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Schistosoma mansoni in IL-4-deficient mice

Edward J. Pearce, Allen Cheever[1,4], Susan Leonard, Maria Covalesky, Raphael Fernandez-Botran[2], Georges Kohler[†] and Manfred Kopf[3,5]

Department of Microbiology and Immunology, NYSCVM, Cornell University, Ithaca, NY 14853, USA
[1]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[2]Department of Pathology, School of Medicine, University of Louisville, KY 40292, USA
[3]Max-Planck-Institut für Immunobiologie, D-79108 Freiburg, Germany
[4]Present address: Biomedical Research Institute, 12111 Parklawn Drive, Rockville, MD 20852, USA
[5]Present address: Basel Institute for Immunology, Grenzacherstrasse 487, Postfach, CH-4005, Basel, Switzerland

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Abstract

Immunopathology and immune responses to *Schistosoma mansoni* were examined in IL-4 –/– mice. IL-5 and IL-10 production by lymphoid cells stimulated with soluble egg antigen (SEA), peripheral eosinophilia and serum levels of soluble IL-4 receptor but not IgE were all significantly elevated over background normal levels in IL-4 –/– mice as a result of infection. Additionally, IL-10 and IL-5 in addition to IL-2 and IFN-γ transcripts were equally evident in diseased liver tissue from infected IL-4 –/– and wild-type mice. Nevertheless, analysis of antigen-stimulated IL-2, IL-4, IL-5, IL-10 and IFN-γ production by lymphoid organ cells from infected or egg-injected IL-4 –/– mice revealed a more T_h1-like pattern of cytokine production (IFN-γ > IL-5) than in (wild-type) mice in which a stronger type 2 response to SEA was detectable (IL-4, IL-5 > IFN-γ). Despite this, at 8 and 16 weeks after infection, liver pathology, as indicated by the size, cellularity, cellular composition and collagen content of granulomas, was similar in IL-4 –/– and wild-type animals. As in wild-type animals, granuloma size at week 16 was smaller than at week 8, indicating that modulation had occurred in the absence of IL-4. Differences in pathology were seen only when eggs were experimentally embolized to the lungs, in which case IL-4 –/– mice made smaller granulomatous responses than did wild-type animals. These data clearly show that IL-4 is not necessary for the hepatic granuloma formation which occurs during experimental schistosomiasis.

Introduction

The major pathologic consequence of infection with *Schistosoma mansoni* is the formation of granulomas around parasite eggs trapped in the sinusoids of the liver, a process which can lead eventually to severe hepatic fibrosis (1,2). While it has been known for many years that the granulomatous response is largely $CD4^+$ cell-dependent (3), the details of which cytokines are central to its orchestration have remained elusive. Indication that cytokines made by the T_h2 subset of $CD4^+$ cells might be involved in pathogenesis came when an examination of the kinetics of the developing T_h response during infection revealed that beginning at weeks 5–6 of infection, coincident with the initiation of egg production and granuloma formation, splenic and mesenteric lymph node (MLN) cells responded to T cell mitogens or antigen by producing, in quantity, IL-4, IL-5, IL-10 and relatively little IL-2 or IFN-γ (4,5). The reactive T cells recovered from lymphoid organs at the time of granuloma formation are therefore strongly T_h2-like, suggesting that these cells play a central role in pathogenesis due to *S. mansoni*.

Two lines of research are supportive of a role for T_h2 cells in granuloma formation. The most recently developed of these is the use of IL-12 to promote a schistosome egg-induced T_h1 response (6,7). Isolated eggs injected into naive mice induce T_h2 responses at the site of lesion development and in draining LN (8–10). When this response was skewed in a T_h1-like direction by co-injecting eggs with IL-12 or by immunizing mice with eggs plus IL-12 i.p. prior to injecting them i.v. with eggs, the granulomas which subsequently formed around eggs trapped in the lungs were very much smaller than those in control animals (6). Moreover, hepatic fibrosis was greatly reduced in mice immunized with eggs

Correspondence to: E J Pearce
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plus IL-12 and subsequently infected (7) The more widely used technique to examine the role of T_h2 cells in granulomagenesis has been the use of neutralizing anti-cytokine mAb. Due to its central role in T_h2 cell development (see 11), IL-4 has been a focus of many such experiments and mAb anti-IL-4 has been shown to reduce considerably the size of granulomas in the lungs of mice injected i.v. with schistosome eggs (10,12). In infected mice, the effect on hepatic granulomas of neutralizing IL-4 has been variously reported as reducing lesion size (13) or having minimal effect on size but significantly reducing hepatic collagen deposition (14) Invariably, in the IL-4 neutralization studies, residual T_h2-like responses have evolved, raising the possibility that neutralization has not been absolute.

Here, we attempt to address in a more defined context the role of T_h2 responses in the immunopathology of schistosomiasis by using mice in which the IL-4 gene has been knocked out by homologous recombination (IL-4 –/– mice, 15); IL-4 has been reported to be the key cytokine promoting T_h2 cell development (16–20).

Methods

Experimental infections and pathology

Schistosoma mansoni were NMRI strain and supplied by Dr F. Lewis (Biomedical Research Institute, Rockville, MD). The IL-deficient (IL-4 –/–) mouse strain was generated by gene targeting in embryonic stem cells (15) Interbred (C57BL/6×129Sv)F_2 IL-4 –/– mice and age- and sex-matched control IL-4 wild-type F_2 breedings were used for experiments. Mice were anesthetized with i.p pentobarbitone and infected by percutaneous exposure of the shaved abdomen to cercariae. Animals were euthanized with heparinized pentobarbitone i p., their spleens were removed aseptically and adult schistosomes were recovered by tissue perfusion with sterile citrate saline. MLN were removed into DMEM containing 10 mM HEPES, 100 U/ml penicillin and 100 µg/ml streptomycin (all from Gibco/BRL, Gaithersburg, MD). Livers were removed and weighed Pieces of small intestine and liver were frozen in liquid nitrogen for subsequent mRNA extraction and fixed in neutral buffered formalin for histology. Additional liver was used for collagen and parasite egg quantitation, as described (21). Using Masson's trichrome-stained liver sections, the diameters of granulomas around single eggs were measured with an ocular micrometer and the volume calculated assuming a spherical shape. Giemsa- and hematoxylin & eosin-stained sections of liver and small intestine were also examined For the study of pulmonary granulomas, mice were injected i.v into the tail vein with 250 µl of sterile PBS containing 5000 eggs (isolated from the livers of heavily infected mice). Fourteen days later the lungs were removed and fixed Giemsa stained sections were examined. Popliteal lymph node (LN) responses were examined in mice injected s.c. into each rear footpad with 2500 eggs in 25 µl PBS, as described (8,9). Peripheral blood eosinophils were enumerated using Unopettes (Becton Dickinson, Rutherford, NJ).

Analysis of cytokine responses

Single cell suspensions were prepared by pressing lymphoid organs through 70 µm nylon mesh (Falcon 2350, Becton Dickinson, Franklin Lakes, NJ) followed by extensive washing with DMEM. Erythrocytes in splenocyte populations were lysed with ammonium chloride. Cells that excluded Trypan blue were counted and resuspended at 10^7/ml in complete tissue culture medium containing: DMEM, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, 30 mM HEPES, 5×10^{-5} M 2-mercaptoethanol and 10% FCS (all from Gibco/BRL). In some experiments, $CD4^+$ cells were depleted using mAb GK1.5 anti-CD4 plus rabbit complement (Accurate Chemical, Westbury, NY), as described (8). FITC-conjugated mAb RM-4-5 anti-CD4 (PharMingen, San Diego, CA) and flow cytometry were used to assess the efficiency of depletion Cytokine assays were performed as previously described (8) Briefly, cells were incubated without or with 50 µg/ml soluble egg antigen (SEA) (22, 23), concanavalin A (5 µg/ml) or plate-bound mAb anti-CD3 (500 ng/well), in 96-well flat-bottom plates in 5% CO_2 at 37°C. Supernatants from 24 h and 72 h cell cultures were removed under sterile conditions and stored at –20°C IL-4, IL-5 and IFN-γ in 72 h culture supernatants and IL-10 in 24 h supernatants were quantitated using highly specfic and sensitive two site ELISA (8, 24) The CTLL proliferation assay, utilizing MTT (Thiazolyl blue; Sigma, St Louis, MO), was used to quantitate IL-2 in 24 h supernatants (25), 11B11 mAb anti-IL-4 was used to confer specificity. In each cytokine assay, recombinant cytokines were used to generate standard curves. For all colorimetric assays color development was monitored on a Bio-Tek EL 309 ELISA reader.

For analysis of cytokine gene transcripts, tissue samples were thawed into RNAzol (Tel-Test, Friendswood, TX), homogenized using a PRO200 (PRO Scientific, Monroe CT) and whole RNA recovered as recommended by Tel-Test. Single-strand cDNA was synthesized from 1 µg RNA using a First Strand cDNA Synthesis kit (Pharmacia, Piscataway, NJ) and 5 µl of the cDNA used for PCR using the cytokine and HPRT (housekeeping gene) primers described by Svetic *et al* (26). PCR conditions were: 95°C for 3 min followed by 34 cycles of 94°C 1 min, 62°C 1 min. PCR products were electrophoresed on 1.5% agarose gels, blotted to Nytran and probed with gene-specific oligonucleotide probes which had been end-labeled with fluorescein. Detection was by a peroxidase-conjugated fluorescein-specific sheep antibody and enhanced chemiluminescence (Amersham, Arlington Heights, IL)

sIL-4R immunoassay

The concentration of sIL-4R in serum samples was measured by a two-site ELISA using the rat anti-mouse IL-4R mAb, M1 and M2 (27), as described (28). The concentrations of sIL-4R were calculated based on a standard curve obtained using recombinant sIL-4R (kindly provided by Dr M. Widmer, Immunex, Seattle, WA)

Quantitation of serum IgE

Total serum IgE was measured using a two-site ELISA Immulon 2 plates (Dynatech, Chantilly, VA) were coated with 25 ng per well of Protein G-purified EM-95 mAb anti-mouse IgE. Bound IgE was detected with FITC-coupled R35-92 mAb anti-mouse IgE (PharMingen) followed by peroxidase-conjugated sheep anti-FITC (Amersham) and ABTS (Kirkegaard and

Table 1. Numbers of parasites recovered from infected IL-4 –/– and wild-type mice[a]

	8 weeks		16 weeks	
	Wild-type	IL-4 –/–	Wild-type	IL-4 –/–
Number of parasites recovered[b]	45 0±12.2	37 7±12 2	17 0±7.2	10.0±6 7
	$P = 0\ 49$		$P = 0\ 14$	

[a] n = 6–10 mice per group
[b] To ensure host survival, the 16 week infections were initiated with ~70% fewer cercariae than the 8 week infections

Perry, Gaithersburg, MD). A purified mouse IgE mAb (the gift of Dr D Holowka, Cornell University) at known concentration was used to generate a standard curve.

Statistics

Comparisons were by Student's *t*-test except for hepatic fibrosis, where analysis of covariance using the log of total liver eggs as the covariate and the log of hydroxyproline per egg was used (14). Values of $P < 0.05$ were considered significant

Results

Parasitology and pathology

To examine whether the lack of the IL-4 gene has any effect on the ability of mice to support a schistosome infection, we infected wild-type and IL-4 –/– mice and perfused groups of animals at 7–8 and 15–16 weeks after infection (hereafter referred to as 8 week and 16 week infection groups) and enumerated the recovered parasites. As shown in Table 1, there were no significant differences in the numbers of parasites recovered from the two types of mice at either time point. Moreover, the numbers of female parasites recovered from wild-type and IL-4 –/– mice were not significantly different (not shown)

Sections of liver and small intestine from killed infected animals were examined for pathologic changes. Hepatic granulomas in IL-4 –/– mice infected for 8 weeks were 13.7% smaller ($p < 0.05$) than those in the livers of wild-type mice (infected for the same amount of time) (Fig. 1A). In both types of mice, granulomas were significantly smaller ($P < 0.001$ in each case) at 16 weeks than at 8 weeks, indicating that modulation had occurred, but there were no significant differences between the IL-4 –/– and wild-type animals at this later time point (Fig. 1A). There were no differences in the numbers of granuloma eosinophils or giant cells between the various groups. When fibrosis was evaluated through the quantitation of hydroxyproline (Fig. 1B) there was no significant difference between the two types of mice at either time point after infection, though fibrosis was greater at 16 weeks than at 8 weeks in both groups ($p < 0.05$ in each case). Examination of Masson's trichrome-stained liver sections, in which collagen was specifically stained, led to a similar conclusion (not shown). Analysis of sections of intestines revealed similar eosinophil-enriched egg-associated lesions in the wild-type and IL-4 –/– animals. However, circumoval granulomas around

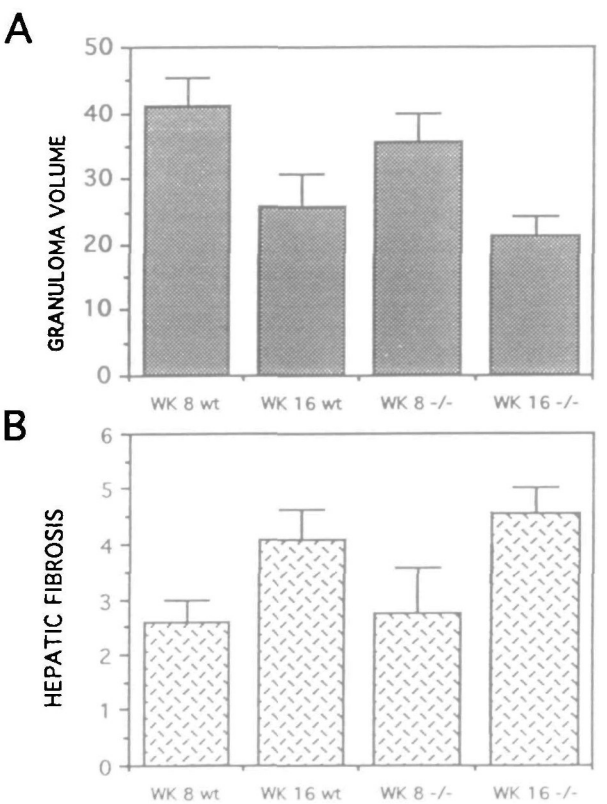

Fig. 1. (A) Volume ($mm^3 \times 10^3$) ± SEM of hepatic circumoval granulomas in wild-type (wt) and IL-4 –/– (–/–) mice at 8 and 16 weeks of infection. (B) Hepatic fibrosis in schistosome infected mice, expressed as μmol hydroxyproline/10,000 eggs ± SEM (after subtracting the mean hydroxyproline per liver of uninfected mice) There were 6–10 mice examined in each group

eggs injected i.v. into mice and entrapped in the lungs, were markedly smaller in IL-4 –/– animals than in wild-type mice (Fig. 2). Taken together, these data indicate that hepatic and intestinal pathology due to *S mansoni* follow a similar course regardless of whether or not IL-4 is produced. However, as reported previously (10,12), the lack of IL-4 has a substantial effect on pulmonary granulomas around experimentally embolized eggs.

Cytokine production in lesions is likely to play a role in pathology. If this is the case in murine schistosomiasis, the histopathologic analyses suggest that local cytokine produc-

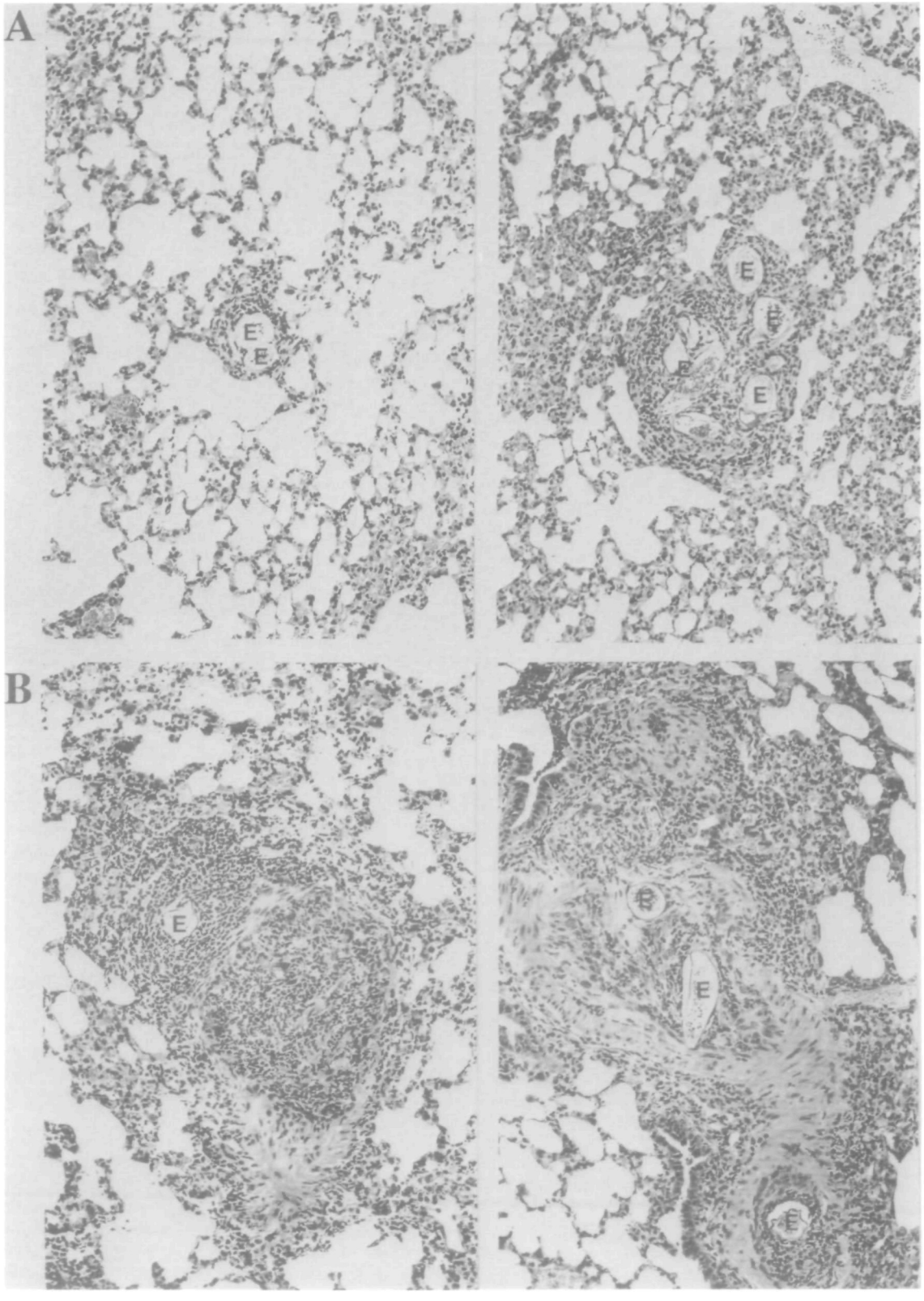

Fig. 2. Pulmonary circumoval granulomas in IL-4 –/– mice (A) and wild-type mice (B) injected i v. with eggs 14 days earlier Fields of view were chosen to illustrate granuloma formation in the presence of few or many eggs Magnification, ×120. E, schistosome egg.

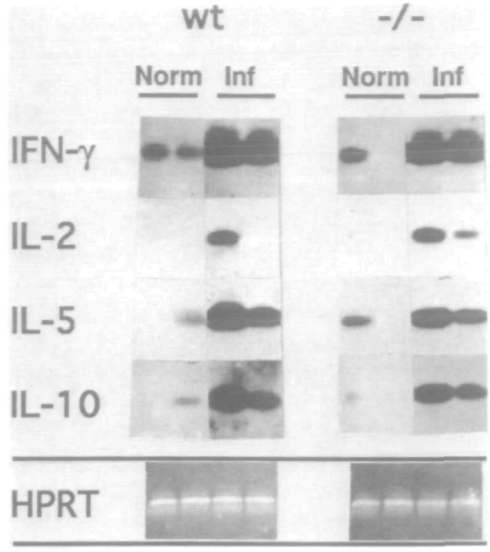

Fig. 3. Cytokine mRNAs, identified using RT-PCR in the livers of uninfected mice (Norm) and mice infected for 16 week (Inf). Results from two wild-type and two IL-4 –/– mice are shown for each time point.

tion in the affected organs of infected wild-type and IL-4 –/– mice is similar. To address this issue, RT-PCR for IFN-γ, IL-2, IL-5 and IL-10 cytokine gene transcripts was performed on RNA from liver and intestinal samples of normal and infected (16 week) wild-type and IL-4 –/– mice. Transcription of each of these cytokines was similar in both livers (Fig. 3) and intestines (not shown) regardless of the ability to produce IL-4; a total of eight infected wild-type and nine infected –/– mice were examined in this way and similar results were obtained in each case, although the liver of one infected IL-4 –/– mouse lacked detectable IL-5 mRNA (not shown). IL-4 transcripts were found only in affected tissues of infected wild-type animals (not shown).

IgE, eosinophils and sIL-4R

In infected mice, eosinophilia and increased serum IgE and sIL-4R levels develop shortly after the onset of parasite egg production, and are considered to be either directly or indirectly under the control of IL-4. In infected IL-4 wild-type mice, IgE levels were markedly increased (>50-fold) by 8 and 16 weeks compared with those seen in normal mice or mice carrying pre-patent (1 week) infections (Fig. 4A). By comparison, uninfected IL-4 –/– mice had little serum IgE compared with normal wild-type animals but did exhibit a slight (<3-fold) increase in serum IgE levels by 8 and 16 weeks of infection. Superimposition of a secondary infection resulted in a further increase to ~5-fold normal levels (Fig. 4A), though at no time was the increase statistically significant compared with background. Peripheral eosinophilia was evident in infected wild-type animals at week 8 (4-fold over background) but was not maximal until 16 weeks (15-fold, Fig. 4B). In infected IL-4 –/– mice the increase in eosinophil levels at week 8 was only 1.3-fold over background but by week 16 a 7-fold increase was noted (Fig. 4B). The increases observed in the wild-type animals were significantly greater ($p < 0.05$) than those recorded for the IL-4 –/– mice at both

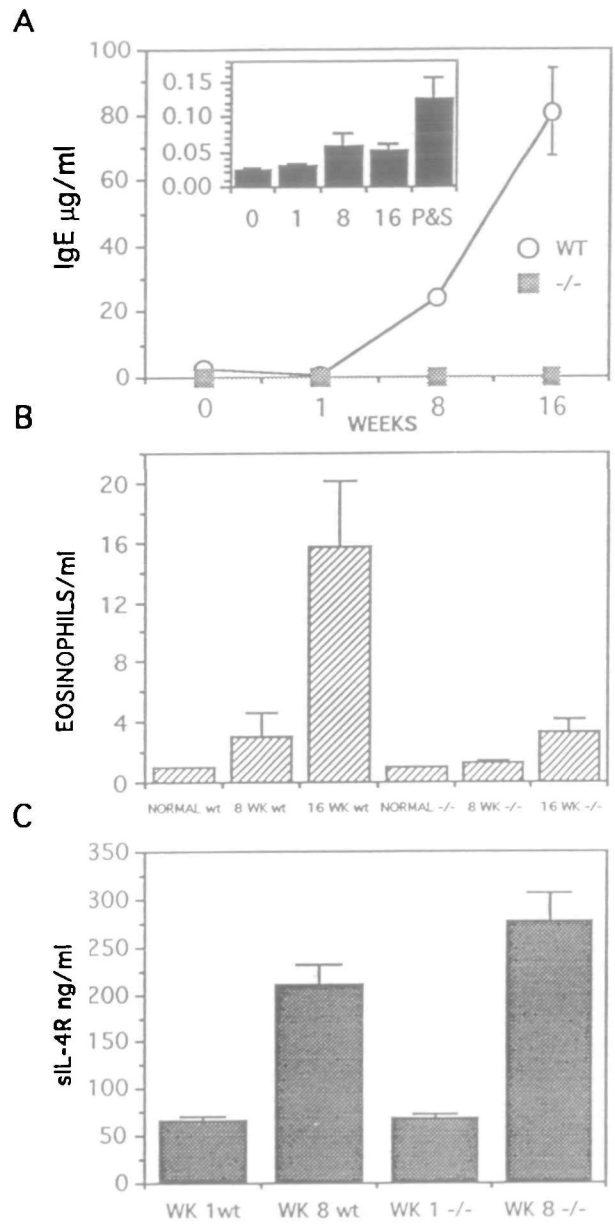

Fig. 4. (A) Serum IgE, (B) blood eosinophil levels (number × 10^{-4} per ml of whole blood) and (C) serum sIL-4R levels in infected wild-type and IL-4 –/– mice. Data are means ± SEM of analyses of six to 10 mice per group. The inset in (A) shows the details of the IgE response in the IL-4 –/– mice, P & S indicates mice which received primary and secondary infections.

times. Soluble IL-4R levels in infected IL-4 –/– mice and wild-type animals were equivalent at week 1 and 8 of infection (Fig. 4C); as shown before, sIL-4R levels are significantly increased ($p < 0.001$ for both) in animals carrying an egg-laying infection compared to those with prepatent infections (Fig. 4C), which were no higher than normal (not shown) (29).

Cytokine production by splenic and MLN cells from infected mice

It would be expected that in the absence of IL-4, schistosome infections would fail to stimulate the differentiation and expan-

sion of antigen-specific $CD4^+$ cells into T_h2 cells. To examine this issue, splenic and MLN cells were cultured *in vitro* with SEA and the cytokines they produced measured. Data from the wild-type mice revealed a cytokine secretion pattern broadly similar to that reported previously for 8 and 16 weeks of infection (Fig. 5). Within the context of the present study two observations about the responses of IL-4 –/– mice are most important. Firstly, in response to SEA, cells from infected IL-4–/– mice make markedly less IL-5 than do cells from infected wild-type animals (Fig. 5). Nevertheless, in the absence of IL-4, infection still results in the development of a population of cells, not detected in uninfected IL-4 –/– mice, which can respond to SEA by producing IL-5. Secondly, cells from infected IL-4 –/– mice produce IFN-γ in response to antigen stimulation *in vitro*, whereas cells from the infected wild-type animals largely fail to do so (Fig. 5). Given the demonstrated role of IL-4 in allowing differentiation of T_h2 cells, we questioned whether or not the IL-5 being made by re-stimulated lymphoid organ cells from the IL-4 –/– mice was coming from T_h cells. That this is the case in the IL-4 –/– mice was suggested by the decrease in the ability of $CD4^+$ cell depleted spleen populations to produce IL-5 or IL-10 in response to SEA (Fig. 6); this was also true for IL-2 and IFN-γ, and additionally for IL-2, IL-4, IL-5, IL-10 and IFN-γ production by spleen cells from the infected wild-type mice (Fig. 6).

Immune responses in IL-4 –/– mice injected with schistosome eggs

In previous studies isolated schistosome eggs induced marked T_h2 responses when injected into naive mice (8–10). This response was associated with the emergence of a population of T_h2 cells in the draining LN. We examined the effect of the lack of IL-4 on this egg-induced response. Popliteal LN cells from wild-type mice injected in their rear footpads with eggs responded to stimulation with SEA *in vitro* by making large amounts of IL-4, IL-5 and IL-10, in addition to IFN-γ and IL-2. Popliteal LN cells from egg-injected IL-4 –/– mice made >10-fold less IL-5, half as much IL-10, over twice as much IFN-γ and a similar amount of IL-2 as the cells from the wild-type animals (Fig. 7).

Discussion

Previous reports have clearly shown that in the mouse, schistosomiasis due to *S mansoni* is accompanied by the development of a strong T_h2-like immune response, with the production of large amounts of IL-4, IL-5 and IL-10, and accompanying elevations in IgE and eosinophil numbers and depressions in type 1 cell-mediated immune responses (2–5,30,31). T_h2 responses develop at the time of the onset of egg production and have been proposed to be responsible for granuloma formation around tissue-trapped parasite eggs. On the basis of such previous work and the established necessity for IL-4 for the differentiation of T_h2 cells (11,16–20), we hypothesized that infected IL-4 –/– mice would fail to develop T_h2 responses and therefore would suffer less severe disease than their infected wild-type counterparts. However, our data show that egg-associated hepatic granulomas in IL-4 –/– mice carrying primary *S. mansoni* infections are substantially similar to those in infected wild-type mice. Moreover, though infected IL-4 –/– mice clearly have enhanced T_h1-like responses compared with infected wild-type animals, they are not devoid of all the characteristics of schistosome-induced T_h2 responses since they exhibit mild eosinophilia, increased plasma sIL-4R concentrations and the expression in diseased tissues of cytokines associated with T_h2 responses.

Our data support in part the results of previous studies in which at 8 week post infection, mice treated since prior to infection with a neutralizing mAb anti-IL-4 had reduced T_h2 responses but circumoval granulomas of only marginally diminished size and similar cellularity and composition to those in infected control mAb treated mice (14). However, the previous report also showed that mAb anti-IL-4 treatment during infection leads to less severe hepatic fibrosis, which was not the case for the infected IL-4 –/– mice. Additionally, mAb anti-IL-4 has been shown to suppress schistosome infection associated elevations in sIL-4R levels (29), a result which contrasts with that reported here which suggests that IL-4 is not essential for this process. These variations and others cited in this paper could be due to genetic differences between the mice used in the different studies or to the expression in the knockout animals of regulatory pathways which are not expressed in the presence of IL-4 If the latter is true, residual un-neutralized IL-4 in the mAb anti-IL-4-treated animals may be sufficient to prevent activation of these putative pathway(s).

In a lung model of granuloma formation, egg-induced T_h2 responses have been clearly shown to play a significant role in immunopathology since presensitization of mice with IL-12 plus eggs, a procedure which leads to the development of an egg antigen-specific T_h1 response, subsequently prevents mice from mounting a T_h2 or granulomatous response to embolized eggs (6). Consistent with this, in egg-injected IL-4 –/– mice, as in mAb anti-IL-4-treated infected mice, pulmonary granuloma formation (though not hepatic granuloma formation) was significantly diminished compared to the situation in egg-injected IL-4 competent mice. This could be due either to IL-4 involvement in lesion development being tissue-dependent or to differences in the quality of injected versus *in situ* produced eggs; recent data from experiments using mAb anti-IL-4 suggest that the latter is most likely to be the case (32).

Through the use of IL-4 –/– mice we have been able to follow the effects of the lack of IL-4 on granuloma modulation, the progressive diminution with time in granuloma size (2). Due to the long time scale over which it occurs, modulation has been difficult to study using mAb anti-IL-4. Histopathologic analyses revealed that at 16 week of infection, granulomas in infected wild-type and IL-4 –/– mice were significantly smaller than at 8 weeks. Though it is clearly a T cell-dependent phenomenon, the exact immunological basis of modulation has not been elucidated. Hypotheses include that modulation is due to suppressor T cells (33) or an IL-10-dependent granuloma macrophage-induced anergy in the egg antigen-specific T_h1 cell population (34). Data presented here eliminate a central role for IL-4 in the process.

It was reported recently that treatment of infected mice with mAb anti-IgE deleteriously affects schistosome survival and fecundity (35). In the present study, where IgE levels in

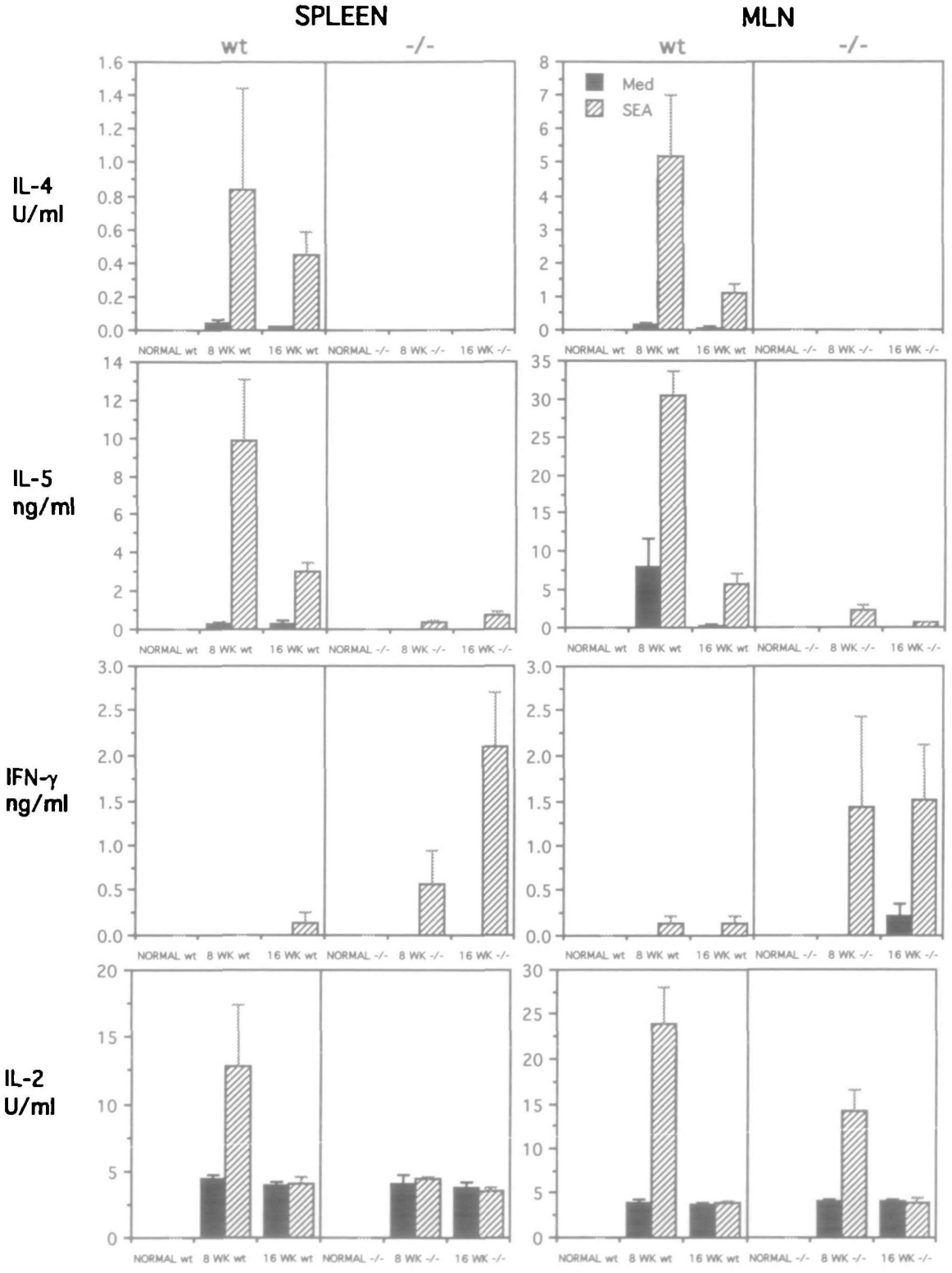

Fig. 5. IL-4, IL-5, IFN-γ and IL-2 production by splenic and MLN cells from normal and infected wild-type and IL-4 –/– mice. Data represent mean values ± SEM of duplicate analyses on cells from three to seven mice per group. Differences in IFN-γ and IL-5 production between cells from wild-type and IL-4 –/– mice are significant ($P < 0.05$ or better) for each time point for both spleen and MLN.

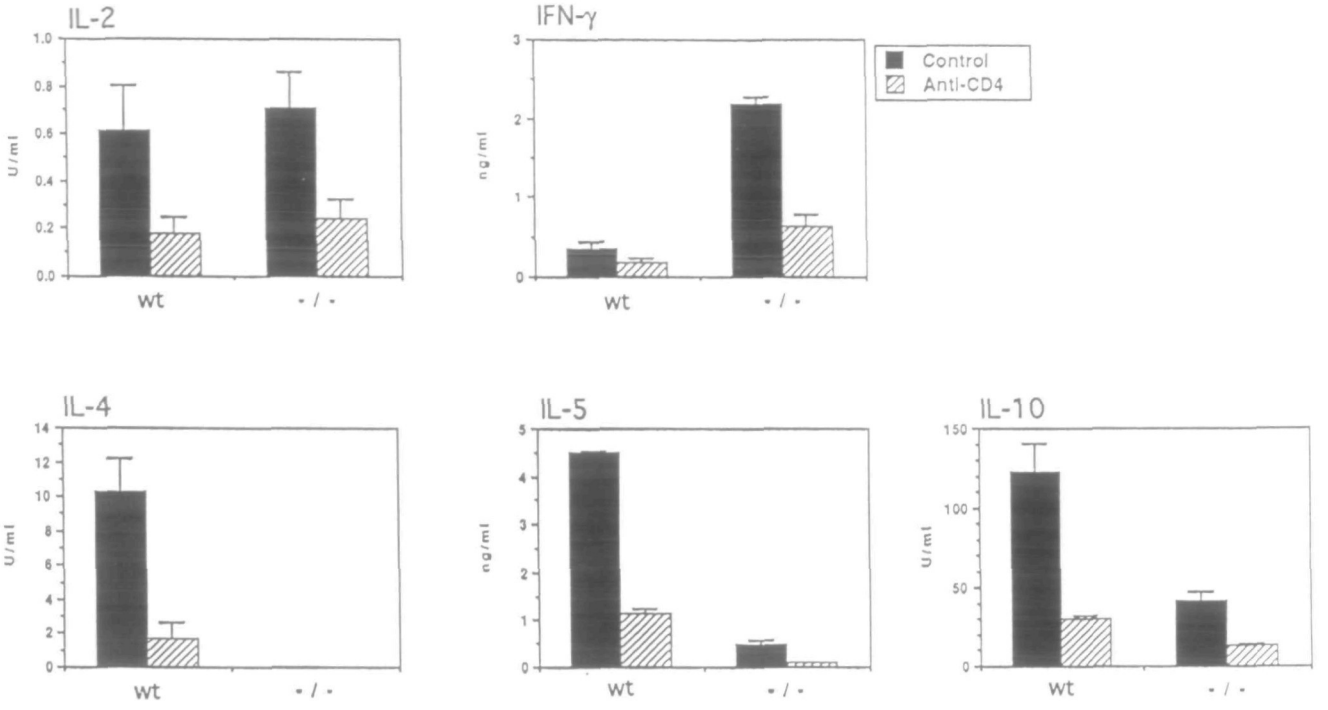

Fig. 6. Effects of *in vitro* depletion of $CD4^+$ cells on cytokine production by spleen cells in response to re-stimulation *in vitro* with SEA. Data are means ± SEM of duplicate analyses on cells from three individual infected mice per group.

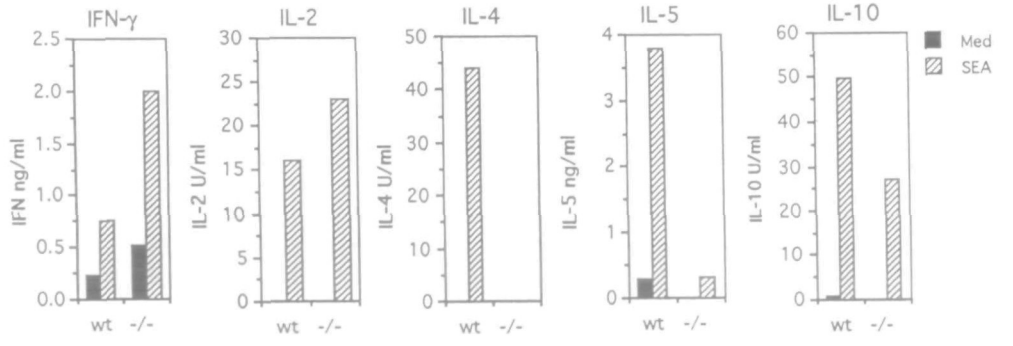

Fig. 7. Cytokine production by SEA re-stimulated popliteal LN cells. The LN were collected from IL-4 –/– or wild-type mice which had been injected in their footpads 10 days earlier with eggs

the infected IL-4 –/– mice were very much lower than in the infected wild-type animals, we did note that the IL-4 –/– mice carried fewer parasites than the wild-type animals, but the differences were not statistically significant at 8 or 16 weeks. Similarly, as determined by liver egg counts, there were fewer eggs per female in the IL-4 –/– mice than in the wild-type animals, but again the differences were not significant (not shown). The slight increase in IgE in the infected compared with normal IL-4 –/– mice is consistent with the recent report of IL-4-independent IgE production in IL-4 –/– mice infected with *Plasmodium chabaudi* (36), though the levels of IgE observed here were far lower than those seen in the malaria model. This is surprising given the propensity of helminth infections to induce IgE.

The defining cell types for T_h1 and T_h2 responses were $CD4^+$ cloned T cells which secreted signature panels of cytokines (37). However, it is now clear that non-T_h cells can secrete these cytokines and so contribute to the overall T_h1 or T_h2-like nature of an immune response developing *in vivo*. A response which is T_h2-like may therefore result from the activation and expansion of T_h cells plus $CD8^+$ cells, mast cells, basophils and/or eosinophils, all of which have been shown to make at least one 'T_h2 cytokine' (38–40). Consistent with this, in schistosome-infected mice, IL-4-dependent non-B non-T, FcεR positive splenic cells make substantial amounts of IL-4 following re-stimulation *in vitro* with antigen (41). In contrast to IL-4, IL-5 production in schistosome-infected animals has been considered to be due solely to T_h cells, a conclusion supported by our observations that in infected wild-type and IL-4 –/– mice the ability of spleen cells to make

IL-5 in response to re-stimulation with antigen is abrogated by depleting $CD4^+$ cells. The similarity in IL-5 and IL-10 mRNAs in the livers of infected and wild-type and IL-4 –/– mice suggest that either T_h2-like cells or other cells capable of making T_h2 cytokines are present in the liver regardless of whether or not IL-4 is being made. Taken together, these data argue for the existence of an IL-4-independent pathway for the generation of T_h2-like responses. An IL-4-independent increase in T_h2-like responses is precedented, having being observed to a greater or lesser extent in IL-4 –/– mice infected with *Nippostrongylus brasiliensis* or *P chabaudi* (15,36) and during *in vitro* cultures where human T_h cells are stimulated with anti-CD3 plus anti-CD28 mAb in the presence of neutralizing levels of anti-IL-4 mAb (42,43)

Our hypothesis when beginning these experiments was that IL-4, due to its role as a key cytokine for T_h2 differentiation, would be central to the pathogenesis of schistosomiasis. The data demonstrate that IL-4 –/– mice produce hepatic granulomas similar to those in wild-type animals and therefore show definitively that IL-4 is not necessary for egg-induced granuloma formation Our observations also support the idea that, given an appropriate stimulus, the induction of weak T_h2-like responses can occur in the absence of IL-4 We are currently investigating this possibility in greater detail.

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Abbreviations

LN	lymph node
MLN	mesenteric lymph node
SEA	soluble egg antigen

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