Helminth Infection Protects Mice from Anaphylaxis via IL-10-Producing B Cells[1]

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Modulation of the immune system by infection with helminth parasites, including schistosomes, is proposed to reduce the levels of allergic responses in infected individuals. In this study we investigated whether experimental infection with *Schistosoma mansoni* could alter the susceptibility of mice to an extreme allergic response, anaphylaxis. We formally demonstrate that *S. mansoni* infection protects mice from an experimental model of systemic fatal anaphylaxis. The worm stage of infection is shown to mediate this protective effect. In vivo depletion studies demonstrated an imperative role for B cells and IL-10 in worm-mediated protection. Furthermore, worm infection of mice increases the frequency of IL-10-producing B cells compared with that in uninfected mice. However, transfer of B cells from worm-infected mice or in vitro worm-modulated B cells to sensitized recipients exacerbated anaphylaxis, which was attributed to the presence of elevated levels of IL-4-producing B cells. Worm-modulated, IL-10-producing B cells from IL-4-deficient, but not IL-5-, IL-9- or IL-13-deficient, mice conferred complete resistance to anaphylaxis when transferred to naive mice. Therefore, we have dissected a novel immunomodulatory mechanism induced by *S. mansoni* worms that is dependent on an IL-10-producing B cell population that can protect against allergic hypersensitivity. These data support a role for helminth immune modulation in the hygiene hypothesis and further illustrate the delicate balance between parasite induction of protective regulatory (IL-10) responses and detrimental (IL-4) allergic responses. *The Journal of Immunology,* 2004, 173: 6346–6356.

The frequency and severity of allergic disorders are steadily increasing, particularly in developing countries where ~20–30% of the population are atopic (1). Atopic individuals may develop a spectrum of allergic diseases, including asthma, allergic rhinoconjunctivitis, atopic dermatitis, food allergies, and anaphylaxis. Mechanistically, atopic conditions induce a type 2 cytokine-polarizing response that is characterized by increased Th2 cell development and production of IL-4, IL-5, IL-9, and IL-13, resulting in IgE production and eosinophilia (2).

The reasons for increases in allergic diseases are multifactorial, but concurrent improvement in sanitation and reduction in childhood infections in developed countries have led to the speculation that early infections may reduce the risk of developing allergy (3, 4). Paradoxically, in the context of this model, the so-called hygiene hypothesis, human populations with high rates of parasitic helminth infections, which induce an immunological shift toward Th2 responses, have a lower prevalence of allergic disorders (5). *Schistosoma* spp. are tropical helminth parasites that have been proposed to ameliorate atopic disorders in humans (5). Schistosomes are characteristically associated with being potent inducers of Th2 cytokine responses, including eosinophilia and IgE responses. Despite type 2 responses being considered necessary for the development of allergies, the presence of schistosome infections in humans may reduce allergic responses in infected populations (6). Thus, *Schistosoma hematobium*-infected school children in Gabon had a lower prevalence of skin reactivity to house dust mites than those free of this infection (7). Therefore, the chronic down-regulation of the immune system during helminth infection evokes a regulatory environment that may impart protection from allergies (8).

It is well characterized that the propensity of schistosomes to induce Th2 cytokines is largely egg-mediated (9). We have previously shown that mice that overexpress IL-13 develop anaphylaxis after sensitization and re-exposure to *Schistosoma mansoni* eggs (10). Anaphylaxis is an acute, life-threatening, allergic reaction that can cause fatalities after exposure to allergenic Ags in sensitized atopic individuals. Being an immediate-type hypersensitivity reaction, anaphylaxis is characterized by an overproduction of Th2 cytokines contributing to elevated levels of IgE and eosinophilia. Classically, anaphylaxis was considered to involve allergen cross-linking of IgE bound to its high affinity receptor, FcϵR1, on mast cells, initiating an intracellular signal transduction cascade that culminates in cell degranulation and the release of potent systemic mediators, including histamine and platelet-activating factor (PAF),[3] which can induce acute bronchoconstriction, tachycardia, hypotension, vascular leakage, and ultimately death (11). In earlier studies, schistosome egg sensitization induced all of these characteristic features in IL-13 transgenic mice, with injection of *S. mansoni* eggs inducing a rapid and highly elevated production of Th2 cytokines and Ag-specific IgE, with subsequent exposure to eggs inducing fatal anaphylaxis with attendant mast cell degranulation and histamine release (10). However, IgE does not account for all anaphylactic pathways because mice deficient in IgE can develop systemic anaphylaxis (12). Indeed, a recent study has identified a

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[3] Abbreviations used in this paper: PAF, platelet-activating factor; Be2, B effector 2; mKO, multiple knockout; mMCP-1, mouse mast cell protease-1; Pen V, penicillin V; TNP, trinitrophenyl.

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distinct role in mice for alternative mechanisms of anaphylaxis involving IgG, FcγRIII, and macrophages (11).

Unexpectedly, despite IL-13 transgenic mice being predisposed to anaphylaxis caused by schistosome eggs (10), these mice had no anaphylactic symptoms during acute or chronic *S. mansoni* infection. Therefore, in this study we evaluated whether schistosome infection renders mice less susceptible to anaphylaxis using an experimental murine model of systemic anaphylaxis. We have identified that the worm stage of *S. mansoni* infection modulates mice so they are completely refractory to active or passive systemic anaphylaxis. We now demonstrate that *S. mansoni* worms up-regulate IL-10-producing B cells that can protect against anaphylaxis.

Materials and Methods

Mice

Female BALB/c or C57BL/6 mice, 6–8 wk of age, were purchased from Harlan (Bicester, U.K.). Outbred CD-1 strain mice were used for egg and worm production. IL-10-deficient mice, on a C57BL/6 background, were obtained from The Jackson Laboratory (Bar Harbor, ME). CBA/N (*xid*) and wild-type (CBA/Ca) mice were obtained from Harlan (Horst, The Netherlands), and age-matched male mice were used in experiments. IL-13 transgenic (13) and IL-4- (14), IL-5- (15), IL-9- (16), and IL-13- (17) deficient mice and combined IL-4-, IL-5-, IL-9-, and IL-13-deficient mice (15) were bred in-house. Mice were housed in a specific pathogen-free facility in individually ventilated and filtered cages under positive pressure (Techniplast, Northants, U.K.).

Parasitology

A Puerto Rican strain of *S. mansoni* was maintained by passage in Tyler's original strain mice and albino *Biomphalaria glabrata* snails. Mice were infected with 350 cercariae percutaneously to establish worm and egg infections for Ag production. Six- to 8-wk-old female BALB/c mice were infected percutaneously with 40 male cercariae for experimental unisexual (worm-only) infections and with 40 male and female cercariae for bisexual (worm+egg) infections. Unisexual male-only snail infections were prepared as previously described (18).

Monoclonal Abs and reagents

The following Abs were used for flow cytometry: tri-conjugated anti-CD4 (clone CT-CD4), tri-conjugated anti-CD19 (clone 6D5), tri-conjugated anti-CD11b (Mac-1; clone M1/70), tri-conjugated anti F4/80 (clone CI: A3-1), PE-conjugated anti-CD25 (clone PC61 5.3), PE-conjugated anti-IgM (μ-chain specific), FITC-conjugated anti-IL-10 (clone JES5-2A5), and PE-conjugated anti-IL-4 (clone BVD6-24G2) (all obtained from Caltag Medisystems, Burlingame, CA).

Depleting mAbs against the IL-10R (clone 1B1.3a), CD25 (clone PC61 5.3), and TGF-β (clone 1D11.16.8) were purchased from American Type Culture Collection (Manassas, VA). The anti-CD4 (clone YTS 191) hybridoma, originally obtained from Dr. H. Waldmann (University of Oxford, Oxford, U.K.), was provided by Dr. A. Cooke (University of Cambridge, Cambridge, U.K.). Murine IgE (clone IGELa2) and murine IgG1 (clone B7.11), both anti-2,4,6-trinitrophenyl (anti-TNP), were also obtained from American Type Culture Collection. The above hybridoma cell lines were cultured under standard conditions, and supernatants were precipitated in 50% ammonium sulfate, followed by extensive dialysis against endotoxin-free Dulbecco's PBS (pH 7.2; Sigma-Aldrich, St. Louis, MO). Ab was purified on protein G separation columns (Sigma-Aldrich), and protein was quantified before use. All Abs were tested for endotoxin contamination and confirmed to have <0.5 endotoxin U/mg (Chromogenic LAL; BioWhittaker, Walkersville, MD). Anti-mouse IgM was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). Control rat IgG was purchased from Sigma-Aldrich. In all in vivo depletion experiments the efficacy of treatment was initially confirmed, with doses of 0.1–0.5 mg of mAb administered per mouse.

Macrophages were depleted by the treatment of mice with liposomes containing dichloromethylene bisphosphonate (clodronate-liposomes), prepared as previously described (19). Clodronate was a gift from Roche (Mannheim, Germany). Macrophages were depleted in Pen V-sensitized mice by two injections of clodronate- or control PBS-liposomes; 24 h before challenge, each mouse received 0.2 ml i.p., and an additional 0.1 ml was injected i.v. 4–6 h before elicitation of anaphylaxis. The efficacy of macrophage depletion was confirmed by flow cytometric analysis of F4/80$^+$Mac-1$^+$ spleen populations.

Induction of active systemic anaphylaxis

For the induction of Pen V-induced active systemic anaphylaxis, Pen V-OVA and Pen V-BSA conjugates were prepared as previously described (20). Mice were first primed by i.p. injection of 500 μg of Pen V-OVA plus 2×10^9 *Bordetella pertussis* (Evans, Liverpool, U.K.; Wako Pure Chemical, Osaka, Japan) and 1.0 mg of Imject alum (Pierce, Rockford, IL). Anaphylaxis was elicited 14 days later by i.v. challenge with 100 μg of Pen V-BSA (21). Rectal temperatures were measured with a digital Thermocouple thermometer linked to a murine rectal probe (Physitemp Instruments, Clifton, NJ) immediately before challenge, then every 10 min for the next 60 min. Activity levels were assessed at the same time the rectal temperatures were obtained. Clinical signs of anaphylaxis were assessed with a 0–4 scoring system (0, normal activity; 1, mild shock including slow movement, dyspnea, ruffling of fur; 2, moderate shock including prostration sluggish gait and slight activity after prodding; 3, severe shock including complete paresis, no activity after prodding; 4, death). Commercial PAF (Calbiochem, La Jolla, CA) was used to induce systemic fatal anaphylaxis (22).

Induction of passive systemic anaphylaxis

Passive systemic anaphylaxis with immune mouse sera was induced as described previously (20). Uninfected or worm-infected BALB/c mice were immunized with 500 μg of Pen V-OVA plus 2×10^9 *B. pertussis* and 1.0 mg of alum. Fourteen days later, blood was recovered, and serum was extracted. To induce passive systemic anaphylaxis, naive mice were injected i.p. with 0.4 ml of anti-Pen V serum from uninfected or worm-infected mice, three times at 10-min intervals. After 24 h, anaphylaxis was induced by i.v. challenge with 100 μg of Pen V-BSA.

Passive IgE or IgG1 mAb-mediated systemic anaphylaxis was induced as described previously (23). Mouse anti-TNP IgG1 (200 μg) or anti-TNP IgE (20 μg) mAbs was administered i.v. in volumes of 200 μl/mouse. Thirty minutes after injection of anti-TNP IgG1 or 24 h after injection of anti-TNP-IgE, mice were i.v. challenged with 1.0 mg of TNP_4-OVA (Biosearch Technologies, Novato, CA) in Dulbecco's PBS. Control mice received OVA in PBS. Rectal temperatures were recorded after challenge, as described above.

In vitro worm culture

Adult worms were cultured as previously described (18). In brief, adult schistosome worms were obtained 8 wk postinfection by portal perfusion as previously described (24). The male and female worms were separated under a microscope, and male worms were washed in fresh medium. For Transwell experiments, spleen cells were plated at a density of 1×10^7/well in a 12-well plate (Greiner Bioscience, Frickenhausen, Germany) in 2 ml of RPMI 1640 with HEPES (25 mM), with 15 male worms placed in each Transwell. Plates were incubated for 48 h at 37°C, after which time the worms were removed, and cells were washed several times. The viability of cells was checked. Worm-modulated spleen cells were used for isolation of B cells or immunofluorescence staining.

Magnetic sorting of cells

B cells were purified by negative depletion using CD43$^+$ magnetic microbeads beads (Miltenyi Biotec, Auburn, CA). Splenic populations were incubated with anti-CD43$^+$ magnetic microbeads, followed by capture with autoMACS separation columns. The resulting B cell populations were routinely >95% pure, as assessed by FACS for CD19 expression. For transfer of purified cells, $0.5–1.0 \times 10^7$ purified B cells were injected i.p. into individual mice 24 h before elicitation of anaphylaxis.

Cell culture and immunological analysis

Single cell suspensions were prepared from splenocytes depleted of erythrocytes by lysis with ammonium chloride solution. Cells were cultured in RPMI 1640 (Invitrogen Life Technologies, Gaithersburg, MD) supplemented with 10% (v/v) heat-inactivated FCS (Labtech, East Sussex, U.K.), 100 mM L-glutamine (Invitrogen Life Technologies), 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma-Aldrich). Cells were unstimulated (medium) or were stimulated with plate-bound anti-CD3 (clone 145-2C11) or LPS (1 μg/ml) in a 24-well plate (Greiner Bioscience) at 37°C for 72 h. Plates were precoated with anti-CD3 mAb at 10 μg/ml for 2 h at 37°C and then washed in sterile Dulbecco's PBS before addition of cells. Supernatants were harvested after 72 h, and cytokine levels (IL-4, IFN-γ, IL-10, and TGF-β) were measured by ELISA.

Sandwich ELISAs were performed to quantify levels of specific cytokines in the supernatants from cell cultures. A commercial kit (Promega, Southampton, U.K.) was used to measure total TGF-β (acidified samples) according to the manufacturer's instructions. Reagents for detection of IL-4 and IFN-γ were obtained from BD Pharmingen (San Diego, CA), and IL-10 reagents were purchased as a DuoSet ELISA development system from R&D Systems (Abingdon, U.K.). Mouse mast cell protease 1 (mMCP-1) was measured in sera using a commercial kit according to the manufacturer's instructions (Moredun Scientific, Midlothian, Scotland). Histamine was measured in plasma samples using a commercial kit (IBL, Hamburg, Germany). Pen V-specific serum IgG1 and IgE levels were detected by direct ELISA (20). In brief, 96-well microtiter plates were coated with 100 μl of Pen V-BSA (10 μg/ml in carbonate buffer, pH 9.6) overnight at 4°C. A serial dilution of serum was added to plates that were then incubated at 4°C overnight. Detection was performed with peroxidase-conjugated IgG1 (1/1000, 2 h; Zymed Laboratories, San Francisco, CA) or biotin-labeled anti-IgE (2.5 μg/ml, 2 h; BD Pharmingen), followed by peroxidase-conjugated streptavidin (1/1000, 30 min; BD Pharmingen) before substrate development using *O*-phenylenediamine.

RT-PCR

Total RNA was isolated from 5×10^6 purified B cells using Tri-reagent (Sigma-Aldrich). cDNA was reverse transcribed, and PCR was performed on samples that were equalized using RT-PCR for β-actin. Primer sequences were: β-actin forward, 5′-GGACTCCTATGTGGGTGACGAGG; β-actin reverse, 5′-TCTTTGCCAATAGTGATGACTTGGC; IL-4 forward, 5′-GAACGAGGTCACAGGAGAAGG; IL-4 reverse, 5′-GGACT CATTCATGGTGCAGCT; IL-10 forward, 5′-CTGGACAACATACT GCTAACCGAC; and IL-10 reverse, 5′-TTCATTCATGGCCTTGTAG ACACC.

Flow cytometry

Surface marker expression and intracellular cytokine phenotyping were assessed by immunofluorescent staining of B cell populations as previously described (25, 26). Data were collected on a FACScan flow cytometer (BD Biosciences, San Jose, CA) and were analyzed using CellQuest software (BD Biosciences). Quadrants were drawn based on isotype-control Ig staining and were plotted on logarithmic scales.

Results

S. mansoni *infection prevents anaphylaxis*

To dissect the underlying mechanism of antiallergic protective responses induced by schistosome infection, an anaphylaxis model using Pen V as the allergen was used. To investigate the relative roles of schistosome worms vs eggs in anaphylaxis, mice were infected with an *S. mansoni* conventional mixed male and female infection where eggs are laid (worm+egg infection) or with a male-only infection where there are no eggs (worm-only infection). After Pen V challenge of presensitized uninfected BALB/c mice, ~10–20% of animals die, with all mice having a marked drop in core body temperature within 10 min of challenge, with associated clinical signs of anaphylactic shock (Fig. 1*A*). Uninfected mice undergoing Pen V-induced anaphylaxis have an attendant mast cell degranulation, as determined by detection of substantial elevations in circulating levels of mMCP-1 and histamine (Fig. 1*B*). In contrast, BALB/c mice that had been infected with *S. mansoni* worm+egg or worm-only infections were both protected from anaphylaxis, with no mortalities, reduced severity of symptoms, and negligible release of mast cell by-products (Fig. 1). Significantly, mice with worm-only infections were completely resistant to anaphylaxis, whereas worm+egg-infected mice showed mild symptoms of anaphylaxis and had detectable, but limited (45 ± 20 pg/ml), release of histamine. These results indicate that the worm stage of *S. mansoni* infection may elicit protection from Pen V-induced anaphylaxis.

Schistosome worm-infected mice are resistant to passive Ab-mediated anaphylaxis, but are susceptible to PAF

The reduced susceptibility of worm-infected mice to Pen V-induced anaphylaxis could be due to the infection modifying the production of anti-Pen V Ab or to the known role for helminth-induced, nonspecific, polyclonal IgE-saturating mast cells (27). However, ELISA for Pen V-specific Ab responses of sera from Pen V-OVA-immunized uninfected and worm-infected mice demonstrated that both groups had comparable titers of allergen-specific IgE and IgG1 (data not shown). Similarly, when sera from Pen V-OVA-immunized uninfected and worm-infected mice were transferred to naive animals, there were comparable levels of anaphylaxis in both groups after subsequent Pen V-BSA challenge (Fig. 2*A*).

To further confirm that resistance of worm-infected mice to anaphylaxis was not due to alterations in the functionality of Ab, a

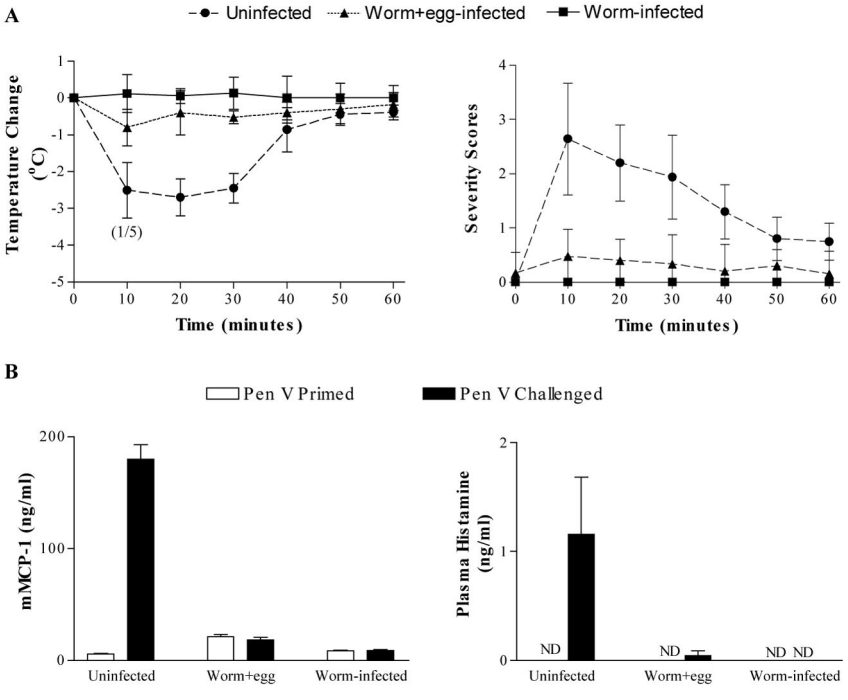

FIGURE 1. *S. mansoni* infection protects mice from systemic anaphylaxis. Uninfected and schistosome worm- or worm+egg-infected BALB/c mice were sensitized with Pen V-OVA on day 1. Infected mice were sensitized 7 wk after infection. Pen V-BSA was injected i.v. 14 days later to elicit anaphylaxis. Rectal body temperature and severity scores (*A*) were recorded before Pen V-BSA challenge (time zero) and every 10 min for 1 h. The deaths of mice are represented in brackets. Serum mMCP-1 and plasma histamine (*B*) levels in uninfected, worm+egg-infected, and worm-infected (□) or Pen V-challenged (■) mice were measured by ELISA (ND, not detected). Data are presented as the group mean ± SEM (n = 5–7) temperature change or severity score and are representative of three separate experiments.

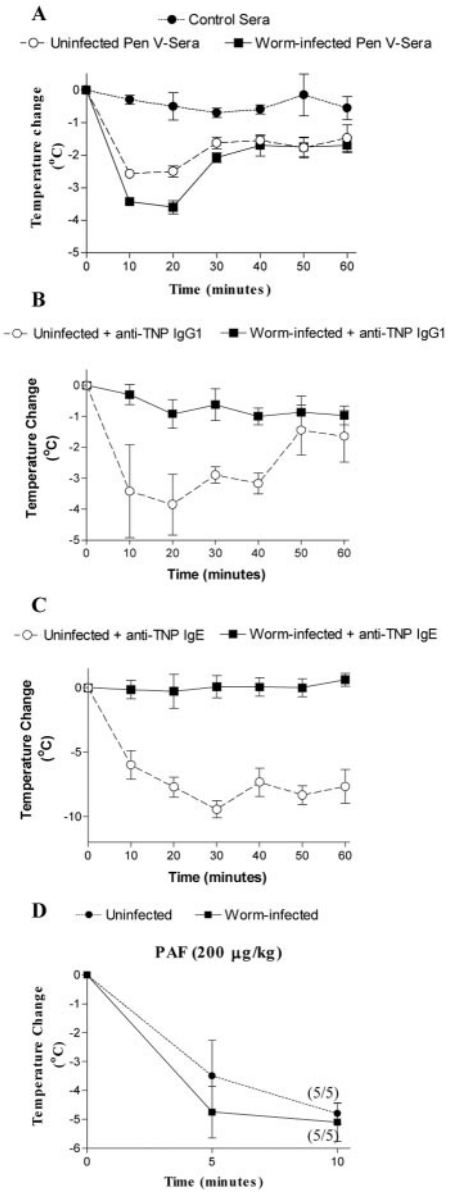

FIGURE 2. *S. mansoni* worm-infected mice produce Pen V-specific Abs capable of inducing passive anaphylaxis, but are themselves protected from direct IgE- and IgG1-passive systemic anaphylaxis and are susceptible to PAF-mediated fatal anaphylaxis. *A*, Passive transfer of systemic anaphylaxis was induced by transfer of sera from Pen V-sensitized uninfected or worm-infected mice to naive recipient mice. Control mice received normal mouse serum. Direct IgE- or IgG1-passive systemic anaphylaxis was induced by IgG1 (*B*) or IgE (*C*) treatment of mice. *D*, Uninfected or worm-infected BALB/c mice were injected with 200 μg of PAF/kg of body weight i.p. Rectal temperature was recorded at 0, 5, and 10 min after PAF administration. There was 100% mortality of all animals 10 min after treatment. Results represent the group mean ± SEM (n = 4–5) change in temperature and are representative of two separate experiments.

model of passive systemic anaphylaxis was used, in which uninfected and worm-infected mice were passively transferred IgG1 or IgE, specific for TNP, before eliciting anaphylaxis by challenge with TNP-OVA. Uninfected mice treated with TNP-IgG1 were susceptible to TNP-induced anaphylaxis, with a drop in body temperature after challenge (Fig. 2*B*). In contrast, the temperature of worm-infected mice was not altered by TNP-IgG1 sensitization. Similarly, worm-infected mice were not affected by anaphylaxis induced by TNP-IgE Ab treatment, which evoked a rapid and severe drop in temperature in uninfected mice within 5 min of challenge (Fig. 2*C*). Therefore, a schistosome worm infection completely protects mice from both IgG1- and IgE-mediated passive systemic anaphylaxis.

Injection of PAF induces rapid fatal anaphylaxis in mice without the need for mast cell degranulation (28). Uninfected and worm-infected mice were treated with PAF to determine whether schistosome worm-infected mice were susceptible to direct PAF-induced anaphylaxis. Both uninfected and worm-infected BALB/c mice were fully susceptible to PAF, with all animals dead within 10 min of treatment (Fig. 2*D*). Worm-infected mice are therefore fully susceptible to direct anaphylaxis induced by PAF.

Protection from anaphylaxis is independent of CD25$^+$ T cells or F4/80$^+$Mac-1$^+$ macrophages

Previously it has been shown that regulatory cells including CD4$^+$CD25$^+$ T cells have a suppressive role in diseases (29, 30), including allergic diseases such as asthma (31). Because the levels of CD4$^+$CD25$^+$ T cells in worm-infected mice (14–16%) are elevated compared with those in naive uninfected (8–11%; Fig. 3*A*),

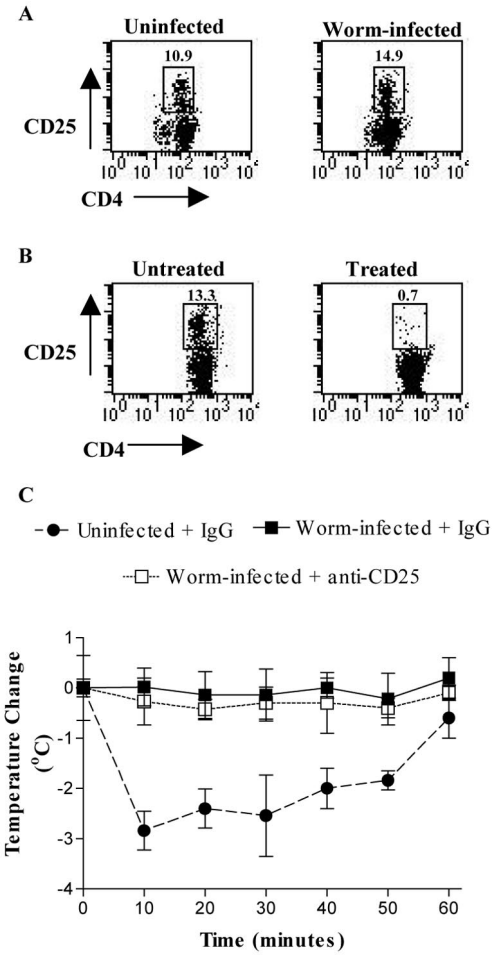

FIGURE 3. CD4$^+$CD25$^+$ regulatory T cell populations are not involved in worm-mediated protection against anaphylaxis. *A*, Levels of regulatory CD4$^+$CD25$^+$ T cells in spleens of uninfected and worm-infected mice were analyzed by flow cytometry. *B*, CD25$^+$ cells were depleted in vivo with anti-CD25 mAb (PC61; 0.5 mg/mouse i.p.) by injection 2 days and 1 day before eliciting anaphylaxis in Pen V-sensitized worm-infected mice. For the control, mice received IgG1. *C*, Temperature changes in Pen V-challenged uninfected and worm-infected mice treated with IgG or anti-CD25 mAb. Results are the group mean ± SEM (n = 7–8) change in body temperature. The data presented are representative of two separate experiments.

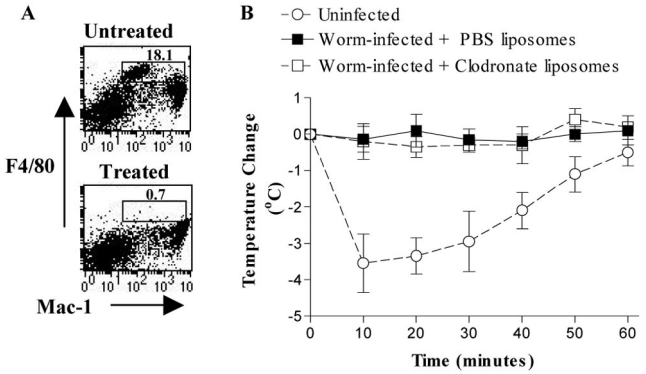

FIGURE 4. Macrophages are not involved in worm-mediated protection against anaphylaxis. *A*, In vivo depletion of macrophages. Percentages of relevant cell type in spleens of untreated or treated worm-infected mice are shown. *B*, Depletion of F4/80$^+$Mac-1$^+$ macrophage populations does not alter the resistance to anaphylaxis of worm-infected mice. Results are the group mean ± SEM (n = 5–7) change in body temperature. The data presented are representative of two separate experiments.

we depleted CD25$^+$ cells to address the role of these regulatory T cells in worm infection and anaphylaxis (Fig. 3*B*). Depletion of CD25$^+$ cells had no effect on the protection from anaphylaxis afforded by worm infection (Fig. 3*C*). This result indicates that CD25$^+$ cells do not mediate the protection against anaphylaxis that is observed in worm-infected mice.

Because macrophages have been implicated in anaphylaxis (11, 32, 33) and are modulated by schistosome worm infection (18), we depleted macrophages in vivo by administration of clodronate liposomes (Fig. 4*A*). The depletion of macrophages had no effect on worm-mediated protection against anaphylaxis, indicating that macrophages are not an effector cell in protection from Pen V-induced anaphylaxis (Fig. 4*B*).

Role for IL-10 in schistosome protection from anaphylaxis

In addition to the known increase in IL-4 production by cells from worm-infected mice (34), the levels of the regulatory cytokines IL-10 and TGF-β were also both elevated in worm-infected mice (Table I). Because both cytokines are implicated in modulation of the immune system by helminth parasites (8), we investigated the roles of these cytokines in the resistance of worm-infected mice to anaphylaxis. Mice received an i.p. injection of 0.5 μg/mouse of anti-IL-10R or anti-TGF-β mAb on 2 consecutive days before anaphylaxis challenge. Sensitized uninfected BALB/c mice treated with a control mAb developed anaphylaxis, with a drop in core body temperature and mortalities (two of seven mice died) after 10 min (Fig. 5*A*). As shown above, worm-infected mice were protected from anaphylaxis, with no drop in core body temperature over time and no mortalities (Fig. 5*A*). Worm-infected mice that had been treated with a depleting TGF-β mAb were also completely protected from anaphylaxis (Fig. 5*A*), indicating that this cytokine had no role in mediating protection. In contrast, worm-infected mice treated with anti-IL-10R mAb were highly susceptible to anaphylaxis, with a significant drop in core body temperature (Fig. 5*A*) and greater mortalities of mice (four of eight mice died) than in uninfected animals (two of seven mice died). These mAb neutralization studies suggested that IL-10, but not TGF-β, has a role in mediating the resistance of *S. mansoni* worm-infected mice to anaphylaxis.

To formally validate that worm resistance to anaphylaxis was mediated by IL-10, we infected IL-10-deficient mice and their C57BL/6 wild-type controls with schistosome worms and tested their susceptibility to Pen V-induced anaphylaxis. Uninfected C57BL/6 were susceptible to anaphylaxis as determined by a drop of 3°C in body temperature (Fig. 5*B*). Worm-infected C57BL/6 mice were resistant to anaphylaxis, but did have a drop in temperature (Fig. 5*B*). As all data shown previously (Figs. 1–4) were for BALB/c strain mice, it is significant that worm infection of both BALB/c (considered Th2-biased) and C57BL/6 (considered Th1-biased) strains renders mice less susceptible to anaphylaxis. Worm-infected IL-10-deficient mice developed anaphylaxis, with a marked drop in core body temperature of >5°C and high mortalities (Fig. 5*B*). These data support a central role for IL-10 in mediating worm-induced protection against anaphylaxis.

Role of IL-10-producing cells in worm resistance to anaphylaxis

Worm-infected mice have elevated levels of IL-10 production after spleen T cell (anti-CD3) or B cell (LPS) activation compared with uninfected mice (Table I). To empirically determine the relative roles of these defined IL-10-producing cell populations in worm resistance to anaphylaxis, we used Abs for selective depletion of each cell type in vivo (Fig. 6, *A* and *B*). Despite worm-infected mice having 2-fold more IL-10-producing CD4$^+$ T cells than uninfected mice (detected by intracellular cytokine staining; data not shown), CD4 depletion had no effect on worm-mediated protection against anaphylaxis (Fig. 6*B*). To assess the role of B cells in anaphylaxis, an anti-IgM Ab was administered to deplete B cells. Although Ab treatment only caused partial depletion of IgM$^+$ B cells (Fig. 6*A*), this reduction in B cells was sufficient to render *S. mansoni* worm-infected mice highly susceptible to anaphylaxis, with the death of all mice after 10 min (Fig. 6*C*). Uninfected and

Table I. *Cytokine response of spleen cells from uninfected and worm-infected mice*

	IFN-γ (ng/ml)[a]	IL-4 (pg/ml)	IL-10 (pg/ml)	TGF-β (pg/ml)
Uninfected[b]				
Medium	ND	ND	ND	10.1 ± 2.0
Anti-CD3	18.9 ± 1.4	18.2 ± 5.5	1400.2 ± 50.4	44.5 ± 6.0
LPS	3.3 ± 0.2	ND	327.4 ± 16.4	32.3 ± 6.0
Worm infected				
Medium	ND	ND	ND	43.4 ± 6.0
Anti-CD3	20.2 ± 1.1	969.8 ± 43.0	1616.1 ± 147.3	64.3 ± 5.0
LPS	3.7 ± 0.2	ND	482.8 ± 92.5	45.7 ± 9.0

[a] ELISA measurements of cytokine levels in the supernatants from spleen cell cultures of uninfected and worm-infected mice were performed as described in *Materials and Methods.* Results are presented as the mean ± SD from triplicate wells. ND, not detected.

[b] Spleen cells from two to three uninfected and worm-infected mice were pooled. Cells were unstimulated (medium) or stimulated with plate-bound anti-CD3 (10 μg/ml) or LPS (1 μg/ml) for 72 h.

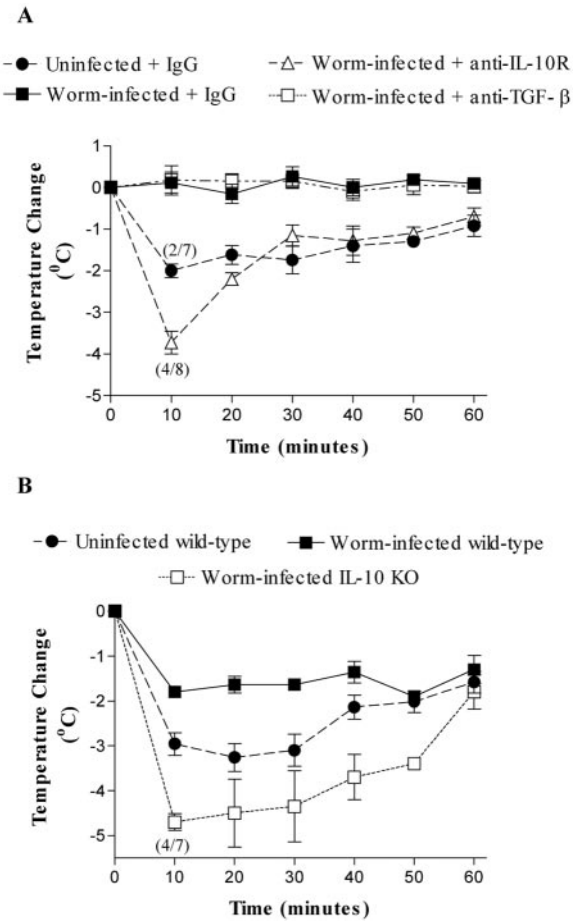

FIGURE 5. IL-10 has a role in protecting *S. mansoni* worm-infected mice from Pen V-induced anaphylaxis. *A*, Depleting mAbs against TGF-β (1D11.16.8) or IL-10R (1B1.3a) were injected (0.5 mg was injected i.p. per mouse at each time point) 2 days and 1 day before eliciting anaphylaxis in Pen V-sensitized mice. Control uninfected and worm-infected mice received irrelevant IgG1. *B*, IL-10$^{-/-}$ (IL-10 KO) and wild-type control C57BL/6 mice were infected with schistosome worms, and Pen V-induced anaphylaxis was performed as described in *Materials and Methods*. Deaths of mice are represented in brackets. Results are the group mean ± SEM (n = 7–8) change in body temperature. The data presented are representative of two separate experiments.

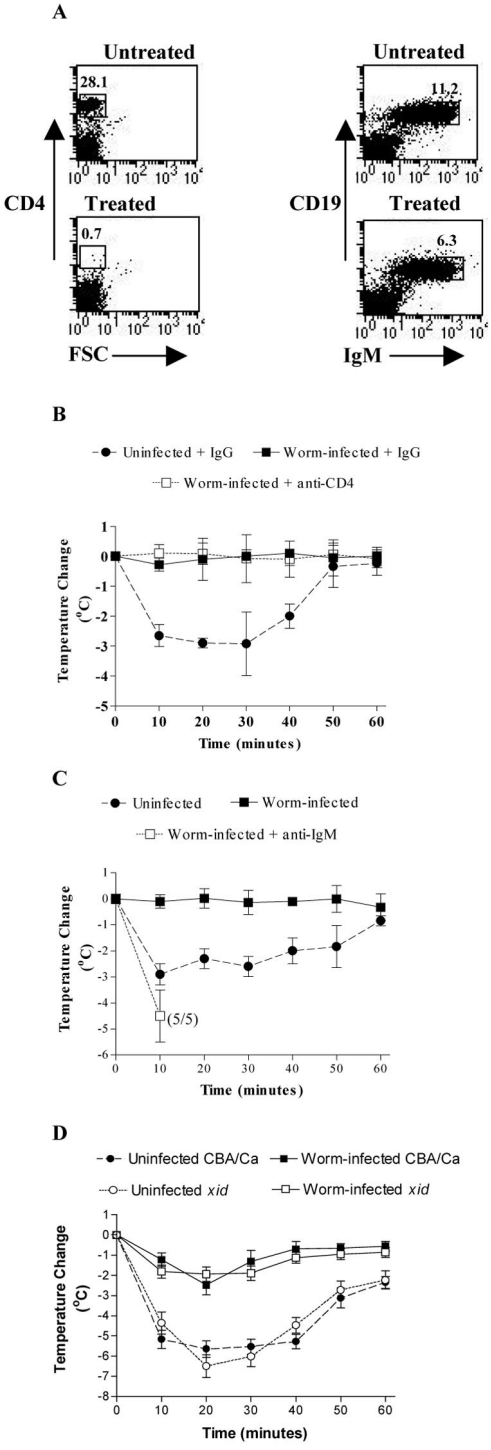

FIGURE 6. Depletion of B cells diminishes worm-induced protection against anaphylaxis. *A*, In vivo depletion of CD4$^+$ T cells and B cells. The percentages of relevant cell type in spleens of untreated or treated mice are shown. *B*, Anaphylaxis in CD4-depleted worm-infected mice. Uninfected and worm-infected mice received YTS 191 or control IgG1. *C*, *S. mansoni* worm-infected mice developed anaphylaxis after depletion of IgM$^+$ cells. Deaths of mice are represented in brackets. *D*, *S. mansoni* worm-infected, B-1 cell-deficient *xid* mice were protected from anaphylaxis. Results are the group mean ± SEM (n = 5–7) change in body temperature. The data presented are representative of two separate experiments.

untreated worm-infected mice showed similar trends of susceptibility and protection, respectively, to anaphylaxis as previously noted.

To specifically investigate the relative roles of B-1 vs B-2 cell subpopulations in anaphylaxis, we used mice expressing the Xid (*xid*) mutation that are deficient in peritoneal B-1 cells. Worm-infected *xid* and wild-type CBA/Ca mice were both equally protected from anaphylaxis compared with the anaphylaxis observed in uninfected *xid* and wild-type mice (Fig. 6*D*). These data indicate that B-1 cells have no role in the B cell-dependent protection from anaphylaxis in worm-infected mice.

Passive transfer of in vivo or in vitro modulated B cells

The fact that IgM depletion renders worm-infected mice susceptible to anaphylaxis, and B cells from these mice produce IL-10 (Table II) suggested that B cells mediate protection. To investigate whether worm-mediated protection against anaphylaxis could be transferred by B cells, B cells were purified from the spleens of worm-infected or uninfected mice and transferred to uninfected mice. B cells from uninfected mice did not alter susceptibility to anaphylaxis (Fig. 7*A*). Unexpectedly, transfer of B cells from worm-infected mice not only did not protect mice from anaphylaxis, but these animals had a greater drop in body temperature,

Table II. *Frequency of IL-4, IFN-γ, or IL-10-producing CD19$^+$ B cells in spleens from uninfected or* S. mansoni *worm- or worm+egg-infected mice or in cells modulated in vitro with worms*

	% IL-4 Positive[a]	% IFN-γ Positive	% IL-10 Positive
In vivo modulated[b]			
Uninfected	0.85 ± 0.19	1.41 ± 1.20	19.13 ± 2.13
Worm infected	4.90 ± 1.67[c]	1.83 ± 1.63	40.30 ± 1.62[d]
Worm+egg infected	6.86 ± 1.38[d]	1.54 ± 1.12	44.50 ± 2.92[d]
In vitro modulated[e]			
Unmodulated	2.00 ± 0.53	0.70 ± 0.36	6.22 ± 1.38
Worm modulated	4.63 ± 0.84[c]	0.52 ± 0.48	12.9 ± 2.14[c]

[a] Cells were gated on CD19 expression and quadrants for IL-4, IFN-γ, or IL-10 set with isotype control mAbs. Data presented are the mean percentage ± SE of cytokine-producing cells from four to six separate experiments.

[b] Spleen cells were isolated from two to three uninfected and *S. mansoni* worm- or worm+egg-infected mice and pooled. Cells were cultured in vitro with brefeldin A (10 μg/ml) for 3 h.

[c] $p < 0.05$ or [d] $p < 0.01$ significant elevation in cytokine-producing cells in schistsome-modulated B cells relative to unmodulated cells. Statistical analysis was performed using the unpaired *t* test with Welch correction.

[e] Spleen cells from naive mice were unmodulated or modulated with 15 male worms for 48 h at 37°C. Cells were cultured in vitro with brefeldin A (10 μg/ml) for 3 h.

increased mortality, and prolonged symptoms of anaphylaxis compared with mice receiving B cells from uninfected mice (Fig. 7*A*). The finding that transfer of B cells from worm-infected mice exacerbates anaphylaxis does not support our initial observation that worm infection induces B cells that mediate protection.

To further determine whether B cells are involved in protection from anaphylaxis, rather than allowing 8 wk of in vivo infection to modulate the cells, an in vitro modulation system was used. We have developed a model of short term in vitro culture of distinct cell populations with live worms (18). Spleen cells from a naive mouse were cultured with 15 live worms in Transwells for 48 h at 37°C. In vitro exposure of spleen cells to worms significantly ($p < 0.05$) elevated the frequency of B cells expressing IL-10 (Table II). Transfer of in vitro worm-modulated B cells made recipient mice more susceptible to anaphylaxis (Fig. 7*B*), data similar to what occurred with transfer of B cells modulated in vivo by worm infection (Fig. 7*A*). It is noteworthy that we have consistently observed that mice receiving in vivo modulated B cells (Fig. 7*A*) had sustained anaphylaxis, with reduced temperature until 50 min after challenge, but animals that were given in vitro worm-modulated cells rapidly recovered from anaphylaxis within 30 min (Fig. 7*B*).

Despite the unexpected observations that in vivo or in vitro worm-modulated B cells exacerbated anaphylaxis, both B cell populations have ~2-fold greater basal levels of IL-10 than uninfected or unmodulated B cells (Table II). Therefore, to confirm that IL-10-producing B cells have a role in worm-mediated protection against anaphylaxis, B cells from IL-10$^{-/-}$ and naive C57BL/6 mice were modulated in vitro and transferred to Pen V-OVA immunized mice before eliciting anaphylaxis. All mice were susceptible to anaphylaxis (Fig. 7*C*). However, mice that received IL-10$^{-/-}$ worm-modulated B cells had a markedly more severe temperature drop and a greater number of mortalities than mice receiving B cells from wild-type animals. These data support a protective role for B cell-produced IL-10 in anaphylaxis.

Exogenous IL-10 or IL-4 alters susceptibility of mice to anaphylaxis

Our initial data from depletion studies suggested that schistosome worm infection induces B cells producing elevated levels of IL-10 that render the mice refractory to experimental anaphylaxis. Unexpectedly, transfer of B cells from worm-infected mice or in vitro worm-modulated cells to naive mice not only did not mediate protection from anaphylaxis, but significantly exacerbated disease (Fig. 7). However, worm-infected mice not only have elevated numbers of IL-10-producing B cells, but by intracellular cytokine staining these mice also have 4- to 5-fold elevations in the frequency of IL-4-producing B cells compared with uninfected mice (Table II and Fig. 8*A*). The elevation in both IL-10 and IL-4 in B cells was confirmed by RT-PCR of purified B cells isolated from both worm+egg-infected and worm-infected mice (Fig. 8, *B* and *C*). Therefore, in the experiments described in Fig. 7, we were not only injecting mice with IL-10-producing B cells, but we were also transferring IL-4-producing B cells. Indeed, the greater ratio of IL-4-producing:IL-10-producing B cells in worm-infected mice (~8:1) compared with that in in vitro modulated B cells (~3:1) could explain the more severe anaphylaxis when B cells from infected mice were transferred into naive animals (Fig. 7*A*) compared with that after transfer of in vitro modulated B cells (Fig. 7*B*). Because IL-4 is known to exacerbate experimental anaphylaxis (35), the increased anaphylaxis in mice receiving B cells from worm-infected mice or in vitro worm-modulated B cells could be due to cotransfer of these IL-4-producing cells. To address this scenario, we first formally examined the relative roles of IL-4 and IL-10 in the Pen V-mediated anaphylaxis model. Sensitized mice were administered rIL-4 with or without rIL-10 before Pen V-BSA challenge. Analysis of the serum levels of IL-4 and IL-10 in these mice (Fig. 9*A*) demonstrated that there were artificially elevated levels of each cytokine in mice during challenge compared with control mice. Administration of rIL-4 exacerbated anaphylaxis in recipient mice, with these mice showing a 2°C greater drop in body temperature compared with control mice and a prolonged lapse in recovery (Fig. 9*B*). In contrast, mice that received rIL-10 were protected from anaphylaxis, with only a slight drop (~1°C) in body temperature at 10 min, followed by a rapid recovery (Fig. 9*C*). Interestingly, mice that were treated with both rIL-4 and rIL-10 showed a similar trend as mice that received rIL-10. These in vivo data demonstrate that IL-10 can reduce the deleterious effects of IL-4 in anaphylaxis.

Transfer of in vitro worm-modulated, Th2 cytokine-deficient B cells

To exclude a role for IL-4 and other Th2 cytokines (IL-5, IL-9, and IL-13) known to induce allergic responses, a B cell transfer experiment was performed with cells from multiknockout (mKO) mice that have a combined deficiency in IL-4, IL-5, IL-9, and IL-13 (15). B cells from mKO and wild-type mice were modulated with worms in vitro and transferred to Pen V-OVA-immunized mice before Pen V-BSA challenge and induction of anaphylaxis. In vitro modulation of mKO splenocytes with worms increased the frequency of IL-10-producing B cells by 32% (data not shown).

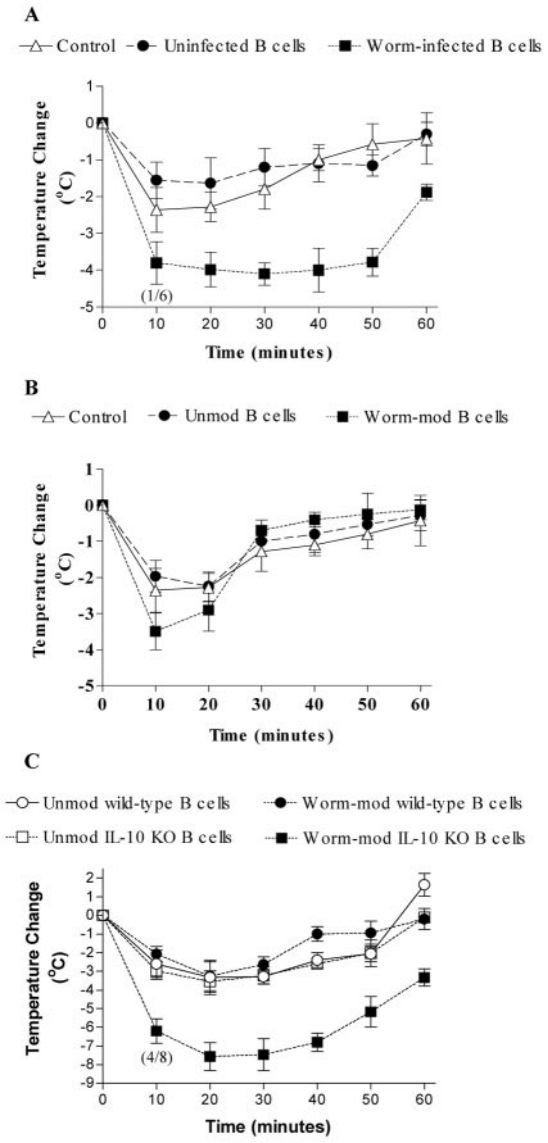

FIGURE 7. Transfer of B cells from worm-infected mice or B cells modulated in vitro with worms does not prevent anaphylaxis in recipient mice, and this effect is exacerbated in the absence of IL-10. *A*, Transfer of B cells from worm-infected mice to Pen V-sensitized mice induces pronounced anaphylaxis. $CD19^+$ B cells were isolated from spleen cells from uninfected and worm-infected mice and injected (total of 1×10^7 cells) into Pen V-sensitized BALB/c mice 2 days and 1 day before inducing anaphylaxis. Data are presented as the group mean ± SEM ($n = 5$–7) temperature change and are representative of three separate experiments. Deaths of mice are represented in brackets. *B*, Transfer of B cells exposed to worms in vitro to Pen V-sensitized mice transiently enhances anaphylaxis. B cells were isolated from spleen cells from BALB/c mice that had been modulated by worms in vitro (mod) or unmodulated (unmod). *C*, Worm-modulated B cells deficient in IL-10 exacerbate anaphylaxis when transferred to Pen V-sensitized mice. Spleen cells from an IL-10-deficient mouse (IL-10 KO) and a wild-type C57BL/6 mouse were worm-modulated (mod) or umodulated (unmod) in vitro. B cells were isolated and transferred to Pen V-sensitized C57BL/6 mice as described above. Deaths of mice are represented in brackets. Data are presented as the group mean ± SEM ($n = 5$–8) temperature change and are representative of three separate experiments.

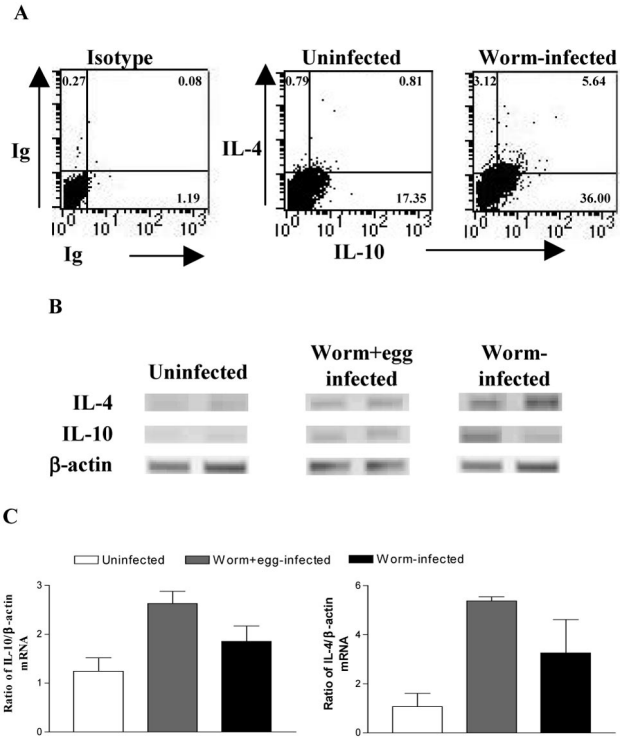

FIGURE 8. Worm-infected mice have elevated levels of B cell IL-10 and IL-4 compared with uninfected mice. *A*, Intracellular cytokine staining to detect the percentages of IL-4- and IL-10-producing B cells in spleen cells from uninfected and worm-infected mice. Quadrants were assigned from isotype controls. Three spleens were pooled, and data are representative of two separate experiments. *B*, IL-4 and IL-10 mRNA levels in purified spleen B cells from uninfected, worm+egg-infected, and worm-infected mice, as determined by RT-PCR. β-Actin was used as a control. *C*, Ratio of IL-4 and IL-10 mRNA expression to β-actin in purified B cells isolated from spleens from uninfected, worm+egg-infected, and worm-infected mice. Data in *B* and *C* are from three separate autoMACS isolations of 5×10^6 B cells purified from three to five spleens.

Mice that received unmodulated B cells from mKO or cells from wild-type animals developed anaphylaxis (Fig. 10*A*). Similar to earlier data (Fig. 7*B*), mice that received worm-modulated B cells from wild-type mice had a more severe initial (first 10 min) drop in temperature, after which they recovered rapidly (Fig. 10*A*). However, passive transfer of worm-modulated B cells from mKO mice completely protected animals from anaphylaxis (Fig. 10).

To formally determine the roles of the individual Th2 cytokines, spleen cells from IL-4-, IL-5-, IL-9-, or IL-13-deficient mice were modulated by worms in vitro, and isolated B cells were transferred to Pen V-sensitized mice. Worm-modulated B cells from mice deficient in IL-4 completely protected recipients from anaphylaxis (Fig. 10*B*). In contrast, animals receiving modulated B cells from mice deficient in IL-5, IL-9, or IL-13 developed a comparable drop in temperature, as seen in animals administered wild-type cells. These results further corroborate a protective role for schistosome worm-modulated B cells in preventing anaphylaxis in the absence of IL-4.

Discussion

In this study we demonstrate that mice infected with *S. mansoni* are refractory to experimental systemic anaphylaxis. It was established that it is the worm stage of infection that elicits the protective phenotype in this model, with worm-infected mice completely protected against anaphylaxis, whereas worm+egg-infected mice were only partially protected. We have identified that schistosome worm infection of mice prevents anaphylaxis via a B cell- and IL-10-dependent mechanism.

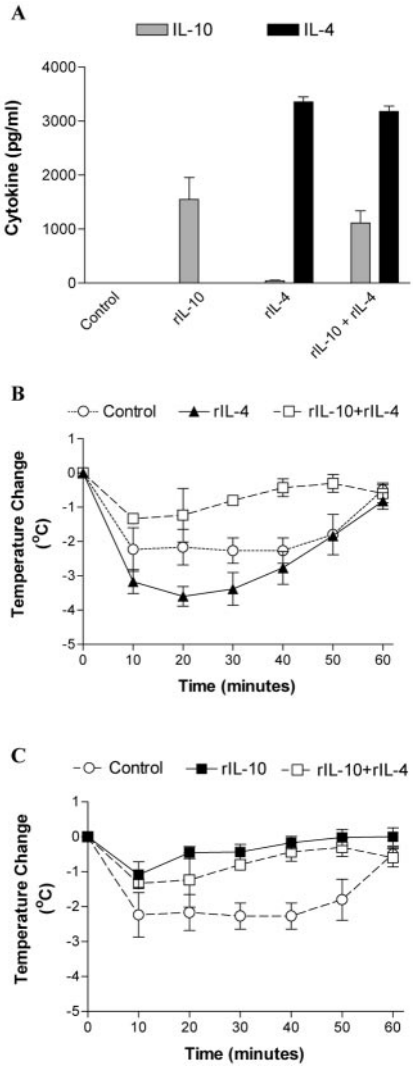

FIGURE 9. IL-10 diminishes IL-4-mediated exacerbation of anaphylaxis in vivo. *A*, Serum levels of IL-4 and IL-10 in mice injected with PBS (Control) or rIL-4 with or without rIL-10. *B* and *C*, Drop in body temperature in Pen V-sensitized mice treated with IL-4 and with or without IL-10. Data are presented as the group mean ± SEM (n = 5–7) temperature change and are representative of two separate experiments.

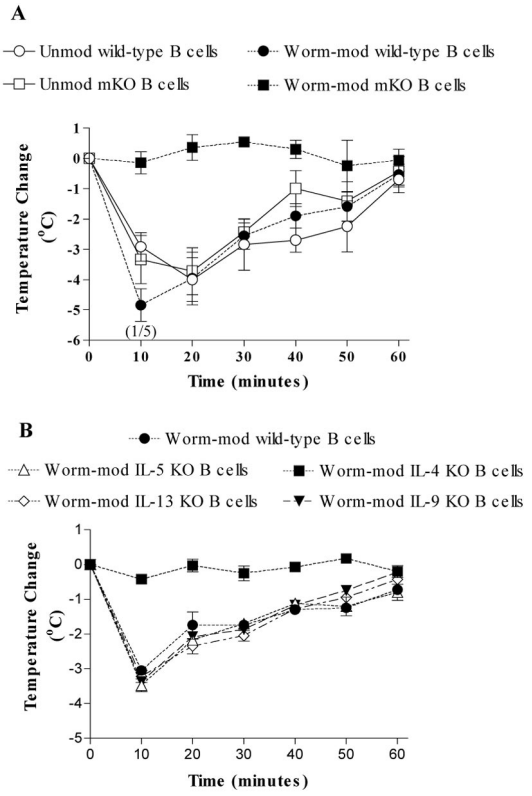

FIGURE 10. Transfer of IL-4-deficient B cells modulated by worms in vitro prevents anaphylaxis. *A*, Pen V-sensitized mice do not develop anaphylaxis after transfer of B cells from a mouse deficient in IL-4, IL-5, IL-9, or IL-13 (mKO) that have been modulated by worms in vitro. Deaths of mice are represented in brackets. *B*, Worm-modulated B cells from IL-4$^{-/-}$ (IL-4 KO) mice, but not modulated cells from IL-5 KO, IL-9 KO, and IL-13 KO mice, prevent anaphylaxis when passively transferred to Pen V-sensitized mice. In vitro modulation and cell transfer are described in Fig. 7. Data are presented as the group mean ± SEM (n = 5–7) temperature change and are representative of two separate experiments.

The central role of B cells in protecting worm-infected mice from anaphylaxis was illustrated by the finding that even partial depletion of B cells caused these animals to be fully susceptible to anaphylaxis. B cells possess a variety of immune functions, including production of Igs, presentation of Ags, and production of distinct cytokines (36). B cells have also been shown to participate in the induction of immune tolerance and suppression of disease, giving rise to the suggestion of IL-10-producing regulatory B cells (37–39). Previously, it has indeed been shown that B cells play an important regulatory role during conventional *S. mansoni* worm+egg infection, with high mortalities of infected μMT mice (40). We show in this study that B cells from schistosome worm-infected mice have 2-fold elevated levels of IL-10-producing B cells, which is consistent with worm-mediated protection from anaphylaxis being dependent on IL-10 and B cells. Interestingly, worm+egg-infected mice also had significantly elevated IL-10-producing B cells. It is well documented that schistosome worm+egg infections induce elevated IL-10 production (41–43). IL-10 production during such infections has an essential function in suppressing egg-induced immunopathology (44–47). Previously, peritoneal B-1 cells from worm+egg-infected mice have been shown to produce IL-10 (48). Therefore, to determine whether B-1 cells were involved in worm infection-induced resistance to anaphylaxis, we infected B-1 cell-deficient *xid* mice. Worm-infected, B-1 cell-deficient mice were resistant to Pen V-induced anaphylaxis, indicating that a B-2 cell population mediates resistance from anaphylaxis. Our future studies will address whether a distinct B-2 cell subpopulation preferentially produces IL-10.

In this study spleen cells from schistosome worm-infected mice also have elevated IL-10 production, with resistance of these mice to anaphylaxis being IL-10 dependent. IL-10 is one of the main cytokines that is implicated in certain regulatory T cell types (49) and, with respect to asthma, has also been shown to suppress immune and inflammatory responses, including allergic airway inflammation (50). In murine schistosome worm+egg infections, a recent study has addressed for the first time the role of IL-10-producing CD4$^+$CD25$^+$ T cell in ameliorating morbidity (47). However, in this study, although natural CD4$^+$CD25$^+$ T cells and IL-10-producing T cells are both elevated in worm-infected mice, depletion studies demonstrated that CD4$^+$ or CD25$^+$ cells had no role in worm-mediated protection against anaphylaxis. The data presented here indicate that T cells are not the only source of IL-10 during a schistosome worm infection, an observation also demonstrated during *S. mansoni* worm+egg infections by Hesse et al. (47). Because schistosome infection of mice stimulates a number

of cells to produce IL-10, it highlights the known significant role for this cytokine in immunomodulation of the host to suppress parasite-induced pathology.

Previously, a protective role for IL-10-producing B cells has been demonstrated in experimental encephalomyelitis and collagen-induced arthritis (26, 37). In experimental encephalomyelitis, IL-10-producing B cells change the recovery of mice from disease through suppression of type 1 autoantigen responses (37). Similarly, transfer of in vitro anti-CD40-activated B cells from spleens of arthritic DBA/1 mice prevented collagen-induced arthritis in recipients, with B cells from IL-$10^{-/-}$ mice failing to mediate this protective function (26). In this study transfer of B cells from schistosome worm-infected mice (in vivo modulated) or in vitro worm-modulated B cells not only did not prevent anaphylaxis, as we expected, but induced more severe anaphylaxis in recipients. Because we attributed this failure to confer protection to cotransfer of IL-4-producing B cells (Fig. 8), it would explain the discrepancy with B cell transfer protecting in the collagen-induced arthritis (26), because in this model IL-4 is protective; treatment of mice with engineered IL-4-producing B cells (51) or IL-4-expressing dendritic cells (52) was shown to reduce or suppress arthritis.

During cytokine phenotyping of B cells in infected mice we had observed that splenocytes from schistosome worm-infected mice had 4- to 5-fold more IL-4-producing B cells than those from uninfected mice. Because schistosome worm infections are type 2 cytokine-biased (34), it is perhaps not unexpected that B cells from worm-infected mice have a type 2, or B effector 2 (Be2), profile. In the more Th2-biased worm+egg-infected mice, there was an even more marked increase in levels of IL-4-producing cells (Table II and Fig. 8). It has previously been shown that mesenteric lymph node cells from mice infected with the Th2-inducing nematode worm *Heligmosomoides polygyrus* have elevated Be2 cells (36). Thus, upon transferring worm-modulated B cells that had elevated IL-10 to recipient mice, we were also transferring IL-4-producing Be2 cells. This contamination with Be2 cells became relevant after Finkelman and colleagues (35) demonstrated that injection of mice with only 65 ng of IL-4 significantly increased the severity of anaphylaxis. Indeed, earlier findings by Park et al. (20) also demonstrated that blockade of IL-4 by anti-IL-4 mAb treatment prevented fatal anaphylaxis in the same antibiotic model as that used in this study. We therefore treated mice with exogenous IL-4 and/or IL-10 and not only illustrated the respective deleterious or protective effects of these cytokines in Pen V-induced anaphylaxis, but also showed that IL-10 can suppress the pathogenic effects of IL-4. Previously, it was reported that treatment of mice with 50 μg of IL-10 had no effect on their susceptibility to anaphylaxis (35), whereas we observed that administration of 1 μg of IL-10 protected mice. Because this discrepancy could be due to the difference in anaphylaxis models used, it would be of interest to evaluate the effects of IL-10 in different models of anaphylaxis. The use of mice deficient in Th2 cytokines permitted confirmation that it was up-regulation of IL-4 in schistosome worm-modulated B cells that was responsible for the exacerbated anaphylaxis in recipient mice given worm-modulated B cells. Therefore, in the experiments described in Fig. 7, in which transferred B cells exacerbated anaphylaxis, we were not only injecting mice with IL-10-producing B cells, but we were also transferring IL-4-producing B cells. In the B cell transfer studies, mice injected with in vivo modulated cells had more severe anaphylaxis than mice receiving in vitro modulated cells, which is consistent with B cells from infected mice having greater relative frequencies of IL-4-producing:IL-10-producing B cells.

Bashir et al. (53) have demonstrated a role for experimental helminth infection in protection against the development of allergy in mice. They assessed the effect of Th2 responses induced by the intestinal helminth infection (*H. polygyrus*) on the development of an allergic response to the food allergen peanut Ag, with protection being mediated at least in part by the production of IL-10. Although we have demonstrated a novel mechanism by which a helminth parasite can prevent anaphylaxis in mice, it is possibly too simplistic to envisage a common mechanism by which different helminth parasites may prevent allergic responses. This can be illustrated by infection of mice with a gastrointestinal nematode (*H. polygyrus*) that has been shown to reduce allergic responses (53) in one study, whereas in a separate study mice infected with another gastrointestinal nematode (*Trichinella spiralis*) had exacerbated anaphylaxis (35). There are defined differences in the infectivity and immunity of these two parasites (54) as well as in the allergy models used that could explain the differences between the studies.

In the context of the hygiene hypothesis, it has previously been proposed that schistosome infection can ameliorate allergic disorders in humans (8). In support of this argument, schistosome-infected Gabonese schoolchildren have fewer allergic responses and elevated IL-10 levels (55), with an increase in atopic responses in the schoolchildren after chemotherapy to eliminate helminth infections (56). Using experimental animal studies, we now formally demonstrate that *S. mansoni* worm infection of mice can protect mice from anaphylaxis via an immunoregulatory response. Using different anaphylaxis models, it is evident that worm infection does not alter Ag sensitization, but protects the mice from anaphylaxis when they are rechallenged with the allergen. Therefore, resistance of schistosome-infected mice to anaphylaxis was not due to the known propensity of helminth infections to alter responses to vaccination (57) or the stimulation of polyclonal IgE, which can saturate FcϵRs and thereby prevent mast cell degranulation (27). Protection from anaphylaxis is thus primarily mediated when the sensitized mice are rechallenged with the appropriate allergen.

It is noteworthy that when a protective factor, i.e., B cells or IL-10, was inhibited, the worm-infected mice had a heightened propensity to develop fatal anaphylactic reactions upon Ag challenge. This highlights that in the absence of regulation by IL-10-producing B cells, schistosome-infected mice are, in fact, highly predisposed to type 2 allergic responses, thereby illustrating the sensitive balance of *S. mansoni* modulation of host immunity to achieve host protective vs detrimental immune responses. Hence, IL-10-producing B cells are necessary to maintain a fine balance of regulation in the allergic-predisposed environment of schistosome infection.

Collectively, these experimental data demonstrate that schistosome worms evoke IL-10-producing B cells during infection, with these cells protecting against anaphylaxis. We show the IL-10-producing B cells are induced during schistosome infection as part of the parasite's regulation of host immunity, with this modulation also influencing unrelated allergic (anaphylaxis) responses. These data offer for the first time conclusive experimental evidence that *S. mansoni* can suppress allergic responses via a novel regulatory cell mechanism.

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