Humoral Response to Mycobacterial Heat Shock Proteins in Patients with Constrictive Pericarditis Caused by Tuberculosis and Its Implications for Pathogenesis

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Tuberculous pericarditis is one of the commonest causes of cardiac failure in Transkei and the surrounding regions in southeast Africa. About 20% of patients with clinically diagnosed tuberculous pericardial effusion go on to develop pericardial fibrosis (i.e., constriction), a complication which is associated with significant mortality and morbidity. The pathological mechanisms underlying this aberrant inflammatory response are poorly understood, and there is a lack of reliable pointers (clinical or laboratory) in predicting the likelihood of development of constriction. We studied the humoral response to mycobacterial heat shock proteins (65 and 71 kDa) in 25 patients with culture-positive tuberculous pericardial effusion and found a significant correlation between high anti-mycobacterial hsps antibody titers (before treatment) and subsequent development of fibrosis ($P = 0.035$ by logistic regression), which is independent of the effect of the use of prednisolone as adjuvant therapy. Possible mechanisms underlying the pathogenesis of pericardial constriction in tuberculosis are postulated.

Mycobacteria are rich in antigens capable of stimulating both T- and B-cell responses in mammals. A significant proportion (up to 20%) of the cell-mediated response generated is directed at the mycobacterial heat shock proteins (M-hsp) (6, 8). The humoral response to some of these proteins such as M-hsp70 (71/70 kDa) is also exaggerated in patients with active tuberculosis (TB) (4). Because of the antigenic cross-reactivity shared between these proteins and their human homologs (reviewed in reference 20), considerable interest has been generated in establishing their role in the pathogenesis of autoimmune diseases such as rheumatoid arthritis. In a previous study (4), we demonstrated that elevated antibody titers against the 71-kDa M-hsp were also present in a small group of patients with nontuberculous lung fibrosis (of unknown etiology) and proposed that this specific serological response could be used as a marker of fibrosis. Chronic inflammation of unknown etiology which results in substantial fibrosis is usually due to an aberrant immune response or hypersensitivity reaction (e.g., in autoimmune diseases). Fibrosis of this type is also seen in tuberculous constrictive pericarditis, which is caused by a hypersensitivity reaction resulting from the rupture of tuberculous lymph nodes (mediastinal or paratracheal) into the pericardial sac. In this study we investigated the serum antibody responses against the 65- and 71-kDa M-hsp by an indirect enzyme-linked immunosorbent assay (ELISA) in 25 patients with microbiologically confirmed tuberculous pericardial effusion and found a significant association between high anti-65-kDa M-hsp antibody titers and subsequent development of pericardial fibrosis and constriction.

MATERIALS AND METHODS

Subjects and sera. Sera from a total of 65 subjects were tested. Of the 65 subjects, 25 patients from a previously reported clinical trial (14) had clinically diagnosed and microbiologically confirmed (by culture of pericardial fluid) tuberculosis pericardial effusion and 40 were controls who did not have TB. Fourteen of these patients with pericardial TB were randomized in the original trial to receive prednisolone (in addition to standard antituberculous therapy), while the remainder 11 received placebo tablets. The sera were collected from Northwick Park Hospital, London, United Kingdom (15 controls), and Umtata Hospital, Transkei (25 pericardial TB patients and 25 controls). Of the 40 control subjects, 15 (from Northwick Park Hospital) had known diagnoses other than TB which were divided into five categories and are summarized in Table 1. The remaining 25 controls (from Transkei) had diseases simulating tuberculosis (unspecified) and no history of recent or earlier TB. All sera from TB patients were collected before the institution of antituberculous therapy. All sera were stored in aliquots at $-20^\circ$C and tested blind to eliminate operator bias.

Purified recombinant antigens. Both the Mycobacterium bovis BCG recombinant 65-kDa (r65-kDa) and Mycobacterium tuberculosis r71-kDa antigens were kindly prepared and purified by J. Kamerbeek and J. van Embden, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. The r65-kDa protein was obtained from Escherichia coli K-12 strain M1164, carrying plasmid pRIB1300 (16, 18), and the r71-kDa protein was obtained from E. coli K-12 strain M1485, carrying plasmid Y3111/8 (11).

Indirect ELISA. Flat-bottomed flexible polystyrene microtiter plates (Falcon 1413 Microtest III flexible assay plates; Becton Dickinson Labware, Oxnard, Calif.) were used. These were coated with 50 $\mu$l of 10-µg/ml recombinant mycobacterial antigen (dissolved in phosphate-buffered saline [PBS]) or PBS
TABLE 1. Known diagnoses of 15 control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putative mycobacterial condition (Putat.)</td>
<td>Rheumatoid arthritis</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary infection/inflammatory condition (PI)</td>
<td>Lung abscess</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pneumonia</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Legionianna's disease</td>
<td>1</td>
</tr>
<tr>
<td>Nonpulmonary infection/inflammatory condition (NPI)</td>
<td>Pelvic abscess</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bacterial epididymitis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sinusitis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Viral meningitis</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Hydatid disease</td>
<td>1</td>
</tr>
<tr>
<td>Malignancy (Malig.)</td>
<td>Lung cancer</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>Diarrhea</td>
<td>1</td>
</tr>
</tbody>
</table>

alone per well and held overnight at 4°C. After being washed once with PBS containing 0.5% Tween 20 (BDH, Poole, United Kingdom) (PBST), the wells were incubated with 200 μl of 2% skimmed milk in PBST at 37°C for 1 h. The liquid was tipped off, and the plates were patted dry. Three dilutions of human sera (1:50, 1:250, 1:1,250; 50 μl each) in 3% bovine serum albumin (BSA) solution containing 0.2% Tween 20 (BSA-Tween) were added to triplicate wells (one coated with PBS only) and incubated for 1 h at 37°C. The wells were then washed five times with PBST and patted dry. Affinity-isolated Fe-specific goat anti-human immunoglobulin G (IgG) peroxidase conjugate (Sigma, St. Louis, Mo.) diluted in BSA-Tween to 1/1,000 was added at 50 μl per well, and the plates were incubated for 1 h at 37°C. After a further five washes, the plates were dried and 50 μl of 2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS) solution (0.5 mg of ABTS per ml in phosphate-citrate buffer [pH 4] with 0.01% hydrogen peroxide) was added per well. The A405 was read (40 min after the addition of ABTS) in a Titertek Multiskan spectrophotometer. Antibody titers were expressed as the dilution of serum giving 50% of the plateau binding value of a standard hyperimmune serum (ABTS0) (4) after the value of nonspecific binding to wells coated with PBS had been subtracted from all values (to keep mean optical density ± standard deviation due to nonspecific binding amounts to 0.1 ± 0.03). Antibody titers of less than 50 were calculated by extrapolation from the linear regression lines (derived from the three serum dilutions; r ≥ 0.9 in most cases). All titers of less than 1 were recorded as zero.

Statistical analysis. Statistical analysis was done by using the SPSS/PC+ Statistics 4.0 (SPSS Inc., Chicago, Ill.) package as specified in Results. Curve-fitting by linear regression was done by using Fig.P (Biosoft, Cambridge, United Kingdom).

RESULTS

Non-TB controls. For the African controls (n = 25), the mean ABTS0 values against the r65-kDa and r71-kDa antigens were 13 (range, 0 to 155) and 52 (range, 0 to 223), respectively. For the British controls (n = 15), the mean ABTS0 values were 59 (range, 0 to 349) and 92 (range, 0 to 267), respectively (Fig. 1 and 2).

Tuberculous pericarditis patients. (i) r65-kDa IgG antibody. The mean ABTS0 value for patients with tuberculous pericarditis (n = 25) was 21 (range, 0 to 280). Twelve (48%) patients subsequently developed signs of pericardial constriction (constrictors) despite standard antituberculous therapy (14) (time to development of constriction ranged from 2 weeks to 4 months [mean, 6 weeks]). Of these, four received prednisolone and eight received placebo tablets initially (among those who did not develop constriction, 10 were given prednisolone and 3 were given placebo). The mean r65-kDa ABTS0 values for the constrictor (n = 12) and nonconstrictor (n = 13) groups were 36.7 (range, 0 to 280) and 6.6 (range, 0 to 50), respectively. The results are shown in Fig. 1.

(ii) r71-kDa IgG antibody. The mean ABTS0 value was 41 (range, 0 to 137). The mean r71-kDa ABTS0 values for the constrictor and nonconstrictor groups were 39 (range, 0 to 106) and 42 (range, 0 to 137), respectively (Fig. 2).

Statistical analysis. The effects of r65-kDa IgG seropositivity and prior prednisolone therapy on the development of constriction were further analyzed by using a logistic regression
model (with the cutoff titer for r65-kDa IgG arbitrarily chosen at 4). The r65-kDa IgG seropositive rates for the constrictor and nonconstrictor groups were 58% (7 of 12) and 15% (2 of 13), respectively ($P = 0.035$ by logistic regression). The effect of adjunctive steroid therapy resulting in a reduction in fibrotic sequelae was also statistically significant ($P = 0.04$). The effects of seropositivity and steroid were found to be independent of each other ($P = 0.3426$ for the interaction by logistic regression). This analysis was not performed on the r71-kDa IgG data because of the similarity in the distribution of antibody titers between the constrictor and nonconstrictor groups.

The use of the r65-kDa ABT$_{50}$ value alone in predicting the likelihood of development of constriction was associated with a positive predictive value of 0.8 and negative predictive value of 0.7.

There was no significant difference in the mean r65-kDa or r71-kDa ABT$_{50}$ values among the African controls ($n = 25$), British controls ($n = 15$), and the patients with tuberculous pericarditis ($n = 25$) ($P = 0.4945$ and 0.5152 by the Kruskall-Wallis test for r65-kDa and r71-kDa values, respectively). The r65-kDa and r71-kDa titers were closely correlated among the pericarditis patients (Spearman correlation coefficient $= 0.6432$; $P < 0.002$) but not in the corresponding African control group (Spearman correlation coefficient $= 0.2974$; $P = 0.07$).

**DISCUSSION**

The 65-kDa M. bovis antigen shares at least four identical regions (of 10 or more amino acids) with the human GroEL protein (also known as the human hsp58 [H-hsp58]) (12). Both are homologous with the E. coli GroEL protein (21), a cytosolic chaperonin protein which functions primarily to mediate the folding of newly synthesized polypeptides into their final conformation before export (10). The surface expression of the human GroEL protein (normally located in the mitochondrial compartment) or its cross-reactive epitopes can be induced by cytokines such as gamma interferon (19) and may serve as a marker of active inflammation in certain tissues such as alveolar lining or synovium (5). It is conceivable (but not demonstrated) that the same process may also occur in chronically inflamed pericardium as a result of tuberculous infection. The surface expression of H-hsp58 or its epitopes may then provide potential targets, by virtue of the cross-reactivity of H-hsp58, for the host cytotoxic T lymphocytes generated in response to the invading mycobacteria (9).

We have shown in this study a previously undocumented association (independent of the effect of steroid) between high IgG antibody titers against the M. bovis 65-kDa antigen and subsequent development of fibrosis (constriction) in patients with tuberculous pericarditis. It remains to be seen whether the antibodies produced are autoreactive and capable of binding to the autologous epitope sites expressed in the diseased pericardium, thereby providing an alternative (antibody-dependent) mechanism of cell-mediated cytotoxicity. These two (T-cell-mediated and antibody-dependent) mechanisms may operate synergistically in bringing about the fibrotic sequelae in pericardial TB.

Regardless of the effector cell type, any aberrant immune response raised against H-hsp58 (a dominant self-antigen) would normally be tightly controlled by the naturally existing anti-idiotypic networks to prevent the development of pnenious autoimmune disease (reviewed in references 1 and 2). Factors which suppress these regulatory networks and hence facilitate the development of pericardial fibrosis are at present unknown.

The measurement of serum antibody titers against the M. bovis 65-kDa antigen may be used to assist in the clinical management of patients with tuberculous pericarditis. Despite antituberculous chemotherapy, about 15 to 20% of patients who present with clinically diagnosed tuberculous pericardial effusion will subsequently develop constriction (13), a complication which is associated with significant mortality (4 to 11%) and morbidity (15). The ability to identify the group of patients with an increased propensity to develop constriction may enable us to tailor the use of immunomodulatory agents to the individual’s “susceptibility.” Diagnostically, the antibody response to neither of the stress proteins could be used to differentiate pericardial TB from other nontuberculous inflammatory conditions. This is somewhat surprising in view of our previous finding of elevated antibody titers against the M. tuberculosis 71-kDa antigen in patients with active TB (4). However, nearly two-thirds of these patients had pulmonary disease. The apparent discrepancy between the results of the two studies may therefore be accounted for by the site-dependent property of B-cell activation in tuberculous infections (7; our unpublished data).

The close correlation between the anti-65-kDa and anti-71-kDa antibody titers in pericardial TB is also found in other nontuberculous mycobacterial lung diseases but not in Crohn’s disease (3). The finding of no detectable IgG antibody titer against the mycobacterial 65-kDa antigen in our control subject with rheumatoid arthritis (a putative mycobacterial condition) is surprising in view of the results of a previous study (17).

The immunopathogenesis of tuberculous pericarditis is not yet fully understood. Clinically, the use of corticosteroids in addition to antituberculous therapy for tuberculous pericardial effusion has led to a reduction in the mortality and morbidity associated with the development of fibrosis (and possibly its incidence as shown in our study) (14, 15). The exact mechanism by which prednisolone, a nonspecific immunosuppressive agent, halts the progression of the aberrant inflammatory response is, however, unknown. A better understanding of these immunopathological processes may enable us to develop more-specific immunomodulatory agents than are currently available.

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**REFERENCES**


HUMORAL RESPONSE TO M-hsps IN TUBERCULOSIS


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