Brief Definitive Report

Cytokine-mediated Regulation of Chronic Intestinal Helminth Infection

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Summary

Most inbred strains of mouse infected with the intestinal nematode *Trichuris muris* are resistant to infection expelling the parasite before adult worms establish. However, a few susceptible strains exist that are incapable of worm expulsion and harbor chronic infections of mature adult worms. Analyses of in vitro cytokine production by cells from the draining lymph node (mesenteric lymph node) have indicated that expulsion phenotype is tightly correlated with the selective expansion of helper T cells (Th) of the Th1 or Th2 cell subset within the mesenteric lymph node, resulting in susceptibility and resistance to *T. muris*, respectively. We have now confirmed and extended our in vitro observations in a series of experiments involving the in vivo manipulation of host cytokine levels. Depletion of interferon (IFN)-γ in normally susceptible mice resulted in expulsion of the parasite, representing the first evidence for a role for IFN-γ in the establishment of chronic helminth infection. Blocking interleukin (IL)-4 function in normally resistant animals prevented the generation of a protective immune response allowing adult stages of the parasite to develop. Conversely the administration of IL-4 to a normally susceptible host facilitated expulsion and indeed enabled established adult worms to be expelled when administered late in infection. In all cases assessment of a variety of in vivo parameters indicative of a Th1- or Th2-type response (parasite-specific immunoglobulin (Ig) G2a and the parasite-specific IgG1, total IgE levels and intestinal mastocytosis, respectively) demonstrated that the in vivo modulation of a Th1- or Th2-specific cytokine allowed the reciprocal Th cell subset to expand and become dominant with dramatic consequences for worm expulsion.

Murine $CD4^+$ Th cells can be divided into two distinct subsets (1); Th1-type cells produce IL-2, IFN-γ, and lymphotoxin, while Th2 cells produce IL-4, IL-5, IL-9, and IL-10 (2, 3). Development of an appropriate Th cell response is critical to the outcome of infection as exemplified by the cecal dwelling nematode parasite *Trichuris muris* in the mouse where Th2-type responses are associated with host protection while Th1 responses are associated with susceptibility to infection (4, 5). Although resistance to other intestinal helminth parasites has also been shown to be associated with the activation of Th2 cells (6, 7, 8), the *T. muris*–mouse model is unique in that it is the only nematode system where reciprocal activation of Th cell subsets in relation to acute and chronic infection has been described. We here assess the roles of key Th1 and Th2 cytokines (IFN-γ and IL-4) in the development of protective immunity by a series of in vivo studies. We demonstrate that IFN-γ plays an important role in the establishment of chronic trichuriasis while the presence of IL-4 is essential in the development of a protective Th2-type response.

Materials and Methods

Animals. Male AKR and BALB/K mice were purchased from Harlan Olac Ltd. (Bicester, Oxon, UK) and infected when 6–8-wk-old.

Parasite. The maintenance of *T. muris* and the method used for infection were as described by Wakelin (9). Mice were killed at various time points after infection and worm burdens were assessed as previously described (10).

Cytokine and Antibody Reagents. IFN-γ levels were depleted in vivo using the rat anti-IFN-γ mAbs XMG-6 (11) or R46A2 (12) injected intraperitoneally as detailed in the text. IL-4 function was blocked in vivo by intravenous injection of the rat anti-IL-4 receptor mAb M1 (13). IL-4 was delivered in vivo in the form of a complex as previously described (14, 15). Briefly, 10 μg IL-4 was complexed with 50 μg 11B11 (16), 1D11.2 (17) (both neutralizing anti-IL-4 mAbs), or BVD6.24G2.3 (18) (24G2.3, a nonneutralizing mAb) before intravenous injection.

ELISAs. Parasite-specific IgG1 and IgG2a levels were conducted as previously described (19). Briefly adult *T. muris* excretory/secretory (E/S) antigen was used as the target antigen at 0.25 μg/well and sera double diluted through eight dilutions from 1/20. IgG1

was detected using alkaline-phosphatase conjugated sheep anti-mouse IgG1 (Serotec Ltd., Oxford, UK) and IgG2a with biotinylated rat anti-mouse IgG2a (AMS Biotechnology, Witney, Oxon, UK) followed by streptavidin peroxidase (Boehringer Mannheim UK, East Sussex, UK).

Total IgE levels were measured by sandwich ELISA using a rat mAb to murine IgE (Serotec Ltd.) as the capture antibody, goat anti-mouse IgE peroxidase (Nordic Immunological Labs, Maidenhead Berks, UK) as the detection antibody and an IgE monoclonal antibody specific for DNP (Serotec Ltd.) as standard.

Histology. Intestinal mast cells were counted after conventional staining of carnoys-fixed tissue with 0.5% toluidine blue, pH 0.3.

Statistical Analysis. Significant differences between experimental groups were calculated using the Mann-Whitney U-test with $p > 0.05$ considered nonsignificant.

Results

IFN-γ Is Critical in Determining the Chronicity of T. muris Infection. To assess the role of IFN-γ in promoting susceptibility to infection we depleted susceptible (Th1-dominated) AKR mice with the anti-IFN-γ monoclonal antibody XMG-6 (11) as detailed in Fig. 1 *a*. Neutralization of IFN-γ in vivo resulted in expulsion of the parasite by day 35. Mice treated with an isotype-matched mAb (GL113) and mice receiving saline harbored full complements of mature adult worms at levels not significantly different from the infectivity level of 88.8 ± 8.7 estimated on day 10 after infection ($p > 0.05$). In vivo depletion of IFN-γ not only altered the expulsion phenotype of AKR mice but also modified their parasite-specific antibody responses as shown in Fig. 2 *a*. IgG1 levels, under the control of Th2 cell cytokines (20), were similar in all infected groups. However the parasite-specific IgG2a response, controlled by the Th1 cytokine IFN-γ (20), was considerably depressed in IFN-γ depleted mice (Fig. 2 *b*) providing further evidence for the dependency of the murine IgG2a response on IFN-γ. Repeat experiments using a different susceptible strain of mouse, B10.BR, and the anti-IFN-γ mAb R46A2 (12) (0.4 mg/injection weekly from day 0 to day 28 after infection) confirmed the effects of depleting IFN-γ on worm expulsion and antibody isotype production (data not shown). These results represent the first demonstration of a critical role for IFN-γ in determining chronic intestinal helminth infection.

Blocking IL-4 Function In Vivo Prevents Expulsion of T. muris. The presence of IL-4 during T cell differentiation has been shown to be important in the development of Th2-type responses both in vitro (21) and in vivo (22). Therefore to assess the Th2 cytokines critical in determining resistance to *T. muris* infection we treated normally resistant (Th2-dominated) BALB/K mice with the anti-IL-4 receptor mAb M1 (13) or an isotype control (GL117) as detailed in Fig. 1 *b*. All mice in which IL-4 functions had been blocked still harbored considerable numbers of adult worms 36 d after infection in contrast to control GL117-treated mice from which no worms were recovered. *T. muris*–specific IgG1 responses were similar in both groups; a very strong specific IgG2a response was seen in mice given M1, while no IgG2a response was detected in control GL117-treated mice (Fig. 2, *c* and *d*). In addition, the intestinal mast cell response and total IgE levels (both Th2-controlled responses [23–26]) were depressed to levels significantly below normal in M1-treated animals ($p < 0.05$ and $p < 0.005$, respectively; Fig. 3, *a* and *b*). These results strongly suggest that blocking IL-4 function in vivo enabled a nonprotective Th1-type response to develop, resulting

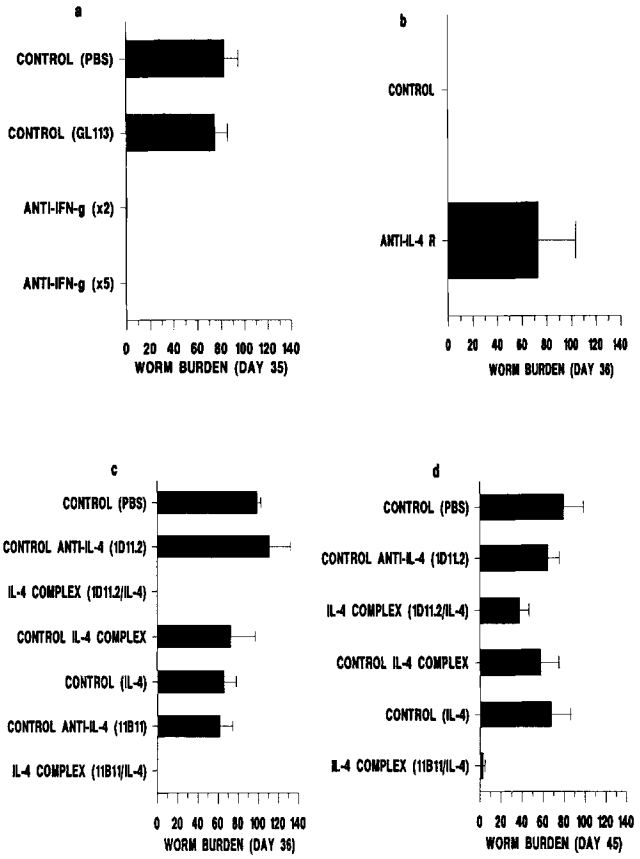

Figure 1. Adult worm burdens recovered from susceptible and resistant strains of mice after in vivo manipulation of cytokines levels. (*a*) Susceptible AKR mice were infected orally with 400 *T. muris* eggs on day 0 and injected with 1 mg anti-IFN-γ mAb XMG-6 intraperitoneally on days 0 and 7 (*anti-IFN-g × 2*), or XMG-6 (*anti-IFN-g × 5*), control mAb GL113 or PBS on days 0, 7, 14, 21, and 28 post infection. Adult worm burdens were estimated on day 35 post infection and are presented as mean values ±SE, six mice/group. (*b*) Resistant BALB/K mice were infected as above and injected with 3 mg anti-IL-4 receptor mAb M1 (*anti-IL-4 R*) or control mAb GL117 (*control*) intravenously on days 0, 3, 6, 9, 12, and 15. Mean adult worm burdens ± SE for five individuals/group on day 36 post infection are shown. (*c*) Susceptible AKR mice were infected as above and injected intravenously on days 8 and 11 post infection with 10 μg IL-4 in the form of a complex with 50 μg of the neutralizing anti-IL-4 mAbs 11B11 (*IL-4 complex*; *11B11/IL-4*), or 1D11.2 (*IL-4 complex*; *1D11.2/IL-4*). Control groups received equivalent amounts of anti-IL-4 mAb or IL-4 as found in the complex (*control anti-IL-4, 11B11*; *control anti-IL-4, 1D11.2*; *control, IL-4*) or a control IL-4 complex consisting of 10 μg IL-4 complexed with 50 μg of the nonneutralizing anti-IL-4 mAb 24G2.3 (*control IL-4 complex*). Results are given as day 36 post infection mean worm burdens ±SE for group sizes of four or five mice. (*d*) Susceptible AKR mice with patent adult infections on day 35 post infection as assessed by the presence of eggs in feces, were injected with IL-4 complexes or control reagents as described in *c* on days 35 and 38 post infection. Mean worm burdens ± SE on day 45 are shown, four or five mice per group except for the 1D11.2/IL-4 group where $n = 3$.

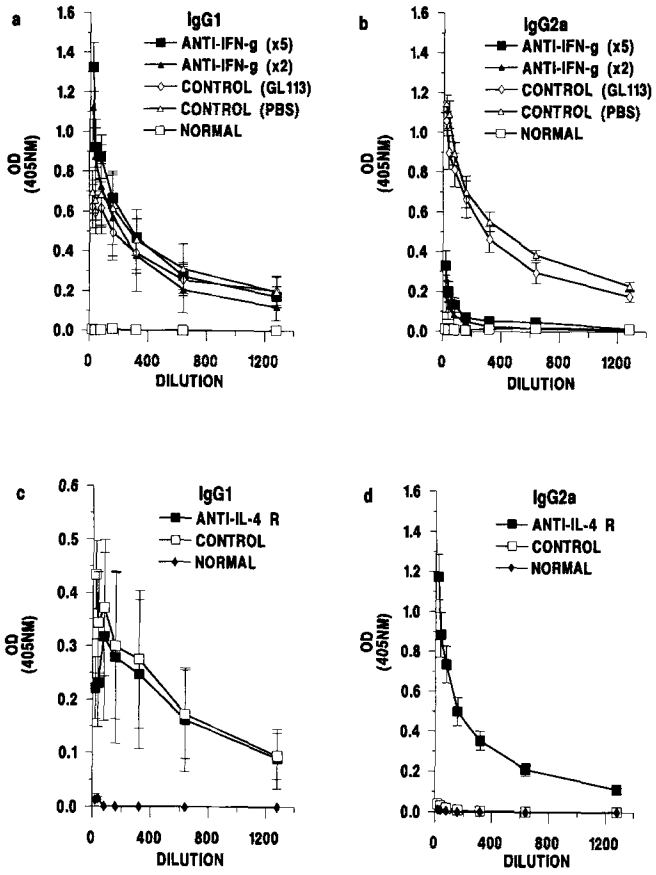

Figure 2. Parasite-specific serum IgG1 and IgG2a responses of susceptible and resistant strains of mouse after the in vivo manipulation of the IFN-γ or IL-4 response. (*a* and *b*) Susceptible AKR mice were infected and depleted of IFN-γ as described in Fig. 1 *a*. Normal uninfected mice received weekly intraperitoneal injections of PBS. Day 35 post infection anti-*T. muris* IgG1 levels are shown in *a*, and the IgG2a response in *b*. (*c* and *d*) Resistant BALB/K mice were infected and their IL-4 response blocked as described in Fig. 1 *b*. Normal mice were given equivalent injections of PBS. Anti-*T. muris* IgG1 levels on day 36 post infection are shown in *c* and IgG2a levels in *d*. Data are represented as the mean OD at λ 405 nm ± SE against dilution of sera.

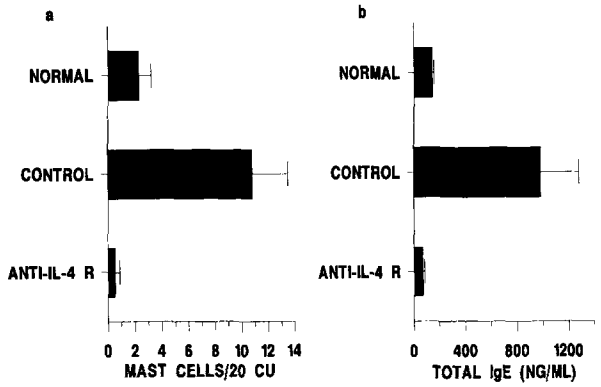

Figure 3. Intestinal mast cell numbers and total IgE levels in BALB/K mice after in vivo blocking of their IL-4 response. Resistant BALB/K mice were infected and their IL-4 response blocked as described in Fig. 1 *b*. Normal mice were given equivalent injections of PBS. Mean numbers of mast cells per 20 crypt units (CU) ± SE on day 36 post infection are shown in *a* and total IgE levels (mean ± SE) in *b*.

in elevated IgG2a levels, depressed IgE levels, depressed mastocytosis, and susceptibility to infection. Repeat experiments confirmed the effects of blocking IL-4 function in vivo using M1 (data not shown).

Administration of IL-4 to Susceptible Mice Facilitates Expulsion. To confirm the importance of IL-4 in mediating resistance to *T. muris* infection, we treated normally susceptible AKR mice with IL-4 in the form of a complex (detailed in Fig. 1, *c* and *d*). The IL-4 complex methodology (14, 15) was selected as injection of mice with complexes of IL-4 and neutralizing anti-IL-4 mAbs increases the duration and magnitude of the IL-4–mediated effects in vivo. Fig. 1 *c* shows the worm burdens recovered from normally susceptible AKR mice when injected early in infection (days 8 and 11) with 10 μg of IL-4 complexed with 11B11 (16) or 1D11.2 (17) (both neutralizing anti-IL-4 mAbs) or 24G2.3 (18) (a nonneutralizing mAb) or a variety of controls including the mAbs and IL-4 alone. Mice treated with 11B11/IL-4 or 1D11.2/IL-4 expelled their infection by day 36. In contrast all the control groups harbored mature adult worms at levels not significantly different from the day 11 infectivity level of 93.3 ± 12.3 ($p > 0.05$). 10 μg of IL-4 alone was insufficient to promote protective immunity underlining the increased effectiveness of cytokine administration using the complex methodology (14, 15). Thus, administration of IL-4 at an early stage after infection is sufficient to allow normally susceptible mice to develop a very effective protective immune response. To determine whether IL-4 could influence an established adult *T. muris* infection, mice with patent infections were injected with IL-4 complexes. As shown in Fig. 1 *d*, 11B11/IL-4 complex treatment induced complete expulsion in most mice, with just a few sterile worms remaining in two of the five individuals. It has recently been shown that treatment of *Leishmania major*-infected mice with a cytokine (IL-12) at the time of parasite inoculation can prevent the development of a chronic infection (27). Data presented here now establish that a cytokine (IL-4) can also be used as effective therapy for an established infection. 1D11.2/IL-4 complexes were less effective against adult parasites than 11B11/IL-4 complexes, although significantly few worms were also recovered in these mice compared with control complex–treated mice ($p = 0.05$). These observations do not exclude the possibility that IL-4 has a direct toxic effect on *T. muris* from the possibility that IL-4 enhances host protection against this parasite. However the latter possibility is supported by recent studies that show that 11B11/IL-4 complexes promote the expulsion of *Heligmosomoides polygyrus* adults during a primary infection and that this effect is blocked by the mAb M1 that inhibits IL-4 receptor function but would not be expected to bind to any existing nematode IL-4 receptor (Urban, J. F., Jr., and F. D. Finkelman, manuscript in preparation). In addition, the administration of IL-4 complexes to nude mice infected with *T. muris* failed to induce worm loss (data not shown).

Discussion

We have previously reported the polarization of the Th cell response in murine trichuriasis towards Th2 cells in resis-

tant strains of mice and Th1 cells in strains of mice susceptible to *T. muris* (4, 5). These observations were based on the cytokine profiles of cells from the lymph node draining the site of infection after restimulation in vitro. The results presented here significantly substantiate and extend these earlier findings demonstrating that the IL-4/IFN-γ balance in vivo is critical in determining the type of Th cell response generated and thus the outcome of infection. The mechanisms involved in polarizing the Th cell response such that some strains of mouse mount a dominant Th2-type response while in others Th1 cells prevail remain unknown. Many suggestions have been put forward including differences in APCs (28–30), differences in antigenic epitopes (31, 32), and the local cytokine environment present at the time of initial T cell activation (21, 33). Clearly, in our system the presence of IL-4 early in infection is a critical component in the generation of a protective Th2-type response, with the "non-B, non-T" cell source of IL-4 being an exciting area of current research (34, 35). Moreover, IL-4 appears to play an important role in modulating the host protective immune response to *T. muris* even in the face of established chronic adult worm infections.

Although Th2 cytokines have been shown to be important in resistance to other intestinal helminth infections (6–8), most notably in resistance to challenge infection with *H. polygyrus* (36), the uniqueness of the *T. muris*–mouse model lies in the ability to study factors promoting both the development of a Th2-type response resulting in resistance to infection and a Th1-type response, resulting in chronic infection. As such, murine trichuriasis provides a powerful model for the study of immune responses to large multicellular helminth parasites and demonstrates the reciprocal relationship between host immunity and cytokine production to that seen in mice infected with the protozoan parasite *L. major*, where selective expansion of the Th1 or Th2 cell subset leads to disease resolution or fatal dissemination, respectively (37, 38).

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