Mechanisms of immunity to the respiratory pathogen *Bordetella pertussis* in normal and gene knockout mice: clearance of primary infection is not enhanced by therapeutic interleukin-12.

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The current understanding of the function of CD4+ T helper (Th) cells in immunity to infectious diseases is that antigen-specific Th1 cells which secrete interferon (IFN)-γ, interleukin (IL)-2 and tumour necrosis factor-β mediate cellular immunity, delayed type hypersensitivity and inflammatory responses, whereas Th2 cells which secrete IL-4, IL-5, and IL-6 are considered to be mainly responsible for the provision of specific T cell help for antibody production. Using a murine respiratory infection model we have demonstrated that CD4+ Th1 cells play a key role in the protective immune response to *B. pertussis* generated by natural infection. [1, 2]. We have also examined the immune responses and outcome of challenge following immunization with whole cell and acellular pertussis vaccines in our murine respiratory model. The rapid bacterial clearance observed in the lungs of mice which received the whole cell vaccine correlated with the induction of a Th1 type response[3]. In contrast an acellular vaccine generated Th2 cells and was associated with delayed clearance.

IL-12 is a key cytokine in the development of Th1 type responses, and is secreted by activated macrophages, B cells and dendritic cells. It has been shown to exert pleiotropic effects on cells of the immune system. IL-12 can induce the secretion of IFN-γ by natural killer cells and by CD4+ T cells and can promote the differentiation and development of Th1 cells from Th0 precursor populations [4]. Recently we demonstrated that intracellular infection of alveolar or other macrophages by *B. pertussis* or exposure to killed *B. pertussis* or purified LPS results in the production of IL-12 [5]. Furthermore the addition of IL-12 to an acellular vaccine switched the T cell response induced from Th2 to Th1 and enhanced clearance of bacteria from the lungs of normal BALB/c mice [5]. In the present study we have examined the effect of therapeutic administration of IL-12 during primary respiratory infection of immunologically naive BALB/c mice.

Mice which received a single intraperitoneal administration of recombinant murine IL-12 before aerosol challenge followed by a daily administration of 1.0 ng IL-12 showed no significant enhancement of bacterial clearance from the lungs (Fig. 1A). Delaying administration of IL-12 until day 11 post aerosol challenge again resulted in no significant alteration in the kinetics of bacterial clearance (data not shown). Indeed administration of higher doses of IL-12 (50 ng) at longer time intervals (Fig. 1B) gave rise to greater numbers of viable *B. pertussis* recovered from the lungs at early time points after challenge. Initial studies on the kinetics of pulmonary clearance of *B. pertussis* in gene knockout mice defective for the IFN-γ receptor revealed increased pathology, but no difference in bacterial elimination from the lungs. Taken together these findings indicate that although IL-12 may play an important role in the induction phase of vaccine mediated immunity against *B. pertussis*, the therapeutic administration of exogenous IL-12 cannot improve the rate of bacterial clearance from the lungs of naive animals.

Our findings do not preclude a role for IL-12 or IL-12-induced IFN-γ in the protective immune response to *B. pertussis*. Indeed it may be that the failure of IL-12 to enhance clearance merely reflects an inability to further stimulate an already dominant Th1 response. However the results do suggest that there is a degree of redundancy in the effector mechanisms employed during bacterial clearance from the lung.

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