A distinct role for interleukin-13 in Th2-cell-mediated immune responses

Grahame J. McKenzie*, Allison Bancroft†, Richard K. Grencis† and Andrew N.J. McKenzie*

Immune responses elicited by allergic reactions and parasitic worm infections are characterised by the induction of T helper 2 (Th2) cells. These cells secrete cytokines such as interleukin-4 (IL-4), IL-5 and IL-13, which induce the production of immunoglobulin E (IgE) and eosinophils [1,2]. Previous studies using gastrointestinal nematodes to elucidate the role of Th2-cell-mediated immune responses have demonstrated a causal relationship between T cells and worm expulsion (reviewed in [3]). Although it has been proposed that IL-4 played a central role in these responses, recent studies demonstrated that IL-4$^{-/-}$ mice expel the parasitic gastrointestinal nematode *Nippostrongylus brasiliensis* normally [4], suggesting that another T-cell mediator is required for efficient worm clearance. Using IL-13$^{-/-}$ mice, we have demonstrated that, unlike wild-type and IL-4$^{-/-}$ mice, the IL-13$^{-/-}$ animals failed to clear *N. brasiliensis* infections efficiently, despite developing a robust Th2-like cytokine response to infection. Furthermore, treatment of the IL-13$^{-/-}$ mice with exogenous IL-13 resulted in a reduction in the numbers of worms recovered. The IL-13$^{-/-}$ animals also failed to generate the goblet cell hyperplasia that normally occurs coincident with worm expulsion. This observation may link IL-13 with the production of intestinal mucus which is believed to facilitate worm expulsion. These data support a unique role for IL-13 in Th2-cell-mediated immune responses and demonstrate that IL-13 and IL-4 are not redundant.

Addresses: *MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK. †School of Biological Science, 3.239 Stopford Building, University of Manchester, Oxford Road, Manchester, M13 9PT, UK.

Correspondence: Andrew N.J. McKenzie
E-mail: anm@mrc-lmb.cam.ac.uk

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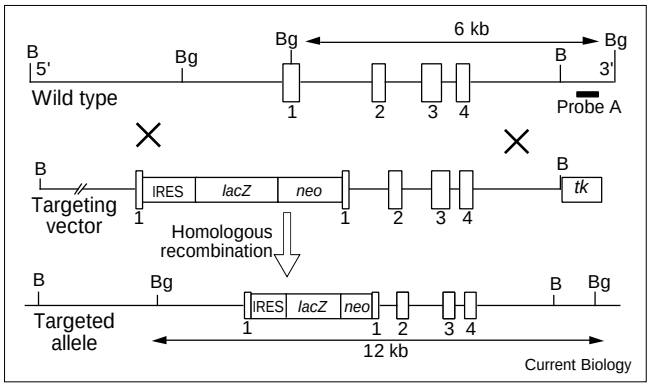
Results and discussion

We disrupted the IL-13 gene by insertion of a cassette containing a *lacZ* reporter gene and a gene encoding neomycin resistance into exon 1 of the IL-13 locus (Figure 1). Homozygous IL-13$^{-/-}$ animals were healthy and displayed no overt phenotypic abnormalities. Lymphoid organs had a normal appearance and no differences were observed in lymphoid or myeloid cell numbers. Expression of the immunoregulatory molecules CD4, CD8, CD3ε and B220 was also normal (data not shown). Enzyme-linked immunosorbent assays (ELISA) specific for IL-13 failed to detect IL-13 protein produced by activated lymphocytes derived from the IL-13$^{-/-}$ mice (see later).

Figure 1

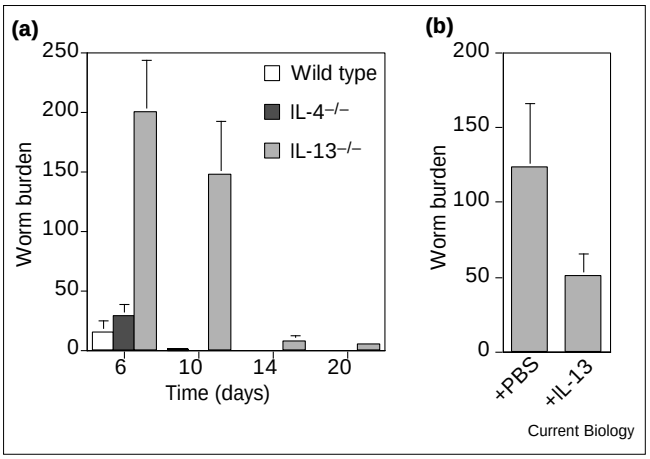


Disruption of the IL-13 gene by gene targeting. The structure of the IL-13 locus, the targeting vector and the predicted homologous recombination event are shown. A replacement gene-targeting construct was generated using a 6 kb *Bam*HI (B) fragment of the mouse IL-13 gene cloned into pUCBM21. An oligonucleotide linker containing *Not*I and *Xho*I sites was cloned into the *Bgl*II (Bg) site in exon 1 of the IL-13 gene using a partial *Bgl*II digest. A *Not*I–*Sal*I cassette containing the following elements (in 5′ to 3′ order) was cloned into the engineered *Not*I and *Xho*I sites: an *Spe*I linker containing stop codons in all three frames, a retroviral internal ribosomal entry site (IRES) element fused to the *lacZ* reporter gene with a 3′ polyadenylation signal, and the gene encoding resistance to neomycin (*neo*). The herpes simplex virus thymidine kinase gene (*tk*) was cloned into the vector 5′ to the IL-13 coding region to yield the completed targeting vector, pIL13lacZKO. The vector was linearized with *Sal*I and transfected into E14.1 embryonic stem cells by electroporation. *Bgl*II-digested genomic DNA from isolated clones were screened by hybridization using flanking probe A. The probe was made by PCR using the primers: 5′-AAGAGCCCGAGGCATGAT-GCG-3′ and 5′-TCTGGGCGCTATTGCTTGGTCTCTTCTGCC-3′. Subsequent hybridization with a probe to the neomycin sequence confirmed that only a single integration event had occurred. Out of the 192 clones screened, 11 had been disrupted by homologous recombination at the IL-13 locus. Correctly targeted clones were injected into C57BL/6 blastocysts to generate chimaeras. Offspring that were heterozygous for the disrupted IL-13 gene were interbred to yield IL-13$^{-/-}$ mice. The IL-13$^{-/-}$ and wild-type animals were maintained on a 129Ola × C57BL/6 (F2) background in a specific pathogen-free environment.

As the production of Th2-specific cytokines is characteristic of immune responses to parasitic nematode infections, we challenged IL-13$^{-/-}$ mice and wild-type mice with the gastrointestinal nematode parasite *N. brasiliensis*, which typically induces a robust Th2-cell-mediated response and is normally expelled rapidly by healthy mice [3,4]. As expected, wild-type animals had expelled all adult parasites from the intestine by day 10 post-infection (p.i.). Similarly, IL-4$^{-/-}$ mice, like wild-type mice, expelled *N. brasiliensis* parasites by day 10 p.i. (Figure 2a). By contrast, IL-13$^{-/-}$ animals still harboured a substantial number of worms at 10 days p.i. (Figure 2a), and worms remained in the intestine until at least day 20 p.i. These results from IL-13$^{-/-}$ mice demonstrate a unique and critical role for IL-13 in T-cell-induced parasitic worm expulsion, and illustrate that IL-4 and IL-13 do not have redundant roles. As the worms are eventually expelled from IL-13$^{-/-}$ mice, however, it is clear that alternative, IL-13-independent mechanisms of expulsion exist.

As the absence of IL-13 resulted in a failure to clear *N. brasiliensis* efficiently, we attempted to reverse the impairment in worm expulsion by treating IL-13$^{-/-}$ animals with recombinant mouse IL-13 (rmIL-13) at the time of infection and again after 3 days. We observed a reduction in the number of worms recovered from the rmIL-13-treated mice compared to those recovered from animals that received carrier alone (Figure 2b). These data strongly support the finding that IL-13 is a major factor controlling the expulsion of gastrointestinal worm infections.

Figure 2



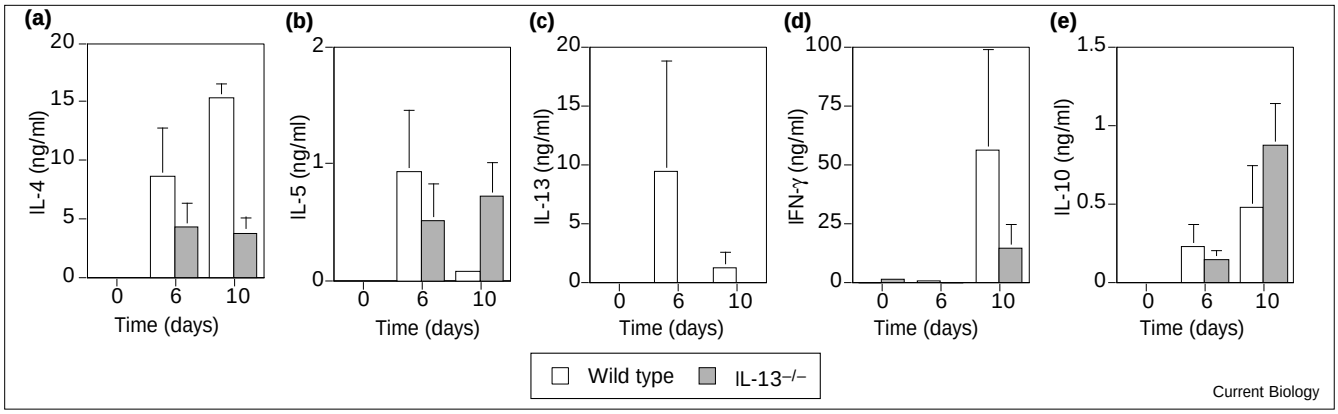
Intestinal worm burdens. (a) Cohorts of five mice were infected subcutaneously with 400 viable third-stage *N. brasiliensis* larvae and sacrificed at the times indicated to obtain intestinal worm counts. Representative data from two repeat experiments are shown. IL-4$^{-/-}$ mice [15] were obtained from B&K Universal Ltd. (b) Intestinal worm burdens of IL-13$^{-/-}$ mice following administration of rmIL-13. IL-13$^{-/-}$ mice were infected as above, and in addition were also injected intraperitoneally (i.p.) with either PBS or PBS containing 0.5 μg rmIL-13 (R&D). Treated mice were given repeat i.p. administrations of PBS or rmIL-13 (0.5 μg) after 3 days and sacrificed 6 days after infection. Data represent the mean and standard deviation from five animals per group.

We determined serum Ig isotype expression from naive and *N. brasiliensis*-infected mice. Interestingly, we detected a reproducible 3–5-fold reduction in the basal levels of IgE in the naive IL-13$^{-/-}$ mice compared to wild-type animals, whereas the levels of other immunoglobulin isotypes were not significantly different (data not shown). As IL-13 has been demonstrated to induce IgE isotype switching by human B cells [2], and as IL-4-independent IgE expression also occurs in the mouse [5], these data suggest that IL-13 may also modulate IgE expression in the mouse. After infection with *N. brasiliensis*, an equivalent induction of Ig isotypes took place in the wild-type and IL-13$^{-/-}$ animals, with the exception of IgA levels, which were significantly greater (10-fold) in the IL-13$^{-/-}$ mice (data not shown). This finding indicates that whereas IL-13$^{-/-}$ mice induced IgA in response to persistent infection, wild-type animals did not, presumably because they eliminated the worms rapidly. We also observed that the magnitude of the antigen-specific IgG1 and IgG2a responses to *N. brasiliensis* at 14 days p.i. was similar in IL-13$^{-/-}$ and wild-type animals (data not shown). Thus, IL-13$^{-/-}$ animals can apparently mount a potent Ig response to worm infection. These data contrast markedly with those from the IL-4$^{-/-}$ mice which produce negligible titres of IgG1 or IgE in response to *N. brasiliensis* [6] yet clear the infection, and support other studies in which antigen-specific Igs were not found to be required for worm clearance [7].

We also assessed cytokine secretion from the draining mesenteric lymph node cells prepared from *N. brasiliensis*-infected wild-type and IL-13$^{-/-}$ mice. We found no gross differences in the levels of IL-4, IL-5 and IL-10 produced by mitogen-stimulated lymph node cells 6 days after infection, at a time when worm expulsion was taking place in the wild-type animals (Figure 3). By day 10 p.i., IL-4 production was reduced by approximately three-fold in the IL-13$^{-/-}$ animals, although this did not appear to affect IgE production. As the complete absence of IL-4 in the IL-4$^{-/-}$ mice does not affect worm expulsion, it seems unlikely that this observed change is significant. Expression of IL-5 remained elevated in the IL-13$^{-/-}$ cells at day 10 p.i. (Figure 3), and it is noteworthy that this correlated with the increased serum concentrations of IgA, because IL-5 has been suggested to enhance IgA production [8]: it should be noted that IL-5$^{-/-}$ mice control *N. brasiliensis* infections normally [9]. Whereas there was considerable variation in the levels of the Th1-specific cytokine interferon-γ (IFN-γ), the trend was towards higher levels of IFN-γ production in the wild-type animals (Figure 3).

Previous studies have identified a correlation between T cells and worm expulsion, with severe combined

Figure 3



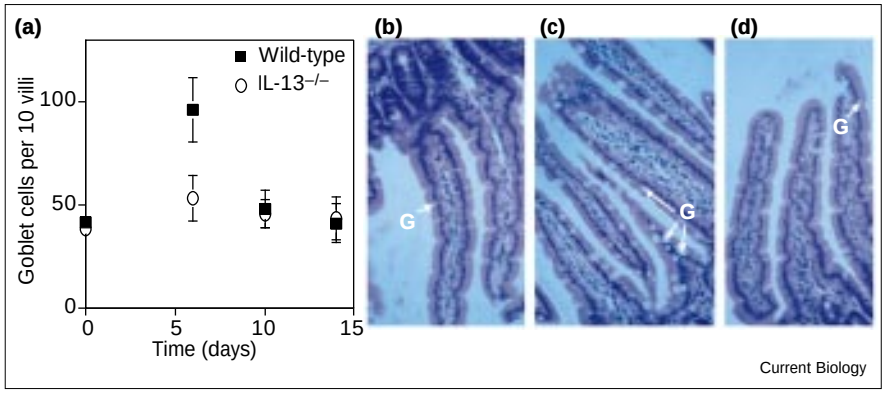
Cytokine expression by activated lymph node cells following infection with *N. brasiliensis*. Lymph node cells prepared from mice either before, or at 6 and 10 days p.i., were plated at 2×10^6 cells per ml and cultured for 24 h in the presence of concanavalin A (2 μg/ml). Supernatants were analysed by cytokine ELISA (Pharmingen), and the IL-13 ELISA was from R&D. Representative data from two repeat experiments containing five mice per group are shown.

immunodeficient (SCID) mice and $CD4^+$ T-cell-depleted mice failing to expel worm infections [3]; a chronological association between goblet cell hyperplasia and worm expulsion has also been reported [10]. Intestinal goblet cells are responsible for the release of mucins into the intestinal lumen and a number of studies have suggested that worm expulsion coincides with the goblet cell response and not the mast cell response [10]. Moreover, recent work has indicated that the quantity of mucus produced as a consequence of *N. brasiliensis* infection affects worm expulsion [11]. Importantly, adoptive transfer of antigen-specific T cells can confer both goblet cell hyperplasia and worm expulsion [10]. We therefore examined intestinal sections to assess the numbers of goblet cells in the intestinal villi of wild-type and IL-$13^{-/-}$ animals before infection and at time points after infection. Significantly, we found that the IL-$13^{-/-}$ mice failed to mount the profound intestinal goblet cell hyperplasia generated by the wild-type animals at the time of worm expulsion (Figure 4a). Coincident with the quantitative increase in goblet cell number in the wild-type animals during infection, we also observed a qualitative change in goblet cell morphology, with the cells appearing considerably enlarged at 6 days p.i. compared with goblet cells from the IL-$13^{-/-}$ mice at a similar time point (Figure 4b–d). Thus, IL-13 may be mediating its essential role by modulating goblet cell differentiation or activation, thereby linking the adaptive immune system to the innate immune response.

We have demonstrated that worm clearance is severely impaired in mice deficient in IL-13. Recently, it has been reported that animals in which components of the IL-4 signaling pathway have been disrupted, notably Stat$6^{-/-}$ mice and IL-4 receptor (IL-4R)$^{-/-}$ mice, also fail

Figure 4

Intestinal morphology following *N. brasiliensis* infection. (a) Intestinal goblet cell numbers were determined at various time points after infection. Data represent the mean number of goblet cells per ten villi from five animals. (b–d) Haematoxylin–eosin staining of intestinal villi sections: (b) wild-type intestine before infection; (c) wild-type intestine 6 days p.i., note the increased numbers of enlarged goblet cells; (d) IL-$13^{-/-}$ intestine 6 days p.i. G denotes goblet cell. Small intestine specimens were fixed in neutral-buffered formalin, embedded, sectioned and stained.



to expel gastrointestinal worms [3]. As Stat6 and one of the subunits of the IL-4R can also be shared by IL-13-mediated signaling [12–14], we must conclude that in certain instances the IL-4R and Stat6 signaling pathways are IL-13-specific and that the Stat6$^{-/-}$ and IL-4R$^{-/-}$ mice display an element of deficiency in IL-13-mediated signaling. Our data highlight the importance of understanding the role of IL-13 in other responses involving Th2 cells, including those provoked in asthma and allergic reactions.

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