ice crystals were based on detailed scattering phase functions and extinction and scattering cross sections and were applied at selected wavelengths in the solar and infrared spectra (*13*).

Figure 3 depicts the net flux and heating rate (per 24-hour interval) profiles for these two cloud models. The results for a cloudless atmosphere are presented for comparison. With regard to the atmospheric heating rates of the pure-ice cirrus cloud, a significant cooling at the cloud top (~9°C per 24 hours) and a pronounced warming at the cloud base (~10°C per 24 hours) are apparent. With the insertion of the 100-m-thick bottom-water layer into the model, the cooling rate is enhanced by about 3°C per 24 hours at the cirrus cloud top. Of more importance, however, is the greenhouse effect caused by an almost twofold increase in the atmospheric heating rate at the cloud base. With respect to the net flux, the thin water layer reduced the net flux available at the surface, the dominant factor determining the surface temperature, by ~12 W (about 15 percent) as compared to the pure ice cloud.

It follows from these relatively simple calculations that, when compared to pure-ice clouds, water-containing cirrus clouds can significantly enhance the atmospheric warming between the surface and the cloud base and, at the same time, can produce a cooling of the surface from the reduced downward solar flux. Although in these exercises we assumed that the cirrus clouds persisted for the 24-hour period over which the net heating rate calculations were averaged, it should be acknowledged that our measurements were insufficient to characterize the cloud layer over such a time scale. Nonetheless, it is clear that the presence of even a thin water layer at the cirrus cloud base will modify the fundamental radiative properties of the cloud, most notably the infrared emissivity and the solar albedo, and will therefore have an impact on attempts to model the climate of the earth-atmosphere system. To aid in the determination of the requisite cloud microphysical inputs for future climate models, it is of paramount importance to obtain climatological data on the frequency and distribution of liquid water within cirrus clouds. It would appear from the findings reported here that ground-based observation programs can be used to provide this information.

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5. A significant change in lidar-measured high-cloud scattering properties was reported to occur at a temperature of −40°C by C. M. R. Platt and A. C. Dilley [*J. Atmos. Sci.* **38**, 1069 (1981)]. More recently, A. J. Heymsfield and C. M. R. Platt [*ibid.* **41**, 846 (1984)] have indicated that small supercooled drops could have contributed significantly to these changes.
6. The ruby laser has a wavelength of 0.694 μm; see Sassen (*4*) for a description of the lidar system.
7. For an example of the application of airborne radiometric measurements for the determination of the radiative properties of cirrus clouds, see K. T. Griffith, S. K. Cox, R. G. Knollenberg, *J. Atmos. Sci.* **37**, 1077 (1980).
8. This interpretation of the gradient in δ values is based on laboratory laser-scattering measurements of artificially "seeded" supercooled water clouds, which demonstrate that frozen cloud droplets are sufficiently nonspherical in shape to provide an ice cloud depolarization signature within 5 to 15 seconds after freezing [K. Sassen and K. N. Liou, *J. Atmos. Sci.* **36**, 852 (1979)].
9. A review of the airborne techniques used for probing cloud microstructure is given by R. G. Knollenberg, in *Clouds: Their Formation, Optical Properties, and Effects*, P. V. Hobbs and A. Deepak, Eds. (Academic Press, New York, 1981), p. 15. The in situ data of Fig. 2 were derived from measurements made with the forward scattering spectrometer probe (FSSP, for cloud droplets) and the two-dimensional ice crystal probe. Although the response of the FSSP to newly frozen cloud droplets is uncertain, an additional in situ probe, the Rosemont icing-rate detector, collected independent data supporting identification of supercooled water.
10. The mixed-phase cloud area centered at a range of 2 km in Fig. 2 resembles a convective cell pattern. Since the aircraft encountered only minute ice crystals at this location about 2 minutes after the lidar scan was performed, it is possible that this portion of the cloud became glaciated in the interim.
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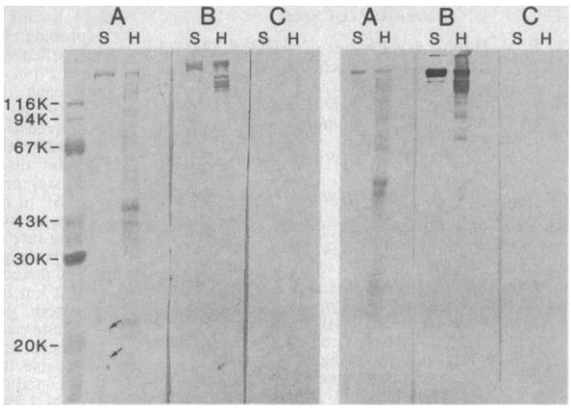
Myosin: A Link Between Streptococci and Heart

Abstract. *Murine monoclonal antibodies to* Streptococcus pyogenes *reacted with skeletal muscle myosin. High molecular weight proteins in extracts of human heart tissue that reacted with an antibody to* S. pyogenes *also reacted with a monoclonal antibody to ventricular myosin. Adsorption of the antibody to streptococci with* S. pyogenes *simultaneously removed reactivity of the antibody for either* S. pyogenes *or myosin. These results indicate that myosin shares immunodeterminants with a component of* S. pyogenes.

Heart-reactive antibodies frequently have been associated with acute rheumatic fever, a sequela of infection with *Streptococcus pyogenes* (group A streptococci) (*1, 2*). These antibodies have been reported in acute post-streptococcal glomerulonephritis and in uncomplicated infections, although less frequently and in lower titers (*2*). Antibodies that cross-react with heart tissue and *S. pyogenes* and *S. mutans* have also been produced in animals after immunization with these microorganisms, but direct evidence for a relation between the antibodies and the disease process has not been forthcoming (*3–5*). Nonetheless, with the advent of streptococcal vaccine studies (*6*), the identification of the cross-reactive components of the streptococcus and human tissue has become a problem.

Evidence has accumulated over the past 30 years suggesting that the cross-reactive antigens in both heart and streptococci are membrane-associated. This suggestion was made primarily because of the sarcolemmal fluorescence observed when antibodies from animals immunized against a group A streptococcus were reacted with heart tissue (*3*). In addition, the membranes of a group A streptococcus were found to be effective adsorbents of the heart-reactive antibodies in the sera of humans or immunized animals (*3, 7*). Skeletal muscle, thymus, kidney, liver, brain, and skin have also been reported to react with antibodies produced after streptococcal immunization or infection (*8, 9*). Other components of group A streptococci have been implicated in the cross-reactivity, including the type-specific M protein and the group-specific carbohydrate (*10, 11*). Although little attention has been given to the cross-reactive antigens of the host tissue, comparison of the structural relation between group A streptococcal M protein and α-tropomyosin has shown that the two proteins share a similar seven-amino-acid residue periodicity that accounts for their high α-helicity (*12*). However, no immunologic cross-reactivity between streptococcal M protein and tropomyosin has been confirmed.

A set of cross-reactive murine monoclonal antibodies to group A streptococci and heart was produced and characterized (*13*). Those monoclonal antibodies that were strongly reactive with compo-



Figs. 1 and 2. Reaction of monoclonal antibodies 54.2.8 (Fig. 1) and CCM-52 (Fig. 2) with rabbit skeletal muscle myosin (S) and SDS-extracted components of human heart (H). Proteins were prepared for SDS–polyacrylamide gel electrophoresis in 1 percent SDS and 1 percent 2-mercaptoethanol and were separated as described (*13*). After electrophoresis, separated proteins were blotted onto nitrocellulose according to the standard Western blot procedure (*13, 21*). Human heart was prepared according to a procedure described for obtaining crude sarcolemmal components (*7*). All heart extraction procedures included the protease inhibitors *N*-α-*p*-tosyl-L-lysine chloromethyl ketone and phenylmethylsulfonyl fluoride ($10^{-3}M$) (*13*). Standards are given as the molecular weight × 1000 (K) at the far left. Fig. 1 (left). (A) Amido black protein stain; (B) monoclonal antibody 54.2.8; (C) culture medium and conjugate control. Fig. 2 (right). (A) Amido black protein stain; (B) monoclonal antibody CCM-52; (C) culture medium and conjugate control.

nents in heart preparations extracted with sodium dodecyl sulfate (SDS) were also reactive with streptococcal membrane-associated components. We now present evidence that monoclonal antibodies to group A streptococcal membranes also react with myosin.

Electrophoretically separated rabbit skeletal muscle myosin (Sigma) and human heart extract were reacted with monoclonal antibody 54.2.8 (Fig. 1). Both myosin and high molecular weight components of the human heart extract reacted with this antibody. Although the light chains of myosin [18,000 (18K) and 25K molecular weight] were evident in the stained portion of the blot (arrows), the antibody reacted only with the heavy chain of myosin and not with the light chains. The separated proteins on the control strip did not react with the antibody conjugate or the culture medium.

The reactivity of monoclonal antibody 54.2.8 for myosin or heart extract components and *S. pyogenes* was adsorbed with whole heat-killed (60°C) *S. pyogenes* M-protein types 5, 6, and 12 (Table 1). After each adsorption it was evident that the reactivities of antibody 54.2.8 for heart components and for *S. pyogenes* decreased simultaneously. Similar results were observed when *S. mutans* strain GS5 was used as an adsorbent (data not shown). The results of Western blot analysis showed nearly complete removal of antibody 54.2.8 reactivity for both myosin and heart extract after adsorption with group A streptococci (data not shown). The reason that 100 percent adsorption of the monoclonal antibody was not seen after three adsorptions may have been either the antibody affinity or the number of exposed antigenic sites on the whole streptococci. Nonimmune mouse immunoglobulin M (IgM; 10 μg/ml) in Iscove's modified Dulbecco's medium was not adsorbed appreciably by the streptococci nor did it bind to streptococci, myosin, or heart components in the enzyme-linked immunosorbent assay (ELISA) or the Western blot procedure.

Although antibody 54.2.8 reacted with rabbit skeletal muscle myosin, it was important to identify the SDS-extracted heart components that reacted with it. For this purpose, monoclonal antibody CCM-52 to rabbit ventricular isomyosin was reacted with the heart extract components. CCM-52 has been shown to react with ventricular myosin from both rabbits and humans with specificity for the light meromyosin portion of the heavy chain (*14, 15*). Antibody CCM-52 reacted strongly with myosin and with a family of protein bands in the heart extract and showed reactivity similar to that of antibody 54.2.8 (Fig. 2). The additional bands seen with CCM-52 may reflect postmortem degradation of myosin. These results suggest that the components in extracts of human heart that reacted with monoclonal antibody 54.2.8 were cardiac myosin. Additional proof that myosin is the heart component that was reactive with this antibody must await studies with purified human ventricular myosin and its peptides.

Monoclonal antibody 54.2.8 reacted both with skeletal muscle myosin and with what appeared to be ventricular myosin. This was not surprising since myofibers of low adenosinetriphosphatase activity are present in skeletal muscle as well as in cardiac muscle (*16*). Primarily peripheral staining of cardiomyocytes was observed when 54.2.8 was reacted with heart tissue sections (*17*). This was similar to staining observed previously with sera from patients with acute rheumatic fever (*18*).

Most important, these data confirm other observations that the cross-reactive antibodies in streptococcal antisera reacted primarily with components of heart and skeletal muscle (*1–3, 5, 7*). At least one of these components now appears to be myosin. Antibodies to myosin have been reported but usually in relation to components of smooth muscle and in other disease states (*19*). In no instance (that we could find) have antibodies to myosin been reported to cross-react with bacterial antigens.

Although the identity of the cross-reactive streptococcal antigen is not known, we have previously shown that monoclonal antibody 54.2.8 does react with several membrane-associated components of *S. pyogenes* approximately 94,000 daltons in size and smaller (*13*). Since group A streptococcal membranes

Table 1. Reactivity of monoclonal antibody 54.2.8 for heart extract components and *Streptococcus pyogenes* M-protein type 5 after adsorption with whole heat-killed *S. pyogenes* M-protein types 5, 6, and 12. Streptococci were grown in 100 ml of chemically defined medium as described (*22*) and were heat-killed at 60°C. Adsorptions were done for 30 minutes at 37°C with subsequent incubation overnight at 4°C. The standardized, washed centrifugate from 50 to 100 ml of organisms grown in chemically defined medium was mixed with approximately 5 ml of culture fluid. Production and cloning of the murine hybridomas to group A streptococci has been described (*13, 21*). All hybridomas studied were the IgM isotype. The ELISA's were used to measure antibody reactivity with heart or streptococcal antigens bound to polyvinyl chloride microtiter plates and were performed as described (*21*).

M-Protein type	Reactivity			
	Heart*		M-Protein type 5	
	A_{405}†	Adsorbed (percent)	A_{405}†	Adsorbed (percent)
5	0.256	77	0.450	68
6	0.431	62	0.542	61
12	0.271	76	0.475	66
None	1.123	0	1.389	0

*Triton X-100 extract of whole human heart as described (*22*). †Absorbance at 405 nm.

and whole cells were found to be reactive with 54.2.8, it is possible that a surface component that extends into the streptococcal membrane is involved in the cross-reactivity. Alternately, it is possible that the membranes contained fragments of cell wall components that reacted with 54.2.8. Adsorption of the reactivity of antibody 54.2.8 with several M-protein types of *S. pyogenes* suggests that the cross-reactivity was not type-specific. Type-specific cross-reactivity of antibodies to *S. pyogenes* type 5 M protein with other heart components has been reported (*10*).

The role that cross-reactions might have in the pathogenesis of streptococcal sequelae is not known. However, several reports of the immunopathology of heart lesions subsequent to rheumatic fever have described the degeneration of the muscle cell (*20*). Whether or not the lesions are a direct result of streptococcal infection is not known. Our discovery that myosin may share immunodeterminants with a component of *S. pyogenes* is apparently the first report of a cross-reactive tissue component. The stimulation of immune clones against myosin by the streptococcus may be an attractive hypothesis for the mechanism of the production of heart-reactive antibodies in streptococcal diseases and the development of sequelae of uncomplicated infections.

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Reversal of Experimental Allergic Encephalomyelitis with Monoclonal Antibody to a T-Cell Subset Marker

Abstract. *Administration of a monoclonal antibody (GK1.5) that recognizes the L3T4 marker present on helper T cells prevented the development of experimental allergic encephalomyelitis (EAE) in mice. Furthermore, treatment with GK1.5 reversed EAE when the antibody was given to paralyzed animals. In vivo injection of GK1.5 selectively reduced the number of L3T4$^+$ cells in the spleen and the lymph nodes. These results suggest that manipulation of the human equivalent of the murine L3T4$^+$ T-cell subset with monoclonal antibodies may provide effective therapy for certain autoimmune diseases.*

Experimental allergic encephalomyelitis (EAE), an autoimmune inflammatory disease of the central nervous system, is a model for the human disease multiple sclerosis (MS) (*1*). Results of several studies suggest that particular T-lymphocyte subsets are implicated in the pathogenesis of both EAE and MS (*2, 3*). In the case of murine EAE, T cells capable of provoking EAE belong to the helper (inducer) subset of T cells that carries the L3T4 surface marker (*4, 5*). Cells belonging to a similar human T-cell subset are associated with the progression of lesions in the brains of MS patients (*3*). Administration of monoclonal antibodies directed against T-cell subset markers might constitute an effective strategy for the treatment of MS and other human disorders, particularly when a specific T-cell subset or an imbalance between regulatory T-cell subsets (*6*) is involved in disease pathogenesis.

To induce EAE, susceptible SJL/J mice were given pertussis vaccine intravenously and immunized with mouse spinal cord homogenate (MSCH) emulsi-

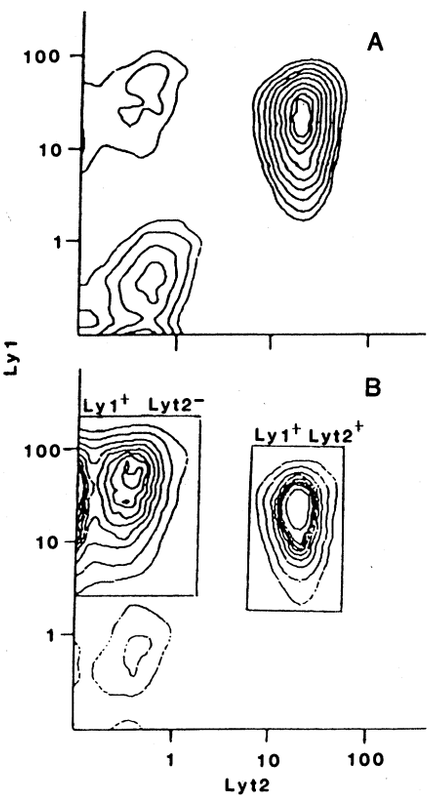

Fig. 1. Monoclonal antibody GK1.5 reduces the number of Ly1$^+$Lyt2$^-$ (L3T4$^+$) lymph node cells. (A) GK1.5-treated; (B) control. Preparation of cells, staining procedures, and data collection were as described (*13*). Lymph node cells were stained with fluorescein-conjugated antibody to Lyt2 (*12*) plus biotin-conjugated antibody to Ly1 (*12*) followed by staining with Texas red avidin and analysis on a dual laser FACS equipped with logarithmic amplifiers (*14*). Axes represent the relative amounts of the respective antigens as revealed by fluorescence intensity. Two-color staining data are presented as "contour plots" that are representations of three-dimensional surfaces in which the levels of green and red fluorescence per cell define locations on a two-dimensional surface, and the frequency of cells with that value of fluorescence defines the elevation at that location. After this surface was smoothed, contour lines were drawn to divide the sample into equal fractions. The integration boundaries used to determine the frequency of Ly1$^+$Lyt2$^-$ and Ly1$^+$Lyt2$^+$ cells are shown on Fig 1B. Treated mice received 100 μg of GK1.5 on each of the 2 days preceding analysis.