Acute rheumatic fever (ARF) and rheumatic heart disease (RHD) are major cardiological diseases of children in developing countries (14, 24, 35). It is widely believed that these debilitating diseases are nonsuppurative autoimmune sequelae of upper respiratory infections with group A streptococci (GAS). To gain a better understanding of the pathogenesis of these diseases, we examined the in vitro proliferative responses of peripheral blood mononuclear cells (PBMC) from RHD patients to human myocardial proteins in a T-cell Western assay. A number of myocardial proteins fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were recognized by PBMC from both patients and controls. However, PBMC from a significant percentage of RHD patients (40%) responded to a discrete band of myocardial proteins migrating with an apparent molecular mass of 50 to 54 kDa while none of the control subject PBMC responded to this protein band ($P \leq 0.0001$). To further investigate the link between infections with GAS and autoimmune carditis, we studied the proliferative responses of PBMC from patients and controls to myocardial proteins before and after in vitro stimulation of the cells with opsonized GAS isolated from ARF patients. Priming of PBMC with rheumatogenic GAS caused the percentage of RHD patients responding to the 50- to 54-kDa myocardial proteins to increase from 43 to 90% ($P \leq 0.0284$). By contrast, PBMC from control subjects failed to recognize the 50- to 54-kDa myocardial proteins even after stimulation with the opsonized streptococci ($P \leq 0.0001$). The assay sensitivity was increased from 40 to 90% after priming of a patient’s cells with opsonized GAS, but the positive predictive value was 100% in both unprimed and primed cultures. Antibodies generated to partially purified 50- to 54-kDa myocardial proteins did not cross-react with either streptococcal homogenates, purified M protein, myosin, laminin, or vimentin, suggesting a lack of cross-reactivity at the humoral level. This study suggests that the 50- to 54-kDa myocardial proteins contain a putative antigen that is preferentially recognized by T cells from RHD patients and demonstrates that exposure to streptococcal antigens enhances the ability of patients to recognize these proteins.
cytoskeletal proteins. Together, these findings suggest that multiple components of the immune response to antigens of GAS may act synergistically in mediating the pathogenesis of ARF and RHD.

We believe that an adequate understanding of the pathogenesis of RHD and the role of GAS in this disease can be achieved only by direct examination of a patient’s immune responses to streptococcal and myocardial antigens. To this end, the ability of T cells from RHD patients and controls to recognize human myocardial antigens was evaluated in this study and the effect of in vitro stimulation with rheumatogenic serotypes of GAS on these responses was analyzed. We report that certain myocardial antigens are preferentially recognized by T cells from RHD patients and that this recognition is enhanced following stimulation with strains of GAS isolated from ARF patients.

MATERIALS AND METHODS

Case definition of patients and controls. All of the RHD patients studied had, by definition, chronic valvular disease (1, 9, 55). The majority of the RHD patients (42/44) had mitral valve disease with regurgitation, stenosis, or incompetence as diagnosed by echocardiography and/or catheterization (56); 36% had involvement of both the mitral and aortic valves or the mitral and tricuspid valves. None of the RHD patients had any evidence of an inflammatory process at the time of blood sample drawing. Control subjects (n = 23) included (i) patients who had non-RHD (NRHD) but had ischemic heart disease (n = 3) as diagnosed by echocardiography and catheterization and (ii) healthy individuals with no history of any heart or rheumatic disorders (n = 20). Heparinized whole blood was obtained from patients and controls following oral consent. Patients and control individuals were matched for age, and they were all recruited from northern Egypt.

Separation of peripheral blood mononuclear cells (PBMC) and a T-cell-enriched population. PBMC from patients and controls were separated from heparinized whole blood by Ficoll-Hypaque density gradient centrifugation, and the cells were resuspended in RPMI 1640 medium supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2 mM l-glutamine, 12.5 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) buffer, 1 mM sodium pyruvate, 24 mM NaHCO3, and 10% heat-inactivated fetal bovine serum (RPMI complete medium).

T cells and antigen-presenting cells (APC) were enriched by erythrocyte rosetting at 37°C as previously described (23). The erythrocyte rosette-positive population is enriched in T cells but not completely depleted of APC. This residual APC contaminant is sufficient to support antigen presentation to T cells and was removed by sequential adherence to plastic dishes as previously described (24). The erythrocyte rosette-negative population in B cells and monocytes (APC) was further purified by incubation with anti-CD3 antibody-coated magnetic beads (Dynal Inc., Lake Success, N.Y.) at a ratio of 107 beads/2.5 × 106 cells for 20 min at 4°C (in accordance with the manufacturer’s instructions) to remove any T-cell contaminant. T-cell-depleted APC were checked for responsiveness to phytohemagglutinin—failure to respond indicated successful depletion of T cells.

The responses of PBMC, APC-depleted T cells, T-cell-depleted APC, and T cells plus added mytomycin C (Mx)-treated APC to myocardial antigens were compared.

Preparation of heart antigens. Total human neonatal hearts were obtained from the morgue of Kasr El Einy Hospital (Faculty of Medicine, Cairo University, Cairo, Egypt). The hearts were collected during the first 3 to 6 h after death that was determined to be unrelated to any form of heart defect. The neonatal heart was kept in ice-cold buffer A, which consisted of phosphate-buffered saline (PBS) containing the protease inhibitors 5 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, Mo.), 5 μg of aprotinin (Boehringer Mannheim, Indianapolis, Ind.) per ml, and 1 mM EDTA disodium salt (Sigma). Human neonatal myocardial tissues (250 mg) were homogenized at 0 to 4°C for 30 min in 1 ml of 1% sodium dodecyl sulfate (SDS) in buffer A using a glass homogenizer. Tissue homogenates were boiled in a water bath for 5 min, immediately cooled in an ice bath, and centrifuged at 4°C for 30 min at 10,000 × g. The supernatant containing SDS-solubilized myocardial proteins (10) was collected and kept on ice, and the protein content was evaluated by the method of Lowry et al. (39) and/or by the Bio-Rad protein assay (7) (Bio-Rad Laboratories, Hercules, Calif.). Aliquots of 100 μg and 1 mg were stored at −70°C until use.

Assessment of the response to myocardial antigen in T-cell Western assays. It has been previously documented that an antigen immobilized on a nitrocellulose membrane can activate T lymphocytes in the presence of APC (64). This procedure is widely used to analyze the response to a complex mixture of antigens that can be fractionated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes which can be cut into small pieces and then incubated with T cells in proliferation assays. This technique, known as the T-cell Western assay, allows direct screening of T cells that recognize individual polypeptides without the requirement for extensive purification of the target antigen (37). Briefly, 2 mg of SDS-solubilized myocardial proteins was boiled in 1 ml of 1× sample buffer (50 mM Tris-HCl, 5% 2-mercaptoethanol, 2% SDS, 10% glycerol, 0.02% bromophenol blue), applied to a well (10 cm by 1.5 mm) of a preparative vertical slab gel (16 by 18 cm; 1.5 mm thick), and subjected to denaturing SDS–10% PAGE. Prestained molecular mass markers ranging from 112 to 20.5 kDa were applied to one side of the gel (36). The separated proteins were electroblotted onto 0.2-μm-pore-size nitrocellulose membranes (Bio-Rad Laboratories) at 50 mA for 45 min at 4°C (58, 59) in 25 mM Tris buffer containing 192 mM glycine and 20% methanol. The blots were rinsed with sterile distilled water and stored at 4°C sandwiched between two sheets of sterile Whatman no. 1 filter paper (35 by 45 cm) until used.

The separated protein bands were visualized on the blot by staining two 0.5-cm vertical strips from both sides of the immunoblot for 15 min with 0.2% Ponceau red in 2% trichloroacetic acid and then washing them extensively in distilled water. With the stained vertical strips as a guide, the immunoblot with proteins ranging from 166 to 23 kDa was cut into 20 horizontal strips (10 cm wide), each representing one to three visible protein bands (Fig. 1); if each horizontal band contained 5% of the total protein, then each 10-cm horizontal band contained approximately 100 μg of protein. The horizontal myocardial protein bands, designated bands 1 through 10, were cut from the top of the gel.

The nitrocellulose pieces from each band were distributed in quadruplicate wells of 96-well flat-bottom tissue culture plates (Costar, Cambridge, Mass.). Similar-size pieces of antigen-free nitrocellulose (band 0) were used as a background control.

PBMC from patients or controls (1 × 105 to 2 × 105 cells/ml of RPMI complete medium) were added to the quadruplicate wells containing the nitrocellulose pieces with immobilized myocardial antigens. The cultures were incubated at 37°C, 5% CO2, and 95% humidity for 48 h. The relatively short incubation time was selected to exclude allogeneic responses that may arise from
processing and presentation of heart donor proteins by recipient APC. During the final 18 h of culture, each well was pulsed with 0.4 μCi of [3H]thymidine (specific activity of 2 Ci/mmol; ICN Biomedicals, Costa Mesa, Calif.). The labeled cells were harvested with an automated cell harvester (LKB Wallac, Wallac Oy, Turku, Finland) and processed with a Wallac Betaplate liquid scintillation analyser (Pharmacia, Alameda, Calif.). The mean counts per minute and the standard error of the mean (SEM) of quadruplicate wells were calculated, and cultures were scored as having a positive response if (i) the stimulation index (SI) was ≥2 times that of the control cultures (band 0) and (ii) the difference between the test and control wells was significant. Statistical significance was determined by using the two-tailed Student t test, and a value of P ≤ 0.05 was considered significant.

**Generation of polyclonal rabbit antisera to rheumatogenic serotypes of GAS.** GAS isolates from Egyptian ARF patients were used in this study. Rabbits (New Zealand White; 7 months old) were each immunized with 50 μl of a standardized suspension (0.05 to 0.06 optical density units at a 540 nm) prepared as follows. The bacteria were streaked on blood agar plates and incubated for 18 to 24 h at 37°C, and pure colonies were inoculated into 50 ml of Todd-Hewitt broth (THB, Difco, Detroit, Mich.). After incubation at 37°C for 5 to 6 h, the primary inoculum was transferred to 500 ml of THB and incubated at 37°C for an additional 18 to 24 h. The growing bacteria were collected by centrifugation at 4°C for 20 min at 600 g, washed three times, resuspended in sterile normal saline (0.9% NaCl) and incubated at 37°C for an additional 5 to 6 h. The bacteria were harvested, washed three times at 4°C, and the growth was determined by assessing [3H]thymidine uptake as described above. The majority of patients tested responded to at least one of the 20 myocardial protein bands; few did not respond to any band, but most responded to several bands (Table 1). The proliferative responses of representative individuals from the patient and control groups are shown in Fig. 2. The response was not confined to patients with RHD, inasmuch as control subjects also showed significant (P ≤ 0.05) proliferation in response to several myocardial protein bands (Fig. 3 and Table 1). Although interindividual variation with regard to the size and number of myocardial protein fractions recognized was seen (Table 1), differences between patients and controls with respect to the response to specific bands were noted (Fig. 3). However, the most striking and only significant difference was seen in the response to band 8 proteins (50 to 54 kDa), which were recognized by 42% of RHD patients but not by any individual in the control groups (P ≤ 0.005).

To further investigate the differential responses of RHD patients and healthy controls to band 8 proteins, we analyzed
the responses of an additional 16 RHD patients and 13 healthy controls to the partially purified (eluted) band 8 50- to 54-kDa myocardial protein fraction (Table 2). Six of the 16 additional patients responded to band 8 proteins, while none of the additional 13 healthy control subjects responded. Therefore, by comparing the number of responders to band 8 proteins among 42 RHD patients and 23 controls tested (Tables 1 and 2), we found a highly statistically significant difference between the two groups ($P = 0.0001$), and this test had a sensitivity of 40% with 100% specificity, a 100% positive predictive value, and a 48% negative predictive value.

**Effect of stimulation with opsonized GAS on recognition of myocardial antigens.** Because of the known association between GAS infections and the development of RHD, we investigated whether exposure to streptococcal antigens potentiates the cellular immune response to myocardial antigens. To best simulate the natural mode of interaction between the streptococcus and immune cells, we stimulated PBMC from patients and controls with opsonized rheumatogenic streptococci. Antisera generated to five isolates of GAS obtained from ARF patients were used to opsonize their respective strains. To avoid bias against a particular serotype, we stimulated PBMC with a mixture of all five serotypes. The opsonized, as well as unopsonized, bacteria were able to induce comparable proliferative responses of PBMC from both patient and control groups (data not shown).

Next, PBMC from seven RHD patients who showed no rheumatic reactivity at the time of blood sample collection and PBMC from seven healthy controls were examined for the ability to recognize the various myocardial protein bands (bands 1 to 20) before and after in vitro stimulation with the mixture of five opsonized rheumatogenic serotypes of GAS (Table 3). Similar to what was observed above, unprimed PBMC from patients and controls responded to numerous myocardial protein bands and 57% of RHD patients recognized band 8 proteins whereas none of the controls responded to them (Table 3). After priming with the opsonized streptococci, 85.7% of RHD patients responded to band 8 proteins but still none of the controls recognized these proteins. Interestingly, an increase in the response to band 18 proteins was also seen in both patient and control groups after stimulation with the bacteria. These data show that exposure of PBMC from either patients or control groups to rheumatogenic streptococci can enhance their ability to recognize myocardial antigens. However, the unique ability of cells from RHD patients to preferentially recognize the band 8 50- to 54-kDa proteins suggests the presence of a unique pool of autoreactive cells in the blood of these patients; this pool may be further stimulated by exposure to streptococcal antigens.

To further focus on the differential responses of RHD patients and healthy controls to band 8 proteins before and after priming with a mixture of opsonized GAS isolates, we analyzed the responses of an additional group of patients and controls to partially purified (band 8) proteins. PBMC from RHD patients (samples 27 to 42) and healthy controls (samples 8 to 20) were either tested directly (unprimed) or stimulated with the mixture of opsonized GAS (primed), rested, and then tested for reactivity against partially purified (eluted) band 8 myocardial proteins (Table 2 and Fig. 4). Figure 4 shows the SIs of RHD patients ($n = 23$) and healthy controls ($n = 20$) tested before and after stimulation with opsonized rheumatogenic GAS. Consistent with our previous results, 10 (43%) of 23 RHD patients responded to band 8 proteins prior to stimulation with opsonized GAS, and after stimulation, 18 (90%) of 20 patients who were tested showed a positive response to this myocardial protein fraction. The mean SIs plus the SEM before and after priming were $2.17 \pm 1.685$ and $3.36 \pm 1.747$, respectively ($P = 0.0284$). None of the healthy controls responded to eluted band 8 proteins regardless of whether or not they were stimulated with the opsonized GAS (Fig. 4). The difference between the responses to band 8 proteins of patients and controls was statistically significant ($P = 0.0075$ for unprimed cells and $P = 0.0001$ for primed cells). Therefore, priming with opsonized GAS markedly increased the test sensitivity from 40 to 90% with 100% specificity and a 100% positive predictive value.

**Identification of a band 8 myocardial protein-responsive cell population.** The above-described studies were conducted with unfractionated PBMC from patients. To determine which population mediates the response to band 8 proteins, we compared the responses of unfractionated PBMC, an APC-depleted T-cell population, a T-cell-depleted APC population, and T cells plus Mx-treated APC to partially purified (eluted) band 8 proteins. As shown in Fig. 5, the T-cell-depleted APC and the APC-depleted T cells failed to respond to band 8 proteins while addition of Mx-treated APC restored their response to band 8 proteins. These results indicate that the response of
RHD patients to band 8 myocardial proteins is mediated by T cells.

Investigation of B-cell epitopes shared between streptococcal antigens and band 8 myocardial proteins. Previous studies have documented the presence of B-cell epitopes shared between streptococcal and myocardial proteins (8, 11, 15, 20, 26, 34, 40). Therefore, it was important to determine whether B-cell epitopes are shared between band 8 proteins and streptococcal proteins from the five isolates of GAS used in this study. The proteins in band 8 were partially purified and used to generate polyclonal antibodies in rabbits. As shown in Fig. 6, the rabbit antibody recognized band 8 proteins in whole myocardial extract, as well as the purified band 8 proteins. However, these antibodies failed to recognize any of the streptococcal proteins when a whole bacterial homogenate was separated by SDS-PAGE. In addition, these antibodies were not cross-reactive with either pepsin-extracted M5 protein, myosin, laminin, or vimentin (data not shown).

DISCUSSION

Previous clinical and experimental studies have suggested that cell-mediated immune responses play a pivotal role in the pathogenesis of ARF and RHD. In 1980, Hutto and Ayoub (22) explored the role of cell-mediated immunity in the pathogenesis of acute rheumatic carditis in humans and documented the presence of heart-specific T lymphocytes circulating in the blood of acute rheumatic carditis patients. Studies demonstrating the preponderance of CD4 T cells in the heart lesions of RHD patients (28, 48) have provided additional compelling evidence for an ongoing cellular immune response in the heart tissues of these patients. Furthermore, the link between infec-

### TABLE 1. Proliferative responses of PBMC from patients and controls to myocardial proteins

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<th>Donor no.</th>
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\(^a\) HC, healthy controls.

\(^b\) PBMC from patients and controls were stimulated with SDS-PAGE-separated myocardial proteins (bands 1 to 20) in T-cell Western assays as described in Materials and Methods. After 72 h in culture, the wells were pulsed with \(^3\)H\textsubscript{thymidine} and harvested 18 h later. The mean counts per minute ± the standard errors of the means of quadruplicate wells were calculated. The SI was calculated by dividing the counts per minute of wells containing myocardial antigens by the counts per minute of those containing blank nitrocellulose paper (band 0). The response was considered significant if the SI was ≥2.0 and the \(P\) was ≤0.05.

\(^c\) Significantly different (\(P ≤ 0.005\)) by Fisher’s exact test.
myocardial proteins which migrated in SDS-PAGE with an addition of control individuals. However, a particular fraction of myocardial and valvular protein fractions. cardiac tissues of RHD patients recognized specific human proteins. However, none of these studies provided information on RHD patients and healthy individuals were cross-reactive with heart addition, Pruksakorn et al. (47) observed that M protein-spe-

Lesions have proliferative responses to heart homogenates. In the mechanism of immune injury in RHD remains an enigma. has been supported by the demonstration of cytotoxic immune cross-reactions between streptococcal antigens and mammalian heart tissues (16, 54, 62). However, despite this evidence, the inability to elicit by heart donor alloantigens and raises the possibility that this 50- to 54-kDa myocardial fraction contains a putative target autoantigen that is recognized by T cells from RHD patients.

The link between exposure to streptococcal antigens and the development of cellular reactivity to specific myocardial proteins was demonstrated in the present study. By stimulating T cells with opsonized rheumatogenic streptococci, we tried to simulate the natural mode of in vivo exposure to streptococcal antigens. With this mode of stimulation, the percentage of RHD patients showing a significant proliferative response to band 8 proteins was increased to 90%. Priming with opsonized GAS increased the test sensitivity from 40 to 90% while maintaining 100% specificity and a 100% positive predictive value. The fact that none of the control subjects studied recognized this myocardial protein fraction, even after stimulation with GAS (P ≤ 0.0001), is consistent with the notion that differences in the T-cell repertoire exist between patients and controls. Indeed, preliminary data from our laboratory showed that RHD patients appear to have a skewed T-cell receptor Vβ repertoire pattern compared to healthy controls (14a). Differences in the T-cell repertoire and/or HLA allotype between patients and controls may allow the former to recognize a putative autoantigen(s) in the 50- to 54-kDa myocardial protein fraction. Alternatively, RHD patients may have a higher precursor frequency of heart-reactive T cells or a pool of memory T cells capable of recognizing specific heart autoantigens.

In this study, we showed that several myocardial antigens with certain strains of GAS and the development of ARF and RHD in a susceptible host has become well established (3, 6, 57, 65), and the role of cell-mediated immune responses to virulence factors of GAS in the pathogenesis of these diseases has been supported by the demonstration of cytotoxic immune cross-reactions between streptococcal antigens and mammalian heart tissues (16, 54, 62). However, despite this evidence, the mechanism of immune injury in RHD remains an enigma.

Earlier studies by Meric and Berkel (43) reported that 40% of ARF patients and 46% of patients with inactive valvular lesions have proliferative responses to heart homogenates. In addition, Pruksakorn et al. (47) observed that M protein-specific T-cell clones generated from peripheral blood of RHD patients and healthy individuals were cross-reactive with heart proteins. However, none of these studies provided information regarding the molecular weights of the heart proteins eliciting the proliferative response. Recently, Guilherme et al. (19) showed that T-cell clones obtained from surgical fragments of cardiac tissues of RHD patients recognized specific human myocardial and valvular protein fractions.

In this study, we showed that several myocardial antigens can be recognized by peripheral T cells from RHD patients, as well as control individuals. However, a particular fraction of myocardial proteins which migrated in SDS-PAGE with an apparent molecular mass of 50 to 54 kDa (designated band 8) was found to be preferentially recognized by T cells from 40% of RHD patients, whereas none of the control subjects tested, including patients with NRHD, responded to these proteins (P ≤ 0.0001). This finding indicates that the observed response is not elicited by heart donor alloantigens and raises the possibility that this 50- to 54-kDa myocardial fraction contains a putative target autoantigen that is recognized by T cells from RHD patients.

The link between exposure to streptococcal antigens and the development of cellular reactivity to specific myocardial proteins was demonstrated in the present study. By stimulating T cells with opsonized rheumatogenic streptococci, we tried to simulate the natural mode of in vivo exposure to streptococcal antigens. With this mode of stimulation, the percentage of RHD patients showing a significant proliferative response to band 8 proteins was increased to 90%. Priming with opsonized GAS increased the test sensitivity from 40 to 90% while maintaining 100% specificity and a 100% positive predictive value. The fact that none of the control subjects studied recognized this myocardial protein fraction, even after stimulation with GAS (P ≤ 0.0001), is consistent with the notion that differences in the T-cell repertoire exist between patients and controls. Indeed, preliminary data from our laboratory showed that RHD patients appear to have a skewed T-cell receptor Vβ repertoire pattern compared to healthy controls (14a). Differences in the T-cell repertoire and/or HLA allotype between patients and controls may allow the former to recognize a putative autoantigen(s) in the 50- to 54-kDa myocardial protein fraction. Alternatively, RHD patients may have a higher precursor frequency of heart-reactive T cells or a pool of memory T cells capable of recognizing specific heart autoantigens.
RHD patients and healthy controls (HC) were stimulated with a 10^2 mixture of opsonized rheumatogenic isolates of GAS. PBMC were incubated at 37°C in 5% CO_2 and 95% humidity with the opsonized GAS in six-well plates in a final volume of 3 ml of RPMI complete medium. After 72 h in culture, the PBMC primed with GAS were harvested, washed, resuspended at 10^6 cells/ml in fresh RPMI complete medium, and allowed to rest by incubation for 48 h at 37°C in 5% CO_2 and 95% humidity. The rested lymphocytes were then harvested and stimulated with band 8 myocardial proteins in a T-cell Western assay at 3 × 10^5 cells/well in a final volume of 100 µl of RPMI complete medium. Data are presented as SIs of the proliferative responses to band 8 myocardial proteins of PBMC from 23 RHD patients and 20 healthy controls before (C) and after (O) priming with the mixture of opsonized GAS. SIs that were ≥2 times those of antigen-free cultures were considered positive (values above the broken line) if the mean counts per minute were also found to be statistically significantly different from the background (P ≤ 0.05). The inset shows the mean SIs plus the standard deviation (SD) for unprimed RHD patients (a), primed RHD patients (b), unprimed healthy controls (c), and primed healthy controls (d). Statistical differences between the various groups were analyzed by Student’s t test (P ≤ 0.05 was considered significant).

This pool may be further expanded following re-exposure to streptococcal antigens. It is not clear whether the enhancement of the anti-band 8 response following exposure of T cells to rheumatogenic streptococci is related to the presence of epitopes shared between a specific streptococcal antigen(s) and a myocardial protein(s). However, the lack of humoral cross-reactivity between band 8 proteins and streptococcal antigens suggests that the enhanced recognition of band 8 myocardial proteins is not due to shared B-cell epitopes. Inasmuch as our data were generated with rabbit antibodies, we cannot rule out the possibility that human antibodies cross-react with band 8 proteins and streptococcal antigens.

The data suggest the existence of T-cell epitopes shared between GAS and band 8 proteins. Alternatively, the enhanced cellular recognition of myocardial antigens following stimulation with opsonized bacteria may be mediated by streptococcal superantigens, including M proteins (61), that stimulate T cells based on the V_β type and regardless of the antigenic specificity of the T-cell receptor. It has been suggested that, through this mode of stimulation, superantigens can potentially activate autoreactive T cells (17, 29, 32, 53). The latter possibility is supported by previous studies in our laboratory demonstrating that stimulation of T cells with peptiderminated M5 protein enhanced their ability to recognize and respond to cardiac myosin (29).

The link between stimulation with streptococcal M protein and the development of T cells with the capacity to kill myocardial cell lines has been reported (29, 31). In addition, Prukasaki et al. (47) generated T-cell lines to different M protein peptides and demonstrated that several of these clones also recognize heart proteins. Guilherme et al. (19) reported that T-cell clones derived from heart biopsies from RHD patients recognized M5 protein-derived synthetic peptides, whereas none of the T-cell clones derived from heart biopsies from patients with chronic Chagas’ cardiomyopathy and heart transplant patients with allograft rejection recognized these peptides. Most of the heart-derived T-cell clones generated by these investigators simultaneously recognized multiple protein fractions from both myocardium and aortic valve tissues. In addition, 95- to 150-kDa and 43- to 65-kDa aortic valve fractions were frequently recognized by M protein cross-reactive T-cell clones, which led the authors to suggest that cross-reactivity may involve proteins such as myosin and vimentin. Yoshinaga et al. (63) also found that T-cell lines established from valvular tissues of RHD patients responded to cell wall and cell membrane antigens of rheumatogenic streptococci. However, in contrast to Guilherme et al. (19), those investigators found that none of the T-cell lines recognized M protein, myosin, or other mammalian cytoskeletal proteins. These seemingly conflicting findings raise the possibility that more than one antigen of GAS and/or more than one cross-reactive heart autoantigen is involved in the pathogenesis of RHD. This possibility may explain why 10 to 14% of the RHD patients studied here failed to respond to band 8 myocardial proteins, even after stimulation with rheumatogenic streptococci. Therefore, further studies examining cellular and humoral immune responses of RHD patients toward specific heart proteins before and after stimulation with specific antigens derived from rheumatogenic strains of GAS will undoubtedly shed light on the mechanism of pathogenesis of this disease.

In summary, we have shown that the 50- to 54-kDa protein fraction from human myocardial tissues is preferentially recognized by T cells from RHD patients and that the ability to recognize these proteins is enhanced by prior exposure to rheumatogenic streptococci. Additional studies are required to...
investigate the nature of the proteins in band 8 and to determine the cellular and molecular basis for the preferential recognition of the target autoantigen by T cells from RHD patients. In addition, other bands of potential interest, including band 18 proteins, which were recognized by 43% of the patients and controls after priming with rheumatococcal GAS, should be investigated also as potential target autoantigens. Despite the need for additional studies, this study shows that examination of immune responses of only healthy individuals and controls after priming with rheumatogenic GAS, underscores the importance of direct examination of patient samples to better elucidate the immunopathogenesis of poststreptococcal autoimmune diseases.

REFERENCES


Editor: R. E. McCallum