The Debate over the Effector Function of Eosinophils in Helminth Infection: New Evidence from Studies on the Regulation of Vaccine Immunity by IL-12

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The production of Th1-type cytokines is associated with strong cell-mediated immunity while Th2-type cytokines are typically involved in the generation of humoral immune responses. In mice vaccinated a single time (1X) with attenuated cercariae of Schistosoma mansoni, *the immunity induced is highly dependent on CD4$^+$ T cells and IFN-γ. In contrast, mice vaccinated multiple times (3X) have decreased IFN-γ expression, develop a more dominant Th2-type cytokine response as well as protective antibodies which can passively transfer immunity to naive recipients. Previously, we demonstrated the ability of IL-12, a potent IFN-γ-inducing cytokine to enhance (1X) schistosome cell-mediated immunity when administered during the period of immunization. More recently, we asked what effects IL-12 would have on the development humoral-based immunity. While multiply-immunized/saline-treated mice demonstrated a 70-80% reduction in parasite burden, 3X/IL-12-vaccinated animals displayed an even more striking >90% reduction in challenge infection, with many mice in the later group demonstrating complete protection. Analysis of pulmonary cytokine mRNA responses demonstrated that control challenged mice elicited a dominant Th2-type response, 3X/saline-vaccinated produced a mixed Th1/Th2-type cytokine response, while 3X/IL-12-immunized animals displayed a dominant Th1-type response. The IL-12-treated group also showed a marked reduction in total serum IgE and tissue eosinophilia while SWAP-specific IgG2a and IgG2b Abs were elevated. Interestingly, animals vaccinated with IL-12 also showed a highly significant increase in total Ig titers specific for IrV-5, a known protective antigen. More importantly, 3X/IL-12 serum alone, when transferred to naive mice reduced worm burdens by over 60% while 3X/saline serum transferred significantly less protection. Nevertheless, animals vaccinated in the presence of IL-12 also develop macrophages with enhanced nitric oxide dependent killing activity against the parasites. Together, these observations suggest that IL-12, initially described as an adjuvant for cell-mediated immunity, may also be used as an adjuvant for promoting both humoral and cell-mediated protective responses.*

Key words: eosinophil - helmint infection - interleukin-12 - vaccine - *Shistosoma mansoni*

CYTOKINE RESPONSES INDUCED BY THE IRRADIATED CERCARIAE VACCINE

It is now well known that with many infectious diseases, protection or progression of disease often depends on which Th cell subtype, Th1 or Th2 is preferentially induced. The cytokine micro-environment which is present during the initiation of an immune response has a major influence on CD4$^+$ T cell subset selection. IFN-γ and IL-12 are the primary cytokines for the differentiation of Th1 cells while IL-4 and IL-10 are involved in Th2 cell development. Th1 cells produce IFN-γ, IL-2, and TNF-β, and are important for the induction of cell-mediated immunity. They are associated with delayed-type hypersensitivity (DTH), macrophage activation, synthesis of complement-fixing antibody (IgG2a), and antibody-dependent cell cytotoxicity (ADCC). In contrast, Th2 cells produce IL-4, IL-5, and IL-10 amongst other cytokines, and are proficient in providing B cell help. Th2 cytokines stimulate the production of IgE, non-complement-fixing antibodies (IgG1 and IgG4), and stimulate mast cell and eosinophil differentiation (Mosmann et al. 1986).

In schistosomiasis, there has been a major effort to identify vaccines capable of inducing high levels of resistance against infection with the parasite. Current experimental vaccines however, provide at best only 30-80% protection from parasite challenge (Bergquist et al. 1994). The reasons for the failure to achieve complete immunity remain unclear. Moreover, there is considerable debate concerning the immunologic effector mechanisms responsible for the rejection of challenge parasites in resistant animals with evidence supporting a function for Th1 dependent cell-mediated immunity and other find-

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ings indicating a role for humoral, Th2 based responses. A strategy which we have employed to begin to improve vaccine efficacy has been to define a mechanism(s) of immunity operating in a given vaccination protocol and to attempt to enhance that response by immunomodulation.

A frequently used model in the study of schistosome immunity is the induction of resistance in mice by immunization with radiation-attenuated cercariae. After a single exposure to this vaccine, animals eliminate 60-70% of the worms ordinarily developing from a challenge infection (James et al. 1984). The resistance is dependent on $CD4^+$ T lymphocytes (Vignali et al. 1989) and appears to be associated with the function of Th1 rather than Th2 type cytokines (Pearce et al. 1991). Thus treatment of vaccinated mice with antibodies to IFN-γ causes a reduction in immunity, while antibodies against IL-4 and IL-5 are without effect (Sher et al. 1990).

Cell-mediated immunity involving IFN-γ-activated effector cells is the major mechanism of protective immunity in this model. The schistosomula may either become trapped in (Smythies et al. 1992) or deflected out of the lung (Dean & Mangold 1992, Kassim et al. 1992) due to vigorous inflammatory foci that form around them as they migrate through the lungs. There is also strong evidence that they may be directly killed in the lungs by toxic factors produced by macrophages or endothelial cells that have been activated with combinations of several cytokines including IFN-γ, TNF-α, IL-1, and IL-2 (Oswald et al. 1994a). This response requires the arginine-dependent production of nitrogen oxides (Oswald et al. 1994b). Our studies have demonstrated that a similar cytokine micro-environment conducive to the activation of effector cells is present in the lungs of vaccinated mice (Wynn et al. 1994, 1995), the proposed site of parasite elimination (Wilson & Coulson 1989). Indeed high levels of NO synthase (the enzyme which drives NO production) were demonstrated in the inflammatory foci surrounding the challenge parasites in vaccinated mice (Wynn et al. 1994). Nevertheless, the significant expression within the same tissues of IL-4, IL-10, and IL-13, cytokines known to inhibit macrophage/endothelial cell activation suggested that these effector mechanisms may not be operating optimally *in vivo* (Wynn et al. 1994, 1995). Therefore we hypothesized that the co-expression of macrophage de-activating Th2-associated cytokines may explain the inability of the attenuated cercariae vaccine to provide complete protection.

We have also analyzed cytokine production during the period of vaccination and compared the response in the lungs to irradiated with normal cercariae in order to begin to understand why attenuated but not normal larvae stimulate protective immunity. As was also observed at the time of challenge (Wynn et al. 1994), both Th1 and Th2-associated cytokine mRNAs were induced following exposure to irradiated cercariae and with similar kinetics (Wynn et al. 1995). In contrast, unattenuated cercariae failed to trigger significant increases in IFN-γ mRNA expression until 8 to 10 days after Th2 responses were first detected. Irradiated parasites therefore appear to induce a mixed Th1/Th2 response *in vivo* that fails to polarize during the initial vaccination period into a dominant Th1 or Th2 mRNA phenotype. Interestingly however, infection with unattenuated parasites is associated with a more rapid progression towards a Th2-dominated response, although IFN-γ mRNA was detected at later times (Wynn et al. 1995). The observed variation in cytokine induction profile may reflect differences in migration, survival, or antigen expression by irradiated versus normal cercariae. Whether this disparate cytokine induction pattern explains the induction of protective immunity by attenuated as opposed to live infection however, still remains unclear.

IL-12 AS AN ADJUVANT FOR THE IRRADIATED CERCARIAE VACCINE

In an attempt to increase the efficacy of the irradiated cercariae vaccine, we have used IL-12, a potent inducer of IFN-γ production, as an adjuvant to drive Th1-responses and simultaneously suppress Th2 cell differentiation. Related studies have shown that administration of IL-12 either just before infection or shortly after exposure to a variety of infectious organisms has proven highly effective at eliminating or decreasing infection intensity. Animals infected with *Leishmania major* (Sypek et al. 1993, Heinzel et al. 1993), *Toxoplasma gondii* (Gazzinelli et al. 1993, Hunter et al. 1995), *Listeria monocytogenes* (Wagner et al. 1994), *Cryptococcus neoformans* (Clemons et al. 1995), *Plasmodium yoelii* (Sedegah et al. 1994), *Mycobacterium tuberculosis* (Cooper et al. 1995), and *Histoplasma capsulatum* (Zhou et al. 1995), have all benefitted from treatment with IL-12 if administered during the initial exposure to the pathogen. In our experiments however, IL-12 had little or no effect on immunity when administered alone. Nevertheless, when combined with irradiated cercariae, IL-12 enhanced the subsequent protection from a challenge infection (Wynn et al. 1995). This enhanced immunity was associated with a significant increase in Th1-associated cytokines at both the mRNA and protein level during the period of immunization. In contrast IL-4 and IL-5 production were markedly suppressed, an observation which was reflected in the significant reduction in serum IgE levels and tissue eosinophilia observed during the vaccination period. These results thus confirmed a lack of

a requirement for eosinophils and IgE in anti-schistosome immunity in mice (Sher et al. 1990).

In agreement with previous studies on post-challenge responses (Wynn et al. 1994), vaccinated mice demonstrated elevated expression of Th1-associated cytokines (IFN-γ, TNF-α, and IL-12 p40) with respect to challenged controls, while expression of the Th2 cytokines IL-4, IL-5, and IL-13 was unchanged or suppressed. Surprisingly, while IFN-γ, IL-12 and TNF-α were increased during vaccination, no significant increase in these cytokine mRNAs was observed at challenge, when compared with the already high levels observed in the vaccinated saline-treated controls except when IL-12 was administered both at the time of vaccination and again during the challenge infection (Wynn et al. 1995). Nevertheless, IFN-γ production was routinely elevated in lung-associated lymph node cultures upon re-stimulation in vitro with a soluble worm extract and IL-5 secretion decreased in both groups of IL-12 treated animals suggesting a reduction in overall Th2 activity and an increase in Th1. Again, the marked decrease in tissue eosinophilia in the same animals was consistent with this hypothesis. Thus the enhancement of vaccine-induced resistance by IL-12 appears to correlate closely with a reduction in Th2-related activity, suggesting that the major effect of IL-12 may be its inhibition of the production of these potent macrophage deactivating cytokines (Wynn et al. 1995) leading to enhanced Th1-associated anti-parasite effector mechanisms.

While the above studies demonstrated that IL-12 can be used as an adjuvant for the development of a strong cell-mediated immune response to the parasite, relatively little was known about its effects on the development of a protective humoral immune response. Cellular immune responses are often associated with low Ab production (Parish 1972). Thus, IL-12 administration might be expected to reduce this arm of the immune response. In agreement with these observations, IL-12 was shown to suppress antibody synthesis in a model where B cells are activated polyclonally with a sheep anti-mouse IgD antiserum (Morris et al. 1994). Interestingly, mice which have been immunized with irradiated cercariae two or more times in the absence of IL-12 develop IgG Abs that passively transfer resistance to naive recipients (Mangold & Dean 1986), indicating that in multiply immunized animals protective humoral immune responses are induced. We therefore wanted to test the effects of IL-12 on the development of protective humoral responses and used the multiple-immunization model (3X) to directly examine this question (Wynn et al. 1996).

In these studies, IL-12 was shown to markedly enhance the parasite specific antibody response induced by the 3X-immunization protocol, while maintaining a highly polarized Th1-type cytokine response (Wynn et al. 1996). Moreover, passive transfer experiments demonstrated that the antibodies induced were protective. Studies examining the type of antibody response stimulated by 3X/IL-12 immunization suggested that the enhanced protective response was associated with increases in IgG1, IgG2a, and most strikingly in IgG2b antibody isotypes. IgE antibodies and tissue eosinophil levels were markedly suppressed in all IL-12-treated animals whether immunized a single or multiple times with the irradiated parasites, again providing little evidence for a role of this effector mechanism in resistance in mice. In addition, to enhancing the humoral immune response against the parasite, IL-12 also enhanced the ability of Ag-elicited macrophages from the 3X-immunized mice to kill schistosomula in vitro (Wynn et al. 1996). Thus, the combined results from these studies suggest that IL-12, when incorporated as an adjuvant, may be useful for enhancing protective Ab responses while at the same time boosting Th1-type CMI.

These findings hold particular importance for schistosomiasis, since existing vaccines provide only 30-80% protection from infection in the mouse (Bergquist et al 1994). Therefore, an immunologically-based strategy which enhances both arms of the immune response may be a highly desirable trait for a vaccine aimed at preventing infection with a parasite which has evolved numerous mechanisms to subvert the host immune response. Regardless of the precise mechanism involved in parasite attrition, the data presented here clearly demonstrate that IL-12 can up-regulate protective immunity against schistosomes, at least in the attenuated vaccine model. Whether the cytokine will have similar effects on immunization with defined antigens has yet to be established and obviously will depend on the particular effector mechanism which each vaccine employs. Clearly, based on its dramatic effects as an adjuvant in vaccination against *L. major* (Afonso et al. 1994), *L. monocytogenes* (Miller et al. 1995) and *S. mansoni* (Wynn et al. 1995, 1996), IL-12 has enormous potential as an agent for modulating the outcome of immunization and may have broad application in the prevention of a variety of different infectious diseases.

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REFERENCES

Afonso LCC, Scharton TM, Vieira LQ, Wysocka M, Trinchieri G, Scott P 1993. The adjuvant effect of interleukin 12 in a vaccine against *Leishmania major*. *Science 263*: 235-237.

Bergquist NR, Hall BF, James S 1994. Schistosomiasis vaccine development: Translating basic research into practical results. *The Immunologist 2*:131-133.

Clemons KV, Brummer E, Stevens DA 1995. Cytokine treatment of central nervous system infection: efficacy of interleukin-12 alone and synergy with conventional antifungal therapy in experimental cryptococcosis. *Antimicrob agents chemother 38:* 460-464.

Cooper AM, Roberts AD, Rhoades ER, Callahan JE, Getzy DM , Orme IM 1995. The role of interleukin-12 in acquired immunity to *Mycobacterium tuberculosis* infection. *Immunol 84*: 423-432.

Dean DA, BL Mangold 1992. Evidence that both normal and immune elimination of *Schistosoma mansoni* take place at the lung stage of migration prior to parasite death. *Am J Trop Med Hyg 47*: 238-243.

Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A 1993. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon g by an intracellular parasite and induces resistance in T-cell-deficient hosts. *PNAS 90*: 6115-6119.

Heinzel FP, Schoenhaut DS, Rerko RM, Rosser LE, Gately MK 1993. Recombinant interleukin 12 cures mice infected with *Leishmania major*. *J Exp Med 177*: 1505-1509.

Hunter CA, Candolfi E, Subauste C, Van Cleave V, Remington JS 1995. Studies on the role of interleukin-12 in acute murine toxoplasmosis. *Immunology 84*: 16-20.

James SL, Natovitz PC, Farrar WL, Leonard EJ 1984. Macrophages as effector cells of protective immunity in murine schistosomiasis: macrophage activation in mice vaccinated with radiation-attenuated cercariae. *Infect Immun 44*: 569-577.

Kassim OO, Dean DA, Mangold BL, von Lichtenberg F 1992. Combined microautoradiographic and histopathologic analysis of the fate of challenge *Schistosoma mansoni* schistosomula in mice immunized with irradiated cercariae. *Am J Trop Med Hyg* 47: 231-235.

Mangold BL, DA Dean 1986. Passive transfer with serum and IgG antibodies of irradiated cercariae-induced resistance against *Schistosoma mansoni* in mice. *J Immunol* 136: 2644-2649.

Miller MA, Skeen MJ, Ziegler HK 1995. Nonviable bacterial antigens administered with IL-12 generate antigen-specific T cell responses and protective immunity against *Listeria monocytogenes*. *J Immunol 155*: 4817-4826.

Morris SC, Madden KB, Adamovicz JJ, Gause WC, Hubbard BR, Gately MK, Finkelman FD 1994. Effects of interluekin-12 on *in vivo* cytokine gene expression and Ig isotype selection. *J Immunol 152*: 1047-1055.

Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL 1986. Two types of murine helper T cell clone. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol 126*: 2348-2356.

Oswald IP, Eltoum I, Wynn TA, Schwartz B, Caspar P, Paulin D, Sher A, James SL 1994a. Endothelial cells are activated by cytokine treatment to kill and intravascular parasite, *Schistosoma mansoni*, through the production of nitric oxide. *Proc Natl Acad Sci USA 91*: 999-1003.

Oswald I P, Wynn TA, Sher A, James SL 1994b. NO as an effector molecule of parasite killing: modulation of its synthesis by cytokines. *Comp Biochem Physiol 108C*: 11-15.

Parish C R 1972. The relationship between humoral and cell-mediated immunity. *Transplant Rev 13*: 35-47.

Pearce EJ, Caspar P, Grzych JM, Lewis FA, Sher A 1991. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a prasitic helminth, Schistosoma mansoni. *J Exp Med 173*: 159-166.

Sedegah M, Finkelman F, Hoffman SL 1994. Interleukin 12 induction of interferon gamma-dependent protection against malaria. *Proc Natl Acad Sci 91*: 10700-10702.

Sher A, Coffman RL, Hieny S, Cheever AW 1990. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J Immunol 145*: 3911-3920.

Smythies LE, Pemberton RM, Coulson PS, Mountford AP, Wilson RA 1992. T cell-derived cytokines associated with pulmonary immune mechanisms in mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *J Immunol 148*: 1512-1519.

Sypek JP, Chung CL, Mayor SEH, Subramanyam JM, Goldman SJ, Sieburth DS, Wolf SF, Schaub RG 1993. Resolution of cutaneous leishmaniasis: Interleukin 12 initiates a protective T helper type 1 immune response. *J Exp Med 177*: 1797-1802.

Vignali DA, Crocker P, Bickle QD, Cobbold S, Waldmann H, Taylor MG 1989. A role for $CD4^+$ but not $CD8^+$ T cells in immunity to *Schistosoma mansoni* induced by 20 krad-irradiated and Ro 11-3128-terminated infections. *Immunol 67*: 466-473.

Wagner RD, Steinberg H, Brown JF, Czuprynski CJ 1994. Recombinant interleukin-12 enhances resistance of mice to *Listeria monocytogenes* infection. *Microb Pathog 17*: 175-186.

Wilson RA, Coulson PS 1989. Lung-phase immunity to schistosomes: a new perspective on an old problem. *Parasitol Today 5*: 274-276.

Wynn TA, Oswald IP, Eltoum IA, Caspar P, Lowenstein CJ, Lewis FA, James SL, Sher A 1994. Elevated expression of Th1 cytokines and nitric oxide synthase in the lungs of vaccinated mice after challenge infection with *Schistosoma mansoni. J Immunol 153*: 5200-5209.

Wynn TA, Jankovic D, Hieny S, Cheever AW, Sher A, 1995. IL-12 enhances vaccine-induced immunity to *Schistosoma mansoni* in mice and decreases T helper 2 cytokine expression, IgE production, and Tissue eosinophilia. *J Immunol 154*: 4701-4709.

Wynn TA, Reynolds A, James S, Cheever AW, Caspar P, Hieny S, Jankovic D, Strand M, Sher A 1996. IL-12 enhances vaccine-induced immunity to schistosomes by augmenting both humoral and cell-mediated immune responses against the parasite. *J Immunol 157*: 4068-4078.

Zhou P, Sieve MC, Bennett J, Kwon-Chung KJ, Tewari P, Gazzinelli RT, Sher A, Seder RA 1995. IL-12 prevents mortality in mice infected with *Histoplasma capsulatum* through induction of IFN-γ. *J Immunol 155*: 785-795.