Extended Abstract

Recombinant antigens and synthetic peptides to characterize *Mycobacterium tuberculosis* proteins for immunological reactivity

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Abstract:

The control and eventual eradication of tuberculosis (TB) requires an effective vaccine and reagents for specific diagnosis. The only available vaccine against TB is the bacillus Calmette Guerin (BCG), but the protection imparted by BCG against pulmonary TB in adults varies between nil to 80%. Moreover, the use of BCG vaccination faces two additional problems: i. BCG vaccination induces a delayed type hypersensitivity response that cannot be distinguished from exposure to *M. tuberculosis*, and therefore it compromises the use of purified protein derivative (PPD) of *M. tuberculosis* for diagnostic purposes. ii. BCG being a live vaccine is contraindicated in HIV infected individuals for fear of causing disease by itself.

For diagnosis, PPD is routinely used as a skin test reagent for detection of *M. tuberculosis* infection. However, a positive PPD test may not distinguish between active disease, BCG vaccination, or sensitization with other mycobacteria. Moreover, antigenic components in PPD are not standardized and therefore PPD from different sources may vary in the skin test responses. Thus there is a need to identify *M. tuberculosis*-specific antigens to develop improved vaccines and specific diagnostic reagents.

The identification of secreted antigens in the culture filtrate of *M. tuberculosis* attracted attention because these are considered to be immunodominant and involved in protective immunity. To identify *M. tuberculosis*-specific antigens actively secreted, the antigens present in the short-term culture filtrate were tested for T cell reactivity with spleen cells obtained from memory immune mice. The results showed that spleen cells from the memory immune mice secreted large quantities of interferon- in response to an antigenic fraction containing a low 6 kDa protein known as ESAT6, relevant for the development of a subunit vaccine against TB.

The advances in biotechnology have greatly facilitated the identification of several major antigens of *M. tuberculosis*. However, the initial attempts to clone and express mycobacterial genes in other systems like *Escherichia coli* were met with failure. The first breakthrough in this field was reported by Young et al. who developed a lambda gt11 phage expression system for expression of mycobacterial genes in *Escherichia coli*.

The testing of recombinant *E. coli* with antibody probes resulted in the identification and characterization of several mycobacterial antigens. Further testing of these recombinant antigens with mycobacterial antigen reactive T cell lines and clones showed that most of the recombinant antigens identified by antibody probes were also recognized by T cells. Among these antigens, the antigens belonging to the family of heat shock proteins (hsp), i.e. hsp18, hsp60 and hsp70 were most frequently recognized by human T cells. However, the antigens recognized by antibodies may not always stimulate T cell responses, we therefore developed a system to directly screen T cell clones with the recombinant libraries to identify new antigens. This approach led to the identification and characterization of a novel 24 kDa lipoprotein antigen. However, all of the above antigens lacked specificity for *M. tuberculosis* as they were also present in BCG and other mycobacteria.

The cloning of specific genes in plasmid vectors and transformation of *E. coli* with the recombinant plasmids allowed the expression and purification of the expressed mycobacterial proteins. Testing of human T cells from tuberculosis patients with recombinant antigens showed that ESAT-6 was the major stimulator of human T cells in proliferation and IFN- assays. In the same assays, other secreted proteins, i.e. Ag85, MPT64 and MPB70 were also found to be better stimulators of human T cells as compared to the antigens of cytosolic origin. However, these proteins cannot be used in specific diagnosis of *M. tuberculosis* because their T cell epitopes are shared with BCG and other mycobacteria.

The full-length naturally purified or recombinantly produced antigens of mycobacteria, although less complex than the whole mycobacterial organisms, are still immunologically quite complex. Most of these antigens may contain up to several hundred amino acids (aa), whereas the T cell epitopes usually range between 8 to 20 aa in length. Thus a complete antigen may have a large number of T cell epitopes; some of which

could be beneficial and others harmful, e.g. heat shock proteins have been shown to have epitopes capable of inducing T cells of helper as well as suppressor/regulatory types. To exclusively identify the epitopes with protective potential, studies have been advanced to identify the epitopes of *M. tuberculosis* antigens recognized by human T cells of protective phenotype.

To identify the epitopes of major T cell antigens of *M. tuberculosis*, i.e. hsp18, hsp60, hsp70, ESAT-6, Ag85B, MPT64 and MPT70, peptides (18 to 25-mer, overlapping by 9 to 10 aa) covering the entire sequence of each protein were chemically synthesized. These peptides were tested with peripheral blood mononuclear cells as well as human T cell lines and clones.

The results showed that T cell epitopes were present in most of these proteins spanning the entire sequence and no single peptide could replace the complete protein. However, in some proteins, dominant epitopes recognized by T cells from majority of the tested donors could be identified. It was of particular interest that all of the 8 peptides covering the sequence of ESAT-6 were stimulatory for T cells, however, the dominant T cell epitopes varied in patients of different geographical locations, most probably due to differences in the genetic background.

Thus, to be universally useful as a diagnostic reagent for TB, a mixture of *M. tuberculosis*-specific synthetic peptides would be required. This has been achieved by using mixtures of synthetic peptides corresponding to ESAT6 and CFP10 in diagnostic applications using interferon-gamma release assays.

In conclusion, recombinant antigens and synthetic peptides have shown their usefulness in identifying immunodominant proteins of *M. tuberculosis*. Furthermore, the pools of synthetic peptides corresponding to *M. tuberculosis*-specific antigens are being extensively used throughout the world in the specific diagnosis of infections caused by *M. tuberculosis*.