DNA BINDING PROTEIN OF MYCOBACTERIA AND HUMAN IMMUNE RESPONSE

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ABSTRACT

A dual step procedure was used to identify a 30 kDa DNA binding protein of mycobacteria (HLPMT) which is a target of human T and B cell response. The immunodominance of HLPMT was established by T cell blot assay as well as by subtractive immunoblot assay. This protein is not secreted into the extracellular culture fluid and is different from the 85 ABC complex of proteins as seen by immunoblots and ELISA. The protein is capable of inducing *in vitro* lymphoproliferation in tuberculin reactors. The protein was purified for the generation of monospecific sera and for amino acid sequencing. The sequence of the 16 amino acid long peptide derived from the 30 kDa protein showed a 100% homology with the translated sequence of a cosmid cY349 (Sanger Centre, Cambridge, UK). The ORF was predicted to code for a protein of 214 amino acids. Oligonucleotide primers were designed against the 5' and 3' end of the gene and the gene was PCR amplified, cloned and expressed in *E.coli*. The protein has unique dual domains which show homology to both bacterial HU proteins and to eukaryotic histones H1.

KEYWORDS : Human immunogenic mycobacterial proteins

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with the translated sequence of a cosmid cY349 (Sanger center Cambridge, UK). The ORF was predicted to code for a protein of 214 amino acids. Oligonucleotide primers were designed against the 5' and 3' end of the gene. T gene was PCR amplified, cloned and expressed in *E.coli*. The protein has unique dual domains which show homology to both bacterial HU proteins and to eukaryotic histones H1.

M.tuberculosis induces humoral as well as cell mediated immune responses in infected humans and experimental animals. In the characterization of mycobacterial antigens associated with human response, several mycobacterial proteins have been isolated using murine monoclonal antibodies that are in addition also recognized by the human immune system. However none of the reported antigens have led to successful designing of a novel vaccine against tuberculosis. This may be attributed to the fact that the perception of the mycobacterial antigens by human immune system thus differs from that in the murine

system. Therefore novel strategies need to be adopted to identify and select antigens capable of stimulating a human response.

Identification of antigens of *M.tuberculosis* with human immune response:

Two immunological assays were used to identify unique antigens of *M.tuberculosis* associated with human immune response namely T-cell blot (1) and subtractive immunoblot assays. **T-cell blot assay** : PBMC's isolated from 44 healthy individuals were co-cultured with nitrocellulose-bound fractionated antigens of *M. tuberculosis*. The fraction corresponding to molecules in the molecular weight range of approximately 30kDa were seen to induce a vigorous in vitro lymphoproliferative response in peripheral blood mononuclear cells (PBMC) derived from 81% (21/26) of the tuberculin reactors (S.I. of 6/ >). None of the fractions were found to be immunodominant by this assay in tuberculin non-reactors.

Subtractive immunoblot assay: Transblots of *M.tuberculosis* were exposed to a panel of characterized murine monoclonal antibodies specific for mycobacterial antigens. In the second step, these blots were exposed to individual sera/pools of sera derived from healthy contacts (15 reactors and 10 non-reactors) and 10 patients. Regardless of the source of the sera/pool of sera, predominant persistent immuno-reactivity was seen with mycobacterial antigens in the molecular weight of 30kDa and less. These observations parallel the result obtained in the T-cell blot assay where fractions corresponding to molecular weights of 30 and 14 to 21kDa antigens generated a vigorous lymphoproliferative response and was the dominant antigen seen in the subtractive immunoblot assays. This antigen(s) was further characterized to establish its relationship, if any, with other known mycobacterial proteins in the similar molecular weight range.

Lymphoproliferative response to the 30kDa antigen : PBMC isolated from healthy contacts were incubated with mycobacterial antigens bound to nitrocellulose. Mycobacterial sonic extract was used as a reference antigen for assessing lymphoproliferation. The 30kDa antigen elicited moderate to high proliferative response in 8 of the 10 tuberculin reactors. The proliferative response seen in cultures co-cultured with the 30kDa antigen was in the S.I. range of 2 - 6 as compared

to 2 - 8 in mycobacterial stimulated cultures. In 4 out of 5 tuberculin non-reactors neither the mycobacterial extracts nor the 30kDa protein was capable of inducing proliferation.

Murine sera specific for the 30kDa antigen

Monospecific serum was generated in mice against purified 30kDa antigen. Transblots of *M. tuberculosis* sonicates and culture filtrate proteins were probed separately. A single band at a molecular weight corresponding to 30kDa was seen with sonic extracts alone, indicating that the antigen is associated with the mycobacterial cell and is not secreted into the culture fluid. Simultaneously blots were probed with rabbit sera specified for the secretory antigens of the 85 ABC complex of mycobacteria (kindly provided by Dr. M. Harboe, Institute of Rheumatology, Oslo, Norway). The three components have molecular weights in the range of 30-32kDa. The antigens were detected both in the culture filtrate and sonic extracts, indicating that the 30kDa antigen is distinct from the secreted 85 complex of antigens. ELISA was carried out with serial dilutions of pre-immune and immune murine sera incubated with wells coated with sonically disrupted and integral tubercle bacilli. The antibody reactivity was seen exclusively with the immunized sera incubated with sonic extracts of the tubercle bacilli, indicating that the 30kDa antigen is not present on the surface of actively growing mycobacteria.

Intracellular localization of the 30kDa antigen:

In order to establish the subcellular distribution of the 30kDa antigen, immuno-gold labeling was carried out. Ultra-thin sections of the tubercle bacilli were treated serially with 30kDa antibody, anti-mouse rabbit sera and protein gold (10nm gold particle size). Control grids were exposed to ascitic fluid from unimmunized mice.

On scanning the probed grids, the gold particles were seen only in bacillary sections treated with immune sera. Rarely one/two particles were seen non-specifically located in the embedding resin/in the control sections. The gold particles were distributed randomly in the cytosol as well along the cytoplasmic face of the mycobacterial wall. The gold particles were absent on the surface. Ninety-one percent of the gold particles were found to be associated with the bacilli. These results provide the direct evidence of the intracellular locations of the 30kDa antigen in the tubercle bacilli confirming the exclusive detection of the antigen in sonically disrupted bacilli by

ELISA and immunoblotting techniques.

Acknowledgements

Summary

The results presented in this study show that the 30kDa protein is exclusively present in the cytoplasm and thus can prove to be an useful marker to monitor mycobacterial lysis mediated in vitro by immunological mechanisms or by antimycobacterial drugs.

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