Measuring cytokine responses to vaccination with live and killed BCG

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The members of the *M. tuberculosis* complex are extremely successful intracellular pathogens of many mammalian species. The ultimate goal of a successful immune response to intracellular pathogens is the activation of exudative macrophages newly arrived from the peripheral blood. Following North's observation that T-lymphocytes were required for this process, data which pointed to a central role for gamma interferon (IFN-γ) in this process began to accumulate. More recently the immunological paradigms that have been developed to explain immunity to infectious disease have been dominated by data which suggests that subsets of T-lymphocytes exist which differ in their ability to synthesise and secrete various cytokines. There is increasing evidence that these subsets are present in many species and that the relative ratio of these subsets determines the type of immune response generated by antigenic stimuli. Cell mediated response are driven by cytokines such as IL-2 and IFN-γ which are produced by *Type 1* T cells, while humoral responses are generated under the influence of *Type 2* cell cytokines such as IL-4 and IL-10. A third subset, called *Type 0* T cells, has also been recognised and these produce a mixture of *Type 1* and *Type 2* cytokines. These are thought to be the precursors of both *Type 1 and 2* T cells.

We have recently shown that killed BCG in oil (KO/BCG) is not protective against challenge with virulent *M. bovis*. This is consistent with work carried out in mice. We have also shown that vaccination with low doses of live BCG (L/BCG) can afford high levels (80-90%) of protection against tuberculosis as suggested by Bretscher (1992). We have therefore used this as an experimental model to attempt to identify differences in the protective and disease related immune responses to tuberculosis. We have measured cytokine levels in animals vaccinated and boosted with either low/medium doses of live BCG ($2x10^6$ cfu) or high doses of killed BCG ($5x10^7$ cfu) in oil adjuvant to determine if cytokine patterns found in animals which have developed protective immunity are consistent with *Type 1* cytokine responses. The data in Table 1 shows that vaccination with L/BCG is necessary to induce a protective immune response against tuberculosis. Parallel experiments using the more sensitive RT-PCR to measure IL-4 production have shown that while IL-4 is present before boosting in animals vaccinated with live or killed BCG. After boosting the IL-4 response is turned off in some of the animals vaccinated with L/BCG but not in those given KO/BCG. Taken to-

gether the data suggests that before boosting a *Type 0* response is generated in both groups of animals but that after boosting this switches to a protective *Type 1* response in some animals vaccinated with L/BCG. Animals given KO/BCG do not switch to a classical *Type 2* response in that IFN-γ and IL-4 are expressed concurrently.

Table 1: Cytokine profiles in deer vaccinated with live or killed BCG.

	Disease Status	IFN-γ Positive	IFN-γ Negative	% Protection
Live BCG	Nondiseased	2/4	0/4	
	Diseased	0/4	2/4	50%
	% Protection	100%	0%	
Killed BCG + Adjuvant	Nondiseased	0/5	0/5	
	Diseased	2/5	3/5	0%
	% Protection	0%	0%	

We have also found that IL-2 may also be present in relatively high concentrations in animals with large amounts of circulating antibody and active tuberculosis. This has led us to hypothesise that in the early part of the disease process an intermediary form of immune response (*Type 0/2*) is generated in which IL-4 is beginning to dominate the response but IL-2 and IFN-γ are still present. It is not until later in the disease process that IL-2, and possibly IFN-γ, are switched off and a more classic *Type 2* response is generated. We have recently found that 'hypervaccination' (3-6 doses) of mice and deer with KBCG results in shutdown of antigen specific IL-2 production. Despite this the antigen specific lymphocyte transformation activity is not affected, suggesting that another growth factor is involved. This phenomenon is also found in tuberculous animals with advanced disease. We are currently investigating whether IL-4 or other T cell growth factors are involved.

The data from animals vaccinated with BCG suggests that while IFN-γ is an important factor in the protective immune response. A single dose of BCG is not sufficient to induce IFN-γ production in all animals. Experimental infection trials on larger numbers of animals that while two doses of BCG can induce 80-90% protection against the development of pathology following challenge with

virulent *M. bovis,* one dose only gives 60% protection. This is an important observation as most vaccine regimes rely on a single dose. This may be a factor contributing to the variable efficacy of BCG which has been reported in the field. We are now addressing the question of how to ensure that all vaccinated animals convert to IFN-γ positive status without producing IL-4. It may require a number of doses with BCG or a recombinant BCG that secretes a cytokine which is immunopotentiating. Such a vaccine is currently being developed in this laboratory in association with Dr Mike O'Donnell (Boston, USA). The second question that arises from the data is whether it is more important that certain cytokines are switched off (e.g. IL-4), rather than others being switched on. The data suggests that IL-4 is immunodominant and overrides the positive effects of IFN-γ, so that switching it off may be crucial.

Bibliography

Bretscher, P. (1992). *Immunol. Today.* **13**: 342-345.