

SUMMARY

Tuberculosis and leprosy continue to persist as major public health problems, and their challenge is becoming formidable with the advent of AIDS. Efficient vaccination is the method of choice for ultimate control and eradication of the infectious diseases. Certain inherent problems of integral vaccines have intensified the quest for molecular vaccines. For example, certain constituents of mycobacteria have been found to induce immunosuppression, autoimmunity and tissue necrosis; which could be the reasons behind less than expected success rates of BCG and other integral vaccines against tuberculosis and leprosy. This pursuit has resulted in the exquisite definition of an array of mycobacterial antigens (Wiker and Harboe, 1992; Young *et al.*, 1992). The focus has obviously been on protein antigens, since these molecules or their subunits are capable of generating a 'cell (T cell)-mediated immunity' (Germain, 1994), which is regarded as the principal host-protective mechanism against the intracellular pathogens, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* (Kaufmann, 1988).

Mycobacterial proteins well characterized to date are mainly the ones derived from the cytosol, cell wall, cell membrane or those released in the medium. Depending on the structural or functional features, these proteins have further been grouped under various categories, such as stress proteins (Young *et al.*, 1988), enzymes (Wheeler and Gregory, 1980) and lipoproteins (Young and Garbe, 1991). A good number of these proteins, particularly the cytosolic ones, have also been genetically cloned, and studies addressing vaccine or diagnostic potentials of some of these proteins are well underway (Young and Cole, 1993; Gelber *et al.*, 1994). Proteins working as vital enzymes or virulence factors for the microbe could serve as suitable targets for new drug development.

Mycobacterium habana (TMC 5135) has shown promising results as a candidate vaccine against tuberculosis and leprosy in the mouse model (Gupta *et*

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al.,1979; Singh *et al.*,1989). It could also prime monkeys for the lepromin skin test (Singh *et al.*,1991). Further, this cultivable, non-pathogenic mycobacterial strain was found to share some of the antigens with *Mycobacterium leprae* (Lamb *et al.*, 1990).

The present study pertains to isolation, purification and immunochemical characterization of a major 23kDa cytosolic protein antigen of the vaccine candidate, *Mycobacterium habana*. The 23kDa protein was the only protein to be salted out from the cytosol at an ammonium sulphate saturation of 80 to 95%. It represented about 1.5% of the total cytosolic protein, appeared glycosylated by PAS staining, and showed a pI of ~5.3. Its native molecular mass was determined as ~48kDa, suggesting a dimeric configuration.

Immunoblotting with the WHO-IMMLEP/IMMTUB monoclonal antibodies mc5041 and IT61, and activity staining after native PAGE established its identity as a mycobacterial superoxide dismutase (SOD) of Fe/Mn type. The sequence of N-terminal 18 aminoacids, which also contained the binding site for mc5041, showed a close resemblance not only with the reported deduced sequences of *Mycobacterium leprae* and *Mycobacterium tuberculosis* Fe/Mn SODs but also with human MnSOD.

In order to study its immunopathological relevance, the protein was subjected to *in vivo* and *in vitro* assays for T cell activation. It induced, in a dose related manner, skin delayed hypersensitivity in guinea pigs and lymphocyte proliferation in BALB/c mice primed with *Mycobacterium habana*. Most significantly, it also induced lymphocyte proliferative responses, in a manner analogous to *Mycobacterium leprae*, in human subjects comprising leprosy patients and healthy contacts.

SODs from pathogens bear considerable structural homology with the host enzyme. Such prominent homologies are viewed as a deterrent for considering the concerned parasite antigen in a subunit vaccine design. However, this

apprehension may appear unrealistic in the light of recently unravelled mechanisms of T cell activation. Further, specificity and stringency of the MHC-peptide interaction is governed by only 2-3 aminoacids, strategically located at 'anchor' positions along the peptide backbone (Germain, 1994). Thus, the non-homologous portions of a 'homologous' protein may still possess enough number of potentially antigenic segments.

Poor immunogenicity of the selected proteins/peptides is often encountered as an impediment in successful development of molecular vaccines. This obstacle can be removed by using an appropriate and biologically safe adjuvant or carrier. Liposomes are an attractive alternative as vehicles as the liposomised antigens can elicit both the humoral and cell-mediated immunity.

For mounting an effective immunity against infectious diseases, it is important to develop vaccines which besides eliciting the CD4⁺ T cell responses could also generate the CD8⁺ T cell response. The CD8⁺ T cell response is elicited only when the antigens are procured in the cytosol, and presented through MHC-I molecules. Exogenous antigen can enter the class-I processing pathway if delivered in the vehicle that has the property of undergoing fusion with the plasma or endosomal membrane.

Earlier studies from our laboratory demonstrated that the yeast lipids vesicles can fuse with the macrophages and consequently, could deliver a variety of the entrapped molecules to the cytoplasm of these cells. In the present study, the immunological responses of OVA have been examined after entrapping it in the yeast lipids vesicles. The results of these investigations confirm the earlier findings by demonstrating that OVA loaded in the yeast lipids DRVs can also generate strong CD8⁺ T lymphocyte responses, besides generating the CD4⁺ T cell response. This is, unlike the OVA loaded egg PC/Chol vesicles which generate only the OVA-specific CD4⁺ T lymphocyte responses. From these results it has been concluded that yeast

lipids vesicles may prove useful as antigen vesicles in designing of the effective vaccines against mycobacterial infections.