL fluid and lung histology

Each mouse was injected intraperitoneally with a lethal dose of phenobarbital (450 mg/kg), as described previously.⁶ After ligation of the left main stem bronchus, the trachea was cannulated; the right lung was then lavaged with 0.4 mL of serum-free medium 3 times and the fluid was pooled. Cells in the lavage fluid were counted, and BAL cell differentials were determined on slide preparations stained with Hansel stain (Lide Laboratories, Florissant, Mo). At least 200 cells were differentiated by light microscopy on the basis

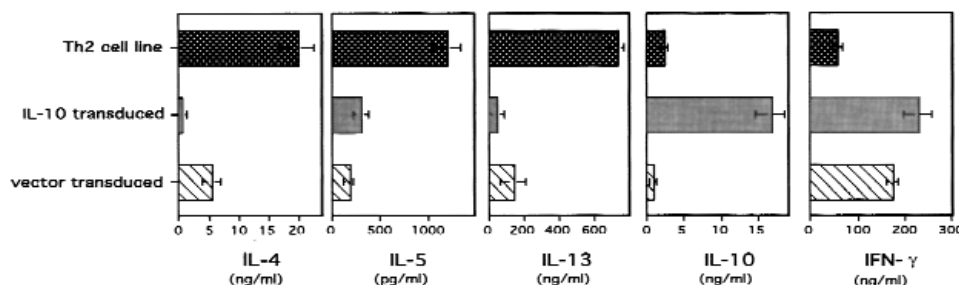


FIG 1. Cytokine profiles of cell lines used in these studies. T-cell lines were generated from DO11.10 mice, as discussed in the Materials and Methods section. OVA-specific T_H2 cells, IL-10 cDNA-transduced cells, and vector-transduced control cells were stimulated with concanavalin A ($1 \mu\text{g}/\text{mL}$) for 18 hours in Dulbecco modified Eagle medium containing 10% FCS. Supernatants were collected and analyzed by ELISA for IL-4, IL-5, IL-13, IL-10, and IFN- γ . Results represent means $\pm$ SDs.

of conventional morphologic criteria. The left lung was inflated and fixed in 10% formalin, routinely processed, and embedded in paraffin wax. Five-micrometer sections were prepared and stained with hematoxylin and eosin.

RESULTS

Characterization of T-helper cell lines

OVA-specific T_H2 cell lines were generated by in vitro stimulation of spleen cells from OVA-specific TCR DO11.10 transgenic mice, as described previously.⁶ The T_H2 lines were used after at least 3 cycles of stimulation once the T-cell lines had achieved a stable committed phenotype.¹⁶ The cytokine profiles of the T_H2 cell lines and IL-10-transduced cell lines are shown in Fig 1. The T_H2 cell lines produced high levels of IL-4, IL-5, and IL-13 but very low levels of IFN- γ , whereas the IL-10-transduced cell lines produced large quantities of IL-10 as well as IFN- γ on antigen stimulation.

IL-10-transduced CD4 T cells inhibit AHR induced by T_H2 cells

To determine the effects of IL-10-transduced CD4 T cells on T_H2 cell-induced AHR and inflammation, we adoptively transferred the different OVA-specific cell lines into histocompatible, immunodeficient SCID mice using an established protocol.^{6,17} By choosing SCID mice, we excluded the contribution of endogenous lymphocytes, which could inhibit the function of transferred T-helper cells or contribute to the development of AHR. Localization of the transferred T-cell lines into the lungs was enhanced by intranasal administration of OVA 1 day before adoptive transfer of the T-cell lines. One and 2 days later, OVA was again administered intranasally. Immunohistochemical staining of lung sections with the anti-TCR clonotypic antibody KJ1.26.1 confirmed that the transferred cell line homed to the lungs of the recipient.⁶

Adoptive transfer of OVA-specific T_H2 cells resulted in significant AHR, as assessed by responsiveness to inhaled methacholine (Fig 2, A). Cotransfer of OVA-specific IL-10-transduced CD4 T cells inhibited T_H2 cell-induced AHR. The inhibitory effect of the OVA-

specific IL-10-transduced CD4 T cells was dependent on the presence of IL-10 because administration of a neutralizing mAb (hybridoma cell: JES5-2A5), specific for active IL-10, abolished the protective effect of IL-10-transduced CD4 T cells against T_H2 cell-induced AHR (Fig 2, B). The IgG2a isotype control antibody (4G10) had no effect in mice receiving OVA-specific T_H2 cells and IL-10-transduced cells. The inhibitory effect of IL-10-transduced CD4 T cells on T_H2 cell-induced AHR was dose-dependent, such that the maximal inhibitory effect of the IL-10-transduced cells occurred when 2.0×10^6 T cells were transferred (Fig 3).

Examination of BAL fluid from mice that received the IL-10-producing T cells demonstrated that IL-10 was present in the lungs. BAL fluid from SCID mice that received both IL-10-transduced T-helper cells and T_H2 cells contained significant levels of active IL-10 (100 pg/mL) and had a 12-fold reduction in eosinophils in comparison with mice receiving T_H2 cells alone. The inhibitory effect of the IL-10-producing cells was dependent on IL-10 production, inasmuch as administration of an anti-IL-10 mAb reversed the reduction of eosinophils in the airways. The increased IL-10 levels in the BAL fluid of mice that received the IL-10-producing cells were associated with reduced levels of IL-4, IL-5, and IL-13 as well as of IFN- γ ; this indicated that the transferred IL-10-producing cells functioned in the lungs and that IL-10 production in the airways inhibited the pulmonary T_H2 -biased response. BAL fluid from SCID mice that received only T_H2 cells contained low levels of active IL-10 (35 pg/mL).

IL-10-transduced CD4 T cells inhibit AHR in immunocompetent BALB/c mice

Although the IL-10-transduced T cells reversed the function of established T_H2 effector cells when adoptively transferred into SCID mice, the mechanisms involved in the induction of AHR by adoptively transferred T_H2 cells might not be the same as those observed during sensitization and challenge of normal mice with allergen. Therefore, we wished to determine whether IL-10-

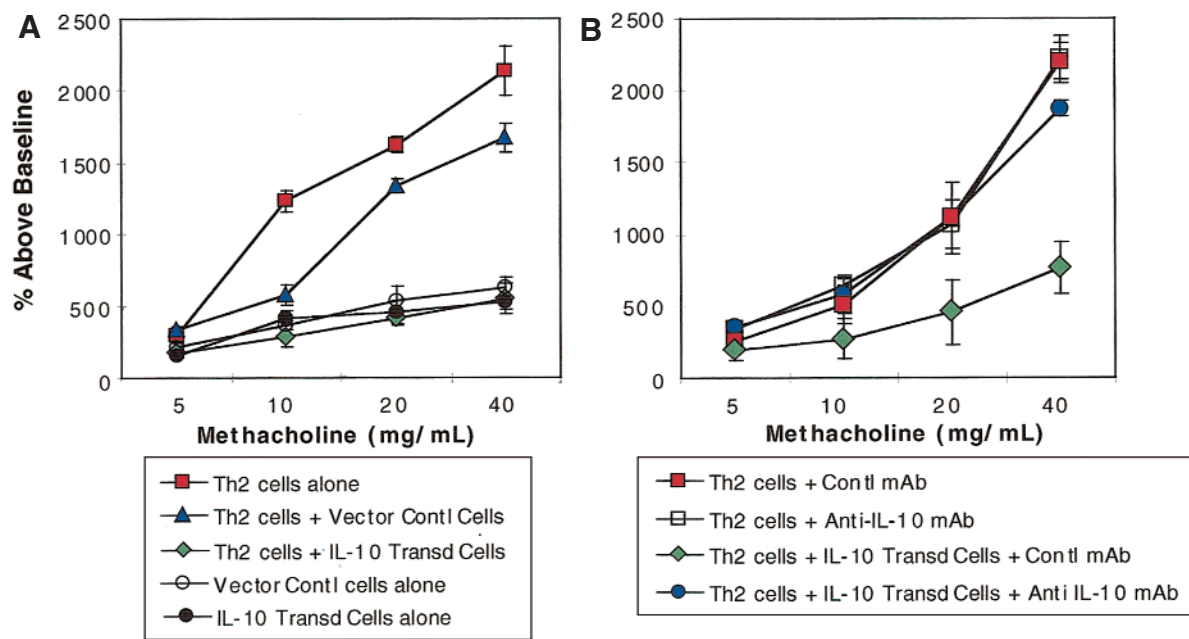


FIG 2. A, IL-10-transduced cells inhibit Th₂-induced AHR in SCID mice. SCID mice received OVA-specific Th₂ (2.5×10^6 cells/mouse), IL-10 cDNA-transduced (2.0×10^6 cells/mouse), or vector-transduced control cells intravenously. OVA was administered intranasally 18 hours before cell transfer and again 24 and 48 hours after cell transfer; AHR was measured 96 hours after cell transfer. **B,** Anti-IL-10 mAb abolishes the inhibitory effect of the IL-10-transduced cells. Neutralizing mAb JES5-2A5 specific for IL-10 (500 μ g/mouse) or control mAb 4G10 was given intraperitoneally to SCID mice 24 hours before adoptive cell transfer. SCID mice received either a mixture of IL-10 cDNA-transduced cells and OVA-specific Th₂ cells intravenously or Th₂ cells alone. OVA was administered intranasally 6 hours before cell transfer and again 24 and 48 hours after cell transfer, and AHR was measured 96 hours after cell transfer. Results are provided as mean percentages ($\pm$ SEMs) above baseline of Penh.

transduced T cells could function in normal immunocompetent mice and inhibit the development of Th₂-driven allergen-induced AHR. Fig 4 shows that intraperitoneal sensitization followed by intranasal challenge with OVA of BALB/c mice resulted in the development of significant AHR. Adoptive transfer of OVA-specific IL-10-transduced T-helper cells significantly reduced this AHR. The effect was antigen-specific, because OVA-specific IL-10-transduced cells failed to reduce AHR induced in BALB/c mice immunized with KLH rather than OVA. These results demonstrated that the IL-10-producing T cells inhibited AHR in normal mice after sensitization and challenge with OVA in a standard model of allergen-induced AHR.

Neutralization of IL-10 in OVA-sensitized mice increases AHR and IL-4 production

To demonstrate that IL-10 was indeed involved in regulating the development of AHR in normal immunocompetent mice, we examined the effects of neutralizing IL-10 with an anti-IL-10 mAb in the development of allergen-induced AHR. In control mice sensitized and challenged with OVA, AHR developed as expected. Fig 5, A shows that treatment of mice with anti-IL-10 mAb

4 days after sensitization and 1 day before intranasal OVA challenge greatly increased AHR. In addition, treatment with anti-IL-10 mAb increased the production of IL-4 in T cells from the bronchial lymph nodes (Fig 5, B) and was associated with increased airway inflammation (Fig 6, A and C). Treatment of naive mice with anti-IL-10 mAb had no effect (Fig 6, B and D). This indicated that IL-10 normally functions to inhibit AHR and inflammation and that in its absence AHR and airway inflammation worsen.

DISCUSSION

In this study, we demonstrated in a murine model of asthma that CD4 T cells engineered to produce IL-10 effectively inhibited the capacity of Th₂ effector cells to induce AHR and airway inflammation. The function of IL-10-producing cells was evaluated by adoptive transfer of well-defined antigen-specific T cells expressing identical TCRs into lymphocyte-deficient SCID mice. Because the recipient SCID mice had no T cells of their own, we can conclude that the transferred IL-10-producing T cells, and not other T cells, directly inhibited AHR and inflammation. The effect of IL-10-producing T cells

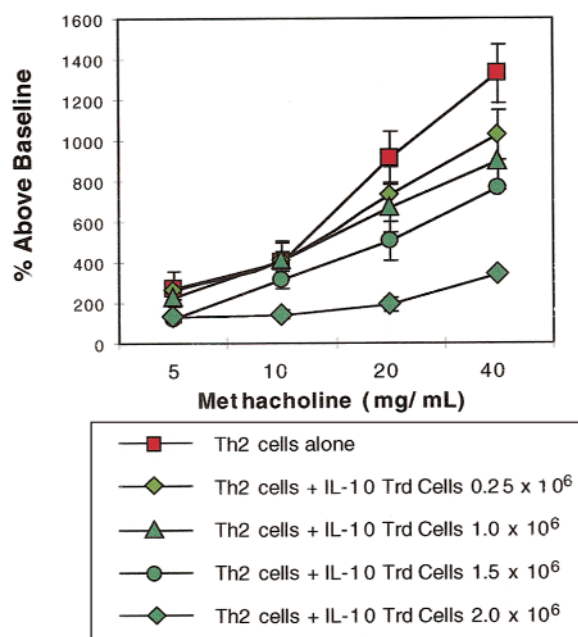


FIG 3. Dose-dependent inhibition of T_H2 -induced AHR by IL-10-transduced cells. SCID mice received OVA-specific T_H2 (2.5×10^6 cells/mouse) with or without IL-10 cDNA-transduced cells (0.25 to 2.0×10^6 cells/mouse). Results are expressed as mean percentages ($\pm$ SEMs) above baseline of Penh. Each group represents the mean results from 4 to 9 mice.

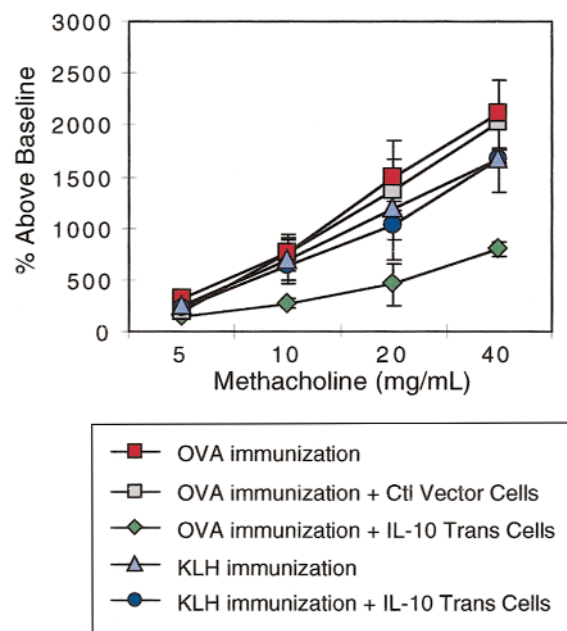


FIG 4. OVA-specific IL-10-transduced cells inhibit the development of AHR in immunocompetent BALB/c mice in an antigen-specific manner. Immunocompetent BALB/c mice were sensitized and challenged with KLH or OVA in a standard fashion to induce AHR. In this protocol, mice were sensitized with antigen in alum on day 0. Seven days later, some mice received IL-10 cDNA-transduced cells or vector-transduced control cells intravenously (2.5×10^6 cells/mouse on day 7). All of the mice were challenged intranasally on days 7, 8, and 9 with OVA or KLH ($50 \mu\text{g}$ in $50 \mu\text{L}$ of PBS). AHR was measured 24 hours after the last intranasal dose of OVA (on day 10). Data are expressed as mean percentages ($\pm$ SEMs) above baseline of Penh.

was antigen-specific and was dependent on secretion of IL-10, inasmuch as a neutralizing mAb to IL-10 abolished the inhibitory effect. Moreover, the inhibitory effect was also observed when the IL-10-producing T cells were transferred into OVA-immunized immunocompetent BALB/c mice, establishing that such cells not only can inhibit the function of effector T_H2 cells but also can prevent the development of AHR in immunocompetent animals. Finally, we showed that neutralization of IL-10 in immunocompetent BALB/c mice sensitized and challenged with allergen seriously exacerbated the development of AHR and inflammation. These results strongly suggest that IL-10-producing T cells play a major inhibitory-immunoregulatory role in asthma.

Our studies directly show that T cells producing IL-10 inhibit the development of AHR and asthma. In contrast, the conventional wisdom indicates that T_H1 cells, by inhibiting the function of T_H2 cells, are the primary cell type that provides protective immunity in asthma. The idea that T_H1 cells are beneficial in allergy and asthma is supported by indirect evidence that is generally based on data developed in infectious disease models, indicating that T_H1 cells mediate resistance whereas T_H2 cells mediate susceptibility to infection.^{4,5,18-20} However, direct examination of the role of antigen-specific T_H1 effector cells in asthma demonstrated that although T_H1 cells were able to reduce mucus production and airway

eosinophilia, they failed to inhibit T_H2 cell-induced AHR and in fact exacerbated airway inflammation.^{6,21,22}

These studies suggest that other forms of immunity might be effective in downmodulating inflammation in asthma. For example, we previously showed that T cells secreting TGF- β , generated with retroviral vectors containing the cDNA for TGF- β ,¹⁷ or T-cell tolerance induced by intranasal exposure to antigen,²³ was very effective in downmodulating inflammation and AHR in asthma. We now demonstrate that IL-10-secreting T cells, generated by IL-10 gene transfer, potentially inhibit the development of airway inflammation and AHR.

The role of IL-10 in limiting airway inflammation and AHR has been controversial, and IL-10 has been shown to be either increased or decreased in the lungs of patients with asthma.^{24,25} IL-10 has been considered to be an essential T_H2 cytokine,²⁶ particularly because IL-10 inhibits T_H1 cytokine production by inhibiting IL-12 synthesis²⁷ and B7 expression.²⁸ In addition, IL-10 plays an important role in inhibiting T_H1 -mediated autoimmune diseases such as experimental allergic encephalomyelitis (a demyelinating disease of the central nervous system widely used as an animal model for multiple sclerosis²⁹),

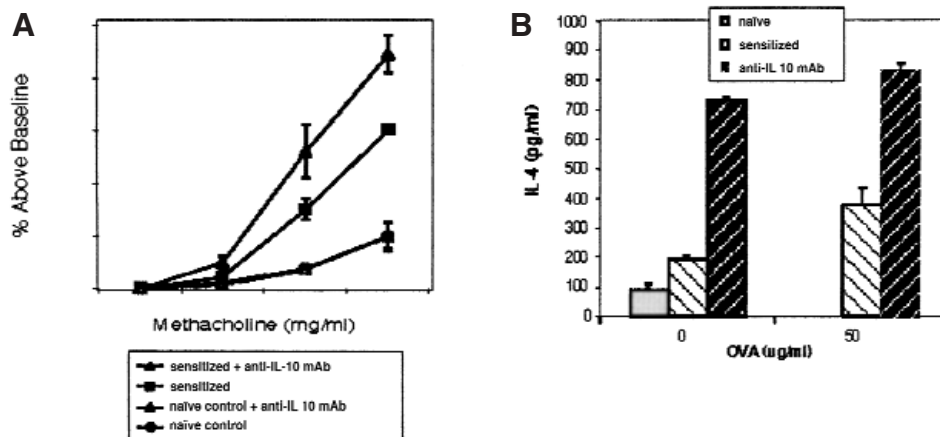


FIG 5. Treatment with anti-IL-10 mAb exacerbates AHR and increases IL-4 production in mice sensitized and challenged with OVA. **A**, BALB/c mice were sensitized with OVA in alum intraperitoneally on day 9 and challenged with OVA (50 µg intranasally $\times$ 3) on days 7, 8, and 9. Some mice received anti-IL-10 mAb (n = 9) or isotype control (n = 10) (500 µg intraperitoneally) 4 days after sensitization and again 1 day before intranasal challenge. Data are expressed as mean percentages ($\pm$ SEMs) above baseline of Penh and are representative of 3 experiments. **B**, Treatment with anti-IL-10 mAb increases IL-4 production. Bronchial lymph node T cells from mice sensitized and challenged with OVA were cultured at 5×10^5 cells/well with 50 µg/mL OVA. IL-4 in culture supernatants was determined after 4 days of culture. Data represent means of triplicate cytokine determinations $\pm$ SDs (5 mice/group). Representative results from one of 2 experiments are presented.

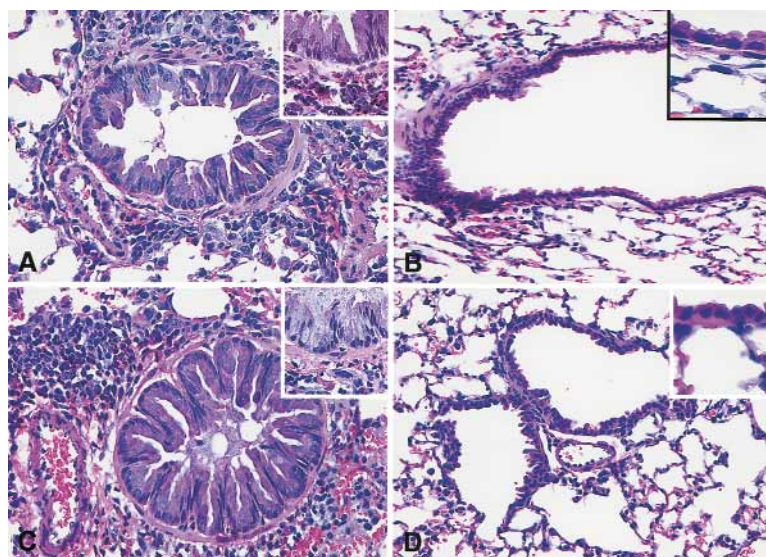


FIG 6. Airway inflammation is increased in mice treated with anti-IL-10 mAb. **A**, Airway inflammation is present in lung tissue from a mouse sensitized intraperitoneally and challenged with intranasal OVA. Lung tissue was removed on day 11. A peribronchiolar mixed inflammatory infiltrate of lymphocytes and eosinophils is present in the peribronchiolar space (hematoxylin-eosin; $\times$ 250). *Insert*: High-power magnification of bronchiolar lining cells shows abundant intracytoplasmic mucin and eosinophils in the peribronchiolar space (hematoxylin-eosin; $\times$ 400). **B**, Nonsensitized (naive) mouse show normal airway histology (hematoxylin-eosin; $\times$ 250). *Insert*: Bronchioles are lined by low cuboidal epithelium (hematoxylin-eosin; $\times$ 600). **C**, Marked airway inflammation is seen in lung tissue of a mouse sensitized and challenged with OVA and treated with anti-IL-10 mAb on day 4 and day 6. The airway lumen is filled and expanded with mucus secretions, and an intense peribronchiolar infiltrate is present (hematoxylin-eosin; $\times$ 250). *Insert*: High-power magnification of the airway epithelium shows abundant luminal and intracytoplasmic mucin in tall columnar epithelial lining cells as well as eosinophils in the peribronchiolar space (hematoxylin-eosin; $\times$ 400). **D**, Lung tissue from a nonsensitized (naive) mouse receiving anti-IL-10 mAb shows normal airway histology (hematoxylin-eosin; $\times$ 250). *Insert*: High-power magnification shows bronchiolar lining cells composed of low cuboidal cells that are devoid of inflammation and intracytoplasmic mucin (hematoxylin-eosin; $\times$ 600).

suggesting that IL-10 might contribute to the pathogenesis of allergic disease. Indeed, administration of IL-10 enhanced AHR or was required for the development of AHR, though it reduced eosinophilia.³⁰⁻³²

However, IL-10 might play several roles in asthma, not only performing a critical function in initiating the development of T_H2 polarized responses but also playing an important regulatory role late during immune responses, by downmodulating T_H2-driven inflammation. Thus, administration of IL-10 cDNA into the lungs of mice via an adenovirus vector inhibited AHR that was induced in the presence of GM-CSF,³³ and IL-10^{-/-} mice developed elevated baseline airway reactivity.³⁴ Furthermore, in a model of allergic lung disease using the antigen *Aspergillus fumigatus*, investigators showed that IL-10^{-/-} mice exhibited exaggerated airway inflammation,³⁵ which suggests that IL-10 potently suppressed inflammatory T_H2-like pulmonary responses. The conflicting results, showing both pro-inflammatory and anti-inflammatory roles for IL-10 in asthma, on one hand, might reflect the pleotropic nature of IL-10, by virtue of which it could enhance the initial development of T_H2 responses but also inhibit established T_H2 biased responses and airway inflammation. On the other hand, the conflicting results might indicate that the effect of IL-10 depends on the amount of IL-10 produced; lower levels of IL-10 might enhance T_H2-mediated inflammation and higher levels of IL-10, as produced by our transduced cells, might inhibit AHR.

Alternatively, the conflicting results might reflect unusual methods of inducing AHR (with GM-CSF)³³ or the use of strains of mice (C57BL/6) that do not easily develop AHR.³¹ Our studies, which focus on the effector phase of asthma, definitively demonstrate an immunoregulatory-suppressive role for IL-10 in asthma, using well-defined T cells producing IL-10 (rather than an IL-10 cDNA-containing adenovirus, as in studies conducted by Stampfli et al³³), using a classical antigen (OVA rather than *Aspergillus*), using BALB/c mice that are predisposed to developing AHR (rather than C57BL/6 IL-10^{-/-} strains), and using established T_H2 effector cells.

Our findings of a regulatory role for IL-10 in asthma are strongly consistent with the idea that IL-10 production mediates antigen-specific tolerance that protects against allergic diseases and airway inflammation. For example, regulatory T cells producing IL-10 are thought to develop after successful bee venom-specific immunotherapy³⁶ and during T-cell tolerance induced with respiratory exposure to antigen.^{37,38} These IL-10-producing cells might be similar to IL-10-producing T_R1 cells, which are generated by culture of T cells in the presence of IL-10 and inhibit the development of T_H1-mediated diseases, such as experimental colitis.³⁹ Whereas others have shown that T_R1 cells might prevent the development of T_H2 responses in naive mice,⁴⁰ we directly showed for the first time that IL-10-producing T cells potently inhibit the development of AHR and inflammation in allergen-sensitized mice and inhibit the function of established effector T_H2 cells. Moreover, we

demonstrated in mice sensitized and challenged with allergen to induce AHR that neutralization of IL-10 exacerbates AHR and inflammation. Although the possibility that IL-10 inhibits asthma has been discussed in the past, our current studies show very clearly and directly for the first time that T cells secreting IL-10 can function to limit and protect against the development of allergic asthma.

Although our results indicate that IL-10-secreting T cells play a significant role in regulating protective immunity in asthma, we believe that the control of allergic inflammation and asthma is complex, involving several different mechanisms and several different cell types. For example, although T_H1 cells do not function in the lung mucosa itself to inhibit allergic asthma (because T_H1 cells exacerbate pulmonary inflammation and cause tissue damage⁶), T_H1 cells might act in peripheral lymphoid organs such as the lymph node to inhibit the early development of T_H2 effector cells. Thus adjuvants such as CpG oligonucleotides⁴¹ and heat-killed *Listeria monocytogenes*⁴² might induce IFN- γ production in T cells and prevent or reverse allergic inflammation and asthma. In addition, TGF- β -secreting T cells might also play a significant role in modulating allergic inflammation, as we have previously shown.¹⁷ TGF- β -secreting T cells, called T_H3 cells, have been shown to play a regulatory role at mucosal sites—eg, in the induction of oral tolerance.⁴³ The precise role in the lungs of regulatory T cells producing TGF- β , in contrast to those producing IL-10, is not clear; it might depend on the specific conditions that induce TGF- β or IL-10 production. Such mechanisms are as yet not fully understood, but they might involve dendritic cells producing IL-10 or TGF- β ^{37,44} and expressing costimulatory molecules such as B7 (CD80 and CD86) and ICOS-ligand, which might preferentially cross-link CTLA-4 or ICOS, respectively, to induce IL-10 production in T cells.⁴⁵⁻⁴⁷ In addition, production of IL-10 by T cells might enhance production of TGF- β by other cells at other sites.⁴⁸ We suggest, however, that T cells producing high levels of IL-10 and not TGF- β (T_R cells) act preferentially in the respiratory epithelium, whereas cells producing TGF- β and IL-10 (T_H3 cells) act preferentially in the gastrointestinal epithelium to counterbalance T_H2 effector cells.³⁷ Further studies are required to affirm this possibility. Finally, it is possible that IL-10-producing regulatory T cells simultaneously synthesize IFN- γ (Fig 1), a combination that might be particularly effective in downmodulating allergic asthma.⁴⁹

In summary, our data, derived from use of several different approaches, clearly demonstrate that IL-10-producing T cells in the respiratory mucosa downregulate airway inflammation and AHR induced by T_H2 effector cells. These studies indicate that modulation of allergic disease and asthma is complex and involves regulatory cells and regulatory mechanisms not predicted by the T_H1/T_H2 paradigm. Studies to more specifically define the role of IL-10-producing T cells in the regulation of asthma and allergies and to elucidate the mechanisms that enhance the expansion of allergen-specific IL-10-producing T cells are ongoing. More complete understanding of

the inhibitory role of IL-10-secreting regulatory cells is particularly important and will be a prerequisite for the development of therapies focused on immunomodulation of deleterious T_H2 -polarized responses in asthma and allergic diseases.

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