Stress proteins are immune targets in leprosy and tuberculosis
(heat shock/dnaK/groEL/vaccines)

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ABSTRACT To understand the immune response to infection by tuberculosis and leprosy bacilli and to develop improved vaccines, the nature of antigens that are involved in humoral and cell-mediated immunity was investigated. We have determined that five immunodominant protein antigens under study are homologues of stress proteins. This finding and observations with other pathogens suggest that infectious agents may respond to the host environment by producing stress proteins and that these proteins can be important immune targets. We postulate that abundant and highly conserved stress proteins may have “immunoprophylactic” potential for a broad spectrum of human pathogens.

Mycobacterium tuberculosis and Mycobacterium leprae are the etiologic agents of tuberculosis and leprosy, respectively. These diseases afflict 20–30 million people and continue to present a significant global health problem (1, 2). To develop more effective tools for the diagnosis and prevention of these diseases, it is important to understand the immune response to infection by mycobacterial pathogens.

The antibody and T-cell responses to infection or inoculation with killed mycobacteria have been studied in humans and in animals. Human patients with tuberculosis or leprosy produce serum antibodies directed against at least 12 mycobacterial proteins. Some of these proteins are also recognized by well-characterized murine monoclonal antibodies. Mice immunized with mycobacterial lysates produce antibodies that are directed predominantly to six M. tuberculosis and six M. leprae protein antigens (3, 4). Genes encoding these 12 mycobacterial proteins have been cloned (5–7), and recombinant proteins produced from these clones have been used to investigate the human T-lymphocyte response to mycobacterial infection.

Protection against mycobacterial disease involves cell-mediated immunity (1, 8). T lymphocytes cloned from patients or from volunteers immunized with killed mycobacteria have been tested for their ability to recognize the recombinant mycobacterial proteins. Lymphocyte-proliferation assays demonstrate that most of the antigens identified with monoclonal antibodies are involved in the T-cell response to mycobacterial infection or vaccination in mice (9, 10) and in humans (11–20). Limiting dilution analysis indicates that 20% of the mycobacterial-reactive CD4+ T lymphocytes in mice immunized with M. tuberculosis recognize a single protein, the 65-kDa antigen (9).

In view of the involvement of these proteins in humoral and cell-mediated immune responses, we wished to obtain clues to the functions of these proteins in the mycobacterial cell and to sequence the DNA encoding several of the M. tuberculosis and M. leprae antigens. We report here that many of these mycobacterial protein antigens exhibit striking sequence similarity to known stress-induced proteins and discuss the implications of this observation.

MATERIALS AND METHODS

Recombinant DNA Clones. The isolation and characterization of M. tuberculosis and M. leprae Agt11 genomic DNA clones with murine monoclonal antibodies have been described (5, 6). DNA was isolated from these clones and was manipulated by standard procedures (21).

DNA Sequence Analysis. DNA was subcloned into vector M13mp18 or M13mp19 (New England Biolabs) as suggested by the supplier. Dideoxynucleotide chain-termination reactions and gel electrophoresis of the sequenced products were as described (22). DNA sequences were determined for both strands of DNA. Computer analysis of sequences with UWGCG programs was as described by Devereux et al. (23).

Immunoblot Analysis. Escherichia coli strain TG1 was transformed with the following plasmids by standard procedures (24) with selection for ampicillin resistance: pNDS, a derivative of pBR325 containing the E. coli groE genes (25); pUC8 (26); pUC8 with the insert DNA from Agt11 clone Y3178 (M. leprae 65-kDa antigen, ref. 6) ligated in the EcoRI site.

Overnight cultures of E. coli strains in Luria–Bertani (LB) medium were centrifuged and resuspended in isosonic phosphate-buffered saline at a cell density corresponding to an absorbance of 20 at 600 nm. An equal volume of sample buffer containing 2% (wt/vol) NaDodSO4 was added, and, after heating on a boiling water bath for 2 min, 5-μl samples were electrophoresed on 12% (wt/vol) polyacrylamide gels in the presence of NaDodSO4. Blots were prepared by electrophoretic transfer of the proteins to a nitrocellulose membrane, and binding of monoclonal antibodies was assayed with a peroxidase-conjugated secondary antibody as described (27).

RESULTS AND DISCUSSION

Six M. tuberculosis and six M. leprae proteins have been implicated in the immune response to the mycobacterial pathogens (Table 1). To obtain clues to the normal cellular function of several of these mycobacterial antigens, DNA clones encoding these proteins, isolated by using monoclonal antibodies to probe Agt11 libraries (5, 6), were subjected to sequence analysis. The sequences elucidated have been submitted to the GenBank sequence database. The Mycobacterial 71-kDa Antigen. The 71-kDa antigen of M. tuberculosis is recognized by human T cells during infection (Table 1). The insert DNA of Agt11 clone Y3271 (5)

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The sequences for these proteins have been deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03838–J03840).
**Table 1. Mycobacterial protein antigens**

<table>
<thead>
<tr>
<th>Protein, kDa</th>
<th>Recognized by human T cells</th>
<th>Subjected to sequence analysis</th>
<th>Homology with known proteins</th>
<th>Ref(s).</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>+</td>
<td>DnaK</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>+</td>
<td>GroEL</td>
<td>17, 19</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>+</td>
<td>None</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>-</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>M. leprae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>ND</td>
<td>DnaK</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>+</td>
<td>GroEL</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>+</td>
<td>-</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>Plant Hsp</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>-</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Mycobacterial protein antigens, their recognition by human T cells, and homology of the deduced mycobacterial protein sequences to known proteins are summarized. ND, not determined. +, Yes; −, no.

*Includes data derived from study of the 65-kDa antigen of *M. bovis* BCG (bacillus Calmette-Guérin), which is identical to the *M. tuberculosis* 65-kDa antigen.


was sequenced to obtain amino acid sequence information for the 71-kDa antigen of *M. tuberculosis*. This clone produces a β-galactosidase fusion protein containing the carboxy-terminal one-third of the 71-kDa antigen. A comparison of the sequence obtained for the carboxy-terminal one-third of the 71-kDa antigen with those in the GenBank database revealed that the *M. tuberculosis* 71-kDa antigen exhibits 40% amino acid sequence identity with the comparable segment of the *dnaK* gene product from *E. coli* (28) (Fig. 1). Fig. 2A shows the extent of sequence similarity between portions of the mycobacterial and the *E. coli* 70-kDa polypeptides. Sequences transcriptionally downstream from the mycobacterial 71-kDa gene predict a 356-amino acid protein homologous to the *E. coli dnaJ* gene product (unpublished data), indicating that the *E. coli dnaK–dnaJ* operon structure is conserved in *M. tuberculosis* and consistent with the conclusion that the mycobacterial 71-kDa antigen is a homologue of the *E. coli dnaK* gene product. The product of the *dnaK* gene is a member of the 70-kDa heat shock protein family that is highly conserved among prokaryotes and eukaryotes (28, 29).

The *leprae* 70-kDa antigen cross-reacts with monoclonal antibodies directed to the *M. tuberculosis* 71-kDa protein (27). The similarity in size and antigenicity indicate that these two mycobacterial proteins are homologues. Thus, we conclude that the 70-kDa antigens of *M. tuberculosis* and *M. leprae* are both members of the 70-kDa heat shock protein family of stress proteins.

The Mycobacterial 65-kDa Antigen. The 65-kDa antigens of *M. tuberculosis* and *M. leprae* are involved in the human T-cell response to mycobacterial infection (Table 1). Genes encoding these proteins have been isolated (5, 6) and sequenced (30, 31), revealing that the amino acid sequences of the 65-kDa antigens of *M. tuberculosis* and *M. leprae* are 95% identical. These protein sequences exhibit no significant sequence similarity to proteins in the GenBank database.

To identify these proteins, we exploited the observation that some monoclonal antibodies directed against the mycobacterial 65-kDa antigens cross-react with an *E. coli* protein of 60 kDa (Fig. 3). We observed that *E. coli* cells transformed

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*EMBL/GenBank Genetic Sequence Database (1986) GenBank (IntelliGenetics, Mountain View, CA), Tape Release 46.0*
proteins. Some stress proteins, including the products of dnaK and groEL, are major constituents of the cell under normal growth conditions and are induced to even higher levels during stress (29, 33). In retrospect, the fact that stress-related proteins are targets of the immune response could have been anticipated. One might expect that immunodominant antigens would be found among such abundant proteins.

Although the function of stress proteins is not entirely clear, it appears that some participate in assembly and structural stabilization of certain cellular and viral proteins (33–36), and their presence at high concentrations may have an additional stabilizing effect during exposure to adverse conditions. Phagocytic host cells produce a hostile environment for foreign organisms, and the ability to produce stress proteins has been implicated in the survival of bacterial pathogens within macrophages (37, 38). We postulate that these abundant proteins are then among the major antigens available for presentation to T lymphocytes and that this may contribute to their antigenicity.

Stress proteins may be common immune targets in a broad spectrum of infectious diseases. Sequence analysis has revealed 70-kDa heat shock protein homologues among major antigens of the protozoan parasites Plasmodium falciparum (39) and Schistosoma mansoni (40) and the filarial parasite Brugia malayi (41). Similarly, homologues of GroEL have been found among antigens involved in the immune response to Salmonella typhimurium and Coxiella (42). The presence of stress proteins among major immune targets in a variety of human pathogens suggests that the stress response may be a general component of infection and that stress proteins should be considered among candidates for subunit vaccines. Moreover, because the stress response is common to prokaryotes and eukaryotes and some stress proteins are highly conserved in sequence (28, 29, 43), it is possible that an antigen from one pathogen could immunize against another pathogen. Exposure to foreign stress proteins early in life might, in fact, induce a degree of immunity to a variety of infectious agents. If so, the presence of this immunity could provide an explanation for the observation that, for many pathogens, only a fraction of infected individuals actually acquire clinical disease.

Immunization with conserved protein antigens, whether during natural infection or during vaccination, might also have negative consequences. Presentation of a self-like determinant may fail to stimulate lymphocytes due to host tolerance. Chronic presentation of such determinants might occasionally result in breaking of tolerance and production of an autoimmune response.

We have observed an intriguing relationship between stress proteins and the immune response to mycobacterial infection. A more detailed examination of stress protein determinants and immune response mechanisms is essential to understanding the relationships among stress proteins, infection, and immunity.

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