

## *Fasciola hepatica* Suppresses a Protective Th1 Response against *Bordetella pertussis*

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**Fasciolosis, like other helminth infections, is associated with the induction of T-cell responses polarized to the Th2 subtype. Respiratory infection with *Bordetella pertussis* or immunization with a pertussis whole-cell vaccine (Pw) induces a potent Th1 response, which confers a high level of protection against bacterial challenge. We have used these two pathogens to examine bystander cross-regulation of Th1 and Th2 cells in vivo and provide evidence of immunomodulation of host T-cell responses to *B. pertussis* by a concomitant infection with *Fasciola hepatica*. Mice with a coinfection of *F. hepatica* and *B. pertussis* exhibited a Th2 cytokine profile in response to *F. hepatica* antigens, similar to those infected with *F. hepatica* alone. By contrast, the Th1 response to *B. pertussis* antigens was markedly suppressed and the bacterial infection was exacerbated following infection with *F. hepatica*. Furthermore, an established Th1 response induced in mice by infection with *B. pertussis* or by parenteral immunization with Pw was also suppressed following infection with *F. hepatica*. This immunomodulatory effect of *B. pertussis*-induced responses by *F. hepatica* infection is significantly reduced, but not completely abrogated, in IL-4 knockout mice. Our findings demonstrate that Th2-inducing parasites can exert bystander suppression of protective Th1 responses to infection or vaccination with a bacterial pathogen and that the modulation is mediated in part by IL-4 and, significantly, is effective at both the induction and effector stages of the Th1 response.**

The identification of Th1 and Th2 cells has provided a useful model for our understanding the selective induction, polarization, and reciprocal regulation of distinct arms of the immune response (1, 25). Th1 cells are normally induced following infection with intracellular bacteria and viruses, whereas Th2 responses are generated in response to allergens and helminth parasites (1, 10, 28). The early decision to polarize the immune response toward type 1 or type 2 is controlled by a number of factors. Gram-negative bacteria and viruses stimulate the production of interleukin-12 (IL-12) and IL-18 by dendritic cells and macrophages, which favors the induction and expansion of Th1 cells (5, 37). Conversely, early IL-4 acts as a potent stimulus for Th2 differentiation (1, 28). Th1 and Th2 cells also produce cytokines that are mutually inhibitory for the differentiation and effector functions of the reciprocal subtype. Thus, once a T-cell immune response begins to develop along either a Th1 or Th2 lineage from a common precursor, it tends to become increasingly polarized in that direction.

This dichotomy into reciprocally regulated Th1 and Th2 cell type responses provides a simple framework in which we categorize immune responses and their role in dealing with distinct pathogens that require different effector mechanisms for their control. However, the real situation, especially in the developing world, is one where individuals may be exposed to multiple infections or where vaccines may be administered in the face of chronic parasitic infection. The aim of the present investigation was to examine the reciprocal influences of a Th1-inducing bacterial pathogen and a Th2-inducing parasite in vivo.

*Bordetella pertussis* is a gram-negative coccobacillus that causes the respiratory disease whooping cough, a significant

cause of morbidity and mortality in infants worldwide. *B. pertussis* associates with respiratory epithelial cells but can also invade and survive within alveolar macrophages and polymorphonuclear leukocytes (12). Respiratory infection or immunization with whole-cell pertussis vaccines (Pw) is associated with the induction of antigen-specific Th1 cells, which are critical in host resistance to infection (23, 32, 33). In particular, the type 1 cytokine gamma interferon (IFN- $\gamma$ ) plays a major role in controlling *B. pertussis* infection and in containing the bacteria to the mucosal site (3, 19).

The parasitic trematode *Fasciola hepatica* infects a wide variety of mammals, including cattle, sheep, and humans, causing liver fluke disease, or fasciolosis. Infection is usually acquired by the ingestion of vegetation on which the infective metacercariae have encysted. The metacercariae excyst in the intestine, burrow through the gut wall of the mammalian host, and migrate across the body cavity to the liver, where the parasite causes extensive damage. Infection with *F. hepatica*, like other helminths, is accompanied by elevated immunoglobulin E levels, eosinophilia, and immune responses associated with the Th2 subtype (10, 26), and we have recently demonstrated that *F. hepatica* infection of mice results in an early and persistently polarized Th2 response (29a). This has provided an ideal model with which to examine the cross-regulatory effect of a Th2-inducing pathogen following prior or simultaneous exposure to a Th1-inducing pathogen.

We demonstrate suppression of the *B. pertussis*-specific Th1 response and delayed bacterial clearance from the lungs in mice coinfecting with *F. hepatica*. In contrast, *B. pertussis* infection had no effect on the *F. hepatica*-specific Th2 response or on liver pathology. The Th1 response induced by immunization with Pw is also downregulated following infection with *F. hepatica*. However, this immunomodulatory effect is almost completely abrogated in IL-4 knockout mice, suggesting that IL-4 plays a major role in the suppressive effect of the parasitic infection.

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## MATERIALS AND METHODS

**Antigens.** A formaldehyde-treated sonic extract of *B. pertussis* (BPS) was prepared as previously described (23). Purified native filamentous hemagglutinin (FHA) from *B. pertussis* was a generous gift from the Swiss Serum and Vaccine Institute, Berne, Switzerland. The third British reference preparation for pertussis vaccine (88/522) was used as the Pw; mice were immunized intraperitoneally with 0.1 to 0.2 human dose. Liver fluke homogenate (LFH) was prepared as described previously (29). Briefly, adult liver flukes were obtained from the infected livers of cattle from a local abattoir. The liver flukes were washed four times with phosphate-buffered saline (pH 7.0) and homogenized in phosphate-buffered saline. After centrifugation at 10,000 rpm for 30 min, the supernatant was removed and stored at  $-20^{\circ}\text{C}$ .

**Mice.** Female BALB/c mice were purchased from Harlan Olac Ltd., Blackthorn, United Kingdom. C57BL/6 and IL-4-defective (IL-4<sup>-/-</sup>) mice were purchased from B+K Universal Ltd., Hull, United Kingdom. The IL-4<sup>-/-</sup> mice (IL-4T strain) (16) were used with the kind permission of Werner Muller (Institute for Genetics, University of Cologne, Cologne, Germany). All mice were bred and maintained according to the guidelines of the Irish Department of Health and were 2 to 3 months old at the initiation of experiments.

**Cytokine assays.** T-cell cytokine production was assessed by culturing spleen cells ( $2 \times 10^6/\text{ml}$ ) in triplicate with *B. pertussis* sonicate, FHA, and LFH. Control stimuli included medium alone (background control) or anti-CD3 (2.0  $\mu\text{g}/\text{ml}$ ) and phorbol myristate acetate (PMA; 25 ng/ml). Supernatants were removed after optimum times for cytokine secretion (24 h for IL-2 or 72 h for IL-4, IL-5, and IFN- $\gamma$ ) and stored at  $-20^{\circ}\text{C}$  until assayed. IL-2 release was measured by the ability of culture supernatant to support the proliferation of the IL-2-dependent CTLL-2 line, and the concentrations of IFN- $\gamma$ , IL-4, and IL-5 were measured by immunoassay using pairs of commercially available monoclonal antibodies (PharMingen, San Diego, Calif.) as described previously (22).

***F. hepatica* and *B. pertussis* infection.** Mice were orally infected with 10 metacercariae of *F. hepatica*, which produced liver fluke infection in 100% of animals. Respiratory infection of mice with *B. pertussis* was performed by aerosol challenge (22). Bacteria from a 48-h culture were resuspended at a concentration of  $\sim 2 \times 10^{10}$  CFU/ml in physiological saline containing 1% casein. The challenge inoculum was administered to mice over a period of 15 min by means of a nebulizer in a sealed container within a class 2 laminar flow cabinet. Groups of four mice were killed at various times after aerosol challenge to assess the numbers of viable *B. pertussis* in the lungs. Lungs were aseptically removed and homogenized in 1 ml of sterile physiological saline with 1% casein on ice. Then 100  $\mu\text{l}$  of undiluted homogenate or of serially diluted homogenate from individual lungs was spotted in triplicate onto Bordet-Gengou agar plates, and the number of CFU was estimated after 5 days of incubation at  $37^{\circ}\text{C}$ . The limit of detection was approximately  $0.5 \log_{10}$  CFU per lung.

**Statistical analysis.** Results are presented as means  $\pm$  standard errors (SE) for cytokine concentrations or CFU counts performed individually (all assays in triplicate) on four mice per experimental group. The statistical significance of difference of the mean values between experimental groups was determined by the two-tailed Student *t* test. *P* values of  $<0.05$  were considered significant.

## RESULTS

***F. hepatica* suppresses the type 1 response induced by respiratory infection with *B. pertussis*.** To examine the effect of *F. hepatica* infection on the immune response induced by infection with *B. pertussis*, BALB/c mice were coinfecting with both parasite and bacteria on the same day. Mice infected with either *F. hepatica* or *B. pertussis* only or naive uninfected mice served as controls. *F. hepatica* or *B. pertussis* antigens did not stimulate cytokine production in spleen cells from naive mice (data not shown). In contrast, spleen cells prepared from mice 3 weeks after infection with *B. pertussis* alone secreted high IFN- $\gamma$  levels, and undetectable IL-4, in response to *B. pertussis* sonicate and to the purified *B. pertussis* antigen FHA (Fig. 1). This finding is consistent with our previous reports (22, 32) that *B. pertussis* infection selectively induces Th1 cell responses. The production of *B. pertussis*-specific IFN- $\gamma$  is almost completely abrogated in mice coinfecting with *F. hepatica*. In contrast, infection with *F. hepatica* results in a polarized Th2 response, with high levels of IL-4 and undetectable IFN- $\gamma$  produced by spleen cells in response to LFH. However, the profile of *F. hepatica*-specific cytokine production was not altered in mice coinfecting with *B. pertussis* (Fig. 1), and there was no effect on the severity of fasciolosis, as determined by liver pathology.

Coincident with the suppression of the Th1 response, con-

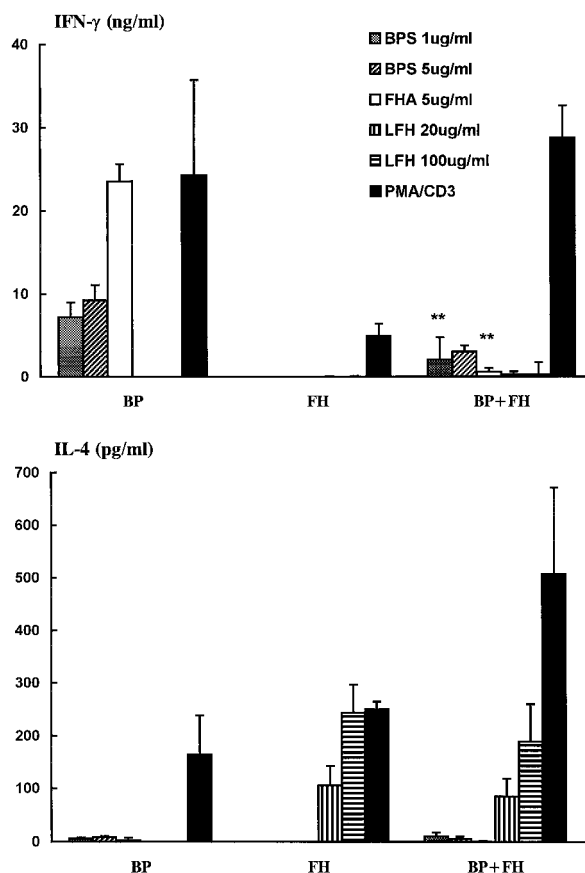


FIG. 1. *F. hepatica* suppresses *B. pertussis*-specific IFN- $\gamma$  production in coinfecting mice. BALB/c mice were infected with either *B. pertussis* (BP) or *F. hepatica* (FH) or were concurrently infected with *B. pertussis* and *F. hepatica*. Three weeks after infection, spleen cells were stimulated in vitro with *B. pertussis* sonicate (BPS), FHA, LFH, PMA and anti-CD3, or medium only, and cytokine levels were assessed in supernatants 3 days later. Cytokine concentrations represent means  $\pm$  SE after subtraction of background control values with medium only (IFN- $\gamma$ , 1.5 to 2.7 ng/ml; IL-4, 11 to 36 pg/ml) for four mice per experimental group and are representative of four experiments. \*\*, *P* < 0.01 versus mice infected with *B. pertussis* alone.

current infection with *F. hepatica* also resulted in delayed *B. pertussis* clearance from the lungs. Mice infected with *B. pertussis* alone began to clear the bacteria at a steady rate after 7 days, whereas clearance was protracted in coinfecting mice. The numbers of bacteria were significantly higher in the coinfecting mice 14 (*P* < 0.01) and 21 (*P* < 0.05) days after challenge (Fig. 2).

***F. hepatica* suppresses an established *B. pertussis*-specific Th1 response.** Having established that *F. hepatica* infection could suppress the *B. pertussis*-specific Th1 response during the induction phase, we decided to determine whether the same suppressive effect could be observed on an established Th1 response. BALB/c mice were infected with *B. pertussis* by aerosol challenge and allowed to recover. After 6 weeks, by which time the *B. pertussis*-specific Th1 response was established and the mice had recovered from infection (the lungs were completely free from bacteria), the mice were infected with *F. hepatica*. Spleen cells from mice infected with *B. pertussis* alone secreted high levels of IFN- $\gamma$  and low levels of IL-4, whereas mice infected with *F. hepatica* alone secreted IL-4 and low levels of IFN- $\gamma$ , typical Th1 and Th2 responses, respectively (Fig. 3). However, IFN- $\gamma$  production in response to *B. pertussis*

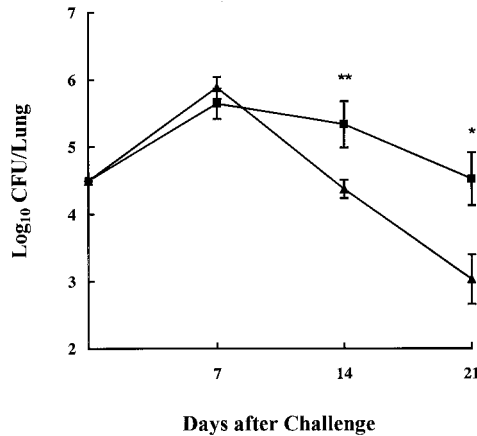


FIG. 2. *F. hepatica* infection delays bacterial clearance in mice infected with *B. pertussis*. BALB/c mice were infected either with *B. pertussis* alone (▲) or concurrently with *F. hepatica* (■). Subsequently, mice were sacrificed at various times to assess the numbers of viable bacteria in the lungs. Results are reported as the mean numbers of *B. pertussis* CFU for individual lungs from four mice from each group at each time point and are representative of two experiments. \*,  $P < 0.05$  versus mice infected with *B. pertussis* alone; \*\*,  $P < 0.01$  versus mice infected with *B. pertussis* alone.

antigens was significantly ( $P < 0.01$ ) diminished in the mice that cleared the *B. pertussis* infection and were subsequently infected with *F. hepatica* (Fig. 3), demonstrating suppression of the already established bacterium-specific Th1 response.

**Infection with *F. hepatica* results in suppression of the *B. pertussis*-specific Th1 response in mice immunized with Pw.** Since immunization with Pw also induces a potent Th1 response and confers a high level of protection against a *B. pertussis* respiratory challenge, we examined the effect of *F. hepatica* infection on this protective vaccination. Mice were immunized twice with Pw (0.8 IU intraperitoneally at 0 and 4 weeks) and 4 weeks later were infected with 10 metacercariae of *F. hepatica*. As expected, mice immunized with Pw alone or infected with *F. hepatica* only developed Th1 or Th2 responses, respectively. The production of IL-4 and IL-5 in response to *F. hepatica* was not affected by prior immunization with Pw. However, *B. pertussis*-specific IFN- $\gamma$  and IL-2 production in Pw-immunized mice was almost completely inhibited following *F. hepatica* infection, demonstrating that infection with *F. hepatica* severely decreases *B. pertussis*-specific Th1 cytokine production (Fig. 4). Furthermore, IFN- $\gamma$  (but not IL-4) production in response to the polyclonal activators PMA and anti-CD3 was also significantly ( $P < 0.001$ ) suppressed in mice infected with *F. hepatica*. Moreover, infection with *F. hepatica* reduced the protective efficacy of the Pw in the respiratory challenge model. The numbers of viable bacteria in the lungs 7 days after *B. pertussis* challenge were 40-fold higher ( $P < 0.05$ ) in immunized mice infected with *F. hepatica* than in mice that received the vaccine only (Fig. 5).

***F. hepatica*-induced suppression of Th1 responses involves IL-4.** IL-4 plays a major role in directing the immune response to the Th2 subtype and has also been implicated in the reciprocal downregulation of Th1 responses. Therefore, we examined the role of IL-4 in the *F. hepatica*-induced suppression of *B. pertussis* specific Th1 responses in IL-4<sup>-/-</sup> mice. As the knockout mice were available only on a C57BL/6 background, we carried out these experiments in a strain different from those reported in Fig. 1 to 5. However, we had already established that the two strains exhibited the same patterns of Th1 and Th2 responses to *B. pertussis* and *F. hepatica*, respectively,

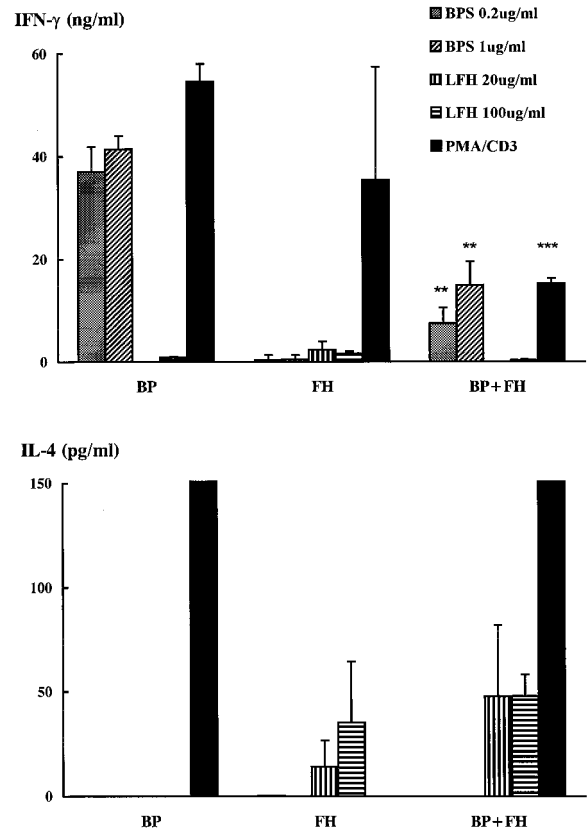


FIG. 3. *F. hepatica* infection suppresses an established Th1 response to *B. pertussis*. BALB/c mice were infected with *B. pertussis* and allowed to recover for 6 weeks. The convalescent mice were then infected with 10 metacercariae of *F. hepatica*. Mice infected with *B. pertussis* (BP) or *F. hepatica* (FH) alone served as controls. Antigen-specific cytokine production by spleen cells was assessed 3 weeks after infection with *F. hepatica*. Cytokine concentrations represent means  $\pm$  SE after subtraction of background controls (IFN- $\gamma$ , 1.5 to 2.7 ng/ml; IL-4, 11 to 36 pg/ml) for four mice per experimental group and are representative of three experiments. \*\*,  $P < 0.01$  versus mice infected with *B. pertussis* alone; \*\*\*,  $P < 0.001$  versus mice infected with *B. pertussis* alone.

with a slight tendency to stronger Th1 responses in the C57BL/6 mice and stronger Th2 responses in the BALB/c mice. IL-4<sup>-/-</sup> and wild-type C57BL/6 mice were immunized with Pw and boosted 4 weeks later. Immunized and control naive mice were then infected with 10 *F. hepatica* metacercariae, and T-cell cytokine production was assessed 2 weeks later. Spleen cells of wild-type C57BL/6 mice immunized with Pw alone exhibited a strong Th1 response, characterized by high levels of IFN- $\gamma$  production and low IL-4 to *B. pertussis* antigens. Interestingly, the levels of *B. pertussis*-specific IFN- $\gamma$  secreted by spleen cells were lower in IL-4<sup>-/-</sup> mice than in wild-type mice. However, this finding is consistent with our previous observations (19) and with a recent report which suggested that IL-4 is required in the priming phase of Th1-associated tumor immunity (34). Following infection with *F. hepatica*, a complete switch from type 1 to a type 2 response was observed. *B. pertussis*-specific IFN- $\gamma$  production was markedly suppressed ( $P < 0.001$  to 0.01), and low but significant levels of IL-4 were now detected in response to *B. pertussis* antigens (Fig. 6). In contrast, *F. hepatica* infection did not suppress IFN- $\gamma$  or elevate IL-4 production by *B. pertussis*-specific T cells from IL-4<sup>-/-</sup> mice immunized with Pw (Fig. 6). We did detect IL-5 in response to *F. hepatica* in IL-4<sup>-/-</sup> mice

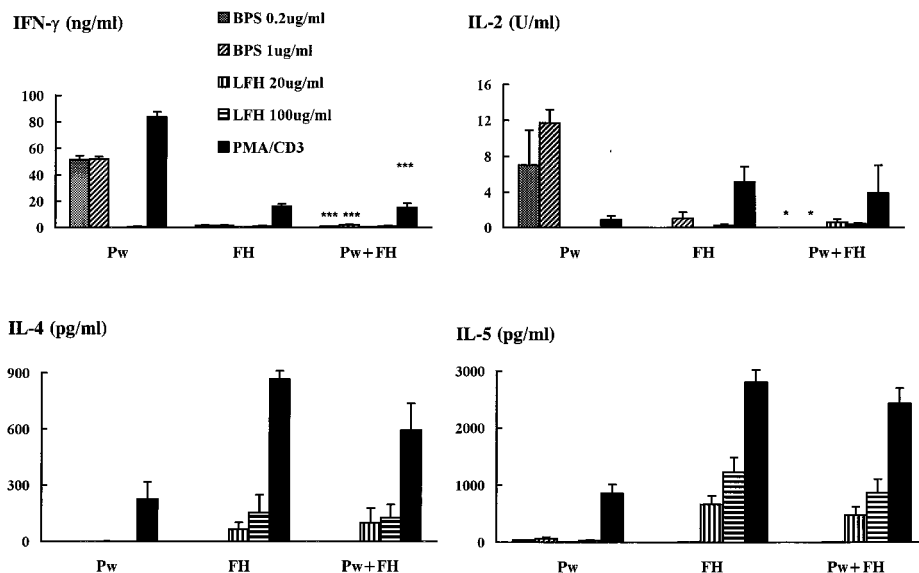


FIG. 4. *F. hepatica* infection suppresses a Th1 response induced with Pw. Mice were immunized with Pw and boosted 4 weeks later. Two weeks after the second immunization, mice were infected with *F. hepatica* (FH). Controls consisted of mice that received either *F. hepatica* infection or pertussis immunization only. Cytokine production by spleen cells was assessed 2 weeks after *F. hepatica* infection, following stimulation in vitro with *B. pertussis* sonicate (BPS) and LFH. Cytokine concentrations represent means  $\pm$  SE after subtraction of background control values (IFN- $\gamma$ , 2.2 to 2.4 ng/ml; IL-2, 0.02 to 0.2 U/ml; IL-4, 11 to 36 pg/ml; IL-5, 12 to 25 pg/ml) for four mice per experimental group and are representative of three experiments. \*,  $P < 0.05$  versus mice immunized with Pw alone; \*\*\*,  $P < 0.001$  versus mice immunized with Pw alone.

(data not shown), suggesting that these mice were still capable of mounting a Th2 response.

DISCUSSION

The results of this study demonstrate that immune responses dominated by one T-cell subtype, evoked at one mucosal surface in the body, can exert bystander modulation on the reciprocal T-cell subtype induced at another site in the body. Fur-

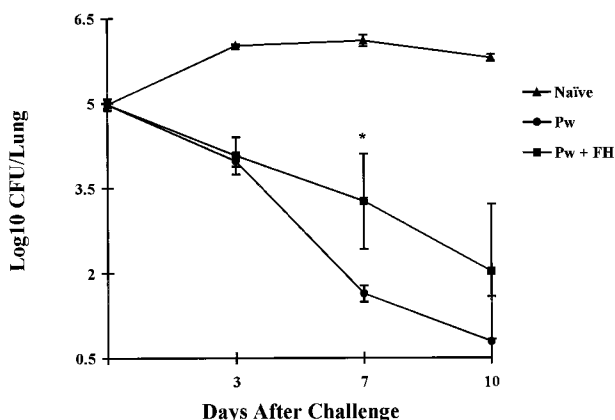


FIG. 5. *F. hepatica* infection reduces the protective efficacy of Pw in mice. BALB/c mice were immunized with Pw (0 and 4 weeks), and 1 week later a proportion of these mice were infected with 10 metacercariae of *F. hepatica* (FH). Respiratory infection of mice with *B. pertussis* was performed by aerosol challenge 1 week after infection with *F. hepatica*. Naive mice and mice that were immunized with Pw and subsequently infected with *B. pertussis* without a preceding *F. hepatica* infection served as controls. Mice were killed from all groups at various times after aerosol challenge to assess the numbers of viable bacteria in the lungs. Results are reported as the mean numbers of *B. pertussis* CFU for individual lungs from four mice at each time point and are representative of two experiments. \*,  $P < 0.05$  versus mice immunized with Pw alone.

thermore, in an experimental exposure to simultaneous Th1- and Th2-inducing stimuli, we observed suppression of Th1 responses, without a reciprocal effect on Th2 responses, suggesting that at least in our model system the Th2 cell may have a dominant effect in Th1-Th2 cross-regulation in vivo. In addition, our results provide the first evidence that the immunosuppressive effect of helminth parasites can also operate on an established Th1 response and that the immunoregulatory mechanism involves IL-4.

In general, parasitic infections do not cause high mortality but counteract the host's immune defenses by developing a variety of strategies to evade protective immune responses (20). It has been well documented that parasitic infection is frequently accompanied by a downregulation in cell-mediated immunity. Inhibition of lymphocyte proliferative responses has been found during nematode (2) and *F. hepatica* (8) infections. Parasitic infections also provide some of the clearest examples of how the nature and protective capacity of the host's immune system are dependent on the polarized development of T lymphocytes of either the Th1 or Th2 subsets. It is well established that the emergence of an immune response dominated by a Th2-type profile is characteristic of many helminth infections, and it has been reported that Th2 responses are essential for resistance to these parasites (10, 14). However, there is also evidence that Th1 stimulation may be associated with protection and that Th2 stimulation is associated with chronic disease (35). The adoptive transfer of a CD4<sup>+</sup> Th1 clone, obtained from mice protectively immunized against the blood fluke *Schistosoma mansoni*, has been shown to convey protection against this parasite (15). In mice, resistance to *Trichinella spiralis* correlates with the early activation of IFN- $\gamma$ -secreting cells and little activation of Th2 cells (31). Although Th1 or Th2 cells may play a role in protection against different parasites, it would be beneficial to the parasite to induce immune responses capable of suppressing the host's immune protective mechanisms.

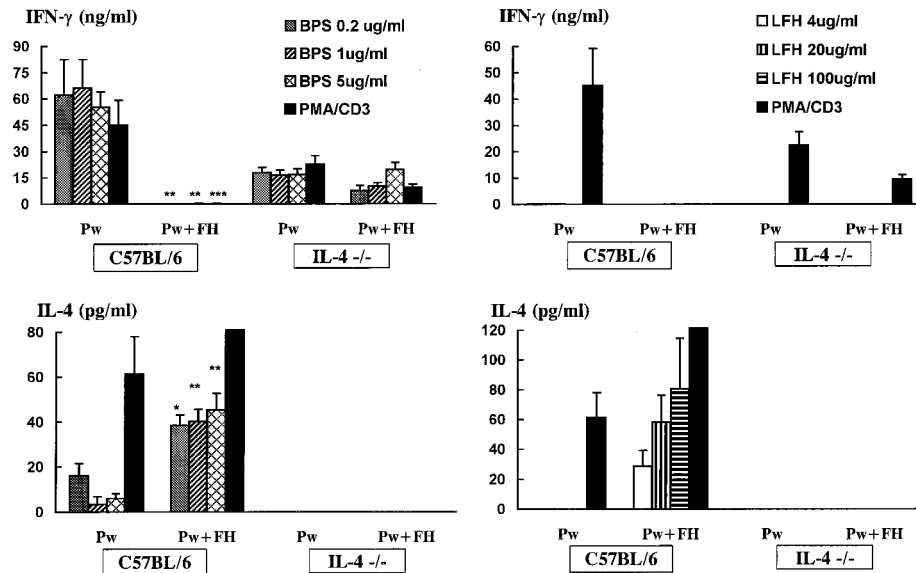


FIG. 6. Effect of *F. hepatica* infection on antigen-specific cytokine production in IL-4<sup>-/-</sup> mice immunized with Pw. IL-4<sup>-/-</sup> and wild-type C57BL/6 mice were immunized with Pw and boosted after 4 weeks. Two weeks after the second immunization, mice were infected with *F. hepatica* (FH). Mice infected with *F. hepatica* or immunized with Pw only served as controls. Cytokine production were assessed 2 weeks after *F. hepatica* challenge by stimulating spleen cells in vitro with *B. pertussis* sonicate (BPS), LFH, or PMA and anti-CD3. Cytokine concentrations represent means  $\pm$  SE after subtraction of background control values (IFN- $\gamma$ , 2.9 to 3.8 ng/ml; IL-4, <10 pg/ml) and are representative of two experiments. \*\*,  $P < 0.05$  versus mice immunized with Pw alone; \*\*\*,  $P < 0.001$  versus mice immunized with Pw alone.

In this present investigation, we exploited two infection models that we have shown to be capable of generating highly polarized Th1 or Th2 responses in mice, in order to examine the cross-regulation of cell subtypes in vivo. Consistent with our previous reports (23, 32, 33), we demonstrated that respiratory infection with *B. pertussis* or immunization with Pw selectively stimulated Th1 responses. In contrast, infection with the parasitic helminth *F. hepatica* evoked a potent Th2 response and was capable of downregulating Th1 responses induced either by respiratory infection with *B. pertussis* or by systemic immunization with Pw. Downregulation of Th1 cytokine responses to both parasite and nonparasite antigens has also been reported during infection with *S. mansoni* (17, 30). This Th2-inducing parasite has also been shown to exacerbate the outcome of *Salmonella typhi* infection in concurrent infections (27). However, since the response has shifted from predominantly Th1 to Th2 at the egg stage of infection with *S. mansoni* (10), this model is limited to an examination of the effects of an established parasite-specific Th2 response on the induction of a Th1 cells to other antigens or pathogens. In the *F. hepatica* model, a highly polarized Th2 response is detected throughout the infection (unpublished observations), providing a model to examine the influence of the Th2-inducing pathogen at different stages of response to the Th1-inducing pathogen.

Our data clearly indicate that the liver fluke has the ability not only to alter the development of a *B. pertussis*-specific Th1 response during infection and vaccination but also to modulate this response after it has become polarized. The modulatory effect of the parasitic infection could be observed when it was delivered either at the induction phase or during an established *B. pertussis*-specific Th1 response. Significantly, our results also demonstrate that the modulation of the cytokine profile by *B. pertussis*-specific T cells was accompanied by a reduction in host resistance to the bacterial infection after challenge. The finding that protective immunity was not completely abrogated

in the mice infected with *F. hepatica* can be explained by the fact that Th2 or a mixed Th1-Th2 response, such as that induced with an acellular pertussis vaccine, can also confer a level of protection against *B. pertussis* challenge by a distinct mechanism (22). Furthermore, we have preliminary evidence that the modulatory effect of the *F. hepatica* infection on cytokine production is not as pronounced in the draining lymph nodes of the lung as in the spleen. We have already demonstrated a degree of compartmentalization of local and systemic immune responses during infection with *B. pertussis* (21). However, these findings together with those of the present study suggest that the systemic response can influence protective effector mechanisms in the lungs.

Our findings suggest that the suppression of antibacterial immunity during *F. hepatica* infection is a consequence of bystander downregulation of the *B. pertussis*-specific Th1 cells by the parasite-specific Th2 cells. Nevertheless, it is possible that the liver fluke infection may have exerted other effects on antibacterial immunity, independent of Th2 cells. It has been suggested that *S. mansoni* may induce apoptosis of IFN- $\gamma$ -producing cells (9). Excretory-secretory components of *F. hepatica* may also exert direct immune suppressive effects through the activity of proteinases on immunoglobulin molecules (6). However, the abrogation of the modulatory effect of the *F. hepatica* infection in IL-4-defective mice argues against these possibilities and points to an important role for IL-4 in Th2-mediated immunoregulation. *F. hepatica* infection of C57BL/6 mice that had been immunized with Pw resulted in significant reduction in *B. pertussis*-specific IFN- $\gamma$  production. In contrast, IFN- $\gamma$  production was not significantly altered following *F. hepatica* infection of IL-4<sup>-/-</sup> mice immunized with Pw. Furthermore, we did not observe a significant difference in the bacterial load in IL-4<sup>-/-</sup> mice coinfecting with *F. hepatica* (data not shown), suggesting that abrogation of the suppressive effect on IFN- $\gamma$  production translates into restoration of full protection. However, interpretation of the effect of IL-4 on the

outcome of infection in IL-4<sup>-/-</sup> mice is complicated by the fact that IFN- $\gamma$  production in the absence of *F. hepatica* infection is also partially suppressed in these mice (references 19 and 34 and this study).

In addition to IL-4, other inhibitory cytokines may also be involved in the Th1 response inhibition by *F. hepatica*. Like IL-4, IL-10 can inhibit cytokine production by Th1 cells (11) and the ability of IFN- $\gamma$  to activate macrophage killing of both intracellular and extracellular parasites (13). It has been suggested that this inhibitory cytokine may be responsible for the suppression of Th1 responses in *S. mansoni* infection (36). IL-4 and IL-10 can act synergistically to inhibit the production of reactive nitrogen oxides, which are known to upregulate IL-12 production and, as a consequence, inflammatory responses (18). It has been shown that the excretory-secretory products produced during *F. hepatica* infection can decrease nitrite production by rat peritoneal cells (7). We have demonstrated that spleen cells from *F. hepatica*-infected mice secrete high levels of IL-4 and IL-10 in response to liver fluke antigens *in vitro* (29a). Thus, *F. hepatica* may, through the induction of IL-4 and perhaps IL-10, inhibit the activation of macrophages and suppress IFN- $\gamma$  production by Th1 cells.

The present investigation demonstrated that *F. hepatica* infection could downregulate *B. pertussis*-specific IFN- $\gamma$  production at both the induction and effector stages of the Th1 response. In C57BL/6 mice immunized with Pw and then infected with *F. hepatica*, the Th1 response completely switched to a Th2 response. The appearance of Th2 cytokines in C57BL/6 but not BALB/c mice was reproducible and is surprising in view of the observations that responses tend to be more polarized to Th1 in C57BL/6 mice. We do not have an explanation for this other than it may reflect complex differences in sensitivity to regulatory cytokines. As well as the recognized role for early IL-4 in directing the immune response to Th2 pathway, there is some evidence that IL-4 is capable of converting Th1 cells to the Th2 subtype. In one study, a highly polarized *Leishmania*-specific Th1 cell population switched to a Th2 phenotype following *in vitro* culture with IL-4, especially when added early in culture (24). Transcripts for IFN- $\gamma$  have been shown to be dominant in the skin-draining lymph nodes of mice vaccinated with irradiation-attenuated cercariae of *S. mansoni*, but following challenge IL-4 becomes dominant and IFN- $\gamma$  message levels are barely detectable (4). Thus, while early IL-4 production probably plays a major role in driving the immune response to a Th2 phenotype and may be important in maintaining the polarization of this response, it can also influence the profile of cytokines secreted in response to unrelated antigens.

In conclusion, our results show that infection with the Th2-inducing parasite *F. hepatica* can suppress a Th1 response induced by *B. pertussis*-infected or immunized mice. As well as suppressing IFN- $\gamma$  and IL-2 production, *F. hepatica* infection also delayed clearance of the bacteria from the lungs following *B. pertussis* challenge. We observed suppression of Th1 responses without a reciprocal effect on Th2 responses, suggesting that at least in the present model system, the Th2 cell may have a dominant effect in Th1/Th2 cross-regulation *in vivo*. The demonstration of bystander immunomodulation of protective type 1 responses during infection with Th2-inducing organisms has profound implications for the outcome of concurrent bacterial infections and on protective efficacy of vaccines against intracellular pathogens.

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