cantly to membrane deformability, an assumption that is probably warranted in tissues in which cytoplasmic viscosity has been measured and found to be low (*6, 12*). However, on the basis of other micrurgy experiments I have the impression that the cytoplasm of collecting duct cells is considerably more viscous than water. Consequently, until proven otherwise the possible role of the subapical cytoplasm in altering surface deformability in response to vasopressin cannot be dismissed. Once the exact location of the hormone-induced mechanical alteration in the cell apex is identified, it may be possible to characterize further the physicochemical nature of the permeability barrier.

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Cross-Reactions between Streptococcal M Proteins and Human Transplantation Antigens

Abstract. *Allogeneic antiserums against human lymphocytes were specifically inhibited by M protein from beta hemolytic group A* Streptococcus pyogenes, *type 1. Analogous M proteins from streptococcus types 3, 4, 5, 6, 12, and 14 had little or no inhibitory activity. The specific inhibition by M protein is not a result of anticomplementary activity or of coating of the lymphocyte surface. Streptococcal polysaccharide and 73 other polysaccharides were inactive. Because all seven HL-A specificities tested were inhibited, it is inferred that M1 protein has a structure common to human histocompatibility antigens.*

Streptococci are known to be associated with many suppurative diseases, such as pharyngitis, mastoiditis, and otitis media. They have also been implicated in triggering nonsuppurative diseases (glomerulonephritis, rheumatic fever) and acceleration of skin allograft rejection in the guinea pig (*1*). Similarities between animal and bacterial antigens have been suspected of being the basis for these phenomena (*2, 3*). Human erythrocyte antigens A, B, O, and Le are closely related in chemical structure to pneumococcal polysaccharide, and substances with blood group activity are found among many bacteria (*4*). Molecular "evolution" may have taken place among human antigens from ancestral substances common to bacteria and man. In addition, pathogenic bacteria may have developed antigens similar to human antigens by convergent evolution (*5*).

Patients with chronic glomerulonephritis have the HL-A2 antigen in a higher proportion than normal persons have (*6*). The HL-A antigens function as transplantation antigens (*7*). With this clue as to the possible association of transplantation and streptococcal antigens, we investigated their cross-reactivities, using the ability of a variety of substances to inhibit antiserums to HL-A, which are cytotoxic to lymphocytes. We used well-characterized human antiserums defining seven HL-A specificities; these were from our laboratory and from the serum bank of the National Institute of Allergy and Infectious Diseases. These antiserums were either from multiparous women or immunized persons. Lymphocytes were typed for HL-A specificities, and antiserums were titrated before use. One microliter of several dilutions of allogeneic antiserums was used above and below the dilution at which 50 percent of the cells are killed. Substances used as inhibitors were dissolved in phosphate-buffered saline generally at a concentration of 5 to 10 μg/μl. One microliter each of antiserum and inhibitor was incubated at room temperature for 1 hour; 1 μl of a lymphocyte suspension (1000 cell/μl) was added, incubated first for another 30 minutes, and then incubated for 1 hour with 5 μl of rabbit complement. For staining the nonviable cells, 2 μl of 5 percent eosin was added; within 1 minute 3 μl of formaldehyde was added (*7*). Each dilution of antiserum, used in control and in experimental tests, was run in triplicate, and the percentage of dead cells was averaged. Each of the two antiserums for each HL-A specificity was tested on lymphocytes from three different persons.

The M proteins were isolated from group A streptococci essentially according to the method of Fox, Wittner, and Dorfman (*8*). Preparations of bacterial cell walls were obtained from group A streptococci, type 12 (*9*). The extraction with hot formamide described by Fuller (*10*) and modified by Zittle and Harris (*11*) was used to obtain the group-specific polysaccharide (APS).

In initial trials, 84 substances (*12*) were screened for their cross-reactivities with transplantation antigens. Among the substances tested were proteins from streptococci, polysaccharides from *Streptococcus pyogenes, Escherichia coli, Diplococcus pneumoniae, Shigella flexneri, Paracolon ballerup, Serratia marcescens, Pasteurella pseudotuberculosis, Salmonella poona,* and human glomerular membrane extract. Most of the substances did not cross-react with HL-A antigens.

Results of a typical inhibition experiment are shown in Fig. 1. Antiserum to HL-A2 was strongly inhibited by M1, but it was not affected noticeably by the six other M proteins or by APS. Inhibition of antiserum to HL-A of five other specificities, HL-A1, -3, -7, -8, and -9, was similar.

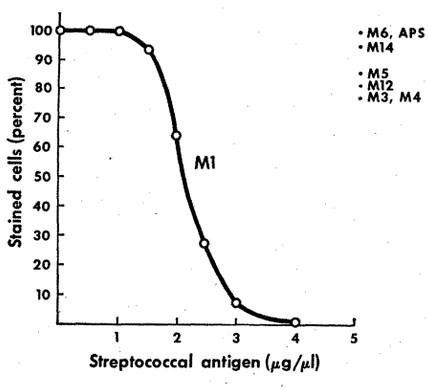

Fig. 1. Inhibition of an antiserum to HL-A2 by streptococcal antigens. The M1 protein was tested at varying concentrations, whereas M6, APS, M14, M5, M12, M3, and M4 were tested only at 5 μg/μl.

One serum of HL-A5 specificity was unusual in that it was inhibited to the extent of only 30 percent when the concentration of M1 was 5 μg/μl; it was also inhibited about 50 percent by 5 μg of APS per microliter. The striking inhibitory capacity of the M1 protein for all the HL-A specificities in contrast to low inhibitory capacity of other types of M proteins is evident. With each of the HL-A specificities, further tests with four different lots of M1 protein preparations confirmed the inhibitory effect. One lot of M1 showed 50 percent inhibition even at a concentration as low as 0.5 μg/μl.

Several experiments were performed to rule out the possibility that M1 was anticomplementary. Complement was first incubated with the M1 protein at 3, 6, and 9 mg/ml in a volume ratio of 5 : 1 for 1 hour at 24°C. Lymphocytes of HL-A2 specificity, which had been sensitized with varying dilutions of antiserum to HL-A2 for 1 hour and washed three times, were then added to this mixture of complement and M1. No decrease of cytotoxicity was observed when the antiserum concentration was high enough to lyse more than 80 percent of cells. Although some decrease in the cytotoxicity was observed at lower concentrations of antiserum, competition between M1 and cells for antibodies during the complement phase of the reaction could account for the decrease. The magnitude of this effect was approximately 20 percent. This level of anticomplementary activity is not enough to account for the consistent, strong inhibitory activity of the M1 protein.

To examine the possibility that M1 was preventing the action of antibodies by coating the lymphocytes, the cells were mixed with the M1 protein, washed, and then used in the cytotoxicity test. There was no indication that cells treated with M protein were less susceptible to the action of antiserums than untreated cells were.

Because M1 was inhibitory to all antiserums with HL-A specificities, the specificity of the M1 inhibition was investigated by substituting two mouse antiserums to human lymphocytes in place of allogeneic serums. No inhibitory activity was found, an indication that M1 does not cross-react with some lymphocyte antigens detected by heterologous antiserums. We have some evidence with antiserums to lymphocytes prepared in other animals that some heterologous serums are inhibited to some extent by M1.

The broad cross-reactivity of the M1 protein suggests that the M1 may possess a structure common to all the histocompatibility antigens. Should this be the case, the question arises as to how antibodies to this common structure could have been produced in immunized humans. One explanation is that the HL-A determinants have a constant and a variable region in their amino acid sequences. The constant region is assumed to represent a structure similar to that of the M1 determinant; and the variable region, together with the constant region, confers the HL-A specificity. Thus allogeneic antibody could react with both the HL-A determinant and the M1 protein. An attempt was made to approach the cross-reactivity problem from another direction, that is, to see whether antiserum made in rabbit against M1 protein would react with human lymphocytes. Hyperimmune rabbit serums against M1 did not have any cytotoxic activity against lymphocytes of seven different HL-A specificities. If we assume that HL-A determinants have variable and constant regions, then it is not unreasonable that the rabbit antiserum to M1 directed, only against a part (M1-like or the constant region) of the HL-A determinants, did not react with lymphocytes.

Unlike the A and B blood group substances, the HL-A antigens now appear to be protein in nature (*13*). The M protein preparations contained, at most, 1 percent carbohydrate. Even at a hundred times this concentration, APS was only mildly inhibitory and then only for one antiserum to HL-A5. In addition, the inhibitory activity of M1 was abolished by prior incubation with trypsin and with pronase. Although the sensitivity of M1 to proteolytic enzymes does not rule out the participation of carbohydrates completely, it does point to the vital role played by the protein moiety in the cross-reactivity of HL-A antigens with the M1.

To our knowledge, our findings provide the first evidence of inhibition of human allogeneic serum with a bacterial protein. This cross-reactivity may aid in chemical characterization studies of the HL-A determinants and in explaining the means by which streptococci might trigger development of autoimmune disorders. The strong cross-reactivity could lead to breakdown of tolerance to autologous transplantation antigens (*14*) and lead to cellular or humoral immunologic response. Antibodies formed against the M proteins may cross-react with tissues and evoke autoimmune-like diseases. Antibodies formed against M proteins could also elicit disorders induced by antigen-antibody complexes, as shown by Dixon and others in subjects with glomerulonephritis (*15*). However it was the M protein from type 1 streptococci which cross-reacted strongly with histocompatibility antigens rather than the M protein from the strongly nephritogenic type 12 streptococci (*3, 16*). The evidence given here provides a basis for understanding the cross-reactions between bacterial substances and human histocompatibility antigens.

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