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An Enteric Helminth Infection Protects Against an Allergic Response to Dietary Antigen[1]

Mohamed Elfatih H. Bashir,* Peter Andersen,* Ivan J. Fuss,[†] Hai Ning Shi,* and Cathryn Nagler-Anderson[2]*

Although helminths induce a polarized Th2 response they have been shown, in clinical studies, to confer protection against allergies. To elucidate the basis for this paradox, we have examined the influence of an enteric helminth infection on a model of food allergy. Upon Ag challenge, mice fed peanut (PN) extract plus the mucosal adjuvant cholera toxin (CT) produced PN-specific IgE that correlated with systemic anaphylactic symptoms and elevated plasma histamine. PN-specific IgE was not induced in helminth-infected mice fed PN without CT. Moreover, when PN plus CT was fed to helminth-infected mice, both PN-specific IgE and anaphylactic symptoms were greatly diminished. The down-regulation of PN-specific IgE was associated with a marked reduction in the secretion of IL-13 by PN-specific T cells. When helminth-infected PN plus CT-sensitized mice were treated with neutralizing Abs to IL-10, the PN-specific IgE response and anaphylactic symptoms were similar to, or greater than, those seen in mice that receive PN and CT alone. Taken together, these results suggest that helminth-dependent protection against allergic disease involves immunoregulatory mechanisms that block production of allergen-specific IgE. *The Journal of Immunology,* 2002, 169: 3284–3292.

T he prevalence of allergic disease and asthma has exploded in the developed world during the last twenty years. Hypotheses to explain the increasing prevalence of the dysregulated mucosal Th2 responses that characterize allergic hyperreactivity have typically been framed in terms of reduced or absent stimulation by Th1-polarizing stimuli. Often called the "hygiene" hypothesis, the predominant model has suggested that this dramatic increase in allergic disease parallels the greatly reduced exposure to childhood bacterial and viral infections brought about by improvements in vaccination and sanitation (1, 2). However, the effect, during this same time period, of the elimination of chronic Th2-polarizing stimuli (induced by infection with helminthic parasites) is virtually ignored by this hypothesis. Many studies have indicated that little allergic disease is found in developing countries where helminth infection is still endemic. Indeed, some recent epidemiological and clinical studies have suggested an inverse association between helminth infection and allergy (3, 4). Yet, there is very little data available to explain how helminth infection might protect against allergy. It is clear that the protective effect of helminth infection on the development of allergy cannot be explained simply in terms of the absence of Th1-inducing stimuli. This has

led, recently, to a reworking of the hygiene hypothesis that emphasizes the counterregulatory mechanisms induced by all types of inflammatory responses (5–7). Prominent among these are regulatory T cells secreting immunosuppressive cytokines like IL-10 and TGF-β.

Dietary Ags are sometimes allergenic, but typically induce systemic nonresponsiveness (8). Experimentally, various microbial products can act as adjuvants and induce systemic and mucosal immune responses to coadministered protein Ags (9). Previous work from our laboratory (Mucosal Immunology Laboratory, Massachusetts General Hospital, Charlestown, MA) has shown that an ongoing enteric helminth infection can also act as an adjuvant to prime for an Ag-specific Th2 response to a model dietary Ag (10, 11). Like helminth infection, allergic diseases are associated with the increased production of IgE Abs. In this report, we examine whether the polarized Th2 response induced by intestinal helminth infection in early life influences the development of an allergic response to a dietary Ag. Although not all helminth infections migrate through the intestine, the helminth used in our study, *Heligmosomoides polygyrus*, has a strictly enteric life cycle. Using a model in which intragastric (i.g.)[3] administration of peanut (PN) Ag in the presence of cholera toxin (CT) (a Th2-inducing mucosal adjuvant) results in the production of Ag-specific IgE and systemic symptoms of anaphylaxis (12), we have compared the response to Ag administered to helminth-infected mice to that seen after sensitization with Ag and CT alone. Our results show that helminth infection does not prime for a PN Ag-specific IgE response; only mice given PN Ag plus CT developed Ag-specific IgE and an allergic response. Moreover, the IgE-mediated anaphylactic response to Ag plus CT was greatly reduced in helminth infected mice, observations that parallel those made in clinical studies. The down-regulation of Ag-specific IgE observed in helminth-infected mice was accompanied by a reduction of Ag-specific T cells secreting IL-13. Treatment of infected mice with neutralizing Ab to

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[3] Abbreviations used in this paper: i.g., intragastric; PN, peanut; CT, cholera toxin; SPL, splenocyte; MLN, mesenteric lymph node.

IL-10 abrogated infection-mediated protection against allergic symptoms. Our results suggest a mechanistic explanation for the absence of Ag-specific IgE in helminth-infected mice and provide a new rationale for the ability of helminth infection to protect against allergy.

Materials and Methods

Mice and parasitic infection

Female C3H/HeJ weanling mice (3-wk-old) were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained under specific viral pathogen-free conditions at an American Association for the Accreditation of Laboratory Animal Care accredited facility at Massachusetts General Hospital (Charlestown, MA). Infective, ensheathed, third-stage larvae of *H. polygyrus* were propagated as previously described and stored at 4°C until use. *H. polygyrus*-infected mice were inoculated orally with 200 third-stage larvae (10, 11).

PN Ag preparation

Ten grams of unshelled ground PN were homogenized in 25 ml PBS (pH 7.2). The suspension was stirred for 4 h and centrifuged at $18,000 \times g$ for 30 min at 4°C to remove insoluble materials. The fatty layer was removed. The clear solution was centrifuged again under the same conditions. The supernatant was dialyzed overnight against distilled water with a 3–5 kDa MWCO membrane (Spectrum Laboratories, Houston, TX). Protein was determined by the Bio-Rad protein assay (Hercules, CA). The dialyzed PN proteins at a concentration of 2 mg of protein per milliliter are referred to as PN Ag. This solution was then sterile-filtered with a 0.22-μm Nalgene syringe filter, and stored at -20°C.

Sensitization with crude PN extract

H. Polygyrus-infected or noninfected female C3H/HeJ mice were sensitized by weekly i.g. gavage with 5 mg of ground whole PN per mouse (previously estimated to be equivalent to 1 mg of PN protein; Ref. 12). Three different sensitization protocols were used in this study (see Fig. 1). In protocol A, mice were sensitized with 5 mg of PN in PBS on days 7, 14, 21, 28, and 35 with or without CT (0.3 μg/g body weight; List Biological Laboratories, Campbell, CA). In protocol B, mice were sensitized with two doses of 5 mg PN in PBS on days 7 and 21 with or without CT (10 μg per animal at a concentration of 1 mg/ml). In protocol C, groups of infected and noninfected mice were treated with three doses of neutralizing rat anti-mouse IL-10 mAb on days 7, 21, and 28. mAb SXC-1 and SXC-2 were purified from ascites fluid as previously described (13). At the indicated times, 0.75 mg of each Ab was administered.

The *H. polygyrus*-infected groups are hereafter referred to as *H. polygyrus*-infected PN-sensitized (HP/PN) and HP/PN plus CT (HP/PN/CT). The noninfected groups are referred to as noninfected PN plus CT-sensitized (PN/CT) and PN. In addition, the noninfected control groups that received PBS alone (naive mice) and PBS plus CT (CT sham-sensitized) are referred to as PBS and PBS/CT, respectively. The *H. polygyrus*-infected group that received PBS alone is referred to as HP/PBS.

All mice were bled weekly, beginning at 1 wk after the initial sensitization by PN. A week after the last sensitization, mice were fasted overnight before two i.g. challenges with PN (10 mg per mouse divided into two doses) at 30–40 min intervals. The mice were continuously monitored for signs of allergic sensitization. Plasma and serum were harvested from each mouse 30 min after the second challenge with PN. Plasma samples were used for measuring histamine levels before and after Ag challenge. In each experiment, there were 5–10 mice per group. Three independent experiments were performed for feeding protocol Fig. 1*A*, two for Fig. 1*B*, and one for Fig. 1*C*.

Measurement of serum total and PN-specific Abs

PN-specific IgE in the sera was measured by ELISA with modification from an assay previously described (14). The upper two rows of a 96-well plate (Maxisorp Immunoplates; Nalge Nunc International, Naperville, IL) were coated with purified goat anti-mouse IgE mAb (2 μg/ml; BD PharMingen, San Diego, CA) and were used to generate a standard curve. The remainder of the plate was coated with PN Ags (5 μg/ml). The plate was incubated at 4°C overnight, washed with PBS/0.05% Tween 20 and blocked with PBS/10% FCS. Diluted serum samples were dispensed in triplicate in the lower portion of the plate and incubated overnight at 4°C. IgE was detected with biotinylated rat anti-mouse IgE (BD PharMingen) and avidin alkaline phosphatase (Sigma-Aldrich, St. Louis, MO). The reaction was developed with the phosphatase substrate (Kirkegaard & Perry

Laboratories, Gaithersburg, MD). The reaction was then stopped by the addition of 100 μl 5% EDTA to each well and absorbance was read at 405 nm (Molecular Devices, Menlo Park, CA). OD values were converted to nanograms per milliliter of IgE by comparison with standard curves of purified IgE (BD PharMingen) by linear regression analysis, and are expressed as the mean concentration of each group of mice $\pm$ SEM.

PN-specific IgG1 was determined by ELISA. The upper two rows of a 96-well (Immunolon II) plate were coated with a goat anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL) and used to generate a standard curve. The remainder of the plate was coated with PN Ag (5 μg/ml) and incubated overnight at 4°C. Following blocking and washing, the plates were incubated with diluted serum samples and standard mouse IgG1 (Southern Biotechnology Associates). Ag-specific IgG1 was detected using HRP-conjugated anti IgG1 Abs (Southern Biotechnology Associates) and substrate *o*-phenylenediamine (OPD; Zymed Laboratories, South San Francisco, CA). The plates were read in an ELISA plate reader at 492 nm.

Total IgE and IgG1 were measured by ELISA as described previously (10). All samples were assayed in triplicate; the limit of sensitivity of all of the ELISAs is <0.5 ng/ml.

Measurement of cytokine production of splenocytes (SPL) and mesenteric lymph node (MLN) cells in vitro

SPL from individual animals (5–10 per group) and pooled MLN (five animals per group) were prepared using a 70-μM nylon cell strainer (Falcon; BD Labware, Franklin Lakes, NJ). Single cell suspensions of SPL and MLN (1×10^6 cells/well) were cultured in 24-well plates (Costar, Cambridge, MA) in the presence or absence of PN (200 μg/ml) in complete DMEM (Life Technologies, Grand Island, NY) containing 10% FCS (Hy-Clone Laboratories, Logan, UT), 10 mM HEPES, 2 mM L-glutamine, 100 U penicillin/ml, 100 μg of streptomycin/ml, 50 μM 2-ME, 0.1 mM non-essential amino acids, and 1 mM sodium pyruvate.

At 72 h after the initiation of the culture, culture supernatants were collected for the assessment of IL-4, IL-5, IL-10, IL-13, and IFN-γ production by ELISA as described previously (10). ELISA capture (BVD4-1D11, IL4; R4-6A2, IFN-γ; TRFK-5, IL-5; and JESS-2A5, IL10) and biotinylated second Abs (BVD6-24G2, IL-4; XMG1.2, IFN-γ; TRFK4, IL-5; and SXC-1, IL-10) were purchased from BD PharMingen. Standard curves were obtained using recombinant murine IFN-γ, IL-4 (Genzyme, Cambridge, MA), IL-10 (R&D Systems, Minneapolis, MN), and IL-5 (BD PharMingen) and are expressed in picograms per milliliter $\pm$ SEM. A DuoSet ELISA development kit for the detection of murine IL-13 was purchased from R&D Systems. IL-13 was assayed according to the manufacturer's instructions and is expressed in picograms per milliliter $\pm$ SEM.

Assessment of hypersensitivity reaction

Anaphylactic symptoms were evaluated for 30–40 min after the second challenge dose by using a scoring system that was modified slightly from previous reports (12): 0, no symptoms; 1, scratching and rubbing around the nose and head; 2, puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth and the tail; 4, no activity after prodding or tremor and convulsion; and 5, death.

Measurement of plasma histamine levels

Plasma samples taken 1 day before PN challenge and 30 min after the last PN challenge were used to determine the histamine level as previously described (15). Histamine levels were determined by using an enzyme immunoassay kit (Immunotech, Marseille, France), as described by the manufacturer. Histamine concentrations were calculated by comparison with a reference standard curve provided by the manufacturer.

Statistical differences in serum Ab levels were determined using a two-tailed Student's *t* test with StatView software (Abacus Concepts, Berkeley, CA). A *p* value < 0.05 was considered significant.

Results

Helminth infection does not prime for PN-specific IgE

It is well-established that i.g. sensitization of mice using PN and CT, a Th2-inducing mucosal adjuvant, leads to a predominant PN-specific IgE response that mimics the clinical and immunologic characteristics of PN allergy in humans (12). We have previously shown that helminth infection, like CT, acts as a Th2-inducing mucosal adjuvant (10, 11). In this report, we have examined the

FIGURE 1. Schematic representation of experimental protocols used for oral sensitization of C3H/HeJ mice with PN Ag and subsequent i.g. challenge. Protocol *A*, PN Ag was administered i.g. either with the mucosal adjuvant CT or in the presence of enteric helminth infection. Protocol *B*, The influence of helminth infection on the allergic response to two oral administrations of PN Ag plus CT was assessed. Protocol *C* was used to assess the role of helminth-induced IL-10 in protection against PN allergy. Neutralizing Ab to IL-10 was injected i.p. 6 hr before i.g. PN sensitization at the timepoints indicated. Thirty-five (protocol *A*), 21 (protocol *B*), or 28 (protocol *C*) days after the initial sensitization with PN plus CT, all mice were challenged i.g. twice with 5 mg PN alone (at half-hour intervals) before the evaluation of allergic symptoms.
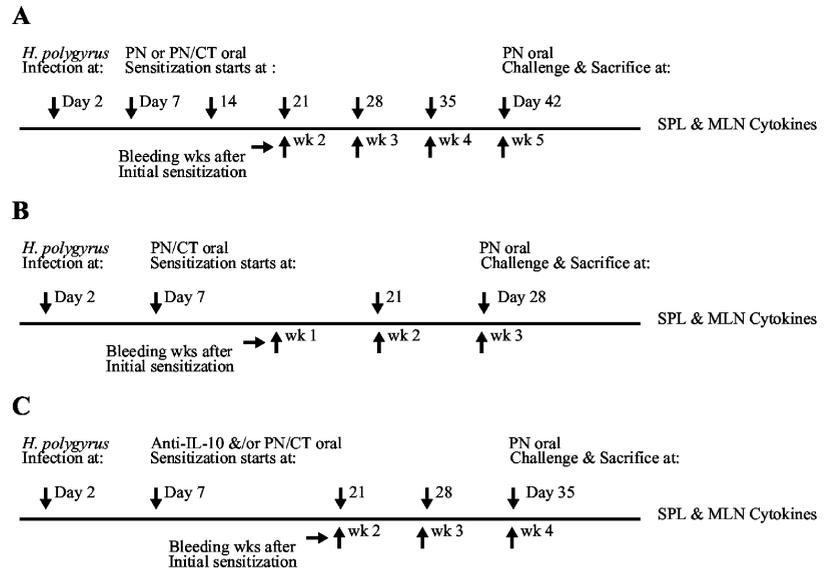
relationship between enteric helminth infection and the development of food allergy. Three different challenge protocols were used (Fig. 1). In the first set of experiments, the kinetics of PN-specific and total IgE and IgG1 isotypes in the serum of HP/PN mice was compared with that of PN/CT mice using protocol *A*. As shown in Fig. 2, *A* and *C*, *H. polygyrus* infection is associated with a vigorous polyclonal IgE and IgG1 serum Ab response in both HP/PN mice and HP/PBS controls. Total IgE levels increased in the two infected groups in a comparable fashion, peaking on day 21 postinfection. In the HP/PN group, total IgE levels increased again after day 28 (Fig. 2*A*). Total IgG1 levels also increased markedly in the HP/PN group and the HP/PBS controls following infection with *H. polygyrus*. No apparent difference was observed between the two infected groups (Fig. 2*C*). Although there was little difference in total IgE or IgG1 isotype production between HP/PN and the control HP/PBS, there was a clear difference when

the HP/PN group was compared with the noninfected PN/CT mice as can be seen from Fig. 2, *A* and *C*. It is evident that only PN/CT sensitization in the noninfected mice induces a PN-specific IgE and IgG1 response, despite elevated serum total IgE and IgG1 levels in helminth-infected mice (see Fig. 2, *B* and *D*). Interestingly, some of the mice that received PN alone also responded with increased total as well as PN-specific IgG1 levels whereas other mice in the same group did not respond. In the HP/PN group, *H. polygyrus* infection failed to induce a PN-specific IgE response in the presence of PN Ags. However, the parasite-infected mice did mount a PN-specific IgG1 response similar to that seen in mice fed PN alone.

PN-specific IgE correlates with anaphylactic symptoms

Ag-specific IgE plays a central role in the pathogenesis of allergic disorders. Cross-linking of high-affinity receptors (FcεR1) on mast
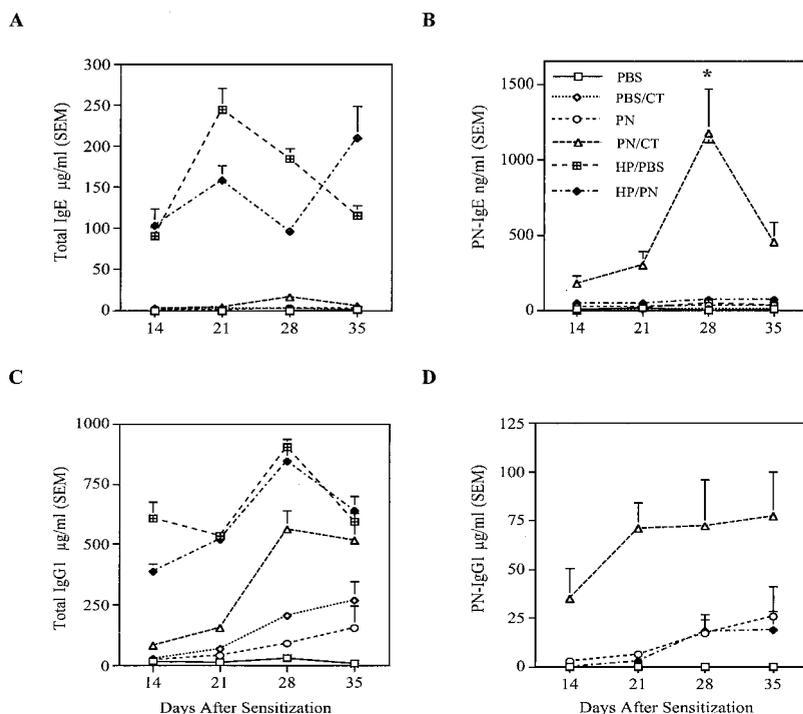
FIGURE 2. PN-specific IgE and IgG1 responses are only elevated in PN plus CT-sensitized mice. Beginning 2 wk after the initial oral sensitization or infection, mice were bled at 1-wk intervals. Total IgE (*A*), PN-specific IgE (*B*), total IgG1(*C*), and PN-specific IgG1(*D*) levels in sera from *H. polygyrus*-infected or noninfected C3H/HeJ mice were measured on the indicated days by ELISA. Individual serum samples (5–10 mice per group) were measured and are expressed as the geometric means ± SEM. One representative experiment of three is shown. The data in Fig. 2–4 are from the same experiment and followed the sensitization protocol shown in Fig. 1*A*.

cells and basophils by allergen-specific IgE leads to the release of mediators that cause allergic reactions (16). Prominent among these is histamine, an intracellular mediator of the immediate hypersensitivity response that exerts potent effects on various target tissues. Plasma histamine levels provide a measure of mast cell and basophil degranulation. To determine how *H. polygyrus* infection and CT affect the plasma histamine response (and to examine whether this measure of systemic anaphylaxis correlates with the Ag-specific IgE response), we examined the difference in plasma histamine levels from samples taken 1 day before and 30 min after i.g. PN challenge. The greatest change in plasma histamine levels was observed in the PN/CT mice (Fig. 3*A*). No difference in histamine levels was noted in the naive (PBS), control (PBS/CT and PN), and infected (HP/PN and HP/PN/CT) mice. The elevated plasma histamine levels observed in the PN/CT group correlated with the symptoms of systemic anaphylaxis (anaphylactic shock, labored respiration, marked decrease in activity) as indicated by the systemic anaphylaxis scores shown in Fig. 3*B* (see *Materials and Methods*). PN challenge elicited anaphylactic shock for one mouse in the PN/CT group within 5–10 min of the first challenge. On an individual basis, a striking association was found between PN-specific IgE levels, the clinical symptoms of allergy as evaluated by systemic anaphylaxis scores, and the difference in the histamine level before and after challenge with PN.

Lack of PN-specific IgE correlates with low PN-specific IL-13

Based on our previous observations, we hypothesized that helminth infection would predispose toward the development of a

Th2-biased immune response to PN Ag. We thought we might be able to uncover a PN responsive T cell population among lymphocytes derived from the spleen or MLN of HP/PN mice upon Ag rechallenge in vitro. To address this issue, we analyzed PN-induced Th1 and Th2 cytokines from PN-stimulated SPL and MLN cells from HP/PN/CT, or PN/CT (Fig. 4). PN Ag restimulation elicited the secretion of elevated levels of IL-13 (Fig. 4*A*), IL-5 (Fig. 4*B*), and IL-10 (Fig. 4*C*) from spleen cells taken from PN/CT mice compared with mice that received PN alone. Only minimal secretion of IL-4 (not shown) or IFN-γ (Fig. 4*D*) above background levels was detectable in these assays. When spleen cells from HP/PN mice were restimulated with PN Ag in vitro, the levels of PN-induced IL-5 and IL-10 detected in the culture supernatant were similar to those seen in mice fed PN alone. Interestingly, however, the amount of PN-specific IL-13 produced by spleen cells from helminth-infected PN-sensitized mice was significantly lower than that produced by cells from PN/CT mice (Fig. 4*A*, $p < 0.0001$). The absence of a PN-specific IL-13 response upon in vitro Ag restimulation correlated with the inability of the helminth-infected, PN-sensitized mice to mount a PN-specific IgE response (Fig. 2). In contrast to spleen cells, in vitro restimulation of MLN cells from PN/CT mice did not elicit an appreciable Th2 response (data not shown).

Helminth infection protects against food allergy

Our first set of experiments showed that mice fed repeatedly with PN Ag plus the Th2 inducing mucosal adjuvant CT developed a PN-specific IgE response and symptoms of systemic anaphylaxis. By contrast, feeding PN Ag to mice with an ongoing enteric helminth infection (which also induces a polarized Th2 response) did not elicit an Ag-specific IgE response or any symptoms of a systemic allergic response. In a second series of experiments (Fig. 1, protocol *B*), we examined the effect of helminth infection on the allergic response to PN plus CT. Fig. 5*A* shows that the marked rise in total IgE that follows *H. polygyrus* infection (HP/PBS) was unchanged by i.g. administration of PN plus CT (HP/PN/CT). A clear but variable Ab response to PN alone was noted, and administration of PN plus CT to noninfected mice elicited both a marked PN-specific IgE and IgG1 response as well as signs of systemic anaphylaxis, as in the previous experimental protocol. Fig. 5*B* shows that significantly lower levels of PN-specific IgE were detectable after oral sensitization of HP/PN/CT compared with PN/CT mice. HP/PN/CT mice showed a significant decrease in PN-specific IgE ($p < 0.01$ and 0.001 at 14 and 21 days after infection, respectively) compared with PN/CT mice.

The first series of experiments showed that, in this model, anaphylactic symptoms are dependent on the production of Ag-specific IgE. Interestingly, both plasma histamine levels (Fig. 6*A*) and systemic anaphylaxis scores (Fig. 6*B*) were also reduced in PN/CT helminth-infected mice. Although enteric helminth infection induced an exuberant polyclonal IgG1 and IgE response, the production of PN-specific IgE (and concomitant anaphylactic symptoms) was down-regulated by infection in the HP/PN/CT group.

Helminth-induced immunoregulation reduces PN-specific IL-13

To examine how the helminth-induced Th2 response might influence allergic sensitization to orally administered PN plus CT, spleen cells from both infected and noninfected mice sensitized with PN plus CT were harvested and stimulated with PN in vitro (Fig. 7). As expected, a marked PN-specific IL-13 (Fig. 7*A*), IL-5 (Fig. 7*B*), and IL-10 (Fig. 7*C*) response was induced by restimulation of spleen cells from PN/CT (PN allergic) mice in vitro. In HP/PN/CT mice, the PN-specific response for each of these cytokines was reduced to the levels seen in mice fed PN alone. This helminth-induced reduction of the

A

B

FIGURE 3. Plasma histamine levels correlate with the onset of anaphylactic symptoms. The difference in plasma histamine levels from samples taken before and 30 min after PN challenge is shown in *A*. Plasma histamine was determined using an enzyme immunoassay kit (see *Materials and Methods*). Clinical systemic anaphylaxis scores induced by PN Ag challenge are shown in *B*. Each symbol represents one mouse in both *A* and *B*. Elevated anaphylactic responses correlated with the plasma histamine response and were observed only in PN/CT mice.

FIGURE 4. T cells develop into PN-specific Th2 cells in the presence of PN and CT. Spleen cells from different groups were removed 35 days after the initial sensitization. Cells were restimulated in the presence or absence of PN (200 μg/ml) in vitro for 72 h. Culture supernatants were collected and the concentration of each cytokine expressed was assayed by ELISA, (*A*) IL-13, (*B*) IL-5, (*C*) IL-10, and (*D*) IFN-γ. Each value shows the average of five mice and is expressed as a geometric mean ± SEM. The results are representative of two independent experiments. Non-Ag-stimulated cultures showed very low cytokine production. The concentration of IL-13 produced by cells from PN/CT mice was significantly higher than that produced by cells from HP/PN mice (∗, $p < 0.0001$).
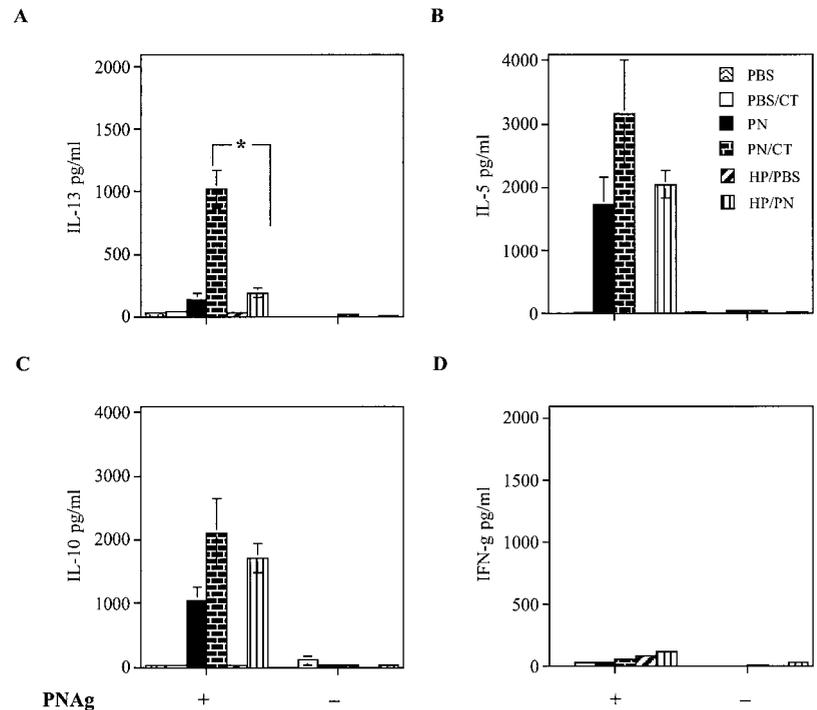
PN-specific cytokine response was most striking for IL-13 (Fig. 7*A*). Spleen cells from helminth infected PN-CT-sensitized mice produced 5-fold less IL-13 than that made by noninfected PN-CT fed mice (mean ± SEM, 2360 ± 145 compared with 650 ± 120 pg/ml, respectively; $p < 0.001$). This was associated with the production of background levels of PN-specific IgE (Fig. 5).

Treatment with neutralizing Ab to IL-10 abrogates the ability of helminth infection to protect against allergy

Although well-known for their induction of a prototypic Th2 type of response, chronic infection with helminthic parasites is also accompanied by the induction of potent immunoregulatory mediators, particularly IL-10 (5–7). Recent epidemiological studies have suggested that helminth-induced IL-10 may play a protective role against allergic disease. Accordingly, we treated PN-sensitized helminth-infected mice with neutralizing Abs to IL-10 according to the protocol shown in Fig. 1*C*. Anti-IL-10 treated helminth-infected mice made much higher levels of PN-specific IgE than nontreated mice (Fig. 8*B*, $p > 0.001$). Interestingly, anti-IL-10 treatment did not increase the PN-specific IgE response in noninfected mice sensitized with PN plus CT. Anti-IL-10 treatment also did not alter the total or PN-specific IgG1 response (Fig.
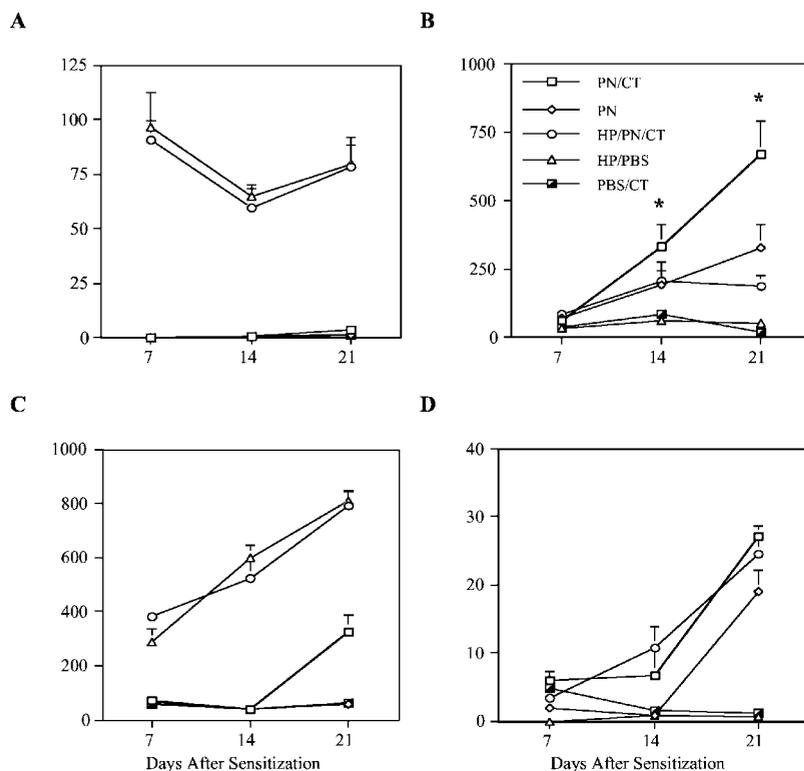
FIGURE 5. Altered PN-specific IgE and IgG1 responses to a Th2-polarizing *H. polygyrus* infection. One week after the initial oral sensitization or infection, animals were bled at 1-wk intervals. Total IgE (*A*), PN-specific IgE (*B*), total IgG1(*C*), and PN-specific IgG1 (*D*) expression in sera from *H. polygyrus*-infected or noninfected C3H/HeJ mice were measured on indicated days by ELISA. Individual serum samples (5–10 mice per group) were measured and are expressed as the geometric means of triplicates ± SEM. HP/PN/CT mice produced significantly less PN-specific IgE than PN/CT mice (∗, $p < 0.01$ and 0.001 at 14 and 21 days after infection, respectively). One representative experiment of two is shown. The data in Figs. 5–7 are from the same experiment and followed the sensitization protocol shown in Fig. 1*B*.

A

B
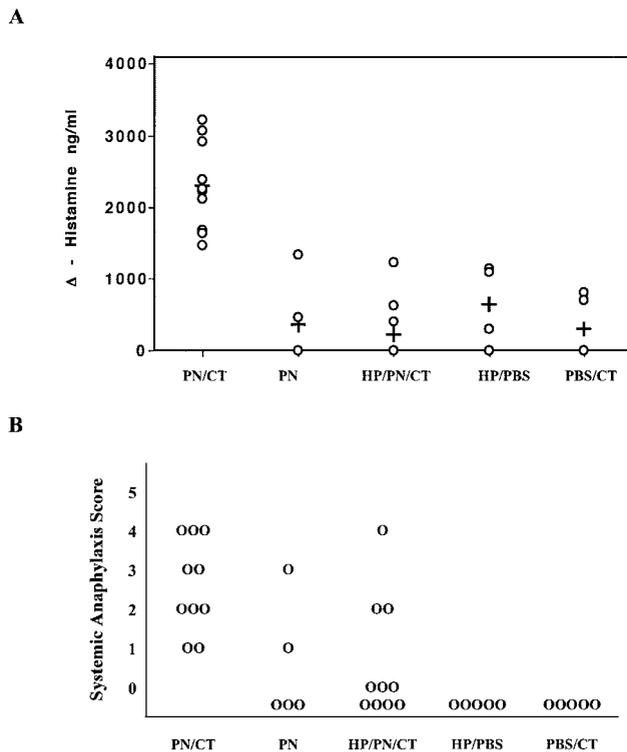
FIGURE 6. Helminth infection inhibits PN-induced systemic anaphylaxis. HP/PN/CT mice had lower plasma histamine levels than PN/CT mice. *A,* The difference in plasma histamine levels before, and 30 min after, PN i.g. challenge. Plasma histamine levels were determined using an enzyme immunoassay kit. Clinical systemic anaphylaxis scores induced by PN Ag challenge are shown in *B*. Each symbol represents one mouse.

8, *C* and *D*). In accordance with its ability to increase the levels of PN-specific IgE, anti-IL-10 strikingly abrogated the helminth-induced down-regulation of plasma histamine levels and systemic anaphylaxis scores in PN plus CT-sensitized mice (Fig. 9).

As in previous experiments, mice that were sensitized with PN plus CT produced high levels of PN-specific IL-13, IL-5, and IL-10 (Fig. 10, *A–C*) upon restimulation with PN in vitro. These cytokine responses were not influenced by in vivo treatment of PN/CT mice with neutralizing Ab to IL-10.
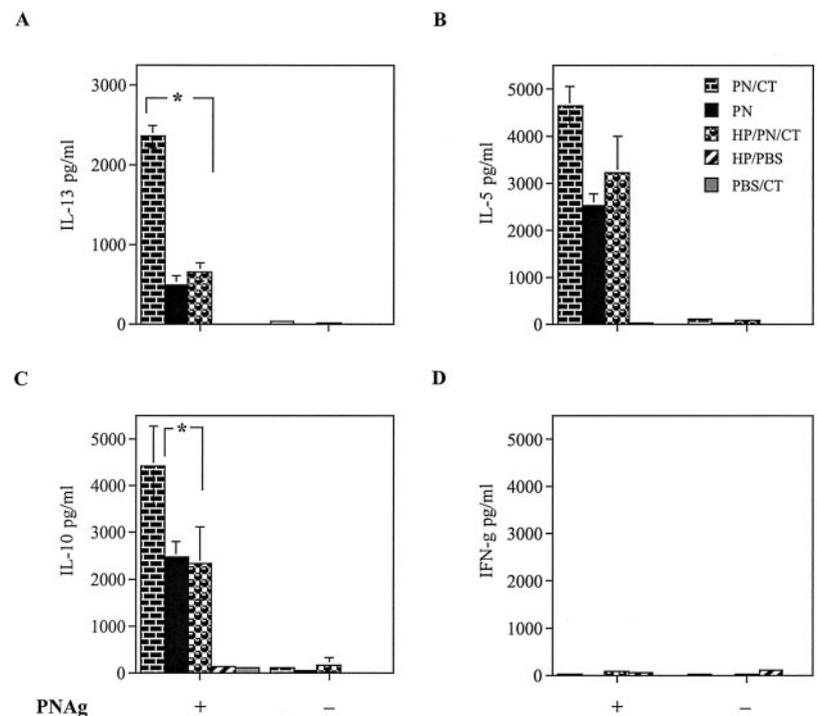
Although not as strikingly reduced as the levels shown in Fig. 7, HP/PN/CT mice produced lower levels of IL-13 and IL-10. Treatment with neutralizing Ab to IL-10 partially restored the PN-specific IL-13 response in these mice. Interestingly, PN-sensitized, helminth-infected mice treated with neutralizing Ab to IL-10 produced about 3-fold less IL-10 upon restimulation with PN Ag in vitro.
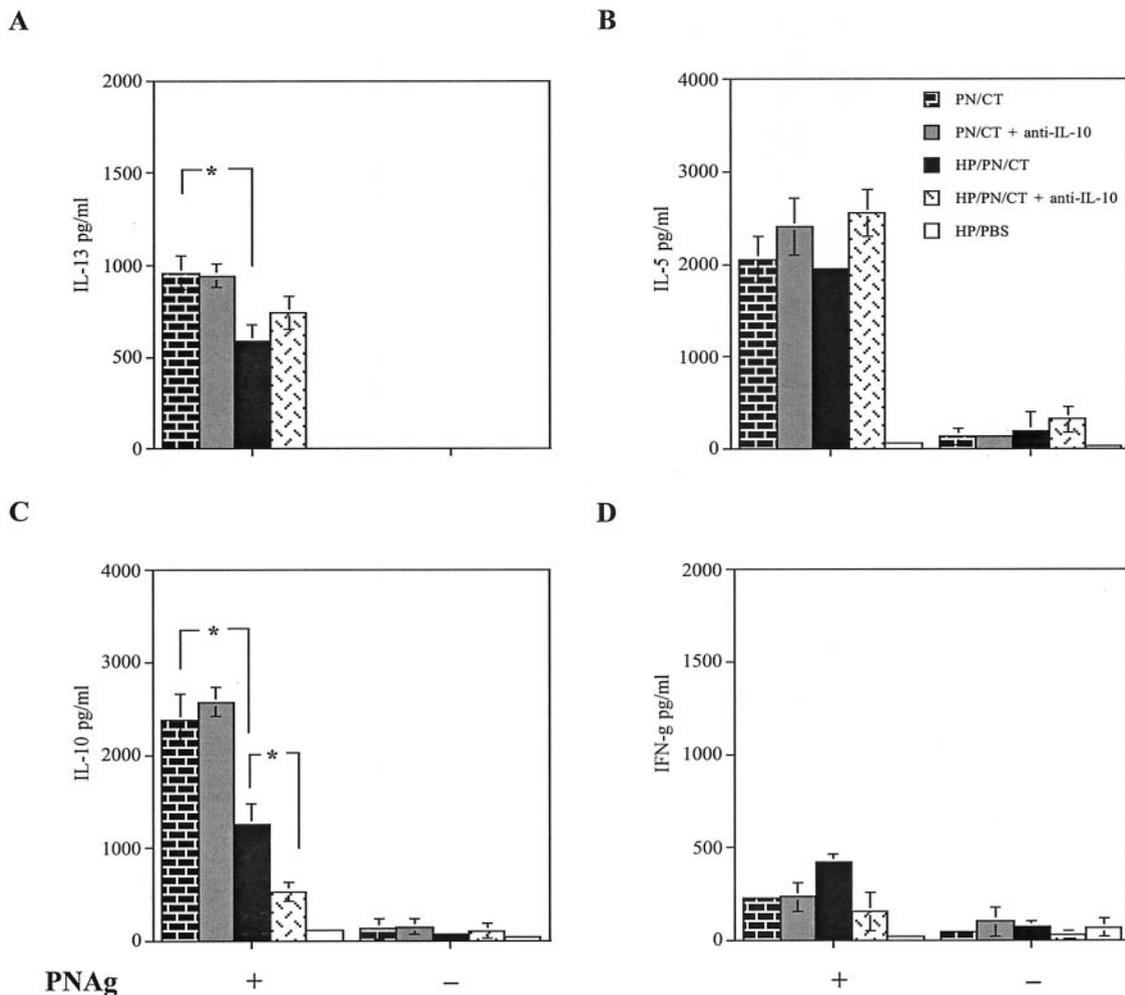
Discussion

Several novel, and somewhat unexpected, observations have emerged from this study. The first is that enteric helminth infection does not elicit an IgE response to an orally administered Ag. Both CT and helminth infection function as mucosal adjuvants to induce polarized Th2 responses to coadministered oral Ags (11, 17). Like CT, chronic infection with helminthic parasites can clearly bias the response to other Ags to a Th2 type of response (18–20). We show in this study that this Th2 bias does not extend to the production of Ag-specific IgE. Moreover, by using a model in which a systemic allergic response to a model food Ag is induced by repeated oral administration of Ag plus CT, we have demonstrated that prior helminth infection protects against the development of allergic symptoms and Ag-specific IgE.

An interesting characteristic of the parasite-induced IgE and IgG1 response is that it is mainly polyclonal, i.e., most of this Ab is not specific for nematode Ags (21). Previous studies in humans and mice have suggested that allergic responses are suppressed by helminth infection. For example, in a study that examined the effects of anti-helminthic treatment on the allergic reactivity of children in a tropical slum, Lynch et al. (22) noted an inverse correlation between the levels of total and Ag-specific IgE. They

FIGURE 7. Helminth-infected Th2 cells make less PN-specific IL-13 than noninfected cells. Compared with PN/CT animals, SPL from HP/PN/CT animals produced significantly lower IL-13 upon in vitro restimulation with PN (∗, *p* < 0.0001). In this set of experiments, spleen cells from different groups were removed 21 days after the initial sensitization. Cells from individual mice were restimulated in the presence or absence of PN (200 μg/ml) in vitro for 72 h. Culture supernatants were collected and the concentration of the cytokines (*A*) IL-13, (*B*) IL-5, (*C*) IL-10, and (*D*) IFN-γ were assayed by ELISA. Each bar graph represents the average cytokine secretion from 10 mice and is expressed as the geometric mean ± SEM. The results are representative of two independent experiments. Non-Ag-stimulated cultures showed very low cytokine production.

FIGURE 8. PN/CT-sensitized, helminth-infected mice treated with neutralizing Abs to IL-10 make high levels of PN-specific IgE. Mice were given three doses of neutralizing Ab to IL-10 according to the protocol depicted in Fig. 1C. Total IgE (*A*), PN-specific IgE (*B*), total IgG1 (*C*), and PN-specific IgG1 (*D*) levels in sera from *H. polygyrus*-infected or noninfected C3H/HeJ mice were measured on the indicated days by ELISA. Anti-IL-10-treated HP/PN/CT mice produced significantly more PN-specific IgE than nontreated mice ($p < 0.01$ and 0.001 at 14 and 21 days after sensitization, respectively). Individual serum samples (five mice per group) were measured and are expressed as the geometric means ± SEM.

suggested that the polyclonal parasite-induced IgE response suppressed the production of Ag-specific IgE and blocked anaphylactic symptoms by saturating high-affinity receptors for IgE (FcεR1) on mast cells and basophils. Parasite-induced suppression of allergic reactivity was reversed by anti-helminthic treatment. More recently, Wang et al. (23) showed that, in a murine model, helminth infection could result in an increased Ag-specific Th2 cytokine response in the bronchiolar lavage fluid accompanied by an apparent reduction in Ag-specific IgE. The authors speculated that high levels of total serum IgE suppressed the synthesis of Ag-specific IgE in the lungs. This would presumably lead to a reduction in anaphylactic symptoms, although it was not measured in this report.

Our studies demonstrate a clear correlation between the induction of Ag-specific IgE and the development of systemic anaphylaxis. By examining the cytokine response to Ag restimulation in vitro we have shown that, in our model, the inability to induce an allergic response to PN Ag in helminth-infected mice is linked to their inability to make PN-specific IL-13. IL-4 and IL-13, the prototypic mediators of the Th2 cytokine response, are produced in abundance both in response to infection with helminthic parasites and during the course of an allergic reaction (5, 24). Both are also central to the regulation of class switching to IgE (25). In keeping with our previous experience with in vitro restimulation assays, the production of Ag-specific IL-4 was difficult to detect in our cultures (10). Therefore, we cannot make any conclusions regarding its role in our model. IL-4 and IL-13 share some biological activities as well as a common receptor subunit, the IL-4Rα chain (25). IL-13 also specifically binds two other cell surface proteins, IL-13Rα1 and IL-13Rα2. Although IL-4 and IL-13 often work synergistically, a role for IL-13 in the induction of an IL-4-independent IgE response has been demonstrated by the generation of IL-13 transgenic mice (26). IL-13 transgenic mice make higher levels of IgE than their wild-type littermates and continue to produce IgE when crossed to IL-4-deficient mice (26). Conversely, reduced levels of IgE have been reported in IL-13-deficient mice, suggesting that IgE levels are responsive to both an increase and a decrease in the expression of IL-13 (27). Moreover, the role of

IL-13 in the development of anaphylactic symptoms is well-documented (28, 29). Sensitization of mice in which IL-13 is overexpressed in lymphoid cells results in the enhanced production of

FIGURE 9. Treatment with neutralizing Ab to IL-10 abrogates the ability of helminth infection to protect against allergy. As in Figs. 3 and 6, *A* shows the difference in plasma histamine levels from samples taken before and 30 min after PN challenge. Clinical systemic anaphylaxis scores are given in *B*. Each symbol represents one mouse.

FIGURE 10. Anti-IL-10 treatment alters cytokine responses in helminth-infected, but not PN/CT, mice. Spleen cells were harvested 28 days after the initial sensitization (35 days postinfection). Cells from individual mice were restimulated in the presence or absence of PN (200 μg/ml) in vitro for 72 h. Culture supernatants were collected and the concentration of the cytokines IL-13 (*A*), IL-5 (*B*), IL-10 (*C*), and IFN-γ (*D*) was assayed by ELISA. Each bar graph represents the average cytokine secretion from 10 mice and is expressed as the geometric mean $\pm$ SEM.

Ag-specific IgE and the rapid induction of fatal anaphylaxis (28). In asthma models, IL-13 has also been shown to be particularly important for the induction of the bronchial constriction and the smooth muscle cell contraction that characterizes the airway symptoms of an allergic response (30).

Recent epidemiological studies have suggested that the ability of helminth infection to protect against allergic disease is linked to its induction of immunoregulatory cytokines (5–7). In one study, various immunological parameters were measured to explain the reduced skin test reactivity to a ubiquitous allergen (house dust mites) observed in helminth-infected children in Gabon (31). Only high-level production of parasite-induced IL-10 significantly correlated with reduced skin test reactivity, even in the face of an equivalent allergen-specific IgE response. Other studies have also suggested that enhanced production of IL-10 by PBL is a characteristic feature of chronic helminth infection (32, 33). In our model, treatment of helminth-infected mice with neutralizing Abs to IL-10 dramatically abrogated the infection's protective effect against allergic sensitization to PN plus CT. Both the anaphylactic symptoms and the PN-specific IgE response in the anti-IL-10-treated mice were similar to or greater than those seen in mice that received PN and CT alone. Therefore, our data provide compelling evidence for the ability of helminth-induced immunoregulatory cytokines to alter an allergic response.

The precise mechanism by which helminth-induced IL-10 protects against allergic symptoms is not yet clear. Although we have noted a strong correlation between the induction of a PN-specific IgE response and the detection of PN-specific IL-13 in vitro, some PN-specific IL-13 is detectable in cultures prepared from mice where both the IgE response and anaphylactic symptoms are clearly reduced. This suggests that there are other cytokines involved in the regulation of the IgE response in this model. Some evidence suggests that IL-10 can down-regulate allergic hyperreactivity via direct effects on both DC (34, 35) and mast cells (36). However, no study has yet demonstrated a mechanism by which helminth-induced immunoregulation directly affects the induction of allergic disease. Recent hypotheses have focused on a role for anti-inflammatory cytokines in down-regulating the effector phase of the allergic response by blocking, for example, mast cell degranulation (6). We show, in this report, that chronic helminth infection can block the induction of allergen-specific IgE by influencing the behavior of the Ag-specific Th cells that are required for this response. This reduction in allergen-specific IgE correlated with the abrogation of anaphylactic symptoms. We speculate that, in our model, the effects of parasite-induced immunoregulation (mediated, at least in part, via IL-10) occur at the level of allergen presentation by APCs, presumably DC. Interestingly, a prominent role for regulatory cells secreting IL-10 has also been attributed to

the gut-associated lymphoid tissue which is exposed to chronic stimulation via both the commensal bacterial flora and food Ags (9). Taken together, our data are consistent with a model in which parasite-induced immunoregulation abrogates the production of allergen-specific IgE. We have shown that helminth infection primes for a Th2 response to an orally administered Ag but fails to induce atopy. Our results demonstrate that, in a murine model, helminth infection protects against the development of allergy.

Acknowledgments

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