Immunology of Herpes Simplex Virus Infection: Relevance to Herpes Simplex Virus Vaccines and Cervical Cancer

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Summary

The immunology of herpes simplex infections has been reviewed, particularly in relation to potential herpes simplex virus (HSV) vaccines, and to the association between HSV and cervical cancer. Relevant data from humans, experimental animals, and in vitro systems implicate both specific immune mechanisms and nonspecific factors in the course of HSV infections. There appear to be complex interactions between the various populations of mononuclear cells, macrophages, and lymphokines or other humoral factors. It is not yet possible, however, to pinpoint the crucial factors determining the different manifestations of the viral infection (primary infection, endogenous recurrence, or exogenous reinfection) in the various human hosts, although genetic factors may be important. While numerous animal models of HSV infection are available for the evaluation of HSV vaccines, models of HSV-induced cervical cancer require further exploration.

Introduction

The past 15 years have witnessed a gradual, then explosive, transition from the purely serological to the more comprehensive immunological aspects of HSV-1 and HSV-2 infections. The stimuli for improved understanding of host immune mechanisms to these viruses have come from several directions. Among these are (a) the large variety of clinicopathological manifestations depending on the type of host, particularly the more severe disease observed in the immunosuppressed individual and in the newborn; (b) the possible role of immune factors in the reactivation or control of herpetic recurrences and the potential use of immunomodulators for the prevention of such recurrences; (c) the association of HSV with human cancers; and (d) the development of HSV vaccines.

Coincidentally, in recent years it has become appreciated that immune mechanisms may operate, not only on the virus itself, but also on virus-infected cells that carry HSV-specific antigens on their surfaces. This has, in turn, expanded the number of assays that can be used to measure humoral and cellular immune mechanisms and their interactions. Although immune responses to HSV may well differ, depending on the type or subtype and whether fresh isolates or laboratory-passaged strains are involved, we believe that restricting the discussion to HSV-2 strains alone would leave out potentially important observations made with regard to other strains. This report reviews current information concerning the immunology of HSV infections in humans. Then follows a presentation of studies relevant to the mechanistic aspects of the various immunological modalities and their interrelationships in animals and in vitro. We conclude with a discussion of certain immunological aspects related to cervical cancer and to HSV vaccines.

Immunology of HSV Infection in Humans

Two lines of approach have been followed in efforts to unravel immune mechanisms that might be operative in humans. The first has been to categorize special hosts who are susceptible to unusually severe HSV infections in an attempt to identify the critical parameters of the immune response. The more recent approach has been to apply a variety of immunological assays to individuals with various types of HSV infection in order to obtain direct information on immune function. Because there may be variations in the immune responses associated with infection in special hosts with different types of HSV infection (primary or recurrent), relevant information is discussed below in its clinical context.

The Immunosuppressed or Immunodeficient Host. It is still unclear whether HSV infections are reactivated with greater frequency in the immunosuppressed host, as is the case with cytomegalovirus or varicella-zoster virus infections. What is clear, however, is that the recrudescences of HSV-1 and/or HSV-2 tend to be more severe and chronic in immunologically compromised individuals. The immunosuppressive agents incriminated include azathioprine, corticosteroids, and antilymphocyte serum (9, 50, 54, 71, 89). Severe local HSV infections are also more common in individuals with cancers, particularly of the lymphoid organs (46, 56). Viral dissemination to visceral organs is infrequent...
in these kinds of patients, but is more commonly observed in individuals with extensive burns (22) and in severely malnourished children, particularly in association with measles (4, 98).

Although several defects in immune responses can be found in these clinical conditions, a common theme is their association with defects in cellular immunity. Defects in cellular immunity are also found in children with the Wiskott-Aldrich syndrome and individuals with circulating lymphocytotoxic antibodies, some of whom develop the more severe forms of herpetic infection (35, 94). In contrast, severe HSV infections appear to be rare in individuals with defects in humoral immunity. In 2 cases of viral encephalitis in children with congenital X-linked hypogammaglobulinemia, HSV was recovered from the brain at autopsy (43). However, the administration of attenuated measles virus vaccine a few days before the onset of symptoms in one child and the concomitant recovery of an enterovirus from the brain of the other child, suggest that viral-associated depression of cellular immunity might have contributed to the development of HSV encephalitis. An active effect of low levels of HSV antibodies in patients with congenital or acquired immunoglobulin deficiencies cannot be ruled out completely, because low titers of antibodies to other viruses can be detected in the serum of such patients.

**Newborns.** The contrast between the high frequency of disseminated HSV (most usually HSV-2) infection which occurs in the newborn and the rarity of viral dissemination in older individuals is consistent with the interpretation that the maturity of the immune system plays an important role in host resistance to the virus (61). However, any such defect in the newborn must be short-lived, since viral dissemination to visceral organs rarely occurs after 3 weeks of age.

Although transplacentally acquired HSV antibodies are not fully protective to the newborn, infection in such cases tends to be localized to the skin, eyes, or central nervous system, without evidence of viral dissemination to visceral organs (61, 66). It is still undetermined whether cases of neonatal herpes occurring in the face of transplacental antibodies are the exception rather than the rule.

We have recently demonstrated that cord blood mononuclear cells of normal newborns have the capacity to act synergistically with HSV antibodies in an antibody-dependent lymphocyte-mediated cytotoxicity assay against HSV-1- or HSV-2-infected target cells (87). Although, compared with adult cells tested at the same effector:target cell ratio, newborn mononuclear cells showed slightly lower cytotoxicity, the total cytotoxicity of cord blood was comparable when adjusted for the higher content of mononuclear cells in newborns. Studies on pairs of maternal and cord blood sera demonstrated the transfer of the lymphocyte-dependent antibody in equivalent titers, providing further evidence for the IgG nature of this antibody.

Infected newborns have been found to produce IgM specific for HSV within 1 to 3 weeks after onset of infection (66). These antibodies continue to increase during the 1st 2 months and may be detectable for as long as 1 year. Some of the IgM antibodies in the serum of infected infants are directed against allotypic determinants on the maternal IgG.

The lymphocytes of some infected newborns have been found to respond to HSV antigens in vitro several weeks after onset of the infection (91). The temporal sequence of the development of cell-mediated responses and various lymphokines in these newborns requires concerted study; such studies are limited by the relatively low survival rate of these patients in any one institution.

**Infection of the Nervous System.** In individuals other than newborns, most cases of HSV encephalitis are caused by HSV-1; cases of meningoitis are related to HSV-2 (12). HSV-2 can be isolated from the buffy coat of peripheral blood and from the CSF in cases of meningoitis, suggesting the potential of HSV-2 to disseminate via the blood. It is still puzzling why HSV encephalitis occurs in apparently normal individuals, particularly in those with evidence of HSV infection before the neurological disease develops. The 2 cases of HSV encephalitis in agammaglobulinemics with concomitant viral infections (43), which might have suppressed cellular immune responses, indicate the need for more intensive investigations along these lines. HSV encephalitis, however, has not been observed with any greater frequency in immunosuppressed or immunodeficient individuals. In contrast, subacute HSV encephalitis has been observed in an anergic patient (72).

High levels of interferon have been detected in the blood and spinal fluid of a severely affected 4-month-old infant with HSV encephalitis (5). The source of the interferon and its role in the disease process are unknown. HSV antigens have been detected by immunofluorescent techniques in CSF leukocytes from patients with encephalitis, although infectious virus could not be isolated from that body fluid (15). HSV antibodies can be measured in the CSF of individuals with HSV encephalitis, although they are demonstrable relatively late after onset (40). The higher titer of HSV antibodies in the CSF, compared with that found in the serum, implies local synthesis of antibodies within the nervous system.

**Primary HSV Infection.** This is defined as an HSV-1 or HSV-2 infection occurring in an individual with no prior infection with either HSV type. There are no data to explain why primary HSV-1 and HSV-2 infections are subclinical in most individuals, yet can be very severe in a few others. Immunogenetic studies would be particularly relevant to this point. The more severe manifestations of primary HSV-2 infection during pregnancy (32) suggest that hormonal factors and/or the relative immunosuppression occurring during pregnancy may be involved.

Serological studies on primary HSV-1 and HSV-2 infections have revealed that IgM antibodies are the first to be demonstrated, usually followed by the development of IgG and IgA antibodies (24, 60). Lymphocyte-dependent antibodies appear around the time IgG antibodies are first detected (86). Complement-dependent neutralizing antibodies can be detected earlier in the serum of individuals with primary HSV infections (112).

Assays for cell-mediated responses have been applied in a very limited number of individuals with primary HSV-1 and HSV-2 infections. Lymphocyte transformation to HSV antigens has been demonstrated between the 2nd and 4th week after onset of infection (91).

**Recurrent HSV Infection.** HSV-2 infection occurs more
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commonly in individuals who have had prior HSV-1 infection than as a primary infection. The clinical manifestations tend to be milder in such cases. Immunological studies of recurrent HSV-2 infection are rendered more complex by several problems: (a) defining individuals with pure HSV-2 recurrences, i.e., without prior HSV-1 exposure; (b) the possibility of exogenous reinfection with the same or different subtype of HSV-2; and (c) the difficulty of ensuring the absence of subclinical recurrences, when sequential studies are performed.

The mechanisms of viral persistence after a primary infection are as yet unknown, but it appears that the viral genome lies dormant in the trigeminal ganglia in patients with labial or ocular infections (3) and in the sacral ganglia in patients with genital herpes (2). The various triggering mechanisms, which include sunshine, menstruation, sexual intercourse, fever, and emotional stress, provide little information about the underlying pathobiology.

Patients with recurrent HSV infections usually possess high levels of serum antibody. Serum titers of 1:100,000 can be detected with an antibody-dependent lymphocyte cytotoxicity assay (86). In most patients there is no change in antibody titers, as measured by neutralization tests, with or without the addition of complement (27), or by complement-fixation assays, following a recurrence. However, with the use of more sensitive serological tests, such as radioimmunoassay and immunofluorescence, rises in titers of IgM, IgG, or IgA antibodies can be demonstrated in some individuals after a recrudescence (60). In contrast to an earlier report (101), other workers have shown that levels of IgA in oral or ocular secretions are not depressed in recurrent herps, and that the titers of HSV antibodies in secretions do not fluctuate with clinical status (8, 17).

Most recent work has centered around the cellular immune responses to herpetic infection, albeit, conducted most often in individuals with recurrent HSV-1 infection. When skin testing with HSV antigens was performed, no differences were found in the response of patients with recrudescences, compared with that of seropositive, asymptomatic individuals (6, 82, 110). Skin test reactivity correlated with the presence of circulating HSV antibodies.

Peripheral blood lymphocytes from seropositive individuals transform when exposed to HSV antigens in vitro (74, 78, 81, 91, 109). In general, responses are higher with the homologous HSV type. Contracting results have been obtained as regards the changes in the lymphocyte stimulation index induced by HSV antigens in relation to recrudescences. Some studies have shown no apparent differences in the lymphocyte stimulation index at different times during or after the herpetic recrudescence (74, 109). Other studies have shown an increase in the stimulation index shortly after the recrudescence, and a fall thereafter (78). Variations in the antigens used by the various investigators might explain some of the differences observed. Lymphocyte responses to phytohemagglutinin, purified protein derivative, streptomycin, vaccinia, and Candida albicans were normal in both seropositive patients without recrudescences and in patients with recrudescence (109).

The role of lymphokines in recurrent herpes is unclear as yet because of seemingly conflicting data. Lymphocytes from patients with recrudescences, when stimulated with HSV antigens, produce less MIF than lymphocytes from seropositive individuals without recrudescences (109). However, production of leukocyte chemotactic factor and lymphotoxin were not found to differ between the 2 groups (78). A sequential study of interferon production by HSV-stimulated leukocytes, in patients with recrudescences, showed that maximum levels were attained 2 to 6 weeks after onset of the recrudescence (74). The leukocytes producing the interferon have been identified as being T-cells (102). Interferon induced by HSV, using combined macrophage lymphocyte cultures, has been found to differ in its pH and temperature lability from human interferon induced in other systems (103). Individuals with the highest levels of leukocyte-produced interferon after a recrudescence tended to have a longer duration between recrudescences. A similar pattern was noted when leukocyte migration inhibitory factor was measured (69).

Direct lymphocyte cytotoxicity has been reported against cells acutely or chronically infected with HSV-1. With the acutely infected cells, lymphocytes from patients with recrudescences taken during the quiescent period were specifically cytotoxic (83). With the chronically infected target cells, lymphocyte cytotoxic responses of patients with recrudescences (during the quiescent phase) were significantly lower than those of seropositive individuals with no history of recurrences (100). Antibody-dependent lymphocyte-mediated cytotoxicity might be confused with direct lymphocyte-mediated cytotoxicity if HSV antibody remains on the membranes of the effector cells after the lymphocytes are washed.

Susceptibility to recurrent herpetic infection may also be associated with genetic factors. Russell and Schlant (84) recently demonstrated a higher frequency of HLA-1 transplantation antigens in patients with frequent herpetic recurrences than in a control population.

**Erythema Multiforme.** It is clinically appreciated that individuals with HSV infections may demonstrate the allergic manifestations of erythema multiforme (85, 95). The immunopathological mechanisms are unclear, although they are likely to be related to the formation of antigen-antibody complexes. The allergic skin manifestation can be induced by the administration of inactivated HSV preparations (85).

**Immunology of HSV in Experimental Animals**

Immunological studies have been conducted in a variety of experimental animals, particularly in mice, guinea pigs, and rabbits. The following factors have been implicated in host responses to HSV infection: genetics, temperature, age, and humoral and cellular immune responses.

**Genetics.** A recent report suggests that genetic factors are operational in the natural resistance of mice to i.p. inoculation with a newly isolated HSV-1 (48). The 11 strains of mice could be categorized as resistant, moderately susceptible, and very susceptible. F1 crosses indicated that resistance is governed by a dominant gene, although it is apparently not linked with a histocompatibility locus.

**Temperature.** Adult mice, inoculated i.c. with HSV-1 or
i.p. with HSV-2, experience significantly lower mortality when housed at 34–36°C than when housed at ambient temperature (22). Although symptomatology was probably modified by metabolic changes, the decreased mortality may have been a consequence of temperature-dependent inhibition of viral replication. Thermal inhibition of HSV replication has been reported in tissue culture systems (13), HSV-2 usually being more temperature labile than HSV-1.

**Age.** Newborn mice have repeatedly been shown to be more susceptible to extraneural HSV inoculation than are adult mice. Johnson (31) has demonstrated that a barrier to the spread of virus inoculated extraneurally developed with age and that the inhibition of viral dissemination depended on the peritoneal and tissue macrophages. Other workers (28) found that syngeneic peritoneal macrophages from adult mice protected neonatal mice from i.p. challenge with HSV. In HSV-infected older mice, specific inhibition of macrophage function by the administration of silica particles or antimacrophage serum led to viral dissemination and early death (113). Proteose peptone-stimulated macrophages were more efficient in conferring protection against HSV infection than unstimulated cells; the enhanced resistance was associated with more efficient phagocytosis and greater production of interferon. In contrast, macrophages from neonatal mice did not respond to proteose peptone stimulation (28). These findings imply that macrophage immaturity is an important factor in the increased susceptibility of neonatal animals to viral dissemination.

BCG has been shown to increase the resistance of newborn mice to subsequent i.p. challenge with HSV-2, probably by activating macrophages. BCG also decreased the mortality of rabbits after corneal or intravaginal inoculation with HSV-2 (36). However, BCG had no protective effect against intravaginal inoculation of HSV-2 in adult mice, except when combined with HSV-2 antiserum (1). Levamizole has been found to confer some degree of protection to newborn rats inoculated with HSV-2 (21).

**Humoral Immune Responses.** HSV can be recovered from the spinal ganglia of previously infected mice in which latency had been established (10, 93, 105). Active immunization with HSV-2 does not affect the establishment of latent ganglionic infection, whereas immunization with HSV-1 confers a moderate degree of protection (105). When ganglia latently infected with HSV-1 were transplanted to noninfected syngeneic mice, both infectious virus and viral antigens could be demonstrated in the recipient animals. Less virus was expressed in ganglia transplanted to latently infected mice or to mice that had been passively immunized with anti-HSV IgG (93). Although a similar phenomenon may be operative in suppressing the reactivation of virus from human ganglia, it is obviously not completely effective in inhibiting recurrences in man.

Serum or γ-globulin containing HSV antibodies can prevent infection if administered before, or within hours after, inoculation of virus (25, 26). Passive immunization thereafter is not usually effective. HSV serum antibody can also act synergistically with peritoneal cells from nonimmune mice to destroy herpes-infected cells in vitro (73). Immunization with inactivated HSV will induce a variable degree of protection, depending on the route of virus challenge, but the mechanisms of protection have not been fully clarified (25, 105). Nevertheless, mice or cebus monkeys genitally infected with HSV-2 could be reinfected with the same virus by the genital route several months later (47).

The physical state of the virus and the virus dose probably influence the type of immune response. Thus, rabbits inoculated with infectious HSV developed both cellular and humoral immunity, whereas those inoculated with UV-inactivated virus developed a predominantly cellular response, with little or no neutralizing antibody (77). Inoculation with an HSV-anti-HSV complex did not elicit either a cellular or a humoral response. The type of assay used to measure antibodies in rabbits following HSV inoculation also appears to be important. Thus, the 1st antibody to appear is best measured with a complement-requiring neutralizing assay (41, 111).

**Cell-mediated Responses.** The development of delayed hypersensitivity is acknowledged to be a cell-mediated immune phenomenon. Guinea pigs sensitized to HSV demonstrate a cutaneous response to viral antigens (38, 75). The timing of the maximal response, the mononuclear nature of the infiltrate, and the ability to transfer reactivity with cells but not with serum, have confirmed that these skin reactions are those of classical delayed hypersensitivity. Related studies have shown that hypersensitivity to HSV antigens can also be induced in the cornea of both guinea pigs and rabbits (52, 53).

The importance of lymphocytes in the immune response has been confirmed by adoptive transfer experiments and in studies with immunosuppressed animals. Spleen cells from HSV-1-immunized mice were shown to confer partial protection to recipient mice challenged with the virus (19). Neonatal thymectomy in mice depressed their resistance to HSV infection, although neutralizing HSV antibodies were produced to almost the same extent in neonatally thymectomized mice as in intact controls (55). HSV-infected mice treated with antilymphocyte serum showed an increase in the magnitude and duration of the viremia and greater dissemination to the central nervous system (113). Mice treated with antithymocyte serum showed different patterns of survival, depending on the route of viral inoculation (62).

Survival was significantly poorer in the antithymocyte serum-treated mice when they were challenged either i.p. or i.v. However, in mice challenged i.c., increased survival and prolongation of survival time were observed, compared with untreated controls.

The specificity of the cellular immune response to HSV has been demonstrated in rabbits by prior inoculation with HSV-1 or HSV-2, followed by stimulation of splenic lymphocytes with the appropriate antigens in vitro (79). Lymphocytes responded more vigorously to the sensitizing than to the heterologous virus type. It was further shown that lymphocyte sensitization occurred within 3 days after infection, with a peak response at 7 days, and that sensitized cells could still be detected up to 120 days after virus inoculation (76). In vitro, specifically sensitized lymphocytes could be

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stimulated by virus alone and by virus bound to cells, but the reaction was abrogated if the cell-bound virus was incubated with antiviral antibody.

The temporal relation of cellular and antibody-mediated immune responses has also been studied in experimental herpetic keratitis in rabbits (51). Lympocyte cytotoxicity and MIF were detected within the 1st 7 to 11 days postinoculation. Detection of neutralizing antibodies peaked at days 11 to 21 and that of complement-dependent cytotoxic antibodies peaked on Day 16. The authors concluded from these observations that the control of ocular HSV infection involves an early inflammatory phase with macrophage reactivity and elaboration of MIF by sensitized lymphocytes. Transient virus-specific T-lymphocytes with effector reactivity, as well as neutrophils with chemotactic activity, occur during the stage of stromal keratitis. Antibody-dependent complement-mediated lysis later provides another phase of restriction of the infection.

HSV is susceptible to interferon and is interferogenic (23, 80, 96), but whether all strains are equally effective interferon inducers is not yet clear. Interferon was detected in the skin of mice 24 hr after the s.c. injection of HSV, and it increased to a peak 5 to 7 days later (96). Administration of interferon inducers was effective in protecting mice only when very small amounts of virus were used as inoculum (7). To date, no conclusive correlation between interferon levels and survival after HSV challenge has been demonstrated.

**Virus-immune Interactions In Vitro**

A fruitful area of investigation has been the examination in vitro of HSV, virus-infected cells, and immune effectors. Such systems have facilitated the dissection of the various specific and nonspecific effectors and their interrelationships. Since HSV can spread to other cells by extracellular transmission or by direct cell-to-cell spread, it follows that immunological intervention can proceed at 3 levels: (a) uptake of virus by leukocytes, (b) neutralization of extracellular virus by humoral factors, and (c) destruction of virus-infected cells by humoral and cellular factors.

**Uptake of Virus by Leukocytes.** HSV is readily taken up by macrophages from both adult and neonatal mice (28, 31, 92). Viral antigens are detectable within 4 hr after infection in macrophages from adult mice, with maximal expression in 10 to 20% of the cells at 6 hr after infection. Infected macrophages from neonatal mice, in contrast to macrophages from adult mice, are able to infect surrounding cells. The titer of virus produced in suckling mouse macrophages is significantly higher than that produced in adult mouse macrophages. The ability of adult macrophages to limit viral replication is not due to lack of viral adsorption, because adult macrophages absorb 100 times more virus than do those of suckling mice (28). There is no block in synthesis of viral DNA or viral proteins, but, instead, a restriction of viral assembly (92). No data are yet available on the activity of macrophages in human HSV infection. It is tantalizing to infer from the mouse studies that a defect in macrophage function might lead to visceral dissemination, such as occurs in newborns.

Whereas macrophages may help to restrict viral growth and dissemination, there is the contrasting finding that peripheral blood lymphocytes may allow replication of HSV (59). Viral replication has been found to be facilitated in human lymphocytes stimulated with phytohemagglutinin, pokeweed, concanavalin A, and antilymphocyte serum (34, 59). The subpopulations of cells capable of sustaining viral replication are yet to be ascertained, as is the in vivo significance of these observations.

**Effect of Humoral Factors.** When moderate concentrations of HSV are exposed to specific antibody in vitro, a so-called "unneutralizable" fraction remains (68, 104, 111). This can be rendered noninfectious by adding either complement or antiimmunoglobulin. Several explanations have been offered for these observations: (a) an actual virolytic action of antibody and complement, as is seen in bacterial systems; (b) immunaggregation of antibody-coated virus; and (c) an enhanced steric effect of antiantibody or complement in actually covering critical virus sites necessary for viral adsorption or penetration. The recent finding (14) that the alternate complement pathway can also assist antibody in virus neutralization may be of relevance to this issue.

The interaction of virus and immune lymphocytes has been shown to trigger the release of migratory inhibitory factor, leukocyte inhibitory factor, lymphotoxin, and interferon (51, 69, 74, 78, 109). Antigen-antibody complexes of HSV with IgG or IgM are also capable of stimulating immune lymphocytes. Rabbit spleen lymphocytes exposed in vitro to HSV antigens demonstrate increased thymidine uptake and interferon production if the donors are previously immunized with HSV (30, 76). Cells infected with virus and then challenged with HSV antibody and complement release a factor chemotactic for mononuclear and polymorphonuclear leukocytes (97). HSV has also been found to depress human monocyte chemotaxis (33).

**Effect on Virus-infected Cells.** HSV-infected cells acquire virus-specific membrane antigens which allow recognition and subsequent attack by various immune effectors (58). Spleen cells from sensitized mice or guinea pigs are able to reduce the size of plaques formed after addition of a known number of HSV plaque-forming units (20, 86). The activity of the mouse spleen cells was not affected by removal of adherent cells, and no lymphotoxin was detectable in the culture supernatants. It has also been demonstrated that high ratios of casein-activated but nonimmune rabbit peritoneal exudate cells cause nonspecific cytotoxicity and prevent viral plaque formation, although the addition of HSV antibody and complement enhanced the reaction (44). With the HSV-1 strain and tissue culture used, complement-mediated antibody cytolysis was found to be active after cell-to-cell viral spread has already occurred. Leukocytes from rabbits immunized with either HSV-1 or complete Freund's adjuvant and stimulated with inactivated HSV-1 or purified protein derivative have been shown to inhibit viral replication in a plaque assay (45). In contrast to cytotoxicity assays, this effect could be demonstrated with effector-to-target cell ratios as low as 1:10. The supernatant fluid from these cultures was shown to contain interferon, which could also inhibit viral replication.

An observation that not only complicates the interpreta-
tion of these results, but also suggests an alternative mechanism for destroying virus-infected cells, is the demonstration that HSV-1 or HSV-2 antibody can act synergistically with immune or nonimmune mononuclear cells to induce cytolysis in both HSV-1- or HSV-2-infected target cells (83, 86, 87). The antibody, which is IgG, is effective at serum dilutions to 1:100,000, so that scrupulous washing of the mononuclear cells is necessary before all of this contaminating antibody is removed. The human mononuclear cells have been partially characterized and appear to be neither T-cells, nor B-cells, nor monocytes; they are most probably K-cells. It has been suggested that the mechanism of target cell damage involves 2 stages: recognition of viral antigens on the infected cell by attachment of specific IgG, followed by interaction of the effector cell with the Fc portion of the bound immunoglobulin molecule (86, 87).

A further complexity of the relationship between HSV and host immune factors is the finding of receptors for the Fc fragment of IgG on the surface of HSV infected cells and some of the HSV transformed hamster cells (107, 108). The importance of this observation is still unclear. However, the presence of the Fc receptor might protect the infected cell from antibody-mediated immune attack either by binding the Fc fragment of IgG antibody so that the Fc is unable to activate complement or cytotoxic effector cells, or by sterically inhibiting the recognition of nearby virus-specific membrane antigen sites.

Notkin’s group has attempted to synthesize the nonspecific and specific control of cell-to-cell spread by virus into a 2-step response encompassing specific recognition and nonspecific execution (44, 45). The response involves specific immunological recognition by previously sensitized leukocytes, antibody and complement, which react with virus or viral antigens on the surface of infected cells. This generates mediators from both complement and immune leukocytes which can attract inflammatory cells to the site of infection. In addition, other mediators, such as lymphokinin and interferon, are produced. The lymphokines and inflammatory cells then act in a nonspecific fashion on infected and adjacent uninfected cells to prevent viral cell-to-cell spread.

Cervical Cancer

If HSV-2 is somehow causally related as an initiator or promoter of cervical carcinogenesis, it would be advantageous to use immunological means to abrogate either the initial infection or possibly the recurrent infection, which may be as important (65). Such an approach would have the added advantage of providing firm evidence for causality. It would therefore be desirable to understand, not only the immunology of the herpetic infections, but also that of cervical neoplasia, and their possible interactions.

There are several levels at which immune factors may affect the development and progression of cervical neoplasia: (a) rejection of the initially transformed cells, (b) prevention of the invasion of the basement membrane by the intraepithelial neoplasia, and (c) prevention of local extension and distant metastasis of the invasive cancer.

The reversion of cervical dysplasia to normal in many women and the slow progression from dysplasia to CIS, and from CIS to invasive cancer, might depend on immune factors which are depressed at critical periods. Coppelson and Reid (11) suggested that host immune factors might operate in this process, on the basis of their morphological studies. These workers detected mononuclear and phagocytic cells in most histopathological sections of cervical dysplasia, CIS, and microinvasive cancer. Although leukocytic cells were usually found along the basement membrane, the foci were occasionally subepithelial and were surrounded by a basement membrane. The superficial epithelium itself was occasionally infiltrated with mononuclear cells, particularly around intraepithelial capillaries. Evidence of death of the cervical neoplastic cells was sometimes associated with an intimate attachment of the mononuclear cells to the neoplastic cells and was most often observed in the stroma at the stage of CIS or microinvasion.

A limited number of studies have been directed specifically towards the immunological aspects of cervical neoplasia. Positive skin test reactions have been obtained with antigens prepared from the membranes of cervical cancer cells (29). In another study (67), skin testing with various antigens, such as Candida or streptokinase-streptodornase, showed a lower frequency of responses in women with the later stages of invasive cervical cancer, compared with those with intraepithelial neoplasia or early invasive cancer. A depression in in vitro lymphocyte transformation responses to HSV antigens has been demonstrated in patients with oral cancer, and increased stimulation in those with precancerous lesions (39). Antibodies to “tumor-specific antigens” of invasive cervical cancer tissues have been detected in the sera of women with cervical cancer by immunodiffusion techniques (42). Lymphocytes from women with cervical cancer have been reported to react against cultured cervical cancer cells in a microcytotoxicity assay (18), and complement-dependent antibody cytotoxicity has also been demonstrated in a similar test system (108). An increase in titers of HSV-2-neutralizing antibody has been associated with a decrease in complement-dependent HSV antibodies with progression of cervical neoplasia (99).

Other immunological studies have used HSV-transformed hamster cells. Spleen cells from hamsters inoculated with HSV-1- or HSV-2-transformed cells, and from hamsters immunized with HSV-1, were specifically cytotoxic for HSV-1-transformed cells in vitro (37). Sera from these animals did not cause complement-mediated antibody cytolysis of the HSV-transformed target cells. In another study (90), spleen lymphocytes from HSV-2 tumor-bearing animals were cytotoxic to both transformed and HSV-infected cells. Immune lymphocytes have also been used to detect the transfer of the HSV genome from transformed cells to HSV-susceptible cells (90).

Herpes Simplex Vaccines

The prospects and problems related to HSV vaccines will be discussed by other speakers in this Symposium. We would like to address ourselves to a few points with immunological overtones.

Of paramount importance are the various types of hosts to which an HSV-2 vaccine might be administered.
The Individual Not Previously Infected with HSV-1 or HSV-2. In this case, the vaccine would have to be administered around the age of 6 months to 1 year, the time of 1st exposure to HSV-1. The possible influence of transplacental antibodies and the need for immunity to persist into adolescence and adult life present significant difficulties.

The Individual Already Infected with HSV-1. The HSV-2 vaccine could be given such individuals before adolescence, prior to the usual 1st exposure to HSV-2. Whether the vaccine would provide sufficient specific immunity is problematic, in view of the well-recognized inability of a prior HSV-1 infection to protect against HSV-2 infection (63).

The Individual Already Infected with HSV-2. Here, the HSV-2 vaccine might be of potential value in preventing recurrences. Since the recurrent infection might be as important or even more important than the initial herpetic infection in causing neoplastic transformations (65), such an approach could prove valuable.

These considerations suggest that, since there may well be specific responses to each of the two virus types, exploration of HSV vaccines should not only HSV-2 but also HSV-1.

In contrast to the human herpesviruses, we are fortunate, with HSV, to have a plethora of experimental animal models for genital or nongenital HSV-1 or HSV-2 infections which can be used to evaluate prospective vaccines. These include nonhuman primates, as well as rabbits and various rodents (21, 36, 47, 52, 64). Furthermore, a mouse model for delineating the effect of active immunization on the latency of HSV-1 and HSV-2 has recently been developed (105). Dose response, mode of administration, types of immunity elicited, and the duration of protection can readily be studied in such systems. In contrast to the ready availability of animal models for HSV infection, a satisfactory animal model for the evaluation of the protective effect of HSV vaccines against cervical neoplasia has yet to be developed. Model systems that might be considered include Cebus monkeys and mice. The utility of the monkey model awaits results of attempts to produce cervical neoplasia after genital inoculation of HSV-2 (70); however, preliminary results in the murine experimental system indicate that cervical neoplasia can be induced after genital inoculation of HSV (57, 60). The establishment of a satisfactory animal model is vital in view of the demonstration that immunization with HSV enhances tumor development in hamsters inoculated with HSV-transformed tumors (18).

Other considerations include not only potential reactogenic side effects of any prospective vaccine, but also long-term immunopathological effects. It has been shown, for instance, that erythema multiforme, which can occur with recurrent HSV infection, can also be precipitated by administration of inactivated HSV (85).

References

29. Hollinshead, A. In: Conference and Workshop on Cellular Immune...
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