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 naïve 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/saline-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 naïve 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 - Schistosoma 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-
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 cercariae. After a single exposure to this vaccine, mice by immunization with radiation-attenuated tosome immunity is the induction of resistance in response by immunomodulation.

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 chal-

lenge (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, Heinzl et al. 1993), *Toxoplasma gondii* (Gazzinelli et al. 1993, Hunter et al. 1995), *Listeria monocytogenes* (Wagner et al. 1994), *Cryptococcus neoformans* (Clemens et al. 1995), *Plasmodium yoelii* (Sedegah et al. 1994), *Mycobacterium tuberculosis* (Cooper et al. 1995), and *Histo-

plasma 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 maintaing 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 antibody isotypes. IgE antibody 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

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