Antigens of Herpes Simplex Virus of Oral and Genital Origin

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The serological distinction between herpesviruses of oral or genital origin has been unequivocally shown by Schneweis (26), Plummer (21), and others (3, 20). Biological studies on the characterization of the 2 viruses have, in general, supported the idea of 2 discrete clusters. The 2 types behave differently in tissue culture held under particular conditions (5, 14, 15, 23) and the behavior of the 2 types on the chorioallantoic membranes of eggs of hens likewise supports the existence of 2 groups (19), but some studies indicate that there may be intermediate types (4). Clear-cut differences are obvious when comparing the acid-extractable proteins of the 2 types of virus (8) and some virus-specific enzymes appear to differ in their physical characteristics (32). Indeed, differences can be detected in the DNA's of the 2 viruses (9), although both seem to be constructed on the same general design (29).

The herpes simplex types can be distinguished serologically but this is a matter of some difficulty since they share common antigens. This fact greatly complicates the interpretation of serological studies. In view of the importance of seroepidemiological investigations of the association between herpes type 2 infection and carcinoma of the cervix, an accurate knowledge of herpes simplex antigens is not merely of academic interest but has become a matter of practical consequence.

A Priori Assumptions

The genome of herpesviruses consists of 2-stranded DNA of a little more than 10⁶ M.W. units. This is sufficient to specify more than a 100 polypeptides of average size. In fact, 24 polypeptides have been reported in the virion alone (28), some of which are glycosylated. This is equivalent to 47% of coding potential. We have every reason to suppose that these virus-specific polypeptides can, in their native state, generate a number of virus-specific antigenic determinants singly, in combination with each other, or even in combination with host-specified polypeptides. It is even possible that the specificity of some antigens involves the carbohydrate moiety of glycoproteins. Finally, one cannot exclude the generation of some new antigenic determinants by modification of host polypeptides by virus infection. However, at least 1 antigenic protein appears to consist of a single virus-specified polypeptide (1, 11). We may assume that virus-specific antigenic determinants are generated by folding polypeptide chains and that this is determined by the primary structure of the polypeptides. We cannot, of course, expect any straightforward relationship between the number of polypeptides and the number of antigenic determinants except that the more virus-specific polypeptides there are, the more virus-specific antigens are to be expected. Nor can we predict the effect on antigenic specificity of amino acid substitution arising from point mutation; thus, a single amino acid difference in the primary structures of an analogous protein of type 1 and type 2 virus may affect its antigenic behavior profoundly or not at all.

Sites of Virus-specific Antigenic Determinants

Virus antigenic determinants can be detected on diffusible proteins extracted from infected cells (10), on the surface of the virion (25), and on cell membranes (24), including those represented at the surface of infected cells (6, 16). Indeed, some sets of determinants, for example, those of "Band 2" (34) can be detected on diffusible proteins, on the surface of the virion, and at the cell surface (see Ref. 36). To date, both type-specific and common antigenic components have been found in all these sites. In general, type-specific reactions may be achieved by absorption of 1 reagent by the heterologous reacting material.

Antigenic Determinants Involved in Virus Neutralization

The most venerable and still the most convenient test for herpes simplex antibodies is by virus neutralization. Indeed, this test in one guise or another has been used in nearly all seroepidemiological studies. Plummer (22) reviews the various methods and shows that they correlate reasonably well in discriminating between virus types. The principal difficulty with these tests has been the virtual impossibility of interpreting the meaning of sera that neutralize both type 1 and type 2 virus. Does such a result mean that the serum comes from an individual who has been infected with both types of virus, or does it merely reflect shared antigenic components? Nahmias et al. (3, 17), using the microneutralization test, obtained results with rabbit and human sera suggesting that the serological properties of the 2 types of herpesvirus were sufficiently distinct for their test to provide a valid discrimination. Indeed, their results indicate that the 2 types of herpesvirus cluster serologically into 2 distinct groups. Skinner et al. (Skinner, G. R. B., Thouless, M. E., Gibbs, A. J., Faulkner, S., and Edwards, J. Serological and Biological Relatedness of Human Herpes Virus, in preparation) compared 47 strains of herpes simplex virus from different sources. These strains fell into 2 discrete groups on the basis of pock size (19), and the 2 groups correlated very well with the sites of origin of the strains. Antisera were tested by the kinetic neutralization test against all the sera. The behavior of each strain was compared with that of each
of the other strains for each serum and a mean similarity coefficient was derived for each pair of strains. These values were used to determine the clustering of strains according to the method of Gower (7). All the strains formed a single cluster with obvious subclustering of genital and oral isolates. In contrast to the findings of Nahmias et al. (3, 17), these subclusters did not form discrete groups; indeed, some of the oral isolates were more similar to some genital isolates than to each other. It was interesting and unexpected to find that the oral isolates had higher mean neutralization rate constants than genital isolates, no matter what immunogens were used to produce the antisera. This situation is similar to that found with herpes simplex type 1 and B virus (37). It suggests that type 1 herpesviruses are peculiarly susceptible to immune neutralization. This fact complicates antigenic analysis by comparative neutralization tests.

In summary, the serological properties of herpes simplex viruses determined by neutralization tests indicate 2 major clusterings, which we call type 1 and type 2. There is general agreement that type 1 and type 2 viruses share common antigens and have type-specific antigens. The area of disagreement is over the discreteness of the serological clusters.

Virus Antigenic Determinants at the Cell Surface. The existence of herpesvirus-specified antigens at the surface of infected cells can be demonstrated by immunofluorescence and cytolytic tests, and these have both type-specific and common components (22, 31, 36).

Virus Antigenic Determinants on Diffusible Proteins. At least 12 separate herpes-specific precipitating antigens can be distinguished in immunodiffusion tests using antisera from rabbits hyperimmunized with extracts of infected rabbit cells. Thouless showed that when these antisera are absorbed with extracts of cells infected with homologous virus, at least 3 type 1- and at least 1 type 2-specific antigens can be detected (36). One of these precipitating antigens (associated with thymidine kinase) has been examined further (31). When antisera were exhaustively absorbed with extracts of cells infected with heterologous virus, at least 3 type 1- and at least 1 type 2-specific antigens can be detected (36). One of these precipitating antigens has been enumerated further (31). When antisera were exhaustively absorbed with extracts of cells infected with a thymidine kinase-deficient mutant of homologous type, they gave but a single precipitin line in immunodiffusion tests against wild-type homologous virus extracts and none at all against those of either mutant or against those of heterologous wild-type virus extracts (Fig. 1). This antigen associated with thymidine kinase activity is evidently type specific.

Another of these diffusible antigens has been thoroughly investigated (33, 34). Extracts of infected rabbit cells were fractionated by polyacrylamide electrophoresis. The fractions were set up in immunodiffusion tests against rabbit antisera. A region (called Band 2) where single precipitin lines were found was used to prepare "monoprecipitin" antisera. These Band 2 preparations contain both common and specific herpes antigenic determinants that are represented on the functional surface of the virion since absorption of antisera with Band 2 antigen preparations reduces their neutralizing activity against both types of virus, leaving only type-specific antibody and anti-Band 2 neutralizes both types of virus. The common antigenic determinants also occur at the surface of infected cells since Geder has shown that anti-Band 2 sera react in both immunofluorescent and cytolytic tests with the surface of cells infected with either type of virus (38).

Other diffusible antigens of herpesvirus are being examined in Birmingham. Some of these possess antigenic determinants shared between the 2 types of virus.

Virus Antigenic Determinants and Enzyme Activity. Some of the enzymatic activities appearing in herpesvirus-infected cells may be neutralized by virus-specific antisera (2, 12, 13). Thouless (31) has shown that antisera to herpes simplex types 1 and 2 neutralize thymidine kinase activity with considerable specificity although there is some degree of intertype cross-neutralization (Table 1). By absorbing these antisera with thymidine kinase-deficient mutants, she found that the antienzyme activity remains while other antibodies (e.g., neutralizing antibodies) are removed.

Antigenic Determinants of Uncertain Situation. The ver-

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**Table 1**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Anti-type 1</th>
<th>Anti-type 2</th>
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<tbody>
<tr>
<td>Type 1 (6)</td>
<td>96 (5-13)</td>
<td>51 (43-63)</td>
</tr>
<tr>
<td>Type 2 (6)</td>
<td>89 (72-100)</td>
<td>10 (3-13)</td>
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* Number of each strain.
* Values indicate percentage mean residual activity with range in parentheses.

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*C. Sim and D. H. Watson. The Role of Type-specific and Cross-reacting Antigens in the Neutralization of Herpes Simplex Virus Types 1 and 2, submitted for publication to the Journal of General Virology.*
The Determination of Type-specific Antibodies

The serological recognition of infection by the different types of herpesvirus has, so far, depended on a comparison of neutralizing activities on 2 prototype strains of virus. Plummer (22) discusses the criteria used to validate the conclusions obtained from such evidence. The findings of Nahmias et al. (3, 17) not only indicated 2 clear serological groupings of herpesviruses but that antisera raised against them also formed clear-cut groups. They showed that rabbits previously infected with 1 type of virus became relatively immune to the other. However, dual infection was possible and in some circumstances the antibody pattern was altered by it. They have evidence that a type 2 virus infection can occur in patients previously immune to type 1 herpes infection (18). Therefore, in evaluating their serological epidemiological studies, they interpret the intermediate antibody pattern as evidence of dual infection. On the contrary, Skinner et al. (Serological and Biological Relatedness of Human Herpesvirus, in preparation) found, using kinetic neutralization, that the antisera of mice did not cluster discretely according to the viruses used as immunogens. Thus, while sera raised in response to type 1 virus strains clustered about a different center of gravity than that of type 2 sera, there was so much overlap among the clusters that it was rarely possible to predict correctly what type of virus had been used to raise any particular serum. These findings suggest that statistical interpretations are possible from such tests but that an interpretation made on an individual serum would be unwise. The mean k value of the 2 groups of antisera (raised against oral or genital isolates) differed significantly only when tested against oral isolates (Table 1).

Clearly, there is an urgent need to develop a robust test for type-specific antibodies of both types of virus. Three approaches seem possible. The 1st approach involves the preparation of pure type-specific antigen that could be used in any of a variety of serological tests. This may eventually be possible since different diffusible antigens with more or less type-specific reactivity can be separated by polyacrylamide electrophoresis (33),2 but we are probably a long way from achieving usable type-specific antigens in this way.

The 2nd approach might make use of an enzyme activity to select a type-specific virus antigen. The type specificity of thymidine kinase determined with rabbit hyperimmune antisera (31) is certainly sufficient to develop a test for specific antibody. Unfortunately, enzyme-neutralizing antibodies have not been found in the sera of a limited number of patients known to be infected with herpesvirus (M. E. Thouless, personal communication).

The 3rd approach involves the absorption of antisera with heterologous antigens. This may be achieved (using neutralization tests) by absorption with extracts of infected cells, “electrofiltrate,” or whole infected cells (6, 16).2 Such a procedure would be extremely cumbersome since each serum to be tested would require 2 types of absorption and 2 types of neutralization test.

A more elegant technique is now possible. After absorption with a preparation of type-shared antigens, the absorbed sera need be tested only with a type 1 and a type 2 antigen. Such a test has been developed making use of the lability of the type-specific “early” antigen and absorbing sera with aged antigen (30). Type-specific antibodies remain that can be detected by complement fixation tests using fresh antigen. Absorption of antisera with Band 2 preparations should offer a more precise method. As mentioned earlier, Band 2 fractions evidently contain all the type-shared antigenic determinants on the functional surface of the herpes virion as well as some type-specific determinants; hyperimmune rabbit sera react type specifically in neutralization tests after such absorptions.2 This procedure has not yet been used on human serum.

Absorption methods represent the only approach immediately open to us for the detection of type-specific antibodies in human sera. They are all cumbersome but the last certainly appears feasible since it uses a defined antigenic preparation, is already relatively simple, and could be applied to individual antisera. Even so, as Sim and Watson2 point out, the success of all these procedures depends upon the nature and proportions of the various antibodies evoked in natural infection in man. It is conceivable, but improbable, that man may produce an abundant species of antibody reacting with shared determinants not present in Band 2 preparations.

In summary, the only practicable approach immediately available for detecting type-specific antibody exclusively is by absorbing the sera with excess type-shared antigen. For the future, it is to be hoped that type-specific antigens will be prepared that may be used directly on human sera.

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References

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