General Discussion

On March 24, 1882 Robert Koch made the landmark observation that the disease tuberculosis was caused by a bacterium *Mycobacterium tuberculosis* Although it was identified 117 years ego, the biology of the bacterium is still poorly understood Despite many decades devoted to research on *M tuberculosis*, and use of antitubercular drugs, tuberculosis remains the world's leading cause of death by a single infectious agent A recent report predicts a total of 225 million fresh cases and 79 million deaths from tuberculosis for the next three decades (Murray and Salomon, 1998) even if DOTS programs are implemented in their current form It also predicts that active case finding and a single contact treatment could save about 23 and 24 million lives, respectively A vaccine with 50 % protective efficacy will reduce the incidence by 36 million and mortality rate by 9 million Although DOTS has proven effective in treating tuberculosis, its implementation by national programs in various countries has been very poor Identification of an effective vaccine therefore may help enormously to reduce the burden of tuberculosis in the coming years

Our attempts to identify immunodominant antigens of *M. tuberculosis* resulted in identification of several genes of the bacterium The majority of these genes represented previously unknown genes of M tb Several immunodominant antigens of *M tuberculosis* were identified by other workers using either mouse monoclonal antibodies or rabbit serum in a similar manner However, the genes we identified using human serum did not include most of the already known antigens suggesting that a different set of proteins may be immunodominant in the host during active infection Earlier studies employed killed preparations to raise antibodies whereas we employed patient sera which is directed to proteins synthesized by live bacteria in tuberculosis patients We also observed that these antigens displayed differential immunogenicity The fact that immunization with live bacteria but not killed preparation can protect from a challenge of virulent *M tuberculosis* points to the important role of live-specific antigens as candidate vaccines, on the other hand, since these antigens are actively seen by the immune system of the host during disease they may also have a role in pathogenesis

Since it is a well established fact that T cell responses mediate protection in tuberculosis, we tested several recombinant proteins from our expression library for their ability to elicit T cell proliferation in PBMC obtained from TB patients as well as healthy contacts Among the antigens we tested, two elicited a strong T cell response only in healthy contacts and this response was absent in patients The responding T cells produced IFN-γ and TNF-α, both of which have been shown to play a major role in protection against tuberculosis in the mouse model (Flynn, et al , 1993, Flynn, et al , 1995, Appelberg, et al , 1994) Incorporation of these genes into BCG may result in a better vaccine for TB and is an approach we are currently exploring Furthermore, ARR213 is present only in pathogenic mycobacteria suggesting an important role for this protein in virulence In order to study these two roles independently, we are currently testing DNA vaccines expressing these proteins to protect guinea pigs against a challenge with virulent

M tuberculosis In addition, we are constructing a mutant of *M tuberculosis* with a disruption in the gene for ARR213 in order to study its virulence phenotype

Many pathogenic bacteria use the strategy of differential gene expression to mediate pathogenesis and modulate host functions (Straley et al, 1993, Menard et al, 1994, Kwaik and Pederson, 1996) Powerful molecular techniques such as subtractive hybridization and DDRT-PCR have been developed in recent years to identify such genes These techniques however require pure populations of messenger RNA to yield meaningful results We developed a technique to specifically polyadenylate messenger RNA from prokaryotes which allowed us to purify this population by oligodT affinity chromatography We further demonstrated that this technique could be used successfully to identify salt inducible genes of *E coli* by DDRT-PCR analysis of polyadenylated messages We are currently using mRNA obtained by this technique from *M tuberculosis* in subtractive hybridization to identify genes differentially expressed within the host Several genes of the bacterium as well as the host that are either up-regulated or down-regulated in response to infection are being analyzed