Immunity, Vol. 9, 423–432, September, 1998, Copyright ©1998 by Cell Press

# Impaired Development of Th2 Cells in IL-13-Deficient Mice

**Grahame J. McKenzie,* Claire L. Emson,* Sarah E. Bell,† Shannon Anderson,* Padraic Fallon,§ Gerard Zurawski,‡ Richard Murray,‡ Richard Grencis,‖ and Andrew N. J. McKenzie*#**

*MRC Laboratory of Molecular Biology
Hills Road
Cambridge CB2 2QH
United Kingdom
†Wellcome Trust Immunology Unit
University of Cambridge
Cambridge CB2 2SP
United Kingdom
‡DNAX Research Institute
901 California Avenue
Palo Alto, California 94304
§Department of Pathology
University of Cambridge
Cambridge CB2 1QP
United Kingdom
‖School of Biological Sciences
University of Manchester
Manchester, M13 9PT
United Kingdom

## Summary

**We report that Th2 cell cultures generated using T cells or splenocytes from IL-13-deficient mice produce significantly reduced levels of IL-4, IL-5, and IL-10 compared with wild-type. In contrast, IL-4 and IL-5 production by mast cells stimulated in vitro with PMA, ionomycin, or IgE cross-linking are unaffected. In vitro Th2 cell differentiation cannot be rescued by the addition of exogenous factors, but in vivo antigen challenge and administration of IL-13 can increase Th2-like cytokine responses as can infection with the parasitic nematode *Nippostrongylus brasiliensis*. IL-13-deficient mice also have lower basal levels of serum IgE and biased antigen-specific immunoglobulin responses. Thus, IL-13 is an important regulator of Th2 commitment and may therefore play a central role in atopy and infectious diseases.**

## Introduction

The identification of a functional polarization of mouse $CD4^+$ T helper (Th) cell clones based on their cytokine secretion profiles has provided a molecular insight into the regulation of immune responses (Mosmann et al., 1986; Mosmann and Coffman, 1989). Th1 cells secreting IL-2, interferon-$\gamma$ (IFN$\gamma$) and lymphotoxin-$\beta$ (LT$\beta$) represent a T cell subset important in enhancing cellular immune responses, while Th2 cells, characterized by their secretion of IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, evoke production of immunoglobulin E (IgE) and the accumulation of eosinophils, both of which are highly detrimental in asthma and allergic reactions (Bousquet et al., 1990; Sutton and Gould, 1993). It is unclear how such Th cell commitment occurs, but factors including the route of antigenic challenge, the type of antigen-presenting cell, the cytokine milieu at the site of challenge, and the dose of the antigen are all believed to influence the skewing of T helper cell phenotypes.

Recent studies have identified multiple pathways that appear to be involved in the initiation of Th2 cell responses. These include the signals induced by TCR ligation, IL-4, the modulation of IL-12R$\beta$2, and ligation of CD4 and CD28 (reviewed by O'Garra, 1998). IL-4-mediated signals are of primary importance in potentiating a Th2 cell response, with addition of IL-4 to in vitro T cell cultures resulting in the induction of a Th2 cell phenotype (Le Gros et al., 1990). Furthermore, deletion of the IL-4 gene in mice results in a diminution of Th2-like responses (Kopf et al., 1993), though residual Th2-like cell lineages can still be isolated from these animals, indicating that IL-4-independent mechanisms for Th2-like cell development exist (Kopf et al., 1993: Brewer et al., 1996). In addition, under certain stimulations, such as infection with malaria parasites or MAIDS, these IL-4-deficient mice can still produce IgE (Van der Weld et al., 1994; Morawetz et al., 1996). Significantly, recent evidence has shown that IL-4 can down-regulate the expression of IL-12R$\beta$2 on Th2 cells, thus making them insensitive to IL-12 signals that would normally invoke a Th1 cell response (Szabo et al., 1997).

IL-13 is a recently described cytokine (Brown et al., 1989; McKenzie et al., 1993a) encoded by a gene mapping to a cytokine gene cluster on mouse chromosome 11 and human chromosome 5 that also includes the genes encoding IL-3, IL-4, IL-5, and granulocyte-macrophage-colony stimulating factor (GM-CSF) (McKenzie et al., 1993b). IL-13 is produced principally by activated T cells, and though expression is primarily associated with the Th2 lineage, human Th1 and Th0 cell clones may also make IL-13 (Zurawski and de Vries, 1994). Using in vitro culture systems, the biological activities of IL-4 and IL-13 have been found to overlap, inducing immunoglobulin isotype switching to IgE in human B cells (Punnonen et al., 1993) and suppressing inflammatory cytokine production in both human and mouse systems (de Waal Malefyt et al., 1993; Doherty et al., 1993).

The relatedness of the responses induced by IL-13 and IL-4 has been elucidated by studies showing that both of these cytokines can interact and cross-compete for IL-4 receptor $\alpha$ (IL-4R$\alpha$) (Zurawski et al., 1993; Smerz-Bertling and Duschl, 1995). However, the exact constitution of the receptor complexes engaging the individual cytokines is poorly understood, though it is apparent that IL-13 has its own primary binding chains (IL-13R$\alpha$1 and IL-13R$\alpha$2), to which IL-4 does not bind (Zurawski et al., 1995; Aman et al., 1996; Caput et al., 1996; Hilton et al., 1996; Gauchat et al., 1997; Miloux et al., 1997). Although IL-4 has been reported to interact with IL-2R$\gamma$c chain (Kondo et al., 1993), no such interaction has been

#To whom correspondence should be addressed (e-mail: anm@mrc-lmb.cam.ac.uk).

shown for IL-13 (He and Malek, 1995; Lin et al., 1995). Thus, while a number of intracellular signaling pathways are common to IL-13 and IL-4, such as Janus kinase 1 (JAK1) and signal transducer and activator of transcription 6 (Stat6) (Lin et al., 1995), others may be more specific. Significantly, recent data generated using parasitic nematode infections of IL-4$^{-/-}$, Stat6$^{-/-}$, IL-4R$\alpha^{-/-}$, and IL-13$^{-/-}$ animals have indicated that IL-13 plays an essential role in Th2 cell-mediated expulsion of these worms (Bancroft et al., 1998; McKenzie et al., 1998; Urban et al., 1998) and indicate that in some instances Stat6 signaling is IL-13-dependent. In addition, studies using IL-4$^{-/-}$ and IL-4R$\alpha^{-/-}$ to study the Th2 cell phenotype induced by *Schistosoma mansoni* implicate IL-13 in the process of granuloma formation (Jankovic et al., 1998). Thus, IL-13 plays a critical role in the development of a number of Th2 cell-mediated responses in vivo.

Interestingly, treatment of mice with a polyclonal antibody raised against mIL-13 resulted in a decrease in the levels of IL-4 being made by antigen-specific T cells in response to immunization (Bost et al., 1996), suggesting that inhibiting IL-13 function results in impaired IL-4 expression. Herein, we present data derived from the study of IL-13$^{-/-}$ mice that demonstrate an important regulatory role for IL-13 in the development of Th2 cell lineages. Using a combination of in vitro assays and in vivo stimulations, we have found that CD4$^+$ helper T cells from IL-13$^{-/-}$ mice display a significant impairment in their ability to develop a Th2 cell lineage following in vitro Th cell differentiation. We found that antigen-specific responses are less overtly affected by the loss of IL-13 expression and that infection with the Th2-phenotype-inducing gastrointestinal nematode *Nippostrongylus brasiliensis* was able to overcome the impairment. Additionally, IL-13$^{-/-}$ mice have significantly reduced IgE levels. These results suggest that IL-13 performs a central role in the development of certain immune responses and that its interrelatedness with IL-4 is complex.

## Results

### Generation of IL-13-Deficient Mice

Targeted disruption of the mouse IL-13 gene was performed by inserting the neomycin resistance (*neo*$^r$) cassette into exon 3 using a replacement targeting construct (Figure 1A). Correctly targeted clones were used to generate chimaeric mice that were bred with 129/J mice and transmitted the disrupted allele through the germline. Interbreeding of heterozygous (IL-13$^{+/-}$) mice gave rise to homozygous gene targeted mice (IL-13$^{-/-}$, Figure 1B). These animals were viable and of normal appearance and behavior. IL-13-specific ELISA failed to detect IL-13 protein in supernatants conditioned by activated Th2 cells from the IL-13$^{-/-}$ mice (Figure 2A). No mRNA transcripts encoding IL-13 could be detected by RT-PCR using RNA isolated from in vitro culture of Concanavalin A (Con A)-stimulated splenocytes from the IL-13$^{-/-}$ mice, though their wild-type littermates displayed normal expression (data not shown).

### CD4$^+$ T Lymphocytes and Splenocytes Display an Impairment in Th2 Differentiation

We used in vitro assays to assess the result of IL-13 deletion on cytokine production. Con A stimulation of

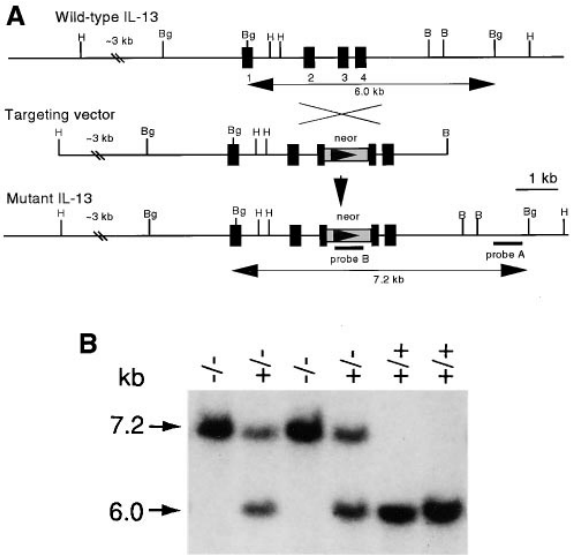


Figure 1. Disruption of the IL-13 Gene by Gene Targeting

(A) The structure of the IL-13 locus, the targeting vector, and the predicted homologous recombination event are shown. A replacement targeting vector containing 6 kb and 1.5 kb of 5′ and 3′ homology, flanking the neomycin resistance gene, was constructed.

(B) Southern blot analysis of $F_2$ tail DNA. BglII-digested DNAs were hybridized with the 3′ flanking probe (probe A). The endogenous and targeted alleles are 6.0 kb and 7.2 kb, respectively.

splenocytes was used initially to determine the levels of IL-4, IL-5, IL-10, and IFN-$\gamma$ being secreted 24–96 hr poststimulation. Profoundly reduced levels of IL-5 and moderately less IL-4 were produced by cells from the IL-13$^{-/-}$ mice, and IL-5 levels remained low even when cells were costimulated with excess IL-4 for 96 hr; no significant differences were found in IFN$\gamma$ production (data not shown). Similar responses were observed with purified CD4$^+$ T cells stimulated with mitogen (data not shown).

As IL-5 and IL-4 are known to be produced predominantly by Th2 cells, we assessed the consequences of IL-13 disruption on T helper cell differentiation. Purified CD4$^+$ T cell cultures were stimulated for 5 days to promote either Th1 or Th2 differentiation. As expected, restimulation of cell cultures supporting Th2 differentiation from wild-type mice resulted in the secretion of IL-4, IL-5, and IL-10 (Figure 2A). In contrast, equivalent cell cultures from IL-13$^{-/-}$ mice produced dramatically reduced levels of IL-4 and IL-5 and a 2-fold reduction in IL-10, despite the inclusion of exogenous IL-4 during the 5-day culture period (Figure 2A). We also noted that Th2 cells from wild-type mice made significant levels of IL-13 and that wild-type Th1 cells also produced moderate amounts of IL-13. As expected, cells from the IL-13$^{-/-}$ animals failed to produce IL-13 (Figure 2A). Interestingly, inclusion of IL-13 in the T cell differentiation assays failed to reverse the observed phenotype (Figure 2A). Following T cell restimulation, levels of IFN$\gamma$ production were approximately 2-fold greater from cells derived from IL-13$^{-/-}$ animals compared to levels from wild-type cells (Figure 2A).

To determine whether the inclusion of additional cell populations into the in vitro Th2 cultures could rescue

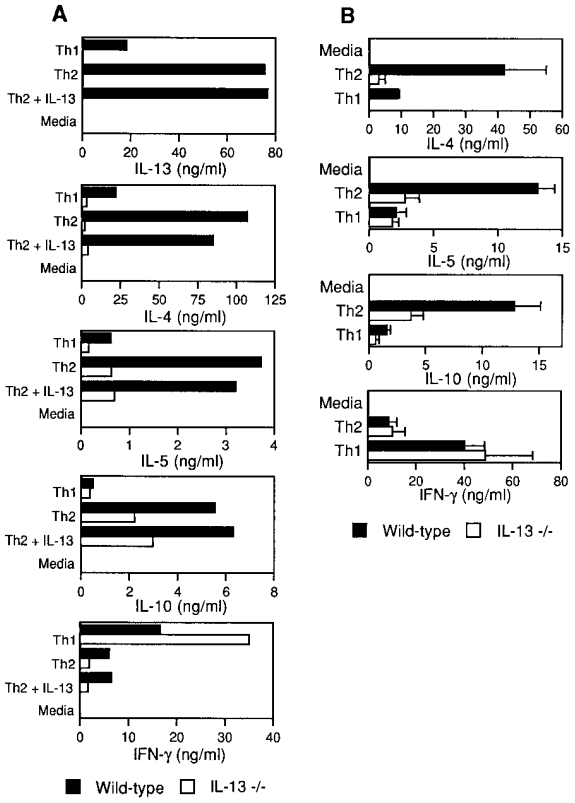


Figure 2. Cytokine Production Following In Vitro Th Cell Differentiation

(A) $CD4^+$ T cells purified from pooled spleens from three wild-type or three IL-$13^{-/-}$ mice were stimulated under Th1 or Th2 culture conditions (or Th2 conditions with the addition of 100 ng/ml IL-13) for 5 days, washed, and restimulated on anti-CD3 antibody coated plates for 24 hr. Supernatants were analyzed by ELISA. Data are representative of four repeat experiments.

(B) Splenocytes were stimulated under Th1 or Th2 culture conditions for 5 days, washed, and restimulated on anti-CD3 antibody coated plates for 24 hr. Supernatants were analyzed by ELISA. Data are representative of five repeat experiments.

the observed phenotype, we set up Th2 cultures with total spleen cell populations from IL-$13^{-/-}$ and wild-type mice. These total splenocyte assays also failed to overcome the impairment in the in vitro differentiation capacity of Th2 cell lineages in IL-$13^{-/-}$ cell cultures, with significant reductions in IL-4, IL-5, and IL-10 production even in the presence of exogenous IL-4 (Figure 2B).

The pattern of Th2 cytokine expression remained consistently lower in cells from the IL-$13^{-/-}$ mice irrespective of whether anti-CD3 or Con A stimulation was used or if cultures included IL-4 or IL-13 (data not shown). IL-13, in the absence of IL-4, also failed to reverse the observed cytokine phenotype in the T cell differentiation assays (data not shown). Furthermore, stimulation of differentiated $CD4^+$ T cells with phorbol myristate acetate (PMA) and ionomycin, which normally induce significant levels of cytokine expression from T cells, yielded similar cytokine profiles to those elicited by anti-CD3 and anti-CD28 (data not shown), indicating that a range of reported cytokine-inducing stimuli were unable to reverse the impairment in cytokine production from IL-$13^{-/-}$ Th2 cell cultures.

The differences in the cytokine profiles were not due to variation in overall cell proliferation, since growth responses to Con A, IL-2, or IL-4 were equivalent in IL-$13^{-/-}$ and wild-type mice (data not shown). Indeed, the up-regulation of activation markers and the proliferation of splenocytes, lymph node cells, and purified T cells in response to IL-4, either alone or in combination with coactivators such as lipopolysaccharide (LPS), anti-IgM, and anti-CD40 were equivalent in both IL-$13^{-/-}$ and wild-type mice (data not shown).

### Antigen Stimulation and Parasitic Infection Induce Production of IL-4 and IL-5

We analyzed the $CD4^+$ T cell populations that developed during in vitro differentiation using intracellular cytokine analysis. This demonstrated that populations of IL-4- and IL-5-producing cells were still present but that there was a significant reduction in the number of IL-5- and IL-4-producing cells in the Th2 cell cultures derived from the IL-13-deficient mice, as compared to those from wild-type cultures (data not shown).

The development of cells capable of producing IL-4 and IL-5 was further illustrated when IL-13-deficient mice were challenged with a complex antigen. We assessed the antigen-specific cytokine response generated against schistosome eggs, an antigen that has been shown to induce Th2 cell responses (Pearce et al., 1991). Following immunization, the levels of IL-5 produced by lymph node cell cultures from both the IL-$13^{-/-}$ and wild-type animals were similar, varying by only 2-fold, and the levels of IL-4 were only approximately 3-fold lower in the IL-13-deficient mice as compared to the wild-types (Figure 3A). However, if the lymph node cells from the immunized animals were cultured using in vitro Th2 cell differentiation conditions and stimulated with anti-CD3 and anti-CD28, then a more severe impairment of IL-5 and IL-4 production was observed (Figure 3B). Thus, the antigen-driven in vivo T cell response was only moderately influenced by the absence of IL-13, in contrast with the profound defect seen in the cell population normally responsive during in vitro Th2 cell differentiation.

We next assessed the affect of infecting IL-$13^{-/-}$ mice with the intestinal parasitic nematode *N. brasiliensis* on the development of Th2 cell populations. Lymph node cells were prepared from wild-type and IL-$13^{-/-}$ animals at 6 and 10 days postinfection with *N. brasiliensis*. These cells were cultured under conditions supporting Th2 cell differentiation, and supernatants were assessed for the presence of Th2 cell cytokines. As shown in Figure 3C, the preinfection IL-$13^{-/-}$ lymph node cultures (day 0) display a significant impairment in IL-4, IL-5, and IL-10 expression, similar to that observed for purified $CD4^+$ T cells and splenocytes using equivalent culture conditions. However, following infection, isolated lymph node cells from IL-$13^{-/-}$ mice cultured in Th2 cell differentiation conditions now generate typical cytokine responses at day 6 postinfection (Figure 3C). These results indicate that antigenic challenge in the form of a sustained parasite infection can overcome the deficit in the signals required to develop Th2 cell responses.

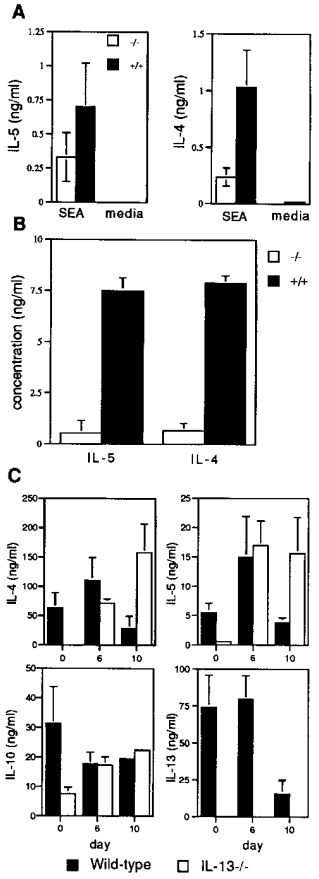


Figure 3. Cytokine Production in Response to Antigen Immunization or Parasite Infection

(A) Mesenteric lymph node cells, isolated from schistosome egg challenged animals, were cultured with soluble egg antigen (SEA) for 5 days before supernatants were analyzed for IL-4 and IL-5 production by ELISA. Representative data from three repeat experiments with five animals per group.

(B) Mesenteric lymph node cells, isolated from schistosome egg-challenged animals, were cultured using Th2 differentiation conditions. Representative data from three repeat experiments with five animals per group.

(C) Mesenteric lymph node cells were prepared from uninfected animals (day 0) or animals 6 days and 10 days postinfection with *N. brasiliensis*. Cells were stimulated under Th2 culture conditions for 5 days, washed, and restimulated on anti-CD3 antibody coated plates for 24 hr. Supernatants were analyzed by ELISA. Data are representative of two repeat experiments consisting of cohorts of five mice at each time point.

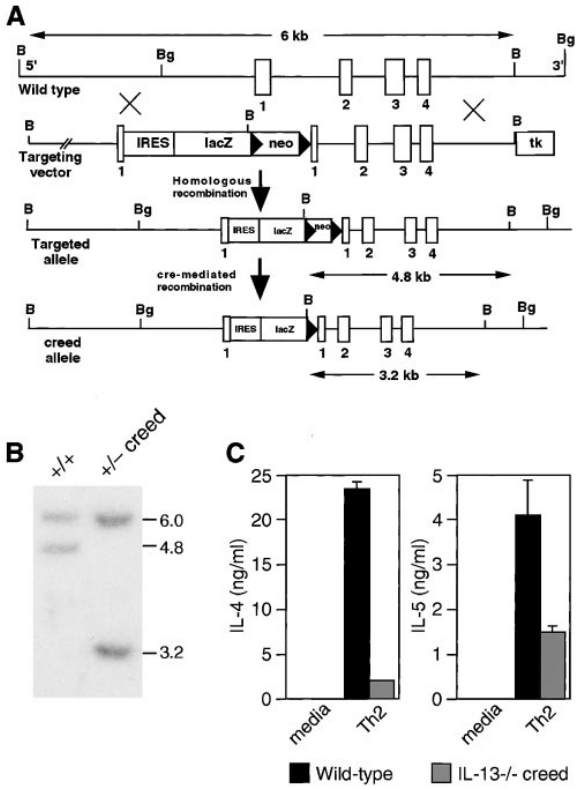


Figure 4. Th2 Cytokine Production Following Removal of the Neomycin Selection Cassette Using cre-Mediated *loxP* Recombination

(A) The structure of the IL-13 locus, the targeting vector, and the predicted homologous recombination event before and after excision of the neomycin cassette are shown (*loxP* sites indicated by broad arrows).

(B) Southern blot analysis of ES cell genomic DNA following cre-mediated recombination. BamHI-digested DNAs were hybridized with the IL-13 cDNA probe. The endogenous allele and targeted alleles are 6.0 kb and 4.8 kb, respectively. The neomycin excised allele is 3.2 kb (+/− creed).

(C) Mesenteric lymph node cells from wild-type and IL-13$^{-/-}$ creed were stimulated under Th2 culture conditions for 5 days, washed, and restimulated on anti-CD3 antibody-coated plates for 24 hr. Supernatants were analyzed by ELISA. Data are representative of two repeat experiments with three mice per group.

## Removal of the PGK/Neomycin Resistance Cassette Does Not Reverse the Impairment in In Vitro Th Cell Differentiation

Since it was possible that the impairment in T helper cell responses might be due to the presence of the exogenous phosphoglycerate kinase (PGK) promoter used to drive the expression of the neomycin resistance gene, we utilized cre-mediated *loxP* recombination to remove both of these elements from targeted ES cells (Figures 4A and 4B). These cells were then used to generate homozygous IL-13$^{-/-}$ mice that no longer contained the neomycin selectable marker or its promoter. Lymph node cells from these animals and wild-type mice were cultured under conditions supporting Th2 cell differentiation, and following restimulation an impairment of IL-4 and IL-5 production was still evident in cultures from the IL-13$^{-/-}$ creed mice (Figure 4C). These results indicate that even with the removal of the exogenous PGK promoter-driven neomycin gene in vitro Th2 cell development is impaired and that promoter interference is not responsible for the observed phenotype.

## Mast Cell Cytokine Production Is Unaffected by IL-13 Deletion

To determine whether there was a global change in the secretion of IL-4 and IL-5, we isolated mast cell populations from spleen and bone marrow and assessed their ability to produce these cytokines. Significantly, IL-13$^{-/-}$ and wild-type mast cells secreted equivalent levels of IL-4 and IL-5 upon stimulation with PMA and ionomycin,

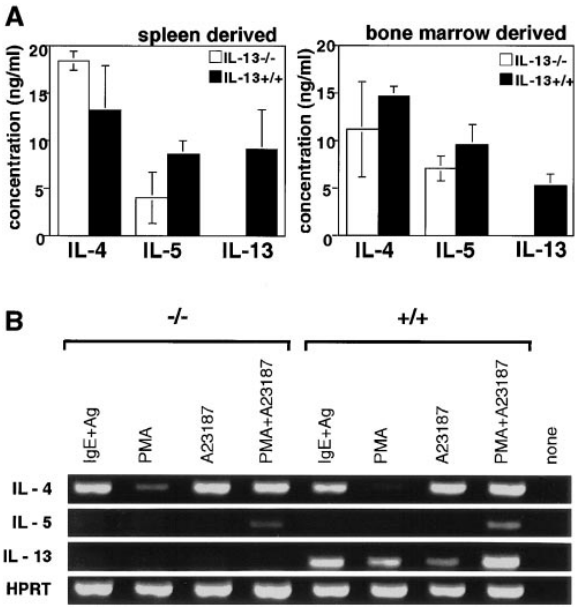


Figure 5. Mast Cell Cytokine Production

(A) Mast cells were stimulated with PMA and ionomycin and supernatants were sampled after 24 hr. Cytokine levels were analyzed by ELISA. Representative data from three repeat experiments with cells prepared from three mice.

(B) RT-PCR analysis of spleen-derived mast cells stimulated with IgE and specific antigen, PMA, and calcium ionophore A23187.

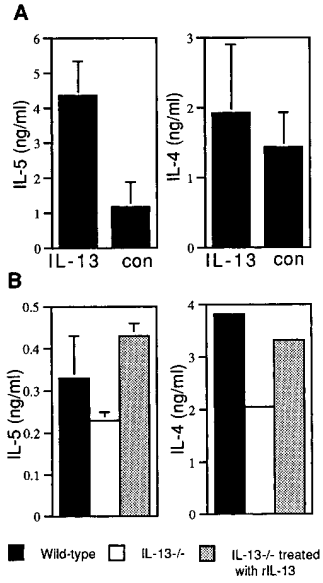


Figure 6. Cytokine Production Following In Vivo Administration of IL-13

(A) Mesenteric lymph node cells from wild-type animals immunized with schistosome eggs and IL-13, or denatured IL-13 (con), were cultured with soluble egg antigen for 5 days. Representative data from two repeat experiments with five animals in each group. Supernatants were assayed for cytokine concentration using ELISA.

(B) Mesenteric lymph node cells, derived from *N. brasiliensis*–infected wild-type animals, IL-13$^{-/-}$ animals, or IL-13$^{-/-}$ animals treated with rmIL-13, were stimulated for 24 hr with Con A. Supernatants were analyzed for the presence of IL-5 and IL-4 by ELISA. Data represent triplicate cultures from five animals per group.

indicating that this cell population is unaffected by IL-13 deletion (Figure 5A); wild-type mast cells also secreted significant levels of IL-13 (Figure 5A). Furthermore, RT-PCR analysis of IL-4 and IL-5 mRNA produced by IL-13$^{-/-}$ and wild-type mast cells, using samples in which competitive PCR was used to determine relative HPRT levels, indicated identical stimulation-specific transcriptional activation in the presence of PMA, ionomycin, or DNP cross-linked anti-DNP IgE monoclonal antibody (Figure 5B). These data demonstrate that disruption of the IL-13 gene does not affect the development of mast cells or their ability to express Th2-like cytokines and indicate that the developmental deficit in Th2-like cytokine production is T cell–specific. Furthermore, since the IL-13$^{-/-}$ mast cell populations are fully responsive to a range of stimuli, including PMA and ionomycin, which were unable to elicit IL-4 and IL-5 production from the T cell population in IL-13$^{-/-}$ mice, these results support the finding that insertion of the neomycin gene into this locus has not affected the regulation of the IL-4 and IL-5 genes.

### Administration of Recombinant IL-13 and Antigen In Vivo Increases Cytokine Production

Since addition of IL-13 to in vitro cultures from wild-type or IL-13-deficient animals failed to influence Th2 differentiation, we attempted to mediate an in vivo effect on cytokine production using recombinant mouse IL-13 (rmIL-13) to inoculate both IL-13-deficient and wild-type mice. The administration of rmIL-13 in the absence of antigen failed to mediate any effect on subsequent in vitro Th2 cell differentiation in wild-type or IL-13$^{-/-}$ mice (data not shown). Thus, animals were coadministered with rmIL-13 and schistosome eggs followed by two further doses of rmIL-13 intraperitoneally over a 10-day period. Mice were then sacrificed and mesenteric lymph node cells isolated and cultured with antigen. Lymph node cells derived from IL-13-treated wild-type animals produced elevated levels of antigen-induced IL-5 when cultured in the presence of schistosome soluble egg antigen (Figure 6A). No such increase was observed in cultures from mice inoculated with denatured IL-13. We were also unable to show an increase in IL-5 produced by cultures of IL-13$^{-/-}$ cells (data not shown). Furthermore, there was no reproducible change in the levels of IL-4 from either set of animals (Figure 6A). Thus, administration of IL-13 with antigen can modulate the expression of IL-5 in vivo.

In addition to the results using a protein immunization strategy, we found that in vivo rmIL-13 treatment of IL-13$^{-/-}$ animals infected with *N. brasiliensis* induced an increase in the levels of IL-4 and IL-5 secreted following 24 hr Con A stimulation of mesenteric lymph node cells (data not shown) or CD4$^{+}$ purified T cells relative to levels from mock treated animals (Figure 6B). Thus, in combination with antigenic challenge, administration of IL-13 can influence cytokine secretion patterns.

### Reduced Serum IgE Levels and Modulated B Cell Expression of CD23 in the Absence of IL-13

No significant differences were detected in the number or composition of cells from spleen, thymus, lymph

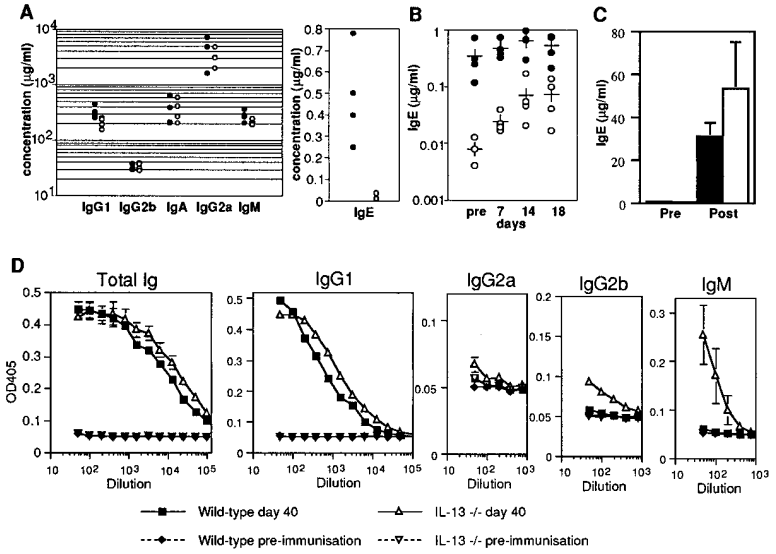


Figure 7. Serum Immunoglobulin Levels from Naive and Immunized Animals

(A) Total serum concentration of immunoglobulin isotypes from naive wild-type and IL-13$^{-/-}$ mice. Representative data from three repeat experiments. (B) Serum IgE levels from mice immunized with ovalbumin/alum. Representative data from two repeat experiments. Closed circle, wild-type; open circle, IL-13$^{-/-}$; plus, mean.

(C) Serum IgE levels detected at day 10 post-infection of mice with *N. brasiliensis* (data represent the mean (± SD) in groups of five mice). Shaded columns are wild-type, open columns are IL-13$^{-/-}$.

(D) Serum ovalbumin-specific immunoglobulin isotype responses to ovalbumin/alum following immunization over a 40-day period. Cohorts of five animals were analyzed.

node, and bone marrow from the wild-type and IL-13$^{-/-}$ mice when we examined the cell surface expression of a panel of immunoregulatory molecules including CD4, CD8, CD3ϵ, CD69, CD25, sIgM, CD28, CD90.2, Mac-3, CD80, CD86, c-Kit receptor, Gr-1, TER-119, CD54, F4/80, B220, CD11b, and IL-4Rα (data not shown). However, we detected significantly reduced levels of CD23 expression (the low-affinity receptor for IgE) on B220$^{+}$ splenocytes from the IL-13$^{-/-}$ mice relative to those from the wild-type animals (data not shown). Culture of splenocytes from either wild-type or IL13$^{-/-}$ animals, in the presence of anti-CD40 antibody, induced similar levels of CD23 expression on both populations (data not shown), indicating that the intrinsic ability to up-regulate expression of CD23 was not impaired in the IL-13$^{-/-}$ cells.

Measurement of serum immunoglobulins in nonimmunized IL-13$^{-/-}$ mice revealed a 5- to 20-fold decrease in total serum IgE levels compared to wild-type animals, while the levels of other immunoglobulin isotypes were not significantly different (Figure 7A). Upon immunization with ovalbumin/alum, increased production of total serum IgE was detected in both the wild-type and the IL-13-deficient animals, although the levels seen in the IL-13$^{-/-}$ mice did not reach those observed in the wild-type controls (Figure 7B). Total serum levels of IgE were also found to increase in the IL-13$^{-/-}$ mice upon infection with *N. brasiliensis* (Figure 7C).

Since total IgE expression was perturbed in naive IL-13$^{-/-}$ mice, their ability to generate a specific antibody response to a protein antigen was assessed. Wild-type and IL-13$^{-/-}$ mice were immunized over a 40-day period with ovalbumin (OVA) complexed to alum, and serum samples isolated pre- and postimmunization were analyzed for OVA-specific immunoglobulin isotype content by ELISA. The total antigen-specific immunoglobulin response in wild-type and IL-13$^{-/-}$ mice was comparable, as was the OVA-specific IgG1 response (Figure 7D). However, while IL-13$^{-/-}$ mice generated small but significant concentrations of OVA-specific IgG2b antibodies and an elevated OVA-specific IgM response, these isotypes were undetectable in wild-type (Figure 7D).

## Discussion

IL-13-deficient mice have highlighted an important regulatory role for IL-13 in the development of T helper cell responses and have also raised the issue of a direct role for IL-13 in the control of IgE production in the mouse system. Using a number of in vitro and in vivo based assays, we have shown that deletion of the IL-13 gene results in attenuated development of certain T cell lineages with a consequent reduction of Th2 cell cytokines. These results are consistent with a study in which anti-IL-13 polyclonal antibodies were used to block IL-13 function, resulting in a significant reduction in T cell–derived IL-4 (Bost et al., 1996), and a recent report in which IL-4Rα$^{-/-}$ mice exhibited a more severe impairment in Th2 development than that observed in IL-4$^{-/-}$ mice (Barner et al., 1998). The molecular mechanism(s) by which IL-13 mediates its effects are unclear, and it remains possible that IL-13 may act indirectly or directly on T cell populations. Intracellular cytokine analysis indicated that significantly fewer IL-13$^{-/-}$ T cells are producing IL-4 or IL-5 and that this population does not expand as is seen in the wild-type cultures. These data correlate well with the cytokine ELISA data and may suggest that cytokine production on an individual cell basis is comparable between wild-type and IL-13$^{-/-}$ mice and that the defect may lie in the generation of these cells.

To date, the addition of exogenous IL-13 to the in vitro differentiation assays has failed to reverse the cytokine secretion profile observed in T cells isolated from IL-13-deficient mice. Thus, commitment or expansion of a responsive cell type apparently fails to occur in the IL-13-deficient mice and cannot be redirected in culture, possibly due to changes in receptor expression and/or intracellular signaling pathways during development or upon derivation of these cells. Since exogenous IL-4 also failed to reverse the phenotype of the cells from the IL-13$^{-/-}$ mice, our results suggest that IL-13 acts upstream of IL-4 in this process. The IL-13$^{-/-}$ phenotype contrasts with that observed in IL-4$^{-/-}$ mice in which

$CD4^+$ T cells produce reduced levels of IL-5 and IL-10 but regain wild-type levels of these cytokines when exogenous IL-4 is added to in vitro differentiation assays (Kopf et al., 1993; A.N.J.M., unpublished data).

As IL-13 is a potent modulator of macrophage function (de Waal Malefyt et al., 1993; Doherty et al., 1993; Muchamuel et al., 1997; Nicoletti et al., 1997), it is possible that in the absence of IL-13 macrophages fail to regulate correctly their expression of cytokines such as IL-6 or IL-12, which have been reported to promote T cell differentiation (Manetti et al., 1993; Rincón et al., 1997). IL-12 is a potent inducer of Th1 cell differentiation (Hsieh et al., 1993) and thus deregulated IL-12 production may influence the subsequent differentiation potential of T cell lineages in the IL-$13^{-/-}$ animals. Indeed, we did observe that activated macrophages from IL-$13^{-/-}$ mice produced elevated levels of IL-12 (G.J.M., unpublished data) and that there was slightly enhanced IFN$\gamma$ production in Th1 cell cultures from IL-$13^{-/-}$ mice (Figure 2A). This indirect role for IL-13 in regulating macrophage function and thereby modulating T cell development may also explain why addition of IL-13 to in vitro cultures failed to reverse the observed phenotype and why by contrast in vivo treatment of animals with IL-13 was able to influence Th2-like cytokine production (Figures 6A and 6B).

However, an alternative explanation involving a direct effect of IL-13 on T cells cannot be ruled out. Observations from the human system have indicated that primary human T cells can respond both functionally to IL-13 (Jinquan et al., 1995) and by phosphorylating Stat6 (Curiel et al., 1997). In addition, it has been shown that upon activation human T cells down-regulate IL-13R$\alpha$1 expression (Gauchat et al., 1997), presumably with an attendant loss of IL-13 responsiveness. Thus, isolated mouse T cells may become insensitive to IL-13 signals in vitro due to down-regulation of receptor subunits, possibly in a manner similar to that described for the IFN$\gamma$ receptor on Th1 cells (Pernis et al., 1995). Certainly, the observed impairment of cellular commitment is not simply due to a deficiency in cell surface expression of IL-4R$\alpha$ receptor, as identical levels were detected on T cells from wild-type and IL-$13^{-/-}$ mice (data not shown). However, IL-13 may be involved in the expression or activation of components of the IL-4/IL-13 signaling pathway such as IRS-2, Jak1, Jak3, or Stat6. It is clear that IL-13 and IL-4 share at least a subset of intracellular signaling pathways, including the localization of Stat6 to the nucleus (Lin et al., 1995), and recent studies using Stat$6^{-/-}$ mice have identified a severe Th2 block (Shimoda et al., 1996; Takeda et al., 1996) possibly due to the combined effect of interrupting the downstream signaling pathways of both IL-4 and IL-13 (Kaplan et al., 1996). The presence of Stat6 binding sites in the IL-4 promoter (Lederer et al., 1996) and IL-4R$\alpha$ promoter (Kotanides and Reich, 1996) presents the possibility that IL-13-induced Stat6 could play a role in modulating IL-4 and IL-4R$\alpha$ expression, thereby influencing T cell expansion. If this were the case, IL-13 produced by naive T cells (van der Pouw Kraan et al., 1996) may represent an early source of cytokine for eliciting T cell function, including the induction of the IL-4 responsible for promoting Th2 cell development.

It is evident that administration of antigen can overcome at least some of the impairment in cytokine production observed in IL-$13^{-/-}$ mice and that concurrent administration of recombinant IL-13 can enhance Th2 cytokine expression still further. It is also apparent that infection of IL-$13^{-/-}$ animals with the gastrointestinal nematode worm, *N. brasiliensis*, circumvents the impairment in Th2 bias seen in vitro. It is noteworthy that at 10 days postinfection, 24 hr Con A stimulation of lymph node cells results in lower levels of IL-4 production by the IL-$13^{-/-}$ cells as compared to wild-type cells (McKenzie et al., 1998), but that in vitro Th2 culture results in a marked increase in IL-4 production by IL-$13^{-/-}$ cells as compared to wild-type cells (Figure 3C), probably as a result of the duration and persistence of the nematode infection. It is possible that infection may enhance other regulatory pathways such as IL-10, thereby suppressing IL-12 and overcoming the deficit in IL-13. Alternatively, pathways including mast cell– and eosinophil-derived IL-4 may result in the development of IL-13-independent T cell subsets.

Athough there is no precedent for the existence of a locus control region for the IL-13/IL-4/IL-5 gene cluster, it was formally possible that the promoter driving the *neo$^r$* gene had affected the transcription of these cytokine genes. We felt this explanation was unlikely due to the cellular specificity of the phenotype with the precise retention of transcriptional control in mast cells, the unaffected IL-4-driven IgE response to *N. brasiliensis*, the lack of effect on the closely linked ($\sim$40 kb) interferon response factor gene (IRF1) (data not shown), and because insertion of the *neo$^r$* gene into the IL-4 gene (Kühn et al., 1991; Kopf et al., 1993) or the IL-5 gene (Kopf et al., 1996) does not mimic the phenotype seen in the IL-13-deficient mice. However, to rule out the possibility of promoter interference, we used cre-*loxP* recombination to remove the PGK promoter/*neo$^r$* gene cassette from the targeted IL-13 locus. Significantly, Th2 cytokine production remained impaired even after the removal of this element, supporting the hypothesis that the observed phenotype results from an absence of responding cells and not from an indirect transcriptional defect.

The IL-$13^{-/-}$ mice display significantly reduced levels of IgE and CD23, and this may be directly attributable to the lack of IL-13 or may also be explained by a failure of the IL-13-deficient T cells to provide sufficient helper functions to generate a robust IgE response. While it is clear that IL-$4^{-/-}$ mice have profoundly reduced IgE levels (Kühn et al., 1991; Kopf et al., 1993) and lower CD23 expression (Hjulström et al., 1995), the attendant reduction in Th2 cytokines including IL-13 may contribute to this phenotype. Interestingly, recent studies have shown that IL-$4^{-/-}$ mice can still switch to IgE secretion after infection with *Plasmodium chabaudi* (Van der Weld et al., 1994) or during the course of a murine acquired immunodeficiency syndrome (Morawetz et al., 1996), thus indicating an IL-4-independent mechanism for the production of IgE. Furthermore, recent data generated in our laboratory have identified IL-13-specific, IL-4-independent, IgE regulation in mice transgenic for IL-13 expression (Emson et al., 1998). Unlike IL-$4^{-/-}$ mice, which display impaired IgG1 responses (Kühn et al.,

1991; Kopf et al., 1993), these were normal in the IL-13$^{-/-}$ animals. However, there were enhanced levels of antigen-specific IgG2a and IgG2b produced by the IL-13$^{-/-}$ animals, indicative of a more biased Th1 type phenotype, correlating with slightly enhanced IFN$\gamma$ expression.

Kopf et al. (1993) suggested that IL-4-independent Th subsets may be responsible for the residual Th2 cytokines detected in IL-4$^{-/-}$ animals, proposing that Thp or Th0 T cells may be IL-4-independent while chronically stimulated Th cells are IL-4-dependent. Thus, our results may go some way to explaining the capacity for IL-4-independent differentiation to a Th2 cell phenotype as reported by others (Brewer et al., 1996; Constant et al., 1995; van der Pouw Kraan et al., 1996). It is evident that complex interrelated roles exist for IL-13 and IL-4 in the development of immune reactions, and though initially thought redundant, these cytokines elicit specific responses (Bancroft et al., 1998; McKenzie et al., 1998; Urban et al., 1998). In light of our findings, the IL-13-deficient mice will prove an important tool in clarifying the relative roles of these closely related cytokines in the generation of immune responses in infectious diseases and allergy.

## Experimental Procedures

### Targeted Disruption of the IL-13 Gene

Three independently derived IL-13-deficient mouse lines have been used in this study. IL-13KO was generated as follows. Genomic DNA clones were isolated as detailed in McKenzie et al. (1993b). The neomycin resistance gene was inserted into exon 3, with stop codons in all three frames 5′ of the selectable marker. The vector was linearized with SalI and transfected into E14.1 ES cells by electroporation. BglII-digested genomic DNAs from isolated clones were screened using a 3′ flanking probe. The probe was made by PCR using the primers 5′-AAGAGCCCGAGGCATGATGCG-3′ and 5′-TCT GGGCGCTATTGCTTGGTCTCTTCTGCC-3′. Subsequent hybridization of Southern blots with a *neo*$^r$ probe (probe B) confirmed the predicted size of the targeted fragment and a single integration event. IL-13$^{-/-}$ was generated as described in McKenzie et al. (1998). IL-13$^{-/-}$ creed was generated essentially as outlined in McKenzie et al. (1998), with the exception that the neomycin resistance gene was excised using cre-mediated *loxP* recombination. A correctly targeted ES cell clone was identified and transiently transfected with plasmid pPGKcrebpA encoding the cre recombinase gene (a gift from Dr. Werner Muller, Cologne). BamHI-digested genomic DNAs from isolated ES cell colonies were screened for cre-mediated recombination using Southern analysis employing the mIL-13 cDNA as a probe. Prospective clones were then replica-plated in the presence and absence of G418, and drug-susceptible clones were subcloned and rescreened using Southern analysis. ES cells containing the correctly recombined targeting event were used to derive chimeric mice that were crossed with C57BL/6 females to generate heterozygous IL-13$^{+/-}$ creed animals. These were interbred to generate homozygous IL-13$^{-/-}$ creed mice and wild-type littermates. With the exception of the mast cell data, which were only generated using the IL-13KO line, all presented experiments were reproduced using the IL-13$^{-/-}$ line described in McKenzie et al. (1998).

### RNA Preparation

Spleen cells were cultured with 2 $\mu$g/ml of Con A for 48 hr. Total RNA was prepared using RNAzolB (Biotecx, Biogenesis, Poole, UK), and polyA+ RNA was isolated with Oligotex (Qiagen, Dorking, UK). RT-PCR primers for IL-13 were 5′-GGGTGACTGCAGTCCTGGCT-3′ and 5′-GCTGGAGACCGTAGTGGG-3′. The internal oligonucleotide used for hybridization was 5′-CTGTGCAACGGCAGCATGGTA-3′. HPRT primers and conditions were as described in Murphy et al. (1993).

### Ovalbumin Immunization

Mice (8- to 10-week-old) were immunized in the peritoneal cavity with 100 $\mu$g of ovalbumin adsorbed to aluminium hydroxide (alum) with subsequent boost injections with 100 $\mu$g of ovalbumin after 7 and 14 days. Alternatively, 8- to 10-week-old mice were immunized intraperitoneally with 100 $\mu$g of ovalbumin/alum with subsequent boost injections with 100 $\mu$g of ovalbumin/alum after 10, 20, and 30 days. Serum samples were assayed for immunoglobulin isotypes.

### ELISA Assays

Serum immunoglobulins were assayed using sandwich ELISA. 96-well plates were coated with monoclonal anti-immunoglobulin isotype capture antibodies, and bound immunoglobulin of diluted serum samples was detected using biotinylated monoclonal anti-immunoglobulin isotype detection antibodies (Pharmingen). Concentrations were calculated using purified immunoglobulin isotypes as standards (Pharmingen). Ovalbumin-specific ELISA were performed by coating 96-well plates with ovalbumin at 2.5 $\mu$g/ml; bound immunoglobulin of diluted serum samples was detected using biotinylated monoclonal anti-immunoglobulin isotype detection antibodies (Pharmingen). Cytokine ELISA also utilized the sandwich format with capture and detection antibodies purchased from Pharmingen. ELISA were performed according to Pharmingen ELISA protocol. The IL-13 ELISA was purchased from R & D (Abingdon, UK).

### Preparation of CD4$^+$ T Cells

Splenocytes were cultured on plastic tissue culture plates for 1 hr at 37°C to remove macrophages. Nonadherent cells were incubated with biotinylated anti-I-A$^b$ antibody (clone AF6-120.1, Pharmingen), biotinylated anti-CD8 antibody (clone 53-6.7, Pharmingen), biotinylated anti-B220 antibody (clone RA3-6B2, Pharmingen), and streptavidin magnetic beads (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) followed by magnetic field separation to remove MHC class II–, CD8-, and B220-expressing cells. Cell purity was determined using FITC-labeled anti-CD4 and PE-labeled anti-CD8 antibodies and was generally 90%–95% CD4$^+$ cells. Purified cells were cultured on anti-CD3$\epsilon$ antibody-coated plates (10 $\mu$g/ml of clone 2C11, Pharmingen) plus anti-CD28 antibody (1 $\mu$g/ml of clone 37.51, Pharmingen) in the presence of exogenous cytokines or anti-cytokine antibody as indicated. IL-2 (10 ng/ml, R & D) was added to all cultures. Th2 cell differentiation was promoted in the presence of 100 ng/ml IL-4 (R & D) and anti-IFN$\gamma$ antibody (10 $\mu$g/ml of clone XMG1.2, Pharmingen), while Th1 differentiation was promoted by anti-IL-4 antibody at 40 $\mu$g/ml (clone 11B11, DNAX Research Institute) and IL-12 (1 ng/ml, Genzyme, West Malling, UK). Cells were cultured for 5 days, washed, and resuspended at 1 $\times$ 10$^6$ cells/ml for 24 hr in the presence of anti-CD3. Supernatants were analyzed by cytokine ELISA performed as above.

### *N. brasiliensis* Infection

Individual mice were inoculated subcutaneously with 400 viable third-stage *N. brasiliensis* larvae.

### Antigen-Specific T Cell Stimulation Using Soluble Schistosome Egg Antigen

Mice were immunized intraperitoneally with 10,000 nonviable *Schistosoma mansoni* eggs (Pearce et al., 1991). After 10 days, mesenteric LN cells were prepared and cultured at 5 $\times$ 10$^6$ cells per ml in the presence of soluble egg antigen (SEA, 20 $\mu$g/ml) for 5 days (Pearce et al., 1991). Anti-IL-4R$\alpha$ antibody (Genzyme) at 0.5 $\mu$g/ml was added for the final 24 hr of culture. Supernatants were analyzed for cytokines using ELISA.

### Mast Cell Preparation and Stimulation

Bone marrow was aseptically flushed from femora and tibias of 8-week-old mice. The cell suspension was cultured at 4 $\times$ 10$^5$ cells/ml in the presence of 5 ng/ml of IL-3 and 10% WEHI-3B conditioned supernatant for 3–4 weeks, with media changes every 7 days. The resultant cell populations were 95% c-kit$^+$ and Gr-1$^+$ when analyzed by flow cytometry (Pharmingen). After washing, cells were resuspended at 1 $\times$ 10$^6$ cells/ml and stimulated either for 4 hr for the analysis of cytokine mRNA production or 24 hr for the determination of secreted cytokine. Cells were stimulated with the polyclonal

activators PMA at 50 ng/ml and/or calcium ionophore A23187 at 500 ng/ml. IgE cross-linking was performed by first incubating the cells for 30 min in the presence of 3 μg/ml of monoclonal mouse IgE anti-DNP (clone SPE-7, from Sigma Chemical Co., Poole, UK), before washing the cells and resuspending in the presence of 10 ng/ml of $DNP_{30–40}$ human serum albumin (Sigma). Cytokine mRNA production was analyzed using reverse transcriptase (RT)-PCR using the pPQRS plasmid and primers described by Reiner et al. (1993), with the exception of IL-13 mRNA, which was detected with the primers outlined above. Cytokine secretion was assessed using ELISA.

### Administration of Recombinant IL-13

The mouse IL-13 cDNA sequence was inserted downstream of an oligonucleotide encoding the FLAG epitope in the mammalian cell expression vector pME18S (Zurawski et al., 1995) to form a fusion protein with the FLAG epitope at the N terminus of the IL-13 protein. IL-13/FLAG was purified, from tissue culture supernatants collected from COS7 cells transfected with the IL-13/flag plasmid vector, using the M2 anti-FLAG monoclonal antibody affinity column (Kodak, New Haven, CT). IL-13 concentration was assessed by ELISA, and bio-activity was determined using the B9 cell line (Aarden et al., 1987). SDS-PAGE analysis indicated that the protein was approximately 80% pure. Mice were inoculated intraperitoneally with 10,000 nonviable *S. mansoni* eggs in the presence of either 2.5 μg of active recombinant IL-13 or 2.5 μg of denatured IL-13 (boiled for 15 min). At 3 and 6 days, the animals were given additional 2.5 μg doses of cytokine or denatured protein intraperitoneally. After 10 days, animals were sacrificed and mesenteric LN cells were cultured for 5 days in the presence of 20 μg/ml of SEA as described above.

Alternatively, IL-$13^{-/-}$ mice were infected with *N. brasiliensis* and in addition were injected intraperitoneally with either PBS or PBS containing 0.5 μg of rmIL-13 (R & D Systems). Repeat administrations of PBS or rmIL-13 (0.5 μg) were given intraperitoneally after 3 days, and the animals were sacrificed 6 days postinfection.

### Acknowledgments

The authors wish to thank David Gray for the gift of anti-CD40 antibody clone FGK-45; Theresa Langford, Gareth King, Tom McNeil, Linda Lucian, and Joy Potter for technical support; and Colin Sanderson, Richard Sever, Richard Turner, John Girdlestone, Bob Coffman, and Michael Townsend for helpful discussions and advice on the manuscript. DNAX Research Institute is supported by Schering-Plough.

Received June 3, 1998; revised August 12, 1998.

### References

Aarden, L.A., DeGroot, E.R., Schapp, O.L., and Lansdorp, P.M. (1987). Production of hybridoma growth factor by human monocytes. Eur. J. Immunol. *17*, 1411–1416.

Aman, M.J., Tayebi, N., Obiri, N.I., Puri, R.K., Modi, W.S., and Leonard, W.J. (1996). cDNA cloning and characterization of the human interleukin 13 receptor α chain. J. Biol. Chem. *271*, 29265–29270.

Bancroft, A.J., McKenzie, A.N.J., and Grencis, R.K. (1998). A critical role for IL-13 in resistance to intestinal nematode infection. J. Immunol. *160*, 3453–3461.

Barner, M., Mohrs, M., Brombacher, F., and Kopf, M. (1998). Differences between IL-4Rα-deficient and IL-4-deficient mice reveal a role for IL-13 in the regulation of Th2 responses. Curr. Biol. *8*, 669–672.

Bost, K.L., Holton, R.H., Kincy Cain, T., and Clements, J.D. (1996). In vivo treatment with anti-interleukin-13 antibodies significantly reduces the humoral response against an oral immunogen in mice. Immunology *87*, 633–641.

Bousquet, J., Chanez, P., Lacoste, J.Y., Barneon, G., Ghavanian, N., Enander, I., Venge, P., Ahlstedt, S., Simony-Lafontaine, J., and Godard, P. (1990). Eosinophilic inflammation in asthma. N. Engl. J. Med. *323*, 1033–1039.

Brewer, J.M., Conacher, M., Satoskar, A., Bluethmann, H., and Alexander, J. (1996). In interleukin-4-deficient mice, alum not only generates T helper 1 responses equivalent to Freund's complete adjuvant, but continues to induce T helper 2 cytokine production. Eur. J. Immunol. *26*, 2062–2066.

Brown, K.D., Zurawski, S.M., Mosmann, T.R., and Zurawski, G. (1989). A family of small inducible proteins secreted by leukocytes are members of a new superfamily that includes leukocyte and fibroblast-derived inflammatory agents, growth factors, and indicators of various activation processes. J. Immunol. *142*, 679–687.

Caput, D., Laurent, P., Kaghad, M., Lelias, J-M., Lefort, S., Vita, N., and Ferrara, P. (1996). Cloning and characterization of a specific interleukin (IL)-13 binding protein structurally related to the IL-5 receptor α chain. J. Biol. Chem. *271*, 16921–16926.

Constant, S., Pfeiffer, C., Pasqualini, T., and Bottomly, K. (1995). Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. J. Exp. Med. *182*, 1591–1596.

Curiel, R.E., Lahesmaa, R., Subleski, J., Cippitelli, M., Kirken, R.A., Young, H.A., and Ghosh, P. (1997). Identification of a Stat-6-responsive element in the promoter of the human interleukin-4 gene. Eur. J. Immunol. *27*, 1982–1987.

de Waal Malefyt, R., Figdor, C., Huijbens, R., Mohan-Peterson, S., Bennett, B., Culpepper, J., Dang, W., Zurawski, G., and de Vries, J.E. (1993). Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. J. Immunol. *151*, 6370–6381.

Doherty, T.M., Kastelein, R., Menon, S., Andrade, S., and Coffman, R.L. (1993). Modulation of murine macrophage function by interleukin-13. J. Immunol. *151*, 7151–7160.

Emson, C.L., Bell, S.E., Jones, A., Wisden, W., and McKenzie, A.N.J. (1998). IL-4-independent induction of IgE, and perturbation of T cell development in transgenic mice expressing interleukin-13. J. Exp. Med. *188*, 399–404.

Gauchat, J-F., Schlagenhauf, E., Feng, N-P., Moser, R., Yamage, M., Jeannin, P., Alouani, S., Elson, G., Notarangelo, L.D., Wells, T., et al. (1997). A novel 4-kb interleukin-13 receptor α mRNA expressed in human B, T, and endothelial cells encoding an alternate type-II interleukin-4/interleukin-13 receptor. Eur. J. Immunol. *27*, 971–978.

He, Y-W., and Malek, T.R. (1995). The IL-2 receptor γc chain does not function as a subunit shared by the IL-4 and IL-13 receptors: implication for the structure of the IL-4 receptor. J. Immunol. *155*, 9–12.

Hilton, D.J., Zhang, J.-G., Metcalf, D., Alexander, W.S., Nicola, N.A., and Willson, T.A. (1996). Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. Proc. Natl. Acad. Sci. USA *93*, 497–501.

Hjulström, S., Landin, A., Jansson, L., Holmdahl, R., and Heyman, B. (1995). No role of interleukin-4 in CD23/IgE-mediated enhancement of the murine antibody response in vivo. Eur. J. Immunol. *25*, 1469–1472.

Hsieh, C.-S., Macatonia, S.E., Tripp, C.S., Wolf, S.F., O'Garra, A., and Murphy, K.M. (1993). Development of Th1 $CD4^+$ T cells through IL-12 produced by Listeria-induced macrophages. Science *260*, 547–549.

Jinquan, T., Deleuran, B., Gesser, H., Maare, H., Deleuran, M., Larsen, C.G., and Thestrup-Pedersen, K. (1995). Regulation of human T lymphocyte chemotaxis in vitro by T cell-derived cytokines IL-2, IFN-γ, IL-4, IL-10, and IL-13. J. Immunol. *154*, 3742–3752.

Kaplan, M.H., Schindler, U., Smiley, S.T., and Grusby, M.J. (1996). Stat6 is required for mediating responses to IL-4 and for the development of Th2 cells. Immunity *4*, 313–319.

Kondo, M., Takeshita, T., Ishii, N., Nakamura, M., Watanabe, S., Arai, K., and Sugamura, K. (1993). Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. Science *262*, 1874–1877.

Kopf, M., Le Gros, G., Bachmann, M., Lamers, M.C., Bluethmann, H., and Kohler, G. (1993). Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature *362*, 245–248.

Kopf, M., Brombacher, F., Hodkin, P.D., Ramsay, A.J., Milbourne, E.A., Dai, W.J., Ovington, K.S., Behm, C.A., Kohler, G., Young, I.G.,

et al. (1996). IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. Immunity *4*, 15–24.

Kotanides, H., and Reich, N.C. (1996). Interleukin-4-induced STAT6 recognizes and activates a target site in the promoter of the interleukin-4 receptor gene. J. Biol. Chem. *271*, 25555–25561.

Kühn, R., Rajewsky, K., and Müller, W. (1991). Generation and analysis of interleukin-4 deficient mice. Science *254*, 707–710.

Jankovic, D., Kullberg, M.C., Caspar, P., Cheever, A.W., Noben-Trauth, N., and Sher, A. (1998). Induction of egg pathology during *Schistosoma mansoni* infection requires IL-4 receptor, but not IL-4 expression. Second Woods Hole Immunoparasitology Meeting Summary Book, Summary no. 52.

Lederer, J.A., Perez, V.L., DesRoches, L., Kim, S.M., Abbas, A.K., and Lichtman, A.H. (1996). Cytokine transcriptional events during helper T cell subset differentiation. J. Exp. Med. *184*, 397–406.

Le Gros, G., Ben-Sasson, S.Z., Seder, R., Finkelman, F.D., and Paul, W.E. (1990). Generation of interleukin-4 (IL-4)-producing cells in vitro and in vivo: IL-2 and IL-4 are required for in vitro generation of IL-4 producing cells. J. Exp. Med. *172*, 921–929.

Lin, J.-X., Migone, T.-S., Tsang, M., Friedman, M., Weatherbee, J.A., Zhou, L., Yamauchi, A., Bloom, E.T., Mietz, J., John, S., et al. (1995). The role of shared receptor motifs and common stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity *2*, 331–339.

Manetti, R., Parronchi, P., Guidizi, M.G., Piccinni, M.-P., Maggi, E., Trinchieri, G., and Romagnani, S. (1993). Natural killer cell stimulatory factor (interleukin-12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing cells. J. Exp. Med. *177*, 1199–1204.

McKenzie, A.N.J., Culpepper, J.A., de Waal Malefyt, R., Briere, F., Punonnen, J., Aversa, G., Sato, A., Dang, W., Cocks, B.G., Menon, S., et al. (1993a). Interleukin-13, a novel T cell-derived cytokine that regulates human monocyte and B cell function. Proc. Natl. Acad. Sci. USA *90*, 3735–3739.

McKenzie, A.N.J., Li, X., Largaespada, D.A., Sato, A., Kaneda, A., Zurawski, S.M., Doyle, E.L., Milatovich, A., Francke, U., Copeland, N.G., et al. (1993b). Structural comparison and chromosomal localization of the human and mouse IL-13 genes. J. Immunol. *150*, 5436–5444.

McKenzie, G.J., Bancroft, A., Grencis, R.K., and McKenzie, A.N.J. (1998). A distinct role for interleukin-13 in Th2-cell-mediated immune responses. Curr. Biol. *8*, 339–342.

Miloux, B., Laurent, P., Bonnin, O., Lupker, J., Caput, D., Vita, N., and Ferrara, P. (1997). Cloning of the human IL-13Ra1 chain and reconstitution with the IL-4Rα of a functional IL-4/IL-13 receptor complex. FEBS Lett. *401*, 163–166.

Morawetz, R.A., Gabriele, L., Rizzo, L.V., Noben-Trauth, N., Kühn, R., Rajewsky, K., Muller, W., Doherty, T.M., Finkelman, F., Coffman, R.L., et al. (1996). Interleukin (IL)-4-independent immunoglobulin class switch to immunoglobulin (Ig)E in the mouse. J. Exp. Med. *184*, 1651–1661.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Gieldin, M.A., and Coffman, R.L. (1986). Two types of murine helper T cell clone: I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. *136*, 2348–2357.

Mosmann, T.R., and Coffman, R.L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. *7*, 145–173.

Muchamuel, T., Menon, S., Piscane, P., Howard, M.C., and Cockayne, D.A. (1997). IL-13 protects mice from lipopolysaccharide-induced lethal endotoxemia: correlation with down-modulation of TNFα, IFN-γ, and IL-12 production. J. Immunol. *158*, 2898–2903.

Murphy, E., Hieny, S., Sher, A., and O'Garra, A. (1993). Detection of in vivo expression of interleukin-10 using a semi-quantitative polymerase chain reaction method in Schistosoma mansoni infected mice. J. Immunol. Methods *162*, 211–223.

Nicoletti, F., Mancuso, G., Cusumano, V., Di Marco, R., Zaccone, P., Bendtzen, K., and Teti, G. (1997). Prevention of endotoxin-induced lethality in neonatal mice by interleukin-13. Eur. J. Immunol. *27*, 1580–1583.

O'Garra, A. (1998). Cytokines induce the development of functionally heterogeneous T helper cell subsets. Immunity *8*, 275–283.

Pearce, E.J., Caspar, P., Gryzch, J-M., Lewis, F.A., and Sher, A. (1991). Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. J. Exp. Med. *172*, 159–166.

Pernis, A., Gupta, S., Gollob, K.J., Garfein, E., Coffman, R.L., Schindler, C., and Rothman, P. (1995). Lack of interferon γ receptor β chain and the prevention of interferon γ signaling in Th1 cells. Science *269*, 245–247.

Punnonen, J., Aversa, G., Cocks, B.G., McKenzie, A.N.J., Menon, S., Zurawski, G., de Waal Malefyt, R., and de Vries, J.E. (1993). Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc. Natl. Acad. Sci. USA *90*, 3730–3734.

Reiner, S.L., Zheng, S., Corry, D.B., and Locksley, R.M. (1993). Constructing polycompetitor cDNAs for quantitative PCR. J. Immunol. Methods *165*, 37–46.

Rincón, M., Anguita, J., Nakamura, T., Fikrig, E., and Flavell, R.A. (1997) Interleukin (IL)-6 directs the differentiation of IL-4-producing $CD4^+$ T cells. J. Exp. Med. *3*, 461–469.

Shimoda, K., van Deursen, J., Sangster, M.Y., Sarawar, S.R., Carson, R.T., Tripp, R.A., Chu, C., Quelle, F.W., Nosaka, T., Vignali, D.A.A., et al. (1996). Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature *380*, 630–633.

Smerz-Bertling, C., and Duschl, A.L. (1995). Both interleukin 4 and interleukin 13 induce tyrosine phosphorylation of the 140-kDa subunit of the interleukin 4 receptor. J. Biol. Chem. *270*, 966–970.

Sutton, B.J., and Gould, H.J. (1993). The human IgE network. Nature *366*, 421–428.

Szabo, S.J., Dighe, A.S., Gubler, U., and Murphy, K.M. (1997). Regulation of the interleukin (IL)-12Rb2 subunit expression in developing T helper 1 (Th1) and Th2 cells. J. Exp. Med. *185*, 817–824.

Takeda, T., Tanaka, T., Shi, W., Matsumoto, M., Minami, M., Kashiwamura, S., Nakanishi, K., Yoshida, N., Kishimoto, T., and Akira, S. (1996). Essential role of Stat6 in IL-4 signaling. Nature *380*, 627–630.

Urban, J.F., Jr., Noben-Trauth, N., Donaldson, D.D., Madden, K.B., Morris, S.C., Collins, M., and Finkelman, F.D. (1998). IL-13, IL-4Rα, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. Immunity *8* , 255–264.

van der Pouw Kraan, T.C., Boieje, L.C., Troon, J.T., Rutschmann, S.K., Wijdenes, J., and Aarden, L.A. (1996). Human IL-13 production is negatively influenced by CD3 engagement. J. Immunol. *156*, 1818–1823.

Van der Weld, T., Kopf, M., Köhler, G., and Langhorne, J. (1994). The immune response to Plasmodium chabaudi malaria in interleukin-4-deficient mice. Eur. J. Immunol. *24*, 2285–2293.

Zurawski, G., and de Vries, J.E. (1994). Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells but not on T cells. Immunol. Today *15*, 19–26.

Zurawski, S.M., Vega, F., Jr., Huyghe, B., and Zurawski, G. (1993). Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. EMBO J. *12*, 3899–3905.

Zurawski, S.M., Chomarat, P., Djousso, O., Bidaud, C., McKenzie, A.N.J., Miossec, P., Banchereau, J., and Zurawski, G. (1995). The primary binding subunit of the interleukin-4 receptor is also a component of the interleukin-13 receptor. J. Biol. Chem. *270*, 13869–13878.