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The Immune Response to Tuberculosis

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Mycobacterial diseases, such as tuberculosis (TB) or leprosy, are characterized by high rates of infection but low rates of disease. While an estimated one third of the world's population is infected with *Mycobacterium tuberculosis*, only a small percentage of infected people will ultimately develop the disease. Some will develop the disease immediately following infection (primary infection), while others will control the infection subclinically for several years before developing disease (secondary infection or reactivation) (Fig. 1). The association between AIDS and TB has highlighted the importance of the immune system, and particularly CD4 T cells, in controlling or eliminating the infection. However, it is clear that there are other components of an effective immune response, and our understanding of the mechanisms involved in protective immunity is far from complete.

Cell-mediated Immunity

The basic cycle of events involved in controlling infection with *M. tuberculosis* is shown in Fig. 2. Bacteria are taken up by macrophages at the site of invasion (usually the lungs). Mycobacterial antigens are then presented in association with major histocompatibility complex (MHC) molecules, either by the macrophages themselves or by other antigen-presenting cells. These antigen–MHC complexes are recognized by specific T cells. Recognition is followed by a cascade of events which includes clonal expansion of T cells, cytokine production, and recruitment of other cells, particularly macrophages, to the site of infection. If the appropriate combination of cytokines is produced, the macrophages are activated to kill or suppress growth of mycobacteria.

Although this cycle of cellular recognition and molecular signalling is known to be important, many of the specific details are poorly understood. For example, we do not know if specific mycobacterial antigens are important for generating protective immunity, or whether many antigens, proteins or non-proteins, are involved. We do not know how the early events following phagocytosis by macrophages may influence the outcome of the various cellular interactions or the roles of the various T-cell subsets involved in the recognition of mycobacterial antigens and the production of cytokines. Although we do know something about which cytokines are important for generating protective immunity, how the correct combination of cytokines is regulated is not clear. And finally, the mechanisms employed by macrophages to kill or suppress growth of *M. tuberculosis* are not well understood (Fig 2b).

T-cell subsets and recognition of mycobacterial antigens

T cells can be subdivided into a series of subsets based on phenotypic characteristics, function and cytokine production. The primary mechanism by which mycobacterial infections are controlled involves $CD4^+$T cells which recognize mycobacterial antigens presented in association with MHC Class II molecules. Although this MHC Class II-restricted, $CD4^+$ T-cell response and activation of macrophages is thought to play a major role in protective immunity, it has become apparent in recent years that a range of other cellular recognition events are involved in the response to mycobacteria. For example, $CD8^+$, cytotoxic T cells have been shown to lyse mycobacteria-infected target cells (Kaleab et al 1990). Other, unusual T-cell subsets have been shown to recognize mycobacterial components. For example, T cells which express $\gamma\delta$ T-cell receptors (as opposed to the more usual $\alpha\beta$ receptor), expand in culture in-vitro when exposed to mycobacteria (O'Brien et al 1989; Pfeffer et al 1992). More recently $\alpha\beta$ T-cell receptor bearing cells which do not express either CD4 or CD8 molecules have been shown to recognize mycobacterial antigens in association with CD1 molecules, unusual non-MHC restriction elements (Beckman et al 1994; Sieling et al 1995). Unlike the conventional $CD4^+$ or $CD8^+$ T cell, both $\gamma\delta$ cells and CD1-restricted $\alpha\beta$ cells appear to recognize non-peptide molecules such as phenyl pyrophosphate derivatives in the case of $\gamma\delta$ (Tanaka et al 1995) and lipoarabinomannan or mycolic acids in the case of $\alpha\beta^+$, $CD4^-$ and $CD8^-$ T cells (Beckman et al 1994; Sieling et al 1995). The role of these unusual T-cell subsets in control of infection is not known. However, work using murine models of TB has emphasized the importance of some of these non-CD4 MHC Class II T-cell

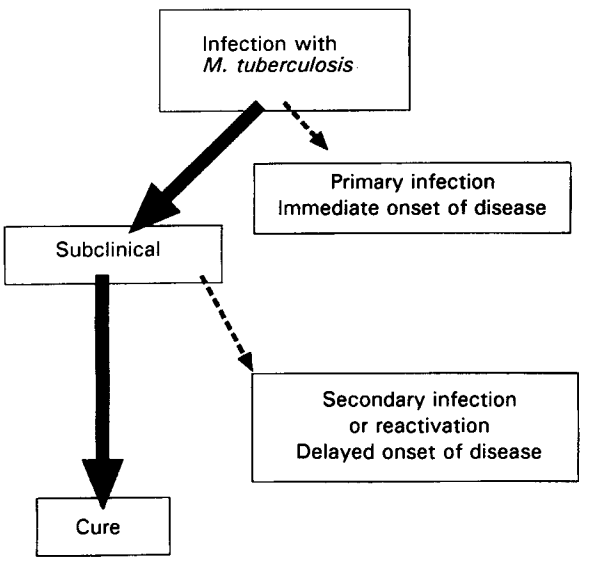

FIG. 1. Pathways following infection with *M. tuberculosis*.

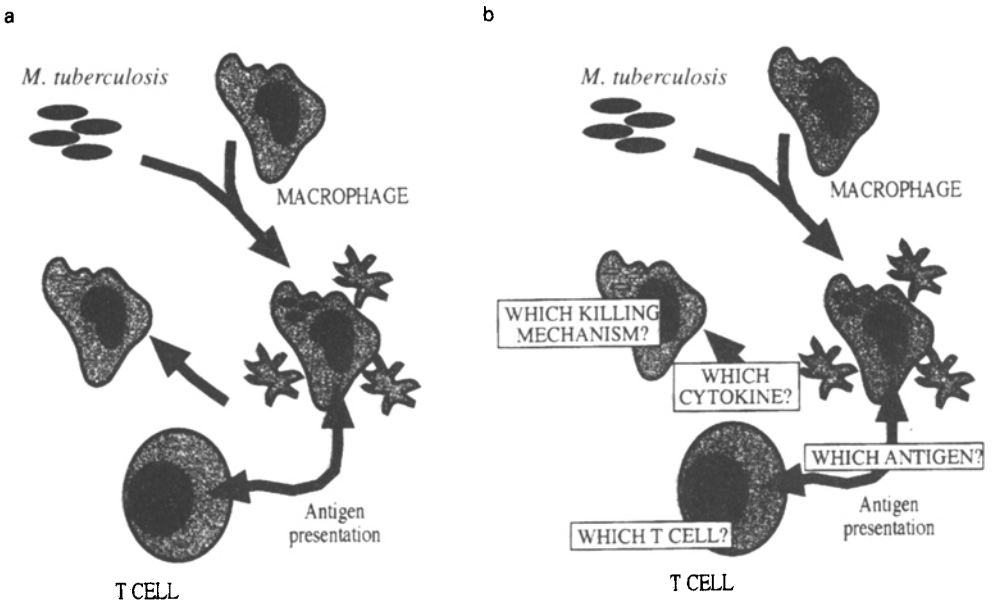

FIG. 2. Cycle of events involved in controlling infection with *M. tuberculosis*. Following phagocytosis by macrophages at the site of infection, growth of *M. tuberculosis* is controlled by the interaction of macrophages, antigen-presenting cells and T-lymphocytes (a). However, many of the details of this interaction remain unknown (b).

mechanisms. For example, mice which lack a CD8 T-cell response are more susceptible to infection than wild-type mice (Flynn et al 1992).

Protective immunity generated by vaccination with DNA

It has proved difficult to generate protective immunity against *M. tuberculosis* using purified mycobacterial proteins. One possible explanation for this is that immunization with purified protein tends to drive the response exclusively towards a CD4 pathway, whereas it may be necessary to activate other components of the response in addition to CD4 cells. In order to investigate this, we compared the protective immunity generated by immunization with a recombinant mycobacterial antigenic protein (hsp65), with that generated by immunization with naked DNA encoding that protein. Vaccination with DNA depends on the encoded antigen being transcribed and translated within tissue cells (Cox et al 1993; Ulmer et al 1993; Xiang et al 1994). The antigen which is thus synthesized intracellularly has the potential to be seen very differently by the immune system than one which is delivered by injection of purified protein, and includes the stimulation of both CD4 and cytotoxic CD8 cells. Our results demonstrate that vaccination with DNA encoding the mycobacterial hsp65 antigen is far more effective in generating protective immunity in a murine model of TB than is vaccination with purified, recombinant hsp65 protein (Tascon et al 1996; results summarized in Table 1).

Table 1. Protection in murine models of TB, afforded by immunization with DNA encoding the mycobacterial hsp65 antigen, control plasmid DNA and BCG (Tascon et al 1996).

Mouse strain	Protection		
	Control plasmid	hsp65 plasmid	BCG
Parkes	–	++	++
CBA/B10	–	++	++
Balb/c	–	+	++

Mycobacterial Antigens

Conventional T cells recognize proteins in the form of short stretches of amino acids presented in the context of MHC molecules. Many protein antigens have been identified and characterized by use of antibodies, particularly murine monoclonal antibodies; a number of laboratories produced monoclonal antibodies from mice immunized with *M. leprae*, *M. tuberculosis* or other mycobacteria (Engers et al 1986; Damiani et al 1988; Ljungqvist et al 1988). Although bacteria consist of several thousand proteins, the range of proteins recognized by antibodies produced in different laboratories was in fact, quite restricted leading to the concept of "immunodominance" (i.e. that there were certain proteins which were selectively recognized by the immune system). When these antigenic proteins were identified by screening expression libraries of mycobacterial DNA (Young et al 1985), several of them were found to belong to a group of molecules whose function is to protect the organism from environmental damage (Young et al 1988); thus the 70-kDa antigen belongs to the heat-shock protein (hsp) 70 family (Garsia et al 1989), the 65-kDa to the hsp65 family (Shinnick 1987), the 18-kDa antigen of *M. leprae* is related to low-molecular-weight hsps of other organisms (Booth et al 1988), the 10-kDa antigen is homologous to the hsp GroES of *Escherichia coli* (Mehra et al 1992) and the 28-kDa of *M. leprae* (23 kDa in *M. tuberculosis*) is the enzyme superoxide dismutase (Thangaraj et al 1990)

which protects organisms from damage induced by superoxide anions which is thought to be a major component of the antibacterial response of macrophages.

Following the identification of protein antigens using monoclonal antibodies to screen expression libraries, other approaches for antigen identification were employed, including screening with serum from infected individuals (Cherayil & Young 1988; Laal et al 1991; Sela et al 1991) or with T cells from immunized individuals (Mustafa et al 1988). Alternatively, attempts have been made to use soluble extracts of mycobacteria, separated according to size by polyacrylamide gel electrophoresis, and then using the fractionated material to drive T-cell proliferation (Abou-Zeid et al 1987; Lamb & Young 1987). Most of these studies served to emphasize the heterogeneity of the T-cell response (Schoel et al 1992). Recent advances in the structural chemistry of mycobacteria have greatly increased our understanding of the ways in which mycobacteria are recognized by, and interact with, the immune system. However, the way in which immunological recognition transfers into a protective immune response is less clear. One particular issue which remains unresolved, is whether there is a single antigen, or small number of antigens, which are capable of generating protective immunity, or whether many antigens are involved. Two papers have been published using DNA encoding different proteins, the hsp65 (Tascon et al 1996) and the 85A antigen (Huygen et al 1996). We also have preliminary data suggesting that four out of five antigens which we have used as DNA vaccines gave successful protection in our mouse model (Tascon, unpublished data). This would seem to suggest that protective immunity is not antigen specific. It appears that the way the antigen is presented to the immune system is the most important factor in determining the outcome of immunization.

The Mycobacteria–Macrophage Interaction

Macrophages play a key role in infection with *M. tuberculosis*. The normal portal of entry of the organism into the host is via alveolar macrophages, where the organism can survive asymptomatically, sometimes for many decades. Occasionally, in a proportion of infected people, after varying periods of time and in response to poorly understood factors, these intracellular bacilli start to divide, escape the macrophage environment and cause clinical disease. Thus, the initial interaction with the alveolar macrophage is likely to play a key role in determining the outcome of infection.

Phagocytosis of mycobacteria

The initial steps of entry of *M. tuberculosis* into host macrophages involve a number of specific receptor–ligand interactions. The most widely studied of these involve complement receptors CR1 (CD35), CR3 (CD11b/CD18) and CR4 (CD11c/CD18) (Schlesinger et al 1990; Schlesinger & Horwitz 1991). In *M. leprae*, the species-specific glycolipid (PGL-1) binds to the C3 receptor and mediates phagocytosis, and it seems likely that components of *M. tuberculosis* play a similar role. In addition to complement receptors, mannose receptors have been found to play a role in mediating uptake of *M. tuberculosis*. Interestingly, it appears that virulent strains involve both mannose and complement receptors, while avirulent strains utilize only complement receptors (Schlesinger 1993).

Most particles which are phagocytosed by macrophages are exposed to the acidic lysosomal compartment by fusion of the phagosome with lysosomes. Mycobacteria have evolved mechanisms for evading this fate by inhibiting phagosome-lysosome fusion (Armstrong & Hart 1971; Hart et al 1987; Crowle et al 1991). The mechanisms by which this is achieved are not well understood, particularly as some components of the endosomal–lysosomal system, in particular the late marker LAMP-1, do appear to be present in the infected vacuole (Xu et al 1994). Mycobacterial components, particularly LAM, can be found in vesicles separate from those containing mycobacteria, providing a mechanism by which such components can interact with regulatory systems in the macrophage (Xu et al 1994).

The macrophage response to invasion

The initial response of macrophages to mycobacterial invasion is likely to play a key role in determining the outcome of the interaction. The initial cytokine response of macrophages could be crucial in the overall regulation of the ensuing, acquired, response. The cytokine response can, for example, influence the initial growth of the mycobacteria. Growth of *M. avium* is stimulated by interleukin-1α and interleukin-6, but restricted by tumour necrosis factor-α and granulocyte–macrophage colony stimulating factor (Denis 1991).

In addition to cytokine responses of macrophages, there is a genetically-determined susceptibility to infection with mycobacteria in mice. The gene involved is called *Bcg*, *Ity* or *Lsh* and determines susceptibility or resistance to a wide range of intracellular pathogens (Skamene & Pietrangeli 1991). The *Bcg* gene has recently been identified and characterized, and has been found to code for a membrane transport protein called Nramp (natural resistance-associated macrophage protein) (Vidal et al 1993). *Bcg* is involved in the early, non-immune phase of infection, operating before the onset of acquired immunity (Gros et al 1981).

The infection of macrophages is likely to involve the regulation of many macrophage genes in addition to those

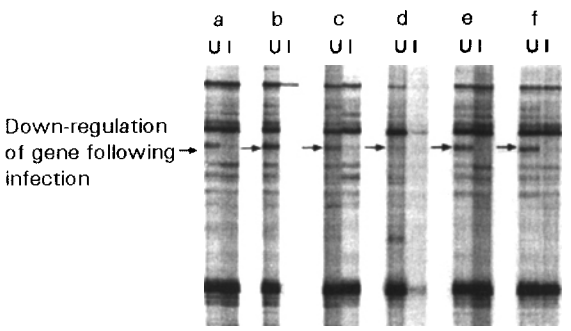

FIG. 3. Changes in macrophage gene expression following infection with *M. tuberculosis*, analysed by differential display reverse transcription polymerase chain reaction. The example shows a gene being down-regulated following infection. Mouse macrophages were infected and cultured at varying time intervals (6 h (a) and 1 (b), 2 (c), 3 (d), 4 (e) and 5 (f) days). Each time interval shows the comparison between uninfected (U) and infected (I) macrophages.

encoding cytokines. We have used a technique called differential display, reverse transcription polymerase chain reaction (DD-RTPCR, Liang & Pardee 1992), in order to investigate changes in macrophage gene expression following infection with *M. tuberculosis*. This analysis shows that there are complex changes in gene expression. An example of a gene being down-regulated in response to infection with *M. tuberculosis* is shown in Fig. 3. This gene has been characterized and shown to encode the enzyme cytochrome C oxidase (Ragno, Estrada-Garcia, Butler, Colston, unpublished data). The significance of this is unclear however, since the cytochrome system is involved in both oxidative metabolism and apoptosis, both of which have been implicated in macrophage killing of mycobacteria (Molloy et al 1994, it is possible that this could represent a mycobacterial evasion mechanism. Thus, it seems that *M. tuberculosis* could contribute to its own survival within macrophages by regulating its own intracellular environment.

Conclusion

The immune response against mycobacterial infection is complex and involves many components. The initial macrophage–mycobacterial interaction, the role of the many different T-cell subsets and how mycobacteria are eliminated by the immune response, are all poorly understood. It seems likely that in order to generate an effective, protective, immune response it is necessary to stimulate a precise cascade of appropriate responses. Unravelling the complexity of these interactions will require extensive dissection of the systems involved. However, already we are starting to see the fruits of this research with the development of novel vaccines, albeit at the early development stage, which could lead to new strategies for immunoprophylaxis and immunotherapy.

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