Impaired Th2 Development and Increased Mortality During *Schistosoma mansoni* Infection in the Absence of CD40/CD154 Interaction[1]

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The role of CD40/CD154 interaction during infection has primarily focused on pathogens that drive inflammatory Th1 responses. In this study, we show that CD40/CD154 interaction is a fundamental requirement for Th2 response development to the parasitic helminth *Schistosoma mansoni*. Compared with infected wild-type mice, greatly reduced levels of Th2-associated cytokines were measured both in vitro and in vivo, and no IgE or IgG1 was detected in infected CD154$^{-/-}$ mice. In the absence of an overt Th2 response, no exaggerated Th1 response was mounted by CD154$^{-/-}$ mice. Infected CD154$^{-/-}$ mice suffered severe morbidity and mortality, even though parasitemias in wild-type and CD154$^{-/-}$ mice did not differ significantly. These data indicate that CD40/CD154 interaction is required to allow development of a Th2-dominated immune response to *S. mansoni* and support the view that failure to develop such a response can have fatal consequences. ***The Journal of Immunology,*** **2002, 168: 4643–4649.**

A member of the TNF superfamily, CD154 (CD40 ligand) is expressed on a wide range of cell types throughout the body that can activate an equally diverse range of CD40-expressing cells (1, 2). Interaction of CD40/CD154 provides a major means for T cells to "help" B cells and, through activation of macrophages and dendritic cells (DC)[7] to make inflammatory mediators such as IL-12, also provides a route for skewing the immune response in a Th1 direction (3). Individuals with hyperIgM syndrome, an X-linked immunodeficiency caused by a mutation of the *CD154* gene, display severely impaired T cell-dependent Ab responses, with no B cell memory, a reduced Ab repertoire, and defective germinal center formation (4, 5). As a result, such individuals suffer from recurrent pathogenic microbial infections (6).

The development of both CD40- and CD154-deficient mice as well as the use of blocking Ab has provided the ability to test the role of CD40/CD154 interaction in a wide range of infectious disease systems (7). This approach has revealed that there is a marked heterogeneity in the requirement for CD40/CD154 for appropriate immune response development depending upon the pathogen investigated, with the majority of research in this area focusing on proinflammatory, Th1-inducing pathogens. However, the outcome of infection with pathogens that induce Th2 responses in such animal models or in individuals with hyperIgM syndrome is much less clearly defined.

The parasitic helminth *Schistosoma mansoni* is a major public health problem on several continents. The immune response to this intravascular parasite is strongly Th2 in nature (8), and it is clear that this response provides protection against potentially life-threatening aspects of ongoing infection as well as against superinfection (9–12). However, the mechanisms that control the development of this response, how it is regulated, and how it serves its protective role are still largely unknown.

We have investigated the importance of CD40/CD154 interaction in the development of an appropriate immune response to *S. mansoni* by infecting mice that are genetically deficient in CD154. Previous work suggested that the CD40/CD154 interaction may be important for Th2 development, because coinjection of *S. mansoni* eggs with anti-CD40 Ab results in down-modulation of Th2 development (13). Our data have revealed a crucial role for CD154 in Th2 response development during active infection with *S. mansoni*. We have found that CD154$^{-/-}$ mice infected with *S. mansoni* mount a greatly impaired Th2 response and suffer from severe morbidity, exhibiting extreme lung pathology and cachexia that eventually lead to death. This sequence of events occurs in the absence of any overt inflammatory response, suggesting that mediators such as NO and TNF-α are not responsible for the morbidity observed in CD154$^{-/-}$ animals.

Materials and Methods

Mice and parasites

CD154$^{-/-}$ mice were obtained from The Jackson Laboratory (Bar Harbor, ME); they were bred in-house and maintained in filter-topped isolator

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[7] Abbreviations used in this paper: DC, dendritic cells; MLN, mesenteric lymph node; SEA, soluble egg Ag; STAg, soluble tachyzoite Ag; WT, wild type.

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cages, with Bactrim (HiTech Pharmaceutical, Amityville, NY)-supplemented water. Wild-type (WT) C57BL/6 mice (Taconic Farms, Germantown, NY) were used as controls and similarly maintained on Bactrim-supplemented water. *Biomphalaria glabrata* snails infected with both sexes of *S. mansoni* and Puerto Rican strain NMRI were obtained from Dr. F. Lewis (Biomedical Research Institute, Rockville, MD). Mice were infected percutaneously with ~70 cercariae, weighed regularly, and euthanized after significant weight loss (>20%) was observed in CD154$^{-/-}$ animals. Adult worms were recovered by perfusion as previously described (14), and samples of lung, liver, and small intestine were collected to quantitate egg deposition (15). Additional samples of lung, liver, and small intestine were fixed in neutral buffered formalin before staining and histologic examination. Liver granuloma areas were measured on Masson's Trichrome-stained sections using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). A minimum of 15 single egg granulomas in clear transverse section were measured per sample. For infection with *Toxoplasma gondii*, mice were injected i.p. with 2×10^5 TS4 tachyzoites.

Reagents

Soluble egg Ag (SEA) was prepared from isolated schistosome eggs as previously described (16, 17). Soluble tachyzoite Ag (STAg) was prepared as described previously (18). SEA (50 μg/ml), STAg (20 μg/ml), and plate-bound anti-CD3 mAb (BD PharMingen, San Diego, CA; 0.5 μg/well) were used to stimulate in vitro cultures.

Cell culture and cytokine measurement

Mesenteric lymph node (MLN) or spleen cells were harvested, and single-cell suspensions were prepared as previously described (19). Cells were counted using trypan blue and resuspended at 10^7/ml in DMEM (Sigma-Aldrich, St. Louis, MO) containing 100 U/ml penicillin plus 100 μg/ml streptomycin (Life Technologies, Gaithersburg, MD), 10 mM HEPES (Life Technologies), and 2 mM L-glutamine (Life Technologies). Cells (10^6/well) were cultured in 96-well flat-bottom plates in 5% CO_2 at 37°C with or without the SEA (50 μg/ml) or plate-bound anti-CD3 (0.5 μg/well). Supernatants were harvested from 24- or 72-h culture and stored at −20°C for subsequent cytokine analysis by ELISA. Cytokine ELISAs were performed on culture supernatants or plasma samples using paired mAb purchased from BD PharMingen or purified from hybridoma supernatants in our laboratory, except for TNF-α, where the TNF-α duoset (R&D Systems, Minneapolis, MN) was used. NO production was measured by Greiss reaction (20). Standard ELISPOT protocols were followed to quantitate the number of IL-4- and IFN-γ-producing MLN cells, using 0.1 M 2-amino-2 methyl-1-propanol buffer containing 0.6% SeaPlaque agarose and 5-bromo-4-chloro-3-indolyl phosphate (1 mg/ml) as a substrate. For peripheral blood counts, thin blood smears were applied to glass slides, air-dried, and fixed in methanol before staining using Hema-3 (Fisher Scientific, Pittsburgh, PA). The proportion of eosinophils was determined by morphologic examination of at least 300 cells/sample in random fields.

Flow cytometry

Expression of surface MHC class II on Mac-1$^+$ or B220$^+$ cells was quantified by flow cytometry using FITC- or PE-conjugated Ab (I-A^b, B220 or Mac-1), purchased from BD PharMingen. Samples were analyzed using a FACSCaliber flow cytometer and CellQuest software (BD Biosciences, Franklin Lakes, NJ). Proliferation was measured by CFSE (Molecular Probes, Eugene, OR) labeling of freshly isolated spleen or MLN cells before culture for 5 days with or without 50 μg/ml SEA or plate-bound anti-CD3. Cells were then fixed and analyzed as described above.

Statistical analysis

The Student *t* test was used to determine the statistical significance between groups. A value of $p < 0.05$ was considered a significant difference.

Results

Morbidity and parasite burden in infected CD154$^{-/-}$ mice

In comparison to infected WT mice, infected CD154$^{-/-}$ mice showed increasingly severe morbidity from around 7 wk postinfection (Fig. 1*A*). Morbidity was characterized by loss of activity, deterioration of coat condition, deterioration of posture, and weight loss. Left unchecked, this eventually culminated in the death of infected CD154$^{-/-}$ mice within ~2 wk of the initiation of weight loss (Fig. 1*B*). Development of these symptoms was dependent upon patent infection and coincided with the onset of egg laying, as CD154$^{-/-}$ mice infected with male parasites alone did not lose weight or die (not shown).

To determine whether cachexia in infected CD154$^{-/-}$ mice might be due to differences in parasitemia, we assessed infection intensities in WT and CD154$^{-/-}$ animals. However, neither adult worm burden (Fig. 1*C*) nor egg burden in the liver and gut (Fig. 1*D*) differed significantly between the two groups. In contrast to this, significantly more eggs were recovered from the lungs of infected CD154$^{-/-}$ mice than from WT mice (Fig. 1*E*; $p < 0.05$). As might be expected of this chronic infection, weight loss by CD154$^{-/-}$ mice correlated with increasing liver egg burden (not shown).

Pathologic changes in target organs

Examination of gross pathology showed that splenomegaly, although significant ($p < 0.05$), was less extreme in infected CD154$^{-/-}$ mice than in infected WT mice (Fig. 2*A*; $p < 0.02$).

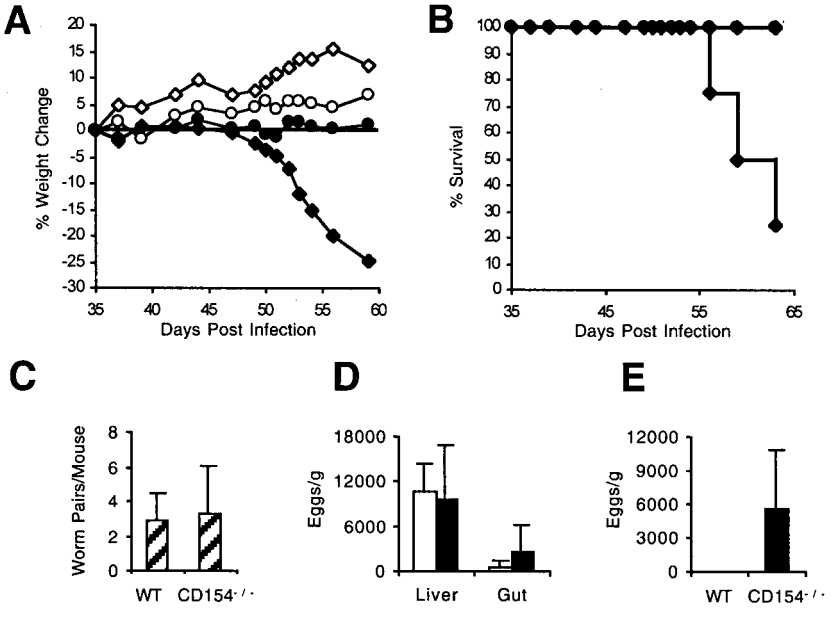


FIGURE 1. Morbidity and parasite burden in WT and CD154$^{-/-}$ mice. Cumulative weight change (*A*) and percent survival (*B*) of uninfected (open symbols) and infected (solid symbols) WT (circles) and CD154$^{-/-}$ (diamonds) mice. *C*, Adult worm burden did not differ significantly between infected WT or CD154$^{-/-}$ mice. Egg burden in liver and gut (*D*) of infected WT (open bars) or CD154$^{-/-}$ (solid bars) mice did not differ significantly. However, egg burden in the lungs was significantly higher in CD154$^{-/-}$ mice (*E*). Data shown are the mean ± SD (where shown) for three to eight mice per group that were individually assayed and are from one representative experiment of six performed.

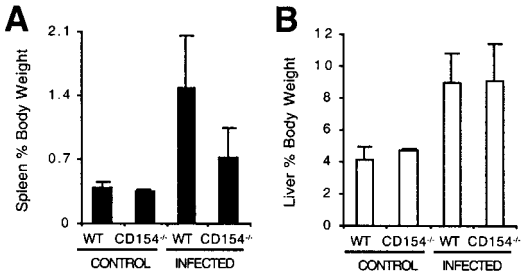


FIGURE 2. Splenomegaly and hepatomegaly in infected WT and CD154$^{-/-}$ mice. Splenomegaly and hepatomegaly were assessed by determining spleen (*A*) and liver (*B*) size in relation to total body weight for uninfected and infected WT and CD154$^{-/-}$ mice. Data shown are the mean ± SD of four to eight mice per group that were individually assayed and are from one representative experiment of six performed.

Pronounced hepatomegaly was evident in both groups of mice and was not significantly different between infected groups (Fig. 2*B*). More detailed histopathological examination of CD154$^{-/-}$ mice revealed several key differences to similarly infected WT mice. Most notably, severe lung pathology was evident in infected CD154$^{-/-}$ animals (Fig. 3). In the lungs, numerous focal granulomas were found in CD154$^{-/-}$ (Fig. 3*D*), but not WT (Fig. 3*C*), mice, coincident with increased numbers of ova and parasite debris in the lungs of these animals. Although there was extensive inflammatory change in the lungs of infected WT mice compared with uninfected controls, granulomas and parasite debris were rare. Liver and gut pathology was similar in both strains of mice, but fewer granulocytes and mature plasma cells were observed in the absence of CD154 (not shown). Additionally, liver granulomas were significantly reduced in size in infected CD154$^{-/-}$ mice compared with WT mice (Fig. 3*E*; $p < 0.001$). Equivalent levels of fibrosis were observed in both groups of infected mice by Masson's Trichrome staining (not shown).

Impaired Th2 responses in infected CD154$^{-/-}$ mice

Adult *S. mansoni* typically reside within the mesenteric veins of the murine host. To determine the role of CD40/CD154 interaction in development of the immune response during helminth infection, we measured the in vitro production of signature Th2 cytokines IL-4, IL-5, and IL-13 by MLN cells isolated from infected CD154$^{-/-}$ and WT animals (Fig. 4*A*). We also assessed in vitro production of the proinflammatory Th1 cytokines, IL-12, TNF-α, and IFN-γ, and regulatory mediators, IL-10 and NO, in these cultures (Fig. 4*A*). Compared with WT mice, MLN cells from infected CD154$^{-/-}$ mice made significantly less IL-4, IL-5, and IL-13 in response to SEA than those from infected WT mice ($p < 0.05$).

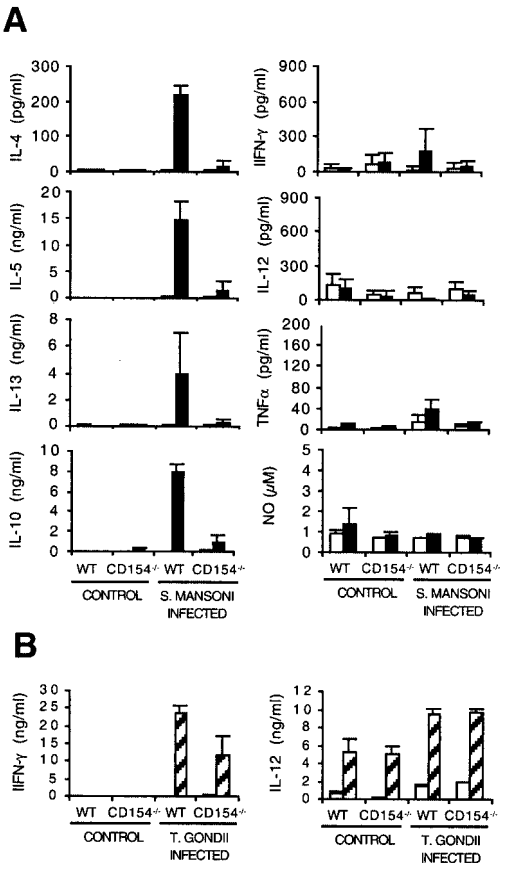
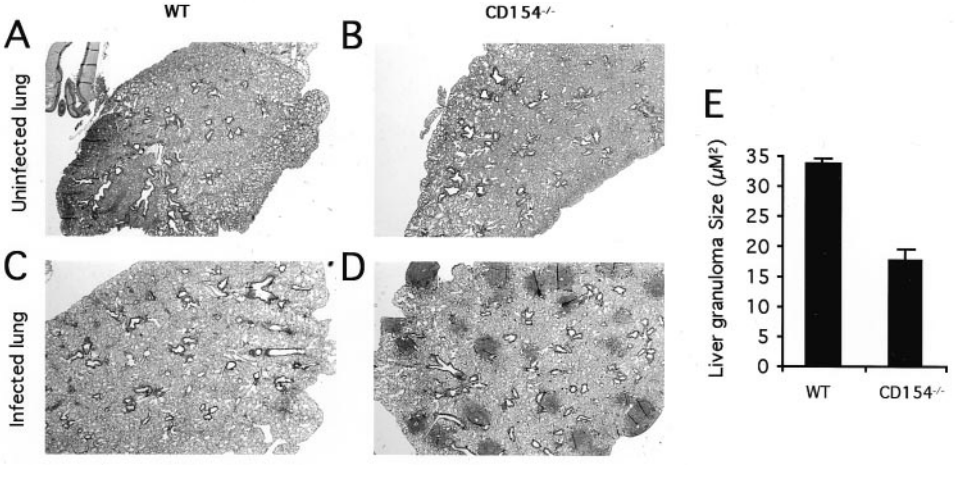


FIGURE 4. Impaired in vitro Th2 cytokine production by infected CD154$^{-/-}$ mice. *A*, Cytokine production as measured by ELISA in culture supernatants from MLN cells taken from uninfected (control) or *S. mansoni*-infected WT or CD154$^{-/-}$ mice. MLN cells were then stimulated in vitro with medium (□) or SEA (■) for 72 h before supernatant harvest. *B*, Cytokine production as measured by ELISA in culture supernatants from spleen cells taken from uninfected (control) or *T. gondii*-infected WT or CD154$^{-/-}$ mice. Spleen cells were then stimulated in vitro with medium (□) or STAg (▨) for 72 h before supernatant harvest. Data shown are the mean ± SD of triplicate wells from three to six mice per group that were individually assayed.

FIGURE 3. Pathologic changes in infected WT and CD154$^{-/-}$ mice. The histologic appearance of lung tissue from uninfected WT (*A*) or CD154$^{-/-}$ (*B*) mice or infected WT (*C*) or CD154$^{-/-}$ (*D*) mice. Note the multiple granulomas present only in infected CD154$^{-/-}$ lung tissue (*D*). Sections were stained with H&E. Original magnification, ×20. *E*, Liver granuloma areas were measured on Masson's Trichrome-stained sections from infected WT or CD154$^{-/-}$ mice. A minimum of 15 single egg granulomas in clear transverse section were measured per sample. Data shown are the mean ± SD of four mice per group that were individually assayed.



No significant difference in levels of production of IL-12, TNF-α, or IFN-γ was seen between the groups, with each of these mediators being produced in low amounts. Additionally, we measured levels of IL-10 and NO to determine whether the reduced production of IL-4, IL-5, and IL-13 might be due to enhanced levels of these regulatory mediators. However, this proved not to be the case, with MLN from infected WT mice actually producing significantly more Ag-specific IL-10 than those from CD154$^{-/-}$ mice ($p < 0.01$), and with no significant difference between the groups in the level of NO detected. Consistent with this, immunohistochemical staining showed that there was no elevation in iNOS levels in lung, liver, or gut from infected CD154$^{-/-}$ mice compared with infected WT mice (not shown). Interestingly, MLN cells from infected CD154$^{-/-}$ mice also showed an impaired ability to produce IL-4, IL-5, and IL-13, but not IL-10, IL-12, or NO in response to polyclonal stimulation with plate-bound anti-CD3 mAb (not shown). Similar results were obtained from splenocyte cultures stimulated with SEA or anti-CD3 (not shown).

Although CD154$^{-/-}$ mice infected with *S. mansoni* appeared to be unable to mount a Th2 response, the same mice were found to capably mount a Th1 response to infection with the protozoan parasite *Toxoplasma gondii*. Parasite-specific IL-12 and IFN-γ were detected in splenocyte cultures from infected CD154$^{-/-}$ mice at levels that did not differ significantly from those in similarly infected WT mice (Fig. 4*B*).

We reasoned that the impaired Th2 cytokine production seen in vitro might be due to the reduced initial numbers of cytokine-producing cells or defective cellular proliferation in response to stimulation. To determine whether either of these possibilities might be the case, we examined the ability of MLN cells from WT or CD154$^{-/-}$ mice to produce IL-4 and IFN-γ ex vivo by ELISPOT (Fig. 5*A*) and to proliferate in response to specific Ag (SEA; Fig. 5*B*) or to a mitogenic stimulus (plate-bound anti-CD3; Fig. 5*C*) using CFSE. Although no significant difference was seen in the number of freshly isolated MLN cells that produced IL-4 or IFN-γ when obtained from either WT or CD154$^{-/-}$ mice (Fig. 5*A*), the subsequent ability of these cells to proliferate was somewhat impaired in CD154$^{-/-}$ animals (Fig. 5*B*). A reduction in the ability of MLN from infected CD154$^{-/-}$ mice to proliferate was seen on stimulation with SEA, but not with anti-CD3, compared with infected WT mice. However, diminished Th2 cytokine production was apparent in cultures exposed to both these stimuli (see above). Therefore, impaired proliferation alone cannot account for the reduced ability of infected CD154$^{-/-}$ mice to mount a Th2 response in vitro, although it may contribute to the reduced Ag-specific response to SEA. We have yet to determine whether the cells isolated from infected CD154$^{-/-}$ mice that proliferate poorly to SEA stimulation are T cells or B cells. The mechanism behind this defective Ag-specific proliferation is not yet known, but is unlikely to be effected by NO or IL-10, because levels of these mediators in culture were not elevated in cells cultured from infected CD154$^{-/-}$ mice compared with WT controls (see above).

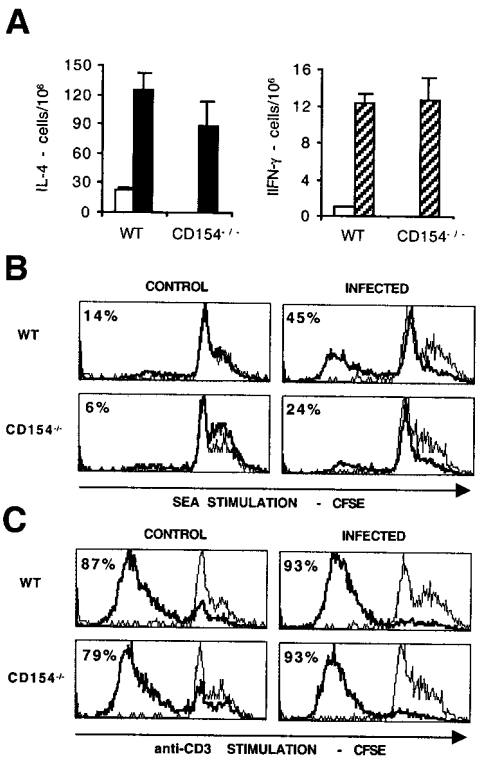


FIGURE 5. Infected CD154$^{-/-}$ mice show defective in vitro proliferative responses. *A*, ELISPOT was used to quantitate the number of IL-4- and IFN-γ-producing MLN cells freshly isolated from uninfected (□) or *S. mansoni*-infected mice (■ or ▨). Proliferation of WT or CD154$^{-/-}$ CFSE-labeled MLN cells from uninfected (control) or infected mice in response to 5-day culture with SEA (*B*) or plate-bound anti-CD3 (*C*) was determined. Histograms represent unstimulated (fine lines) and Ag-stimulated (bold lines) lymphocytes, as gated by scatter, and figures represent the percentage of cells that proliferated, based on markers set on unstimulated samples.

Impaired Th2 response in infected CD154$^{-/-}$ mice in vivo

To ensure that the impaired Th2 response observed in infected CD154$^{-/-}$ mice in vitro was also apparent in vivo, especially given the diminished Ag-specific proliferation we had observed in vitro, we measured plasma levels of IL-4 and IL-5 by ELISA (Fig. 6, *A* and *B*). Supporting our in vitro data, levels of both IL-4 and IL-5 were significantly less in infected CD154$^{-/-}$ mice than in infected WT mice ($p < 0.01$ and $p < 0.03$, respectively). A further indication of the impaired ability of CD154$^{-/-}$ mice to mount a Th2 response in vivo was illustrated by the significantly reduced peripheral blood eosinophilia observed in infected CD154$^{-/-}$ mice compared with WT mice (Fig. 6*C*; $p < 0.001$).

In addition to their defective ability to produce Th2 cytokines in vitro and in vivo, infected CD154$^{-/-}$ mice displayed impaired Ab production, with disrupted isotype switching (Fig. 7). We measured plasma levels of SEA-specific IgM, IgG1, and IgG2a and total levels of IgE. No elevated IgE or IgG1, both of which are indicative of a Th2 response in mice, were measured in infected CD154$^{-/-}$ mice. IgG2a, which is indicative of IFN-γ production

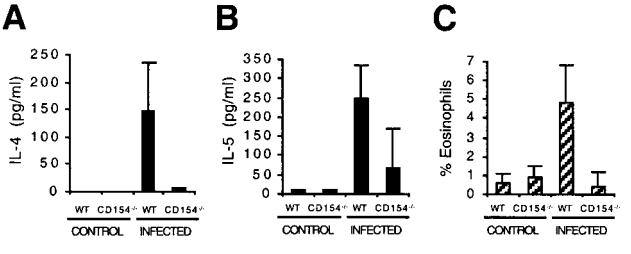


FIGURE 6. Impaired in vivo Th2 responses in infected CD154$^{-/-}$ mice. IL-4 (*A*) and IL-5 (*B*) production in plasma samples from control (uninfected) or *S. mansoni*-infected WT or CD154$^{-/-}$ mice. *C*, Eosinophilia in peripheral blood from control (uninfected) or *S. mansoni*-infected WT or CD154$^{-/-}$ mice. Data shown are the mean ± SD of five to eight mice per group that were individually assayed and are from one experiment representative of three performed.

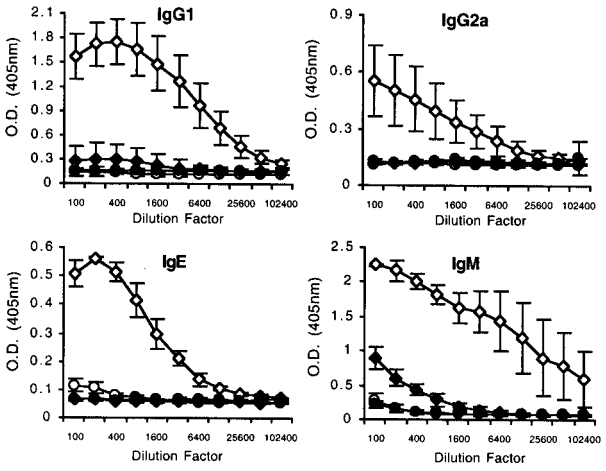


FIGURE 7. Disrupted isotype switching in infected CD154$^{-/-}$ mice. SEA-specific IgG1, IgG2a, and IgM and total IgE in plasma samples from uninfected (circles) or *S. mansoni*-infected (diamonds) WT (open symbols) or CD154$^{-/-}$ (solid symbols) mice. Data shown are the mean ± SD of three to eight mice per group that were individually assayed.

and Th1 response development in mice, was not found in infected CD154$^{-/-}$ animals and was found only at low levels in infected WT animals. Parasite-specific IgM was measured in both groups of infected mice, but was significantly less in CD154$^{-/-}$ than in WT animals ($p < 0.05$).

Impaired MHC class II expression in infected CD154$^{-/-}$ mice

In many experimental systems CD40/CD154 has been shown to be important for optimal activation of B cells (3). One indicator of such activation is the level of MHC class II on the surface of these cells. To determine whether CD40/CD154 interaction was required for normal B cell activation in our system, we compared the levels of expression of MHC class II on the surface of B cells (B220$^+$) and other APC (Mac-1$^+$ cells; Table I). B cells and other APC in infected CD154$^{-/-}$ mice displayed significantly lower levels of surface MHC class II than WT mice following infection with *S. mansoni*, although they showed some up-regulation compared with uninfected controls.

Discussion

Taken together our data provide compelling evidence that CD40/CD154 interaction is necessary to allow appropriate immune response development during infection with the parasitic helminth *S. mansoni*. This requirement is most evident in the significantly impaired ability of these animals to produce Th2 cytokines in vitro and in vivo, their inability to make Th2-associated Ab IgG1 and IgE, and the reduced numbers of granulocytes evident in their peripheral blood and granulomas. Furthermore, this inability to mount a Th2 response has fatal consequences for infected animals.

Table I. *MHC class II expression on Mac-1$^+$ and B220$^+$ cells*[a]

	Spleen		MLN	
	Mac-1$^+$	B220$^+$	Mac-1$^+$	B220$^+$
WT				
Control	47	141	ND	311
Infected	199	564	285	701
CD154$^{-/-}$				
Control	59	164	ND	218
Infected	57	208	82	375

[a] Mean fluorescence intensity of staining for MHC class II on spleen or MLN cells from control (uninfected) or infected WT or CD154$^{-/-}$ mice, gated for expression of Mac-1 or B220. ND, Too few cells present to accurately assess this parameter.

In light of the greatly reduced levels of Th2 cytokines in their plasma and that accumulate in culture supernatants of Ag- or aCD3-stimulated lymphoid organ cells of infected CD154$^{-/-}$ mice, it is interesting that the frequency of cells that can produce Th2 cytokine is equivalent in infected CD154$^{-/-}$ and WT mice as assessed by ELISPOT. This discrepancy may reflect a lower level of cytokine production by CD154$^{-/-}$ animals on a per cell basis. Alternatively, the impaired ability of cells isolated from CD154$^{-/-}$ mice to produce Th2 cytokines after in vitro culture may be due to a defect in the expansion or survival of responder cells after activation. Supportive of this, we have found that SEA-specific proliferation was reduced in CD154$^{-/-}$ animals compared with WT, and others have shown that T cell persistence as well as priming are defective when CD40/CD154 interaction is disrupted (21, 22). However, the reduced ability of CD154$^{-/-}$ mice to mount a Th2 response in vivo, characterized by reduced plasma IL-4, IL-5, IgG1, and IgE levels and reduced peripheral blood eosinophilia in these animals, suggests that our in vitro findings are physiologically relevant.

One interpretation of our data is that B cells play an important role in Th2 response induction, because in the absence of CD40/154 interaction, B cell responses are severely impaired (3). Our data indicate that B cells are not activated appropriately in CD154$^{-/-}$ mice during infection, showing reduced activation status in terms of MHC class II expression and defective isotype switching. Both these outcomes may at least in part be due to the reduced levels of IL-4 produced by infected CD154$^{-/-}$ mice, because IL-4 can act to induce MHC class II up-regulation and facilitate isotype switching to the production of IgE and IgG1 (23). Additionally, granuloma cellularity was somewhat different in infected CD154$^{-/-}$ mice, which had a reduced proportion of plasma cells compared with WT mice. The role of B cells in schistosomiasis is intriguing, but as yet not fully resolved. Somewhat conflicting reports have suggested that mice lacking B cells either fail to mount a Th2 response during infection (24) or mount a normal Th response but develop more severe disease, failing to regulate granulomatous pathology (25). Our data resemble the outcome of infection in B cell-deficient J_H mice, in that they fail to mount a Th2 response, but also exhibit impaired granuloma formation and severe pathology. The emerging role of specific B cell subsets that secrete specific cytokines in immune response polarization (26) raises the interesting possibility that a defective B cell response may also directly influence the resultant cytokine profile during schistosome infection, a possibility that remains to be investigated.

DC represent another CD40-bearing cell type that can be activated through ligation by CD154 (3, 27, 28). It is possible that defective DC function could contribute to the impaired Th2 development we have observed in infected CD154$^{-/-}$ mice. Supportive of this hypothesis, we have shown that CD40-deficient, bone marrow-derived DC exhibit a diminished ability to induce Th2 responses in vivo (29). We have yet to investigate the activation status of DC isolated from *S. mansoni* infection in the absence of CD154. An additional possibility that remains to be addressed is whether signaling to the T cell via CD154 might directly drive Th2 development during infection (30–32).

Costimulation has previously been shown to be important for the development of a Th2 response to *S. mansoni* infection, as mice doubly deficient for both CD80 and CD86 fail to mount a Th2 response to the parasite (33). However, in this case the T cell cytokine profile was reversed, with CD80/86-deficient mice secreting higher levels of IFN-γ than WT mice coincident with the

loss of IL-4 production. This contrasts what we have seen during infection of CD154$^{-/-}$ mice, where no such compensatory Th1 response is noted on loss of the Th2 response. Nevertheless, our data suggest that one outcome of CD40/CD154 interaction might be to initiate events that lead to up-regulation of costimulatory molecules such as CD80 and CD86, although the low levels of these molecules measured during infection of even WT mice has made it difficult to show this experimentally (unpublished observations). Nevertheless, it seems that the sequence of events that leads to Th2 response development originates with and is dependent upon CD40 and CD154 interaction.

The rapid severe weight loss seen in infected CD154$^{-/-}$ mice bears similarity to the outcome of infection of IL-4$^{-/-}$ mice infected with *S. mansoni* (19). In the case of IL-4$^{-/-}$ mice, however, in the absence of Th2 response development an inflammatory response is observed, characterized by elevated production of inflammatory mediators such as NO and IFN-γ. This contrasts with what we have noted in infected CD154$^{-/-}$ animals, in which no such elevated inflammatory response is seen. It is difficult to address whether the absence of CD154 might impair Th1 response development to *S. mansoni*, because infection does not induce a marked Th1 response even in WT mice. Thus, we cannot discount the possibility that infected CD154$^{-/-}$ animals may also mount a defective Th1 response to the parasite.

An additional unexpected finding in infected CD154$^{-/-}$ mice was the presence of large numbers of eggs and severe pathologic changes in the lungs. This occurred before wk 8 of infection at a time when it is rare to find eggs in the lungs of WT mice. Shunting to the lungs reflects the development of portal hypertension and the formation of varices that allow blood to leave the portal system without passing through the liver. Eggs produced by the parasites in the portal system are thus able to pass through the venous system to the heart and be pumped on to the lungs where they become trapped in the capillaries. This indication that infected CD154$^{-/-}$ mice develop severe portal hypertension shortly after egg production begins suggests that there is a failure of vascular regulation in these animals. Infected WT mice exhibit low, but detectable, levels of iNOS in and around granulomas in the liver, gut, and lungs, but this is not seen in infected CD154$^{-/-}$ mice. Therefore, it is possible that reduced production of mediators such as NO in this context might result in defective vascular regulation, perhaps due to impaired vasodilation, that could, in turn, lead to more rapid development of portal hypertension and its detrimental sequelae.

It has previously been shown that IL-4 and IL-13 play important roles in granuloma formation and development of fibrosis during schistosome infection (11, 34). Interestingly, even in the face of a severely impaired Th2 response and a virtual absence of production of IL-4 and IL-13 to the parasite, infected CD154$^{-/-}$ mice did not show significantly different levels of fibrosis from those in WT mice. It is possible that the very low levels of these mediators measured in vitro might be sufficient to allow the development of fibrosis in vivo, or that other mediators are involved. Moreover, the fact that a granulomatous response was mounted by CD154$^{-/-}$ mice indicates that there is a CD4 T cell component in the response to infection by these animals (35).

In summary, our data show that CD40/CD154 interaction is required to allow Th2 response development to the parasitic helminth *S. mansoni*, and that the absence of this response has fatal consequences for the host. These novel observations have implications for understanding the underlying mechanisms of Th2 response initiation, highlighting the pathophysiological relevance of this receptor/ligand pair in this process, and may impact the development of future immunotherapeutic strategies for other Th2-dominated diseases.

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