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Recent Progress in Herpes Simplex Virus Immunobiology and Vaccine Research

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INTRODUCTION

Medically serious complications of herpes simplex virus (HSV) infection are rare but constitute a significant burden, given the high rates of HSV seropositivity in the population (58). Many prophylactic and therapeutic vaccination approaches have been explored for the prevention or treatment of HSV infection. Thorough reviews have included a historical perspective on HSV vaccines and descriptions of preclinical work (28, 41, 42, 71, 98, 180, 254, 255, 291, 292). The basic virology, pathogenesis, epidemiology, and clinical syndromes due to HSV infections are also the subjects of recent excellent reviews (218, 292). This review emphasizes vaccines reaching clinical trials in humans and recent findings relevant to the immunobiology of HSV. Both acquired and innate immune responses are discussed; while classic vaccines influence only acquired immunity, it has been increasingly realized that adjuvants affect the outcome of vaccination in large part by influencing innate immunity.

ETIOLOGIC AGENT

The DNA genomes of HSVs contain about 85 open reading frames. Five of the open reading frames are diploid. Initiation at internal methionine residues, mRNA splice variants, autocatalysis, and extensive posttranslational modifications such as phosphorylation, ribosylation, nucleotidylation, ubiquitination, and glycosylation add complexity to the proteome. Approximately half of the genes of HSV-1 are dispensable for replication in cultured cell lines (218). Fewer studies have been performed for HSV-2, but it is likely that most of the homologous HSV-2 genes are similarly dispensable or required. Deletion mutants with lesions in essential genes can also usually be propagated in vitro using *trans* complementation. Many HSV genes, especially those that are dispensable in vitro, are involved in immune evasion and pathogenesis. Both essential and nonessential genes are targets for modification in whole-virus vaccine formats.

HSV-1 and HSV-2 are members of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genera *Simplexvirus* (219). Their genomes are relatively stable compared with those of RNA viruses such as human immunodeficiency virus type 1 (HIV-1) or hepatitis C virus (HCV) (23, 54). HSV-2 has an inherently higher mutation rate than HSV-1 (225). Mutant strains can readily be selected in vivo by antiviral drug therapy (80). While patient-derived strains have specific nucleotide sequences, few data are available concerning variability within specific T- or B-cell epitopes (40, 100, 269).

Given the overall stability of the genome, it has been assumed that the viral strain chosen as the genetic data source for a subunit vaccine or as the parental strain for whole-virus

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approaches is relatively unimportant. Recent evidence that inactivated (or live) virus, or isolated viral proteins, can activate programs of innate immunity (4, 202) indicate that strain selection may still be important even if the epitopes recognized by the acquired immune system are relatively constant. Also, examples of variations in neutralizing epitopes among wild-type HSV-2 isolates have been documented (29). It is not known if immune escape variants arise in response to acquired immune responses and possibly reascend the axon, superinfect the ganglia, and reestablish latency, leading to endogenous reinfection. The related issue of exogenous reinfection is reviewed below. Mutant strains with a temporary, local replication advantage might also be more likely to be shed and transmitted. With the recent definition of many CD4 and CD8 epitopes and their HLA-restricting alleles (120, 122, 138), this hypothesis can be addressed.

HERPES SIMPLEX VIRUS CELLULAR REPLICATION CYCLE AND IMMUNE RESPONSE

The HSV replication cycle is central to understanding immunity to HSV and vaccine design. Interactions between HSV-encoded glycoproteins and cellular glycosoaminoglycans, such as heparan sulfate, are involved in virion binding to the cell surface (234). The HSV envelope glycoproteins gB, gD, and gH-gL are required for HSV binding and entry into cells. The neutralizing activity of antibodies directed against these proteins (83, 88, 104, 105, 187, 208) was one of the major rationales for the use of HSV glycoproteins as immunogens for HSV subunit vaccines.

After initial binding, interaction between gD and one of several high-affinity receptors is required for viral entry (45, 247, 248). The first receptor protein to be characterized was HveA (herpesvirus entry mediator A), a trimeric transmembrane tumor necrosis factor (TNF) receptor family member also known as TNFR14 or HVEM (191). Physiologic ligands of HveA include the TNF-like molecules LIGHT and lymphotoxin-α3 (168, 227). HveA can mediate costimulatory or pro-apoptotic (248) signals through its cytoplasmic domain (110, 162).

HveA is expressed by lymphoid and monocyte-dendritic lineage cells (75, 140, 191, 222). These cells types are permissive for HSV entry and replication in vitro (183, 215, 295), and some are present in lesions (63). Formal studies demonstrating that infection of lymphoid and myeloid cells is dependent on HveA have not been reported. While blood leukocyte cultures are occasionally positive in neonates or immunocompromised subjects (256), leukocytes are not currently appreciated as major targets of productive HSV infection in vivo. However, in vitro infections can be established in these cells (183) and modulation of the cellular functional activities by HSV could still influence local immune responses.

The second class of gD receptors to be described are several members of the immunoglobulin superfamily termed nectins, including nectin-1α (HveC). These transmembrane proteins may serve as homophilic adhesion molecules and localize to cadherin-based cell junctions. The nectins occur as several isoforms based on mRNA processing. A third type of receptor is generated by specific sulfation of heparan sulfate by certain 3-*O*-sulfotransferases (234). The relative importance of these classes of receptors in vivo is uncertain, but the expression of HveC by sensory neurons (96, 167) implicates this receptor in the pathogenesis of neuronal infection.

Elements within gD essential for interaction with HveA and the nectins have been identified (226, 227, 289). Reciprocal studies have defined host structures that interact with gD (45, 164, 248), and a crystal structure of gD bound to HveA (47) has been solved. Not unsurprisingly, a gD domain recognized by potent neutralizing antibodies is essential for interaction with these receptors (290). These recent insights raise the possibility that improved neutralizing antibodies might be elicited by subunit vaccines containing gD or other glycoproteins that present optimized neutralizing epitopes (290). Neutralizing epitopes might potentially include fusion intermediate structures formed during viral entry, as recently defined for HIV-1 (141). Receptor-blocking compounds based on gD interactions have already shown proof of principle in vitro.

Compelling evidence for the protective effect of antibodies in humans is available from studies of exposed neonates (37). If cervicovaginal HSV infection is present, the risk of serious neonatal infection after vaginal delivery is much lower if the mother has recurrent as opposed to primary HSV infection and is therefore able to provide the neonate with antibodies. The risk is not zero, however, and because more vaginal delivers occur during recurrent than primary HSV infection, a considerable proportion of cases of neonatal herpes do occur as a result of recurrent infection.

Because HSV appears to enter using proteins that function in cellular communication and adhesion, it is also possible that vaccination could elicit primary antibodies, or anti-idiotypic antibodies, with pathologic effects. However, no such events have been noted in a series of subunit vaccine trials which have enrolled over 10,000 subjects. Homozygous mutations of HveC are known to be associated with congenital abnormalities (265), and overexpression of the HveA ligand, LIGHT, leads to abnormal lymphoid tissue (233). Further research into the normal physiological functions of HSV receptors and ligands may allow rational prediction of the toxicities of vaccines or therapeutics directed at inhibiting HSV entry.

Anti-gH antibodies also have potent neutralizing activity (208). The exact role of gH and the identity of the host structures that it interacts with (if any) during viral entry with are unknown. gH forms an obligatory complex with gL (287). Deletion of gH (82) is the basis of the DISC series of discontinuously replicating HSV strains that have been tested as human vaccines (see below). These gH-deleted viruses must be grown on complementing cell lines. After successful entry into, and replication in, wild-type cells, gH-deficient progeny virions are incapable of completing entry into a second generation of cells.

After envelope fusion, the tegument and nucleocapsid are delivered to the cytoplasm. These "virion input proteins" gain access to the HLA class I pathway of antigen processing to CD8 T cells without a requirement for de novo synthesis (120, 272). Viral genes are then expressed in a temporally regulated program. Immediate-early proteins are expressed shortly after viral entry. Some, such as ICP4, are required for subsequent events in replication, so that cells infected with an ICP4 deletion mutant will express only the other immediate-early proteins and can be used to probe HSV-specific cellular immune responses (120). Others, such as ICP27, are associated with

high-titer replication and animal virulence and have been deleted in candidate replication-competent vaccine strains (173). The US12 gene encoding ICP47 is expressed with immediate-early kinetics. ICP47 is an inhibitor of the transporter associated with antigen processing (106, 297), a mandatory cellular intermediate in the presentation of most viral epitopes to CD8 T cells. Deletion of ICP47 leads to decreased neurovirulence in mice (95) and increased recognition of infected cells by human $CD8^+$ CTL (272). Viruses with deletions of multiple immediate-early genes have also been studied and have proven immunogenic in animal vaccination studies (35).

Immediate-early gene products in turn activate the transcription of early genes, which are expressed prior to DNA replication. Deletion of UL29, an early gene encoding the DNA binding protein ICP8, prevents DNA replication and true late-gene expression, while deletion of UL39, an early gene encoding the large subunit of ribonucleotide reductase, is associated with decreased virulence. An HSV-2 strain with a deletion in ICP8 has been used as a live attenuated vaccine and has been shown to attenuate experimental challenge with virulent HSV-2 (64). Double deletions within HSV-2 of both UL29 (ICP8) and UL5, which encodes a second protein required for DNA replication, have been constructed to increase safety for possible vaccine use (65, 66). In general, the breadth and magnitude of anti-HSV immune responses were found, in mice, to increase as viruses expressing additional temporal subsets of HSV proteins were used as vaccines (195). Late genes are expressed mostly after DNA replication and are required for viral assembly and egress. Mutations that reduce or block viral assembly or egress can be complemented by engineered host cells (68, 84). Other than gH-deleted strains (82), these viruses have seen little investigation as vaccines. Deletion of a late gene such as the one encoding gH has the theoretical advantage of expressing an almost full set of viral proteins. Knockout of genes in multiple kinetic classes has also been investigated. For example, lesions in the late *vhs* gene (UL41, see below), which has immune evasion properties, increase the immunogenicity of ICP8-deleted HSV-1 in mice (91).

PATHOGENESIS AND POINTS OF INTERACTION WITH INNATE AND ACQUIRED IMMUNITY

HSV is thought to be transmitted from person to person as cell-free virus. Abrasions help HSV gain access to living cells below the keratinized debris on skin surfaces. Theoretically, local antibodies could neutralize virus prior to cell entry. In this scenario, no initial replication would occur, preventing both neuronal infection and horizontal transmission. This form of sterilizing immunity has been achieved in mice but requires vaginal immunization with live HSV (205). More typically, vaccines which fully protect from morbidity and mortality in animals still allow local replication at genital mucosal sites of inoculation (135). While HSV-specific antibodies are detectable in the cervical secretions in HSV-infected persons (11) and can be elicited by vaccination (12, 205), it is doubtful if sterilizing immunity can be achieved in humans by current vaccine approaches.

Subsequent to epithelial infection, HSV enters sensory nerve endings and ascends in a retrograde manner to neuronal cell bodies in ganglia. It is not known if a level of immunity exists that differentiates between peripheral sterilizing immunity (205) and protection against initial entry into sensory neurons. The neurons become productively infected, and virus is transmitted across synapses. This phenomenon is thought to account for the HSV DNA that is widespread in the human central and peripheral nervous system (94, 112), although other explanations (hematogeneous spread) are also possible. Resolution of lytic ganglionic infection is dependent on (236) and temporally associated with (249) the infiltration of CD8-bearing cells. At the same time, the transcription and expression of HLA class I increases, even for neurons, which are typically negative for these proteins (209). Control of ganglionic infection does not seem to be associated with cytotoxic T-lymphocyte (CTL) killing of neurons, implicating cytokine-mediated nonlytic halt of replication (165, 210). Recently, interest in the role of NK cells in the control of primary HSV infection has been renewed by the mapping of a susceptibility locus in mice to within or near an NK cell receptor locus (211), although the role of NK cells in the control of primary HSV in humans is unclear.

A further consequence of ganglionic infection is the establishment of latency in a variable number of neurons. Latency is associated with transcription of an RNA species termed LAT (latency-associated transcript). While the major LAT contains open reading frames (218), expression of the predicted proteins has been difficult to document in vivo. Recently, it has been appreciated that the levels of cytokines of lymphoid origin are persistently elevated in HSV-infected ganglia (in animals) (50). Acyclovir treatment reduces this local immune activation (97). Additionally, low levels of lytic gene expression can be documented in "latently" infected ganglia by reverse transcription-PCR (51, 133) and may drive local activation of antigen-specific T cells or innate immunity cells. Reactivation of latent HSV from isolated ganglia ex vivo is promoted by anti-CD8 or anti-gamma interferon (IFN-γ) treatments (149, 150), implying that resident CD8 cells and/or lymphocyte-derived cytokines contribute to the control of reactivation. Taken together, these data imply that acquired immunity in general, and CD8 T cells in particular, may be important in both initial control and suppression of reactivation at the level of the ganglia.

Prevention of the establishment of latency is difficult, although many vaccines have been shown in animal models to reduce the levels of acute ganglionic replication and/or the establishment of latency, as assessed by explant cultures or molecular methods (18, 48, 93, 192, 250, 283). It is not known how the mechanisms of immunologic control of acute ganglionic replication, the establishment of ganglionic latency, and reactivation are related. Dose-ranging studies in mice and guinea pigs have demonstrated that the peripheral inoculum, the extent of initial ganglionic replication, the number of latently infected neurons, and the frequency of reactivation are all directly related (146, 228, 229). Data on these issues are not available for experiments with humans. The animal data do give rise to optimism that vaccines that reduce, without eliminating, initial peripheral or ganglionic replication may still have a positive impact on the natural history of the chronic phase of infection.

After reactivation from latency, viral components travel

down the axon and assemble for transmission to epithelial cells (108, 189). Antibodies have been shown in an elegant human two-chamber ex vivo model to inhibit transmission from dorsal root ganglion neurons to keratinocytes (187). Polyclonal and monoclonal antibodies to gD and gB were active, while antibodies to gC, gE, and gG were not. These data were interpreted to be consistent with transit of free virus between neuron and keratinocyte. Both IFN-α and IFN-γ are also active in this model if administered to the keratinocytes early enough after inoculation of the dorsal root ganglion neurons (1842). Possibly, vaccines that elicit T cells capable of rapid homing to incipient lesions might lead to early, local IFN-γ secretion. While HSV-specific T cells are approximately 100-fold enriched in lesions compared with their levels in blood (118), little is known concerning the expression of candidate skin or mucosal homing receptors by these cells.

Reactivation of HSV can lead to either subclinical shedding or symptoms and lesions. Because most sexual transmission occurs during asymptomatic shedding (176, 178), it is critical to measure the effect of proposed preventative or therapeutic immunologic interventions on subclinical shedding. Among immunocompetent persons, rates of subclinical and clinical reactivations are directly related (285), consistent with a model in which similar mechanisms are involved in their control. Therefore, full evaluation of the public health benefits of candidate HSV preventative and therapeutic vaccines requires measurement of their effects on subclinical shedding.

When lesions occur, there is a brisk infiltration of NK and CD4 cells followed by CD8 T cells (63, 123). High levels of β-chemokines and both Th1 and Th2 T-cell cytokines are present (185, 278). Antigen-specific CTL activity typically peaks after antigen-specific CD4 proliferative activity, and is correlated with viral clearance. Both CD4 and CD8 cells contribute to this CTL activity (123). Subclinical shedding in the absence of lesions or symptoms may be the outcome of particularly early or effective local cellular immune responses, but no experimental data support this hypothesis.

IMMUNE EVASION

Chronic pathogens coexist with ongoing innate and acquired immune responses. Microbial agents have developed means of subverting host responses, as recently reviewed for herpesviruses (2, 152, 276). Some of the immune evasion functions of HSV are mentioned above. Studies of the immune correlates of disease severity, and the process of choosing a vaccine compound and format, must take immune evasion into account.

Dendritic cells (DC) are important for priming cellular immune responses. Specialized DC reside in both the skin (Langerhans' cells) and the dermis (151). HSV has profound effects on myeloid-lineage DC derived in vitro by treating monocytes with cytokines. These cells express HSV receptors (222) and can be productively infected with HSV depending on their maturation status (134, 183). HSV infection reduces the maturation, expression of costimulatory molecules, and antigen-presenting capacity of these DC (134, 222). CD83 may be specifically degraded in HSV-infected DC (134, 222). Interestingly, DC infected with the HSV-1 DISC vaccine strain (see below) could prime naive T-cell responses to HSV in vitro, a fairly stringent test of DC function (107). Thus far, no group has identified the viral function(s) responsible for inhibition of DC function.

HSV is a profound inducer of IFN-α by unfractionated human peripheral blood mononuclear cells (PBMC) (224). This innate response influences acquired immunity, for example, by enhancing T-cell survival and supporting Th1-like responses (161, 217). Herpetic vesicle fluid contains very high levels of IFN (199). A DC variously termed plasmacytoid or pDC2, with a characteristic $CD4^+$ $CD11c^-$ $CD123^+$ phenotype in humans, can be purified from PBMC. These cells respond to HSV by secreting large amounts of IFN-α (235). Cells with this phenotype can accumulate in cutaneous blister fluid (232). The viral and cellular structures involved in the induction of IFN-α are undefined, although gD and specific chemokine receptors were implicated in a study using whole PBMC (4). It has recently been demonstrated that resiquimod and imiquimod, small molecules which induce IFN-α secretion (1, 30, 33, 43, 79, 279) and have clinical activity against HSV (253), act via Toll-like receptor 7 (TLR7) (102). pDC2 expresses TLR7, and studies may soon determine whether the innate IFN-α response to HSV also uses this pathway.

HSV evades IFN-α action in several ways. The *vhs* protein, encoded by the UL41 gene and also delivered preformed on cell infection as a virion component, has endoribonuclease activity (153). *vhs* antagonism of the antiviral effect of IFN-α (266) may reflect accelerated turnover of IFN-α-induced transcripts. A more specific effect has been shown for the product of the $\gamma_1$34.5 gene, which in *trans*-complementation assays with IFN receptor-, PKR-, and RNase L-deficient mice, controls HSV virulence through a PKR-dependent but RNase L-independent pathway (145). The $\gamma_1$34.5 protein inhibits IFN-induced phosphorylation of eIF-2α by redirecting protein phosphatase 1 to dephosphorylate eIF-2α. By doing so, $\gamma_1$34.5 reduces the shutoff of host protein synthesis normally caused by eIF-2α phosphorylation. Viruses with the $\gamma_1$34.5 gene deleted have extremely attenuated neurovirulence in animals (53), and deletion of both copies of this diploid gene is a rational component of the design of live attenuated vaccines (250).

As mentioned above, HSV can also infect a variety of immunocompetent cells. HSV infection of human T cells can lead to their lysis by HSV-specific CTL (215). This phenomenon, termed "fratricide," could influence the homeostasis of the CTL response to HSV. Exposure of monocytes to HSV causes the release of multiple cytokines (157, 200–202). These actions are not entirely dependent on live virus, and some can be mediated by soluble gD (4, 202). The molecules released by HSV-infected monocytes may have deleterious effects in animal models of HSV eye infection (116), although their net contribution to pathogenesis is uncertain.

HSV inhibits antigen processing and presentation in both the HLA class I and class II pathways. Inhibition of HLA class I antigen presentation by interaction of the US12 gene product, ICP47, with the transporter associated with antigen processing has been thoroughly described (106, 297). Cell types display differing sensitivity to this effect in vitro: fibroblasts (127, 272) and keratinocytes (127) are sensitive, while B cells (127) and T cells (215) are less sensitive. The *vhs* gene (UL41) is also involved in evasion of HLA class I-restricted responses. Infection of immature monocyte-derived DC with the discon-

tinuously replicating (gH-deleted) HSV-1 down-regulated class I, while wild-type HSV-1 did not (183, 222). Cells infected with *vhs* knockout viruses are recognized by CD8 CTL better than are cells infected with wild-type virus (272). IFN-γ can overcome HLA class I down-regulation in keratinocytes and fibroblasts in vitro (62, 120, 272), and high levels of IFN-γ are present in symptomatic HSV lesions (63, 275, 278). The dynamics and net effect of HSV and IFN-γ on class I expression have not been well studied in genital lesions. Limited human data from HSV encephalitis show net up-regulation of HLA class I in vivo (245).

Inhibition of TAP shortly after infection, by the immediate-early protein ICP47, would be predicted to favor CD8 recognition of virion input and immediate-early proteins. The available data (120, 186, 271) are consistent with this hypothesis. Restoration of HLA class expression on keratinocytes by IFN-γ secreted by infiltrating leukocytes may be required for any CD8 CTL recognition of infected keratinocytes. The spectrum of HSV antigens capable of being recognized may also be broadened in vivo by local cytokine effects. Knockout of ICP47 might increase the immunogenicity of a live or even discontinuously replicating HSV vaccine, although this has not yet been tested.

Less is known concerning HSV evasion of $CD4^+$ T cells. The immediate-early protein of HSV-1, ICP22, inhibits antigen presentation to CD4 T-cell clones in vitro. HSV-infected or ICP22-transfected EBV-transformed B cells were found to inefficiently present antigenic epitopes to CD4 T-cell clones (22). The mechanisms involved are unknown, and these cells may not be representative of functional antigen-presenting cells (APC) in vitro. HSV was observed not to reduce HLA DR levels on monocyte-derived DC, although key costimulatory molecules were down-regulated (183), as discussed above.

ACQUIRED IMMUNE RESPONSE TO HERPES SIMPLEX VIRUS

CD4 T-Cell Responses

TCR $\alpha\beta^+$ $CD4^+$ cells are strongly stimulated in vivo by HSV infection. Responses are generally undetectable in HSV seronegative persons. Recently, Posavad and coworkers reported a group of HSV-seronegative persons with proliferative responses to whole HSV-2 antigen (263; F. Struyf, E. Keyaerts, C. Posavad, L. Corey, M. Van Ranst, and P. G. Spear, Proc. 25th Int. Herpesvirus Workshop, abstr. 2.35, 2000). Whether this represents true acquired immunity to HSV-2, as reported for HIV-1 and HCV in exposed but seronegative populations (115, 231), remains unknown.

The integrated CD4 memory response to HSV-1 appears to occupy about 0.2% of circulating CD4 T cells when measured by intracellular cytokine flow cytometry for IFN-γ (7). Results of similar magnitude were obtained by enzyme-linked immunospot (230) and limiting-dilution (213) assays. To measure responder cell frequencies for specific HSV-2 peptides, we combined secondary in vitro restimulation of carboxyfluorescein succinimidyl ester (CFSE)-labeled PBMC with HLA class II tetramer staining. This technique led to the estimate that 0.002% of circulating CD4 cells responded to a specific DQB1*0602-restricted epitope in HSV-2 VP16 (139).

CD4 responses are typically assessed in vaccine trials or natural history studies by performing simple [^{3}H]thymidine incorporation proliferation assays, using whole PBMC as the source of APC and responders and using killed HSV as antigen. Limiting-dilution variants of this assay provide some degree of quantitation (143, 299). The predominant cell type secreting IFN-γ in response to HSV-1 antigen is the CD4 lymphocyte (60). However, caution is required in ascribing proliferative or cytokine responses to inactivated HSV antigen solely to $CD4^+$ responders. TCR γδ-bearing cells in the PBMC can proliferate to HSV; these responses are quite variable from person to person and do not segregate with HSV seropositivity (155, 156). Occasional CD8 T-cell clones that proliferate and secrete cytokines in response to killed HSV have been documented (111, 124). It has recently been appreciated that cross-presentation of the cytomegalovirus tegument protein, pp65, in apoptotic bodies has the ability to restimulate pp65-specific CD8 T-cells (5, 268). It is therefore also possible that the typical UV-inactivated HSV preparations used in typical "CD4" lymphoproliferation experiments also stimulate CD8 memory cells.

Given these caveats on proliferation and cytokine secretion studies, no consistent correlations between CD4 responses and disease severity have been observed in natural history studies. A small study indicates that brisk spontaneous (61) IFN-γ secretion correlates with a longer time to the next symptomatic recurrence of HSV-1. Comparison of Th1 (IFN-γ and interleukin-2 [IL-2]) and Th2 (IL-4 and IL-10) responses by PBMC between HSV-1-infected persons with and without a history of symptomatic infection failed to detect statistically significant differences, although trends were present consistent with a protective effect of strong Th1 responses (251). In phase II studies of prophylactic vaccination with gB2 and gD2, the vaccine-induced CD4 responses were as high as those measured in specimens from persons with natural HSV-2 infection (143). However, this vaccine was not protective against infection (57). Moreover, in a cross-sectional study of HIV-1- and HSV-2-infected men, the clinical severity of recurrent HSV-2 infection did not correlate with HSV-2-specific CD4 cell number as measured by the limiting-dilution assay (214).

HSV-specific $CD4^+$ T-cell clones are heterogeneous with regard to CTL activity (296), but broad CD4 CTL activity has not been measured as a correlate of disease severity. CD4 CTL are capable of killing infected, IFN-γ-treated keratinocytes in vitro (182), and HSV-2-specific CD4 CTL are present in the human cervix (125) and in herpetic skin lesions (121, 123). Some, but not all, research groups have reported that CD4 cells are required for the protection of immune mice from vaginal HSV-2 challenge (188, 206). These data, along with the important roles of CD4 cells in supporting B-cell (294) and CD8 T-cell function, make it likely that a successful vaccine will elicit CD4 responses.

The CD4 response to HSV in humans is broadly directed. Targets include immediate-early (D. M. Koelle et al., unpublished data), early (dUTPase [122, 126]), and late proteins. At the structural level, virion proteins from the envelope (gB, gC, gD, gE, and gH [121, 126, 181, 274, 288, 300]), tegument (VP11/12, VP13/14, VP16, VP22, and the UL21 gene product [122, 280]), and capsid (VP5 [126]) are all recognized, as are nonstructural proteins that are only present within infected

cells (dUTPase and ICP8 [246]). These studies with defined antigens and T-cell clones reinforce earlier studies with fractionated viruses and PBMC (114). The large number of proven epitopes in VP16 and VP22 (122, 124, 138) is likely to reflect the intensive investigation of these proteins rather than bona fide immunodominance. A relatively large proportion of HSV-2-specific CD4 clones, including lesion-infiltrating clones, have been HLA DQ or DP restricted. Since lesional keratinocytes primarily up-regulate HLA DR (63), while Langerhans' cells express high levels of HLA DR, DP, and DQ (32), the broad use of class II-restricting loci may indicate that memory CD4 T cells are restimulated by professional APC in vivo.

CD8 T-Cell Responses

Investigation of antigen-specific CD8 responses has been difficult for both experimental and biological reasons. HSV-specific CD8 T cells occur at low frequency in PBMC and require secondary in vitro restimulation with antigen prior to the use of ^{51}Cr release cytotoxicity assays. The immune evasion mechanisms targeting the CD8 response, mentioned above, complicate restimulation, as does the need for autologous or HLA-matched APC. The most frequently used systems involve the use of autologous HSV-infected lymphoid cells as stimulators (treated to reduce HSV infectivity), CD8 cell selection prior to stimulation or readout, and autologous immortalized B cells as target cells. IFN-γ-treated keratinocytes are also useful as APC (62, 120, 181, 182, 186). It will be important to determine how the results are influenced by the type of APC used.

Several lines of evidence indicate that CD8 responses are functionally important. A cross-sectional study of HSV-2- and HIV-coinfected men, using a limiting-dilution variant of the above system, showed an inverse correlation between CD8 responder number and severity of genital herpes lesions (214). Infiltration of $CD8^+$ cells occurs after the infiltration of $CD4^+$ cells in recurrent HSV lesions (63). We found that HSV-specific CD8 T- cells were locally enriched in recurrent genital HSV-2 lesions (118, 127). In subsequent serial lesion biopsy studies, the infiltration of CD8 T- cells and CTL, but not CD4 or NK cells, was found to correlate temporally with the clearance of infectious virus (123). Data from murine models concerning ganglionic infection and CD8 were was mentioned above.

The data available to date suggest that the $CD8^+$ T-cell response to HSV is more narrowly focused than is the $CD4^+$ response. Whether this is due to methodological issues or to a true biological difference is not clear. Early studies isolated single HLA class I-restricted CD8 clones specific for gB2 and gD2 (271, 272) but could not define the specificity of most CD8 CTL clones. A keratinocyte APC model revealed a high population prevalence of PBMC CD8 CTL responses to the immediate-early proteins ICP4 and ICP27 (186). These studies used in vitro restimulation methods, which could potentially favor the detection of responses to certain proteins.

To avoid secondary in vitro restimulation, we have used the in situ lesional enrichment of CD8 CTL. HSV-2-specific CD8 CTL clones isolated from lesions were cocultivated with Cos-7 cells cotransfected with the cDNA encoding the restricting HLA class I heavy-chain molecule and a library of HSV-2 DNA. T-cell activation was detected by IFN-γ secretion using an enzyme-linked immunosorbent assay (ELISA). While the novel specificities were uncovered using Cos-7 as APC, in each case the HSV-2 reactivity was initially detected using the human Epstein-Barr virus lymphoblastoid cell line (EBV-LCL) as APC and in most instances was also confirmed using skin-derived fibroblasts and/or keratinocytes as APC (120). With these methods, epitopes recognized by $CD8^+$ T cells were uncovered in the immediate-early protein ICP0 and the tegument proteins VP13/14 (encoded by UL47) and VP22 (encoded by UL49) (120). Limited population prevalence studies using HLA class I peptide tetramers revealed that most HLA-appropriate persons had responses to UL47 (120). For the tegument-specific clones, protein transferred into cells on virion entry was sufficient to sensitize target cells to lysis. Further work is required to rank HSV-2 antigens in a hierarchy of immunodominance.

Antibody Responses

Neutralizing antibodies to HSV were documented in the 1930s (reviewed in reference 292) and formed the basis for early viral typing methods. Antibodies also cooperate with cells, including polymorphonuclear leukocytes, monocytes, and NK-like cells, in mediating antibody-dependent cell-mediated cytotoxocity (128). As noted above, the natural history of infection after neonatal exposure implies that antibody alone can be protective against infection and disease in some situations. Another indication of the functional importance of antibodies is the development of HSV-encoded immune evasion functions directed at the humoral response.

The HSV-encoded gE-gI high-affinity Fc receptor expressed by infected cells may absorb and inactivate anti-HSV antibodies. Animal pathogenesis experiments with viral FcR knockouts clearly show that this function is associated with virulence (193). In this analysis, HSV-specific antibodies do not protect because they are "neutralized" by the virus. Friedman has suggested that immunization strategies which generate anti-gE or anti-gI antibodies, which block this FcR function, might enhance the activity of vaccine-induced neutralizing antibodies (H. M. Friedman, Letter, JAMA 283:746, 2000).

Serum and local antibody responses are typically broad, reacting with envelope glycoproteins and tegument, and capsid proteins (10, 11). Immunoblots using whole virus antigen have a very complex pattern. To date, no consistent correlations between levels of antibodies, their subclasses, their functional activities, or their fine specificities and the severity of HSV infection have been demonstrated. Limited data suggest that symptomatic HSV-infected persons may have higher levels of antibody than asymptomatic but seropositive persons (251), consistent with a model in which antibody levels are driven by antigen load.

Cervical antibodies to HSV-2 are mostly immunoglobulin G (IgG), although HSV-specific IgA is also detectable (11, 12, 14). Immunized IgA knockout mice were not particularly susceptible to HSV-2 in a murine vaginal challenge model (220). The greater activity of therapeutic (260) and prophylactic HSV-2 vaccines in women than in men raises the possibility that the induction of mucosal antibody responses may be clinically important.

Vaccines can induce neutralizing antibodies. The Chiron gB2/gD2 vaccine used with alum (below) was able to elicit HSV-2 neutralizing antibodies in HSV-seronegative subjects, but the levels were lower than those measured in HSV-2 infected persons (259). Among HSV-1-infected subjects, this vaccine regimen increased HSV-2 neutralizing-antibody titers to levels above those seen in natural HSV-2 infection. Among HSV-2-infected subjects, vaccination raised levels significantly above baseline (258). Use of MF59 adjuvant in HSV-seronegative subjects produced neutralizing-antibody levels at or above the levels measured in HSV-2-infected persons (57, 143). However, this vaccine did not protect against infection, and subgroup analyses failed to show any correlation between neutralizing-antibody titers and protection against sexual HSV-2 acquisition (57). Antibody data from the GlaxoSmithKline (GSK) gD2-MPL vaccine, which did protect subsets of subjects from HSV-2 infection and disease, may clarify the possible role of antibody in vaccine-induced protection against HSV-2.

Complement in normal serum has potent virus-neutralizing activity. The HSV-1 gC binds several components of complement and reduces the antiviral activity of complement. This effect is at least in part antibody independent (87, 154). Complement can also enhance antibody-dependent lysis of infected cells (223). Blockade of the complement receptor activities of gC has also been proposed as a measure to enhance the antiviral effects of antibody (154).

Type-Common and Type-Specific Responses

The influence of preexisting immunity to HSV-1 (type-common responses) on the pathogenesis and natural history of HSV-2 infection is highly relevant to HSV-2 vaccine design. If HSV-1 protects against HSV-2, type-common responses in HSV-1-infected persons become rational benchmarks for vaccine-induced immunity. Type-specific seroprevalence data are quite unclear and inconsistent in this area. If prior HSV-1 infection protected against HSV-2 acquisition, geographic areas with a high seroprevalence of HSV-1 might have relatively low seroprevalences of HSV-2 infection. HSV-1 is invariably acquired during early childhood in many locations. For example, despite the nearly universal presence of HSV-1 antibodies, HSV-2 seroprevalences in Africa are among the highest in the world (92, 284). Similar data have been obtained in Mexico (55). In contrast, in other geographic areas, such as Japan, the seroprevalence of HSV-1 is moderately high but the seroprevalence of HSV-2 is very low (99).

Small, prospective studies of couples suggested that prior HSV-1 infection protects against HSV-2 acquisition (39, 178). In contrast, prospective study of 6,107 pregnant women showed no difference in HSV-2 seroconversion between HSV-seronegative and HSV-1-seropositive persons (38). Observation of 1,135 HSV-2-seronegative persons receiving a placebo vaccine also failed to show a protective effect of prior HSV-1 infection (57). Curiously, HSV-1-seropositive recipients of placebo did have a lower attack rate for HSV-2 than did HSV-1-seronegative persons in the GSK trial (257), with results reaching statistical significance in women, but not men.

While the data are unclear on whether prior HSV-1 protects against HSV-2 infection, HSV-1-infected persons have shorter and milder symptoms associated with the acquisition of HSV-2 infection (56) and are more likely to have asymptomatic HSV-2 seroconversions (144) than are HSV-seronegative persons. However, the subsequent rates of symptomatic recurrences (24) and of asymptomatic shedding (285) are similar. These data warn that prophylactic vaccines that ameliorate symptomatic genital herpes, but do not limit infection, may have only limited public health benefit.

Type-common responses elicited by HSV-2 infection protect against HSV-1 infection (144). Given the serious medical sequelae of HSV-1 infection (292), administration of an active HSV-2 vaccine to HSV-seronegative persons might have clinical benefit by reducing HSV-1 infection and/or disease. In addition, since up to 30% of first clinical episodes of genital herpes are caused by HSV-1 (52, 58), administration of an "HSV-2-based" vaccine with cross-protection against HSV-1 would be desirable.

Type-common responses also have a practical influence on HSV-2 vaccine design and protocols for administration. Systemic reactogenicity of the Chiron gB2/gD2/MF59 was higher in the HSV-1-infected persons than in seronegative controls in a dose-finding phase II study (143). It is possible that cross-reactivity may limit the administration of potent vaccines to HSV-1-infected persons.

Definition of T-cell epitopes permits the direct assessment of the type specificity of clonal CD8 and CD4 responses. As expected, many of the CD4 T-cell clones specific for gB and gD described in the 1980s recognized type-common epitopes, since these proteins are >85% identical at the amino acid level (72, 169, 170). However, we have found more recently that the CD4 response to HSV-2 recognizes a large number of polypeptides (see below). More than 50% of the CD4 epitopes found to date are type specific for HSV-2 (118, 121, 122, 138). Similarly, the great majority of the known CD8 epitopes in HSV-2, including the only described epitope in the vaccine compound gD2 (271), are type specific. Recent efforts by our group to clone HSV-specific CD8 CTL from blood and herpetic lesions have yielded almost exclusively HSV-2 type-specific responses (118, 120, 123, 212, 213; Koelle et al., unpublished). Both the type specificity of the cellular immune response to HSV-2 and the degree to which prior HSV-1 infection protects from HSV-2 infection and disease require further study.

VACCINE DEVELOPMENT

Experimental vaccine formats have included peptides, proteins, mixtures of viral proteins, whole and split killed virus, replication-defective viruses, and attenuated replication-competent viruses. The advantages and disadvantages of these formats have been reviewed (255). Delivery of peptide and protein antigens has been performed in a variety of formats including recombinant heterologous viruses and bacteria and as DNA predicted to encode these antigens. Many adjuvants have been explored with these various forms of antigen, and a large number of animal species, routes, schedules, and doses of vaccine and variety of virus challenges have been evaluated. Major histocompatibility complex-inbred mice have been preferred for their uniformity and the related ability to analyze peptide-specific responses. After vaginal inoculation, guinea pigs experience locally recurrent disease that mimics some

features of human genital herpes. Therefore, the "natural history" of modern vaccines entering clinical trials typically includes extensive work in these models, which will not be reviewed in detail. We review selected recent human and animal trials, moving from simpler to more complex antigens and highlighting demonstrations of immunologic principles which may move into human trials.

Peptide Vaccines

The hypothesis that immune responses to a single epitope can protect against disease has been tested in major histocompatibility complex haplotype-identical inbred mice. When administered in a suitable context, immunization with a single immunodominant CD8 CTL epitope (31) or neutralizing epitope (179) can be protective. These systems are of interest in that adjuvants such as T-helper epitopes (220) and heat shock proteins (137), "string-of-pearls" sets of epitopes expressed as minigenes (298), and expression formats such as recombinant viruses (31, 302) and bacteria (49) can be quantitatively compared. The outbred nature of the human population obviously complicates the development of peptide-based vaccines, especially since immunodominant and disease severity-associated peptide-specific responses have not yet been defined. A phase I trial of immunization with a type-common, HLA A*0201-restricted epitope in gB2 (272) and a heat shock protein adjuvant has recently begun. This study is designed to determine if the peptide "concept" can elicit high levels of $CD8^+$ T cells to an HSV antigen. Both HSV-seronegative and HSV-2-seropositive subjects will be studied.

Subunit Vaccines

This type of vaccine presents a more complex antigen to the immune system, potentially including CD4, CD8, and B-cell epitopes. Recombinant protein formats have been extensively tested in humans. Conceptually similar, the introduction of complete or near-complete HSV open reading frames into heterologous viruses (3, 26, 76, 159, 163), bacteria (81, 136), or expression vectors for DNA vaccination (76–78, 89, 194, 237–240), has been extensively tested in animals. As with peptides, subunit vaccines in these formats have allowed comparison of a large variety of adjuvants and vaccine formats. A phase I trial of a DNA vaccine using the gene for gD of HSV-2 has been completed, and immunogenicity analyses are under way.

Two similar subunit vaccines have recently completed phase III clinical trials. The Chiron vaccine contained gB2, truncated at amino acid 696, and gD2, truncated at amino acid 302, expressed in transfected CHO cells. The truncations of cytoplasmic tail and transmembrane domains were made to help the proteins exit the producing cells, allowing the manufacturing process to yield large amounts of protein. Phase I studies with gD2 and alum adjuvant established that this vaccine could both induce ELISA and neutralizing antibodies in seronegative subjects and boost preexisting responses in HSV-1- and also HSV-2-infected subjects. Maximal neutralizing-antibody levels in seronegative subjects were somewhat below baseline neutralizing-antibody levels in HSV-2-infected persons. Lymphoproliferative responses to gD2 were also induced (259).

A novel adjuvant containing MF59 (see below) and the muramyl dipeptide derivative MTP-PE (198) was then tested with recombinant gD2 and gB2. This adjuvant and gD2 were active in prophylactic and therapeutic guinea pig models of genital HSV-2 (42). While this vaccine preparation was immunogenic in humans, it had a high level of local and systemic reactivity, particularly in HSV-1-infected subjects (C. L. Dekker, S. F. Adair, R. Sekulovich, N. Niland, and R. L. Burke, Prog. Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother, abstr. 1431, 1992). Phase III studies, therefore, used MF59 alone as the adjuvant.

Adjuvant MF59 is a submicron-sized oil-in-water emulsion containing squalene, sorbitan monoleate (Tween 80), and sorbitan trioleate (Span 85) (197). In randomized human trials with HBV and cytomagalovirus antigens, MF59 was superior to alum with regard to induction of antibodies (101, 207). Labeled MF59 is internalized by macrophage-like and DC-like cells after intramuscular injection and is transported to draining lymph nodes (73, 74). Some data indicate that MF59 may produce a Th2-like CD4 response (241, 277).

The pivotal phase III experiments compared injection of 30 μg of truncated gB2 and gD2 with MF59 at 0, 1, and 6 months to injection of MF59 alone in 1:1 randomized double-blind trials. Subjects were monitored for 1 year after the last vaccination. One trial administered vaccine to the HSV-2-seronegative partner in stable couples disparate for HSV-2 infection status as assessed by serologic testing. The study population in the second trial were sexually transmitted infection clinic attendees with multiple partners or a history of a sexually transmitted infection.

The primary end point was seroconversion to HSV-2 as assessed by sensitive and specific immunoblotting (8). To clarify testing, type-common gB- and gD-specific antibodies were reduced by absorption from the serum of HSV-1-infected subjects. Seroconversions were confirmed by gG-based assays. Neutralizing antibodies and ELISA antibodies to the administered glycoproteins were also tested.

Vaccination appeared to have a partial and transient protective effect against HSV-2 seroconversion, which was most apparent during the initial 150 days of the trial, in women, and in HSV-1-seronegative persons. However, there was no overall protection regardless of sex or HSV-1 serostatus. While the study was not powered to uncover differences in HSV disease, there were no differences in the proportions of asymptomatic and symptomatic seroconversions between vaccine and placebo recipients in any subject group. There was also no difference in the duration of first-episode genital HSV-2 disease or the recurrence rate between vaccine and placebo recipients.

Overall, the peak neutralizing-antibody and ELISA titers exceeded those observed in natural HSV-2 infection. In a nested case-control study, immunized HSV-2 seroconverters were compared to matched, immunized nonseroconverters. There was no correlation between the peak levels of vaccine-induced neutralizing or anti-glycoprotein antibodies and seroconversion to HSV-2.

GSK has conducted trials with a vaccine containing a similar, truncated form of gD of HSV-2. The vaccine was administered at a dose of 20 μg intramuscularly at 0, 1, and 6 months. The protein was compounded with an adjuvant containing alum and 3-*O*-deacylated monophosphoryl lipid A (3d-MPL). MPL is derived from the gram-negative bacterium *Salmonella en-*

terica serovar Minnesota R595 (21). Forms of MPL have been reported in human studies to favor the induction of Th1-like CD4 responses and delayed-type hypersensitivity responses (M. Koutsoukos, G. Leroux, P. Vandepapeliere, M. Slaoui, and P. Pala, Program Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M. 1994; G. Leroux-Roels, E. Moreau, I. Desombere, O. Crayne, M. Francotte, P. Pala, M. Slaoui, and P. Vandepapeliere, Program Ab 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. H57 1994) and to be superior to alum for the induction of antibodies to hepatitis B surface antigen (270). There are no human data available concerning the induction of primary CD8 responses by 3d-MPL. MPL, based on its structural similarity to the lipid A component of lipopolysaccharide, may provide a "danger signal" to APC by interaction with CD14 and/or toll-like receptors to enhance immunologic priming.

The GSK vaccine results have recently been reported, 257) and suggest approximately 75% efficacy in the prevention of clinically apparent genital herpes. Only HSV-seronegative women were protected. Protection against HSV-2 infection among HSV-seronegative women was on the order of 40%. No statistically significant efficacy was seen in men, although the number of cases of genital herpes disease and HSV-2 seroconversion were small. It is unclear what accounts for the differences in the results of the Chiron and GSK trials. The gD2 proteins were very similar. Different adjuvants were used. No direct comparisons of the immune responses of vaccines containing MPL or its derivatives versus MF59 are available, and detailed immunogenicity data from the GSK vaccine are not yet available.

Subunit vaccines containing truncated HSV-2 glycoproteins have also been evaluated as immunotherapy in adults with symptomatic genital HSV-2 infection. Rational design of HSV-2 immunotherapy is difficult because we do not have consistent immune correlates of disease severity among naturally infected persons. Two doses of 100 μg of gD2 with alum were administered 2 months apart in a blinded, placebo-controlled trial. A statistically significant reduction in the number of genital herpes recurrences was detected during the 12-month observation period. Neutralizing-antibody and lymphoproliferative responses were increased in vaccine recipients, but no correlations were noted between immune responses and vaccine efficacy among the treated group. In a follow-up study, a smaller dose (10 μg) of both gD2 and gB2 was administered with MF59 adjuvant (see above). While there were suggestions of clinical activity, especially with regard to the severity of the first postvaccination recurrence, there was no overall difference in the monthly rates of HSV recurrences between the vaccine and MF59-alone control groups. Again, no relationship between baseline or vaccine-induced antibody levels and vaccine efficacy was noted (258, 260).

Killed-Virus Vaccines

Many variations of the killed-virus vaccine format have provided protection in animals. Early vaccines were made by phenol treatment of infected animal tissue or UV light treatment of cell culture-derived virus. These preparations were evaluated as immunotherapy; unfortunately, as reviewed by Whitley, many reports did not include placebo controls. Placebo-controlled studies failed to establish long-term benefit (292), although some immunotherapeutic studies have shown short-term benefit similar in magnitude to that provided by suppressive acyclovir (166). We now know that both asymptomatic (119) and symptomatic (25) genital HSV-2 decrease over time, so that some improvement is part of the natural history of infection. Many studies were performed before accurate type-specific serologic testing or typing of viral isolates became available, and not all subjects had culture-confirmed disease, placing some doubt on the assignment of subjects. Subject-reported disease severity, without clinician examination or cultures during suspected recurrences, was used in many studies as the primary clinical end point.

An update on experience using this type of vaccine to prevent genital herpes was provided by Skinner et al. (242). Cells infected with a mixture of clinical HSV-2 isolates were sonicated and clarified. The resulting supernatant, which was likely to contain virions and viral protein, was treated with formaldehyde to make the vaccine. The dosage, number of administrations, and adjuvants varied. While the vaccination appeared safe, no conclusions about efficacy are possible from the available data.

Fractionated-Virus Vaccines

Several groups have prepared HSV vaccines by subjecting infected cells to procedures to inactivate virus and to partially purify subsets of viral proteins. Randomized, blinded, placebo-controlled trials were conducted in the mid-1980s by Merck with a mixture of envelope glycoproteins. HSV-2-infected cells were treated with detergent and DNase to inactivate virus, and glycoproteins were enriched by binding to a plant lectin. The vaccine contained gD2, gB2, and other HSV-2 glycoproteins (13). The bound fraction was further inactivated with formalin and compounded with alum. Most seronegative vaccinees developed neutralizing and antibody-dependent cell-mediated cytotoxicity antibodies and lymphoproliferative responses to HSV-2, but HSV-1-seropositive vaccinees had no increase in preexisting responses (177). The HSV-reactive T-cell clones recovered from vaccinated, initially HSV-seronegative donors were mostly $CD4^+$ and $CD8^-$; a subset of these clones had CTL activity (299). A dose of 50 μg was administered at weeks 0, 4, and 22 in a blinded, placebo-controlled trial enrolling both HSV-uninfected and HSV-1-infected subjects. No protection from HSV-2 infection or disease was noted in a double-blind, placebo-controlled trial of 161 subjects (175).

A vaccine enriched in HSV-2 envelope glycoproteins by detergent extraction and sucrose density centrifugation was studied by Cappel et al. in the 1970s and 1980s. The vaccine was immunogenic and appeared to decrease the severity of recurrent HSV-2 disease (46), but it did not undergo further development. Experience with a preparation emphasizing viral proteins expressed in the cytoplasm of infected cells has been recently updated by Skinner et al. (243). The preparation of a recent HSV-1 vaccine involved the reduction of nuclear material by detergent lysis, treatment with formaldehyde, centrifugation to remove virions, and harvest of a protein fraction of the remaining material by acetone precipitation. A 300-μg quantity of this material was injected three times with alum adjuvant into subjects with clinical diagnoses of genital herpes

in a randomized, double-blind, placebo-controlled trial. There was a trend toward modest increases in neutralizing and total antibodies and HSV-stimulated proliferation (bulk PBMC) stimulation indices in the vaccinees. While safe, there was no strong evidence that vaccination had clinical benefit.

Discontinuously Replicating Viruses

The deletion of one or more genes required for productive viral replication was discussed in an earlier section in the context of the viral replication life cycle. This type of HSV strain is typically grown in a complementing cell line genetically engineered to provide the required function in *trans.* When viruses with a deletion of UL22, the late gene encoding gH, infect a noncomplementing cell, the progeny virions can exit the cell but cannot infect a secondary cell. This type of HSV strain has been termed DISC (for "discontinuously replicating virus"). Other, conceptually similar HSV-2 deletion strains developed by Knipe and coworkers (192, 195) and Aurelian et al. (18), with blocks at a variety of points in the replication cycle, are discussed earlier in this review and have not yet entered human trials for HSV indications. All of these vaccine candidates share the potential disadvantage that they will not periodically restimulate immunity due to recurrent, peripheral lytic replication.

Replication-deficient vaccines elicit a classic CD8 CTL in mice after a single injection (85). Both HSV-1 (172) and HSV-2 (34) DISC viruses were protective in a guinea pig vaginal challenge model of HSV-2 infection. Immunotherapy experiments with the HSV-1 DISC virus in HSV-2-infected guinea pigs showed a trend toward improvement (171). With regard to safety, the HSV-2 DISC virus was avirulent in the normally susceptible BALB/c and nude athymic mouse strains. The establishment of neuronal latency could be detected after high-dose vaccination, but no reactivation from latency of the vaccine strain was noted.

The replication-deficient gH-deleted HSV-2 vaccine has entered clinical trials and has been safe and immunogenic. No serious adverse effects were noted during receipt of two doses of 2×10^3 to 3×10^5 PFU 8 weeks apart, regardless of HSV-1 or HSV-2 serostatus. No live virus was recoverable from sites of inoculation. Among HSV-seronegative subjects, dose-dependent induction of PBMC [^{3}H]thymidine incorporation in response to inactivated HSV antigen, typically ascribed to CD4 T-helper responses, was noted 4 weeks after a single infection and was detectable 16 weeks after the second infection. Responses were not increased in HSV-seropositive subjects. IFN-γ secretion was also induced in HSV-seronegative persons in a dose-related fashion, and IL-5, as an indicator of Th2 responses, was also induced in this population. No increases in cytokine production were detected in HSV-infected persons. De novo antibody responses were not noted in seronegative persons, and increases in preexisting titers were not seen in HSV-infected subjects. CTL responses were noted in one of six subjects receiving the highest dose but were not characterized as CD4 versus CD8 mediated. Follow-up studies used up to three doses at up to 7×10^6 PFU/dose. The higher-dose regimens induced antibody responses. In addition, proliferative (probably CD4) and cytokine secretion responses were induced and most of the higher dose recipients acquired CTL activity. The phenotypes of the CTL were not evaluated (J. K. Hickling, S. E. Chisholm, I. A. Duncan, E. J. Taylor, C. Boswell, C. S. McLean, J. Uttridge, J. S. Roberts, A. Tomasi, L. Stanberry, D. J. Bernstein, M. E. G. Boursnell, and S. C. Inglis, Proc. 8th Int. Congr. Infect. Dis., abstr. 20.008, 1998.)

A large phase 2 trial of HSV-2 DISC as an immunotherapeutic agent was performed. The study population included 483 HSV-2 seropositive adults with symptomatic infection. Recent analyses of data showed no clinical improvement (Xenova Group; http://www.cantab.co.uk/pr2001101001.html).

Replication-Competent Live-Virus Vaccines

The replication-competent live-virus vaccine approach has the advantages of stimulating a broad (antibody, CD4, and CD8) response and presenting a complex mixture of epitopes from the entire genome, lacking only those encoded by the purposely deleted genes. If the virus can establish latency and reactivate without harming the host, endogenous boosting may occur. Experience from experiments with varicella virus indicates that a single vaccination can be effective and that responses are quite durable (6). Disadvantages of live-virus vaccination include the inherent neurovirulence of HSV, the possibility that vaccine strains could reactivate and either recombine with wild-type virus or be transmitted to immunocompromised persons, the potential instability of the genotype of the vaccine strain during production, and interference with serologic testing for HSV infection.

Which genes should be deleted in a live attenuated vaccine? As comprehensively reviewed by Roizman and Knipe (218), many HSV-1 genes that are nonessential in culture alter virulence in animal models. Fewer HSV-2 genes have been studied, and there are almost certainly type-specific effects. In addition, neither murine acute lethality, or reactivation models, nor the guinea pig vaginal model, closely mimics the pathogenesis of HSV in humans. The effect of alterations in thymidine kinase provide an example of species and virus type-specific effects. Thymidine kinase-deficient mutants of HSV-1 have reduced acute lethality in mice, and while these strains can establish latency, they reactivate in vitro very poorly (113). However, thymidine kinase-deficient strains of HSV-2 can cause severe acute disease in mice and guinea pigs and can reactivate in guinea pigs similarly to wild-type strains (27). A case of recurrent human genital HSV-2 disease caused by thymidine kinase-deficient, mouse-virulent strain has been reported (267). Mutations in thymidine kinase, in isolation, clearly do not attenuate HSV-2 sufficiently for human vaccines.

Deletion of a portion of the ribonucleotide reductase large-subunit gene (RR1 protein, ICP10 of HSV-2, UL39 gene) with protein kinase activity yields an HSV-2 strain with attenuated replication in vivo and in vitro (18). This tegument (244) protein can self-phosphorylate; the cellular target(s) of the protein kinase is unknown (218). Interestingly, the kinase domain seems to have been captured from a cellular kinase expressed in keratinocytes (19). Removal of the kinase domain reduces the transforming activity of HSV-2 (reviewed in reference 16). Human studies with RR1 mutants have not been reported.

A second live, attenuated HSV strain developed for vaccine use is RAV 9395 (250). This virus is based on HSV-2 strain G and contains deletions of both copies of the virulence factor

$\gamma_1$34.5 mentioned above, UL55, and UL56. Clinical results have not been reported for this agent. Attenuated HSV with deletions in multiple genes are also being developed for local therapy of cancer (59).

The most extensive experience in humans with an attenuated live HSV vaccine is with strain R7020, created by Roizman and coworkers. This virus, based on HSV-1 strain F, is attenuated by a deletion extending from UL54 (encoding ICP27) through the promoter region of ICP4. A portion of the unique short region of HSV-2 encoding gD2, gG2, gI2, and a part of gE2 was inserted in this deletion to make R7020. This virus was very strongly attenuated in rodents and primates (173, 174). In a dose escalation study, local reactions were noted in HSV-1-infected persons. A dose-dependent induction of antibodies occurred in HSV-seronegative subjects (M. Cadoz, M. Micoud, J. M. Seigneurin, M. R. Mallaret, C. Baccard, P. Morand, B. Meignier, R. Whitley, and B. Roizman, Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 341, 1992), but the virus was thought to be poorly immunogenic overall, and development has been stopped (291).

POTENTIAL TOXICITIES OF VACCINES

As noted above, administration of subunit glycoprotein vaccines to HSV-seropositive persons has been associated with local pain and induration and with fever, especially when potent adjuvants have been used (260). This has generally been ascribed to restimulation of antigen-specific T cells or to the formation of immune complexes, with subsequent release of cytokines and inflammation, although reactions have also been noted in HSV-seronegative persons (257). Unless vaccination is preceded by serologic testing, an expensive and imperfect procedure (9), it is likely that any mass vaccination program would expose HSV-infected persons to vaccine. The competing requirements for high antigenicity and low reactogenicity may be difficult to satisfy in this population.

Over the last decade, a role for HSV-specific immunity has been hypothesized for two medical disorders, HSV-associated erythema multiforme (HAEM), and herpetic interstitial keratitis (HSK). No toxicity related to these diseases has been recognized in trials of over 10,000 persons, but brief review is warranted, given the increasing use of potent adjuvants and targeting of cellular immunity in contemporary vaccination trials. HAEM refers to episodic erythema multiforme skin lesions that occur in a pattern that is temporally, although not spatially, correlated with typical recurrent HSV infection (109). An estimated 50 to 80% of cases of recurrent erythema multiforme are HSV- associated (109). A causal role for recurrent HSV infection has been strongly suggested by the clinical improvement that occurs when suppressive doses of nucleoside antivirals are given (147). The pathogenesis is controversial. Evidence of HSV protein (129, 158, 196), DNA (15, 36, 131, 190), and RNA (129) in HAEM lesions has been obtained. However, live HSV has seldom been recovered in culture (131), and inclusions typical of lytic HSV replication are not seen. If immunity to HSV proteins is involved in the pathogenesis of HAEM, as implied by some data (17, 130, 131, 204), caution is warranted in therapeutic or prophylactic vaccination of individuals with a history of this disorder

Corneal HSK is a serious HSV-1-related ocular disorder. Because HSK is responsive to corticosteroids and cyclosporin and occurs in animals after clearance of all infectious virus, its pathogenesis is thought to include tissue damage by a dysregulated inflammatory response. Athymic mice fail to develop HSK, implicating T cells. Tissue and draining lymph nodes contain HSV-specific CD4 T cells and are enriched in Th1-like cytokines (261). Detailed, elegant animal models have shown a possible role for cross-reactive CD4 T cells recognizing HSV-1 UL6 and a corneal autoantigen (20, 203, 301), although contrasting data have also been presented (69, 70, 90). CD4 clones reactive with HSV-1, and in some cases cross-reactive with HSV-2, have been obtained from human HSK specimens (124, 281, 282). It is therefore reasonable to carefully monitor for exacerbations of HSV-1 ocular inflammatory conditions in persons receiving highly antigenic HSV-2 vaccines.

IMPACT OF SPECIFIC IMMUNITY ON CELL AND GENE THERAPY

Replication-competent and -incompetent HSV strains have entered human clinical trials for several conditions including cancer. Some protocols include repeated administration of recombinant viruses or cells that express HSV-encoded proteins. Preexisting immunity to HSV, perhaps boosted by administration of gene or cell therapy products, might limit the duration of the expression of therapeutic genes inserted into recombinant HSV, the antitumor activity of specialized HSV constructs injected into tumors, or the longevity of cells expressing HSV gene products. Similarly, primary immune responses to HSV antigens in seronegative persons might influence the biological activity of these types of therapies. Administration of cloned CD8 T cells which express the HSV-1 thymidine kinase protein has been documented to stimulate thymidine kinase-specific CD8 CTL responses, which may be responsible for the short biological survival of these cells after passive transfer to patients (216). Seroconversion to HSV-1 has been reported after the local injection of recombinant HSV-1 into a brain tumor (160). Further research is required to determine if preexisting or primary immunity to HSV or HSV-encoded proteins will have a significant impact on these promising areas.

UNANSWERED QUESTIONS AND PROSPECTS

Most pathogens which have been controlled with vaccinations elicit immune responses after natural, wild-type infections that are sufficient to lessen the severity and transmissibility of subsequent infection with the same viral type. The rationale for prophylactic vaccination against HSV begins with the assumption that natural infection protects against exogenous reinfection with the homologous viral type, or at least ameliorates it. Molecular fingerprinting of serial genital isolates has occasionally yielded more than one HSV-2 strain (40, 221). On some occasions, each of two or more distinct strains have been recovered more than once, consistent with the establishment of latency of more than one strain (264). While these subjects could have been inoculated with multiple strains prior to the development of immunity, it is likely that exogenous reinfection can occur. Some data indicate that this is rare (142, 221), but more research is needed to clarify this important point. We lack sufficient data to evaluate how acquired

immune responses, be they type specific to HSV-2 or the summation of type-specific and type-common responses in the case of HSV-1- and HSV-2-coinfected persons, influence the acute and long-term outcomes of exposure to a second strain of HSV-2. As a corollary, it is not clear if vaccines that elicit responses matching or even exceeding the levels seen after natural infection will prove adequate for prophylactic vaccines.

"Original antigenic sin," in which prior immunity to a virus (HSV-1 in this case) changes or limits immune responses to a vaccine for a related virus (HSV-2), could influence the efficacy of HSV-2 vaccines in HSV-1-infected persons (117). Detailed analyses of epitope-specific responses are required to determine if this occurs in human HSV infection. The durability of immune responses to an HSV-2 vaccine is also important. Intermittent sexual exposure over a relatively long period (decades) is likely. Even some live-virus vaccines, such as the older measles vaccines, provided only temporary protection. Vaccination for HSV-2 would most logically be performed prior to sexual activity. However, preadolescents are harder to vaccinate than are infants and school-entry-age children. If a vaccine were found to give type-common protection against both HSV-1 and HSV-2, early childhood vaccination would be optimal. The durability of responses and the need for booster doses will have to be carefully assessed for any vaccine reaching licensure.

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REFERENCES

1. **Ahonen, C. L., S. J. Gibson, T. M. Smith, L. K. Pederson, J. M. Lindh, M. A. Tomai, and J. P. Vasilakos.** 1999. Dendritic cell maturation and subsequent enhanced T-cell stimulation induced with the novel synthetic immune response modifier R-848. Cell. Immunol. **197:**62–72.
2. **Alcami, A., and U. H. Koszinowski.** 2000. Viral mechanisms of immune evasion. Trends Microbiol. **8:**410–418.
3. **Allen, E. M., J. P. Weir, S. Martin, C. Mercadel, and B. T. Rouse.** 1990. Role of coexpression of IL-2 and herpes simplex virus proteins in recombinant vaccinia virus vectors on levels of induced immunity. Viral Immunol. **3:**207–215.
4. **Ankel, H., D. F. Westra, S. Welling-Wester, and P. Lebon.** 1998. Induction of interferon-alpha by glycoprotein D of herpes simplex virus: a possible role of chemokine receptors. Virology **251:**317–326.
5. **Arrode, G., C. Boccaccio, J. Lule, S. Allart, N. Moinard, J. P. Abastado, A. Alam, and C. Davrinche.** 2000. Incoming human cytomegalovirus pp65 (UL83) contained in apoptotic infected fibroblasts is cross-presented to $CD8^+$ T cells by dendritic cells. J. Virol. **74:**10018–10024.
6. **Arvin, A. M.** 2001. Varicella vaccine: genesis, efficacy, and attenuation. Virology **284:**153–158.
7. **Asanuma, H., M. Sharp, H. T. Maecker, V. C. Maino, and A. M. Arvin.** 2000. Frequencies of memory T cells specific for varicella-zoster virus, herpes simplex virus and cytomegalovirus determined by intracellular detection of cytokine expression. J. Infect. Dis. **181:**859–866.
8. **Ashley, R. A., J. Militoni, F. Lee, A. Nahmias, and L. Corey.** 1988. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunoblot for detecting antibodies to herpes simplex types 1 and 2 in human sera. J. Clin. Microbiol. **26:**662–667.
9. **Ashley, R. L.** 2001. Sorting out the new HSV type specific antibody tests. Sex. Transm. Infect. **77:**232–237.
10. **Ashley, R. L., J. Benedetti, and L. Corey.** 1985. Humoral immune response to HSV-1 and HSV-2 viral proteins in patients with primary genital herpes. J. Med. Virol. **17:**153–166.
11. **Ashley, R. L., L. Corey, J. Dalessio, P. Wilson, M. Remington, G. Barnum, P. Trethewey.** 1994. Protein-specific cervical antibody responses to primary genital herpes simplex virus type 2 infections. J. Infect. Dis. **170:**20–26.
12. **Ashley, R. L., F. M. Crisostomo, M. Doss, R. E. Sekulovich, R. L. Burke, M. Shaugnessy, L. Corey, N. L. Polissar, and A. G. Langenberg.** 1998. Cervical antibody responses to a herpes simplex virus type 2 glycoprotein subunit vaccine. J. Infect. Dis. **178:**1–7.
13. **Ashley, R., G. Mertz, H. Clark, M. Schick, D. Salter, and L. Corey.** 1985. Humoral immune response to herpes simplex type 2 glycoproteins in patients receiving a glycoprotein subunit vaccine. J. Virol. **56:**475–481.
14. **Ashley, R., A. Wald, and L. Corey.** 1994. Cervical antibodies in patients with oral herpes simplex virus type 1 (HSV-1) infection: local anamnestic responses after genital HSV-2 infection. J. Virol. **68:**5284–5286.
15. **Aslanzadeh, J., K. F. Helm, M. J. Espy, S. A. Muller, and T. F. Smith.** 1992. Detection of HSV-specific DNA in biopsy tissue of patients with erythema multiforme by polymerase chain reaction. Br. J. Dermatol. **126:**19–23.
16. **Aurelian, L.** 1998. Herpes simplex virus type 2: unique biological properties include neoplastic potential mediated by the PK domain of the large subunit of ribonucleotide reductase. Front. Biosci. **3:**237–249.
17. **Aurelian, L., H. Kokuba, and J. W. Burnett.** 1998. Understanding the pathogenesis of HSV-associated erythema multiforme. Dermatology **197:**219–222.
18. **Aurelian, L., H. Kokuba, and C. C. Smith.** 1999. Vaccine potential of a herpes simplex virus type 2 mutant deleted in the PK domain of the large subunit of ribonucleotide reductase (ICP10). Vaccine **9:**1951–1963.
19. **Aurelian, L., C. C. Smith, R. Winchurch, M. Kulka, T. Gyotoku, L. Zaccaro, F. J. Chrest, and J. W. Burnett.** 2001. A novel gene expressed in human keratinocytes with long-term in vitro growth potential is required for cell growth. J. Investig. Dermatol. **116:**286–295.
20. **Avery, A. C., Z.-S. Zhao, A. Rodriguez, E. K. Bikoff, M. Soheilian, C. S. Foster, and H. Cantor.** 1995. Resistance to herpes stromal keratitis conferred by an IgG2a-derived peptide. Nature **376:**431–434.
21. **Baldridge, J. R., Y. Yorgensen, J. R. Ward, and J. T. Ulrich.** 2000. Monophosphoryl lipid A enhances mucosal and systemic immunity to vaccine antigens following intranasal administration. Vaccine **18:**2416–2425.
22. **Barcy, S., and L. Corey.** 2001. Herpes simplex inhibits the capacity of lymphoblastoid B cell lines to stimulate CD4+ T cells. J. Immunol. **166:**6242–6249.
23. **Bartenschlager, R., and V. Lohmann.** 2000. Replication of hepatitis C virus. J. Gen. Virol. **81:**1631–1648.
24. **Benedetti, J., L. Corey, and R. Ashley.** 1994. Recurrence rates of genital herpes after symptomatic first episode infection. Ann. Intern. Med. **121:**847–854.
25. **Benedetti, J. K., J. Zeh, and L. Corey.** 1999. Clinical reactivation of genital herpes simplex virus infection decreases in frequency over time. Ann. Intern. Med. **131:**14–20.
26. **Bernstein, D. I.** 2000. Effect of route of vaccination with vaccinia virus expressing HSV-2 glycoprotein D on protection from genital HSV-2 infection. Vaccine **18:**1351–1358.
27. **Bernstein, D. I., J. Ireland, and N. Bourne.** 2000. Pathogenesis of acyclovir-resistant herpes simplex type 2 isolates in animal models of genital herpes: models for antiviral evaluations. Antiviral Res. **47:**159–169.
28. **Bernstein, D. I., and L. R. Stanberry.** 1999. Herpes simplex virus vaccines. Vaccine **17:**1681–1689.
29. **Bhattarakosol, P., C. Yoosook, and P. Matangkasombut.** 1991. Intratypic variations in neutralizable epitopes among herpes simplex virus type 2 isolates. Microbiol. Immunol. **35:**525–533.
30. **Bishop, G. A., Y. Hsing, B. S. Hostager, S. V. Jalukar, L. M. Ramirez, and M. A. Tomai.** 2000. Molecular mechanisms of B lymphocyte activation by the immune response modifier R-848. J. Immunol. **165:**5552–5557.
31. **Blaney, J. E., E. Nobusawa, M. A. Brehm, R. H. Bonneau, L. M. Mylin, T. M. Fu, Y. Kawoaka, and S. S. Tevethia.** 1998. Immunization with a single major histocompatibility class I-restricted cytotoxic T-lymphocyte recognition epitope of herpes simplex virus type 2 confers protective immunity. J. Virol. **72:**9567–9574.
32. **Bos, J. D., and M. L. Kapsenberg.** 1993. The skin immune system: progress in cutaneous biology. Immun. Today. **14:**75–78.
33. **Bottrel, R. L., Y. L. Yang, D. E. Levy, M. Tomai, and L. F. Reis.** 1999. The immune response modifier imiquimod requires STAT-1 for induction of interferon, interferon-stimulated genes, and interleukin-6. Antimicrob. Agents Chemother. **43:**856–861.
34. **Boursnell, M. E., C. Entwisle, D. Blakeley, C. Roberts, I. A. Duncan, S. E. Chisholm, G. M. Martin, R. Jennings, D. NiChallanain, I. Sobek, S. C. Inglis, and C. S. McLean.** 1997. A genetically inactivated herpes simplex virus type 2 (HSV-2) vaccine provides effective protection against primary and recurrent HSV-2 disease. J. Infect. Dis. **175:**16–25.
35. **Brehm, M., L. A. Samaniego, R. H. Bonneau, N. A. DeLuca, and S. S. Tevethia.** 1999. Immunogenicity of herpes simplex virus type 1 mutants containing deletions in one or more alpha genes: ICP4, ICP27, ICP22, and ICP0. Virology **256:**258–269.
36. **Brice, S. L., D. Krzmien, W. L. Weston, and J. C. Huff.** 1989. Detection of herpes simplex virus DNA in cutaneous lesions of erythema multiforme. J. Investig. Dermatol. **95:**183–187.
37. **Brown, Z. A., J. Benedetti, R. Ashley, S. Burchett, S. Selke, S. Berry, L. A. Vontver, and L. Corey.** 1991. Neonatal herpes virus infection in relation to asymptomatic maternal infection at the time of delivery. N. Engl. J. Med. **324:**1247–1252.
38. **Brown, Z. A., S. Selke, J. Zeh, J. Kopelman, A. Maslow, R. L. Ashley, D. H. Watts, S. Berry, M. Herd, and L. Corey.** 1997. The acquisition of herpes simplex virus during pregnancy. N. Engl. J. Med. **337:**509–515.

39. **Bryson, Y., M. Dillon, D. I. Bernstein, J. Radolf, P. Zakowski, and E. Garratty.** 1993. Risk of acquisition of genital herpes simplex virus type 2 in sex partners of persons with genital herpes: a prospective couple study. J. Infect. Dis. **167:**942–946.
40. **Buchman, T. G., B. Roizman, and A. J. Nahmias.** 1979. Demonstration of exogenous reinfection with herpes simplex virus type 2 by restriction endonuclease fingerprinting of viral DNA. J. Infect. Dis. **140:**295–304.
41. **Burke, R. L.** 1992. Contemporary approaches to vaccination against herpes simplex virus. Curr. Top. Microbiol. Immunol. **179:**137–158.
42. **Burke, R. L.** 1993. Current developments in herpes simplex virus vaccines. Semin. Virol. **4:**187–197.
43. **Burns, R. P., B. Ferbel, M. Tomai, R. Miller, and A. A. Gaspari.** 2000. The imidazoquinolines, imiquimod and R-848, induce functional, but not phenotypic, maturation of human epidermal Langerhans' cells. Clin. Immunol. **94:**13–23.
44. Reference deleted.
45. **Campadelli-Fiume, G., F. Cocchi, L. Menotti, and M. Lopez.** 2000. The novel receptors that mediate the entry of herpes simplex viruses and animal herpesviruses into cells. Rev. Med. Virol. **10:**305–339.
46. **Cappel, R., S. Sprecher, F. De Cuyper, and J. De Braekeleer.** 1985. Clinical efficacy of a herpes simplex virus subunit vaccine. J. Med. Virol. **16:**137–145.
47. **Carfi, A., S. H. Willis, J. C. Whitbeck, C. Krummenacher, G. H. Cohen, R. J. Eisenberg, and D. C. Wiley.** 2001. Herpes simplex virus glycoprotein D bound to the human receptor HveA. Mol. Cell **8:**169–179.
48. **Caselli, E., P. Grandi, R. Argnani, P. G. Balboni, R. Selvatici, and R. Manservigi.** 2001. Mice genetic immunization with plasmid DNA encoding a secreted form of HSV-1 gB induces a protective immune response against herpes simplex virus type 1 infection. Intervirology **44:**1–7.
49. **Chabalgoity, J. A., C. M. Khan, A. A. Nash, and C. E. Hormaeche.** 1996. A *Salmonella typhimurium htrA* live vaccine expressing multiple copies of a peptide comprising amino acids 8–23 of herpes simplex virus glycoprotein D as a genetic fusion to tetanus toxin fragment C protects mice from herpes simplex virus infection. Mol. Microbiol. **19:**791–801.
50. **Chen, S. H., D. A. Garber, P. A. Schaffer, D. M. Knipe, and D. M. Coen.** 2000. Persistent elevated expression of cytokine transcripts in ganglia latently infected with herpes simplex virus in the absence of ganglionic replication or reactivation. Virology **278:**207–216.
51. **Chen, S. H., M. F. Kramer, P. A. Schaffer, and D. M. Coen.** 1997. A viral function represses accumulation of transcripts from productive-cycle genes in mouse ganglia latently infected with herpes simplex virus. J. Virol. **71:**5878–5884.
52. **Cheong, W. K., T. Thirumoorthy, S. Doraisingham, and A. E. Ling.** 1990. Clinical and laboratory study of first episode genital herpes in Singapore. Int. J. STD AIDS **1:**195–198.
53. **Chou, J., E. R. Kern, R. J. Whitley, and B. Roizman.** 1990. Mapping of herpes simplex virus 1 neurovirulence to gamma 1 34.5, a gene nonessential for growth in cell culture. Science **252:**1262–1266.
54. **Colgrove, R., and A. Japour.** 1999. A combinatorial ledge: reverse transcriptase fidelity, total body viral burden, and the implications of multiple-drug HIV therapy for the evolution of antiviral resistance. Antiviral Res. **41:**45–56.
55. **Conde-Glez, C. J., L. Juarez-Figueroa, F. Uribe-Salas, P. Hernandez-Nevarez, D. S. Schmid, E. Calderon, and M. Hernandez-A vila.** 1999. Analysis of herpes simplex virus 1 and 2 infection in women with high risk sexual behaviour in Mexico. Int. J. Epidemiol. **28:**571–576.
56. **Corey, L., H. G. Adams, Z. A. Brown, and K. K. Holmes.** 1983. Genital herpes simplex virus infection: clinical manifestations, course, and complications. Ann. Intern. Med. **98:**958–972.
57. **Corey, L., A. G. M. Langenberg, R. Ashley, R. E. Sekulovich, A. E. Izu, J. M. Douglas, H. Handsfield, T. Warren, L. Marr, S. Tyring, R. DiCarlo, A. A. Adímora, P. Leone, C. L. Dekker, R. L. Burke, W. P. Leong, and S. E. Straus.** 1999. Two double-blind, placebo-controlled trials of a vaccine containing recombinant gD2 and gB2 antigens in MF59 adjuvant for the prevention of genital HSV-2 acquisition. JAMA **282:**331–340.
58. **Corey, L., and A. Wald.** 1999. Genital herpes, p. 285–312. *In* K. K. Holmes, P. F. Sparling, P. A. Mardh, S. M. Lemon, W. E. Stamm, P. Piot, and J. M. Wasserheit (ed.), Sexually transmitted diseases, 3rd ed. McGraw-Hill, Inc., New York, N.Y.
59. **Coukos, G., A. Makrigiannakis, S. Montas, L. R. Kaiser, T. Toyozumi, I. Benjamin, S. M. Albelda, S. C. Rubin, and K. L. Molnar-Kimber.** 2000. Multi-attenuated herpes simplex virus-1 mutant G207 exerts cytotoxicity against epithelial ovarian cancer but not normal mesothelium and is suitable for intraperitoneal oncolytic therapy. Cancer Gene Ther. **7:**275–283.
60. **Cunningham A. L., and T. C. Merigan.** 1984. Leu-3+ cells produce gamma-interferon in patients with recurrent herpes labialis. J. Immunol. **132:**197–202.
61. **Cunningham, A. L., and T. C. Merigan.** 1983. Gamma interferon production appears to predict time of recurrence of herpes labialis. J. Immunol. **130:**2397–2400.
62. **Cunningham, A. L., and J. R. Noble.** 1989. Role of keratinocytes in human recurrent herpetic lesions. J. Clin. Investig. **83:**490–496.
63. **Cunningham, A. L., R. R. Turner, A. C. Miller, M. F. Para, and T. C. Merigan.** 1985. Evolution of recurrent herpes simplex lesions: an immunohistologic study. J. Clin. Investig. **75:**226–233.
64. **Da Costa, X. J., N. Bourne, L. R. Stanberry, and D. M. Knipe.** 1997. Construction and characterization of a replication-defective herpes simplex virus type 2 ICP8 mutant and its use in immunization studies in a guinea pig model of genital disease. Virology **26:**1–12.
65. **Da Costa, X. J., C. A. Jones, and D. M. Knipe.** 1999. Immunization against genital herpes with a vaccine virus that has defects in productive and latent infection. Proc. Natl. Acad. Sci. USA **96:**6994–6998.
66. **Da Costa, X., M. F. Kramer, J. Zhu, M. A. Brockman, and D. M. Knipe.** 2000. Construction, phenotypic analysis, and immunogenicity of a UL5/UL29 double deletion mutant of herpes simplex virus 2. J. Virol. **74:**7963–7971.
67. Reference deleted.
68. **Desai, P., G. L. Sexton, J. M. McCaffery, and S. Person.** 2001. A null mutation in the gene encoding the herpes simplex virus type 1 UL37 polypeptide abrogates virus maturation. J. Virol. **75:**10259–10271.
69. **Deshpande, S. P., S. Lee, M. Zheng, B. Song, D. Knipe, J. A. Kapp, and B. T. Rouse.** 2001. Herpes simplex virus-induced keratitis: evaluation of the role of molecular mimicry in lesion pathogenesis. J. Virol. **75:**3077–3088.
70. **Deshpande, S., M. Zheng, S. Lee, K. L. Banarjee, S. Gangappa, U. Kumaraguru, and B. T. Rouse.** 2001. Bystander activation involving T lymphocytes in herpetic stromal keratitis. J. Immunol. **167:**2902–2910.
71. **Dix, R. D.** 1985. Prospects for vaccine against herpes simplex virus type 1 and type 2. Prog. Med. Virol. **34:**89–128.
72. **Dolan, A., F. E. Jamieson, C. Cunningham, B. C. Barnett, and D. J. McGeoch.** 1998. The genome sequence of herpes simplex virus type 2. J. Virol. **72:**2010–2021.
73. **Dupuis, M., K. Denis-Mize, A. LaBarbara, W. Peters, I. F. Charo, D. M. McDonald, and G. Ott.** 2001. Immunization with the adjuvant MF59 induces macrophage trafficking and apoptosis. Eur. J. Immunol. **31:**2910–2918.
74. **Dupuis, M., T. J. Murphy, D. Higgins, M. Ugozzoli, G. Van Nest, G. Ott, and D. M. McDonald.** 1998. Dendritic cells internalize vaccine adjuvant after intramuscular injection. Cell. Immunol. **25:**18–27.
75. **Eling, D. J., P. A. Johnson, S. Sharma, F. Tufaro, and T. J. Kipps.** 2000. Chronic lymphocytic leukemia B cells are highly sensitive to infection by herpes simplex virus-1 via herpesvirus-entry-mediator A. Gene Ther. **7:**1210–1216.
76. **Eo, S. K., M. Gierynska, A. A. Kamar, and B. T. Rouse.** 2001. Prime-boost immunization with DNA vaccine: mucosal route of administration changes the rules. J. Immunol. **166:**5473–5479.
77. **Eo, S. K., S. Lee, S. Chun, and B. T. Rouse.** 2001. Modulation of immunity against herpes simplex virus infection via mucosal genetic transfer of plasmid DNA encoding chemokines. J. Virol. **75:**569–578.
78. **Eo, S. K., S. Lee, U. Kumaraguru, and B. T. Rouse.** 2001. Immunopotentiation of DNA vaccine against herpes simplex virus via co-delivery of plasmid DNA expressing CCR7 ligands. Vaccine **19:**4685–4693.
79. **Erard, F., J. A. Garcia-Sanz, R. Moriggl, and M. T. Wild.** 1999. Presence or absence of TGF-beta determines IL-4-induced generation of type 1 or type 2 CD8 T cell subsets. J. Immunol. **162:**209–214.
80. **Field, H.** 2001. Herpes simplex virus antiviral drug resistance-current trends and future prospects. J. Clin. Virol. **21:**261–269.
81. **Flo, J., S. Tisminetzky, and F. Baralle.** 2001. Oral transgene vaccination mediated by attenuated salmonellae is an effective method to prevent herpes simplex virus-2 induced disease in mice. Vaccine **19:**1772–1782.
82. **Forrester, A., H. Farrell, G. Wilkinson, J. Kaye, N. Davis-Poynter, and T. Minson.** 1992. Construction and properties of a mutant of herpes simplex virus type 1 with glycoprotein H coding sequences deleted. J. Virol. **66:**341–348.
83. **Forrester, A. J., V. Sullivan, A. Simmons, B. A. Blacklaws, G. L. Smith, A. A. Nash, and A. C. Minson.** 1991. Induction of protective immunity with antibody to herpes simplex type 1 glyoprotein H (gH) and analysis of the immune response to gH expressed in recombinant vaccinia virus. J. Gen. Virol. **72:**369–375.
84. **Foster, T. P., and K. G. Kousoulas.** 1999. Genetic analysis of the role of herpes simplex virus type 1 glycoprotein K in infectious virus production and egress. J. Virol. **73:**8457–8468.
85. **Franchini, M., C. Abril, C. Schwerdel, C. Ruedl, M. Ackermann, and M. Suter.** 2001. Protective T-cell-based immunity induced in neonatal mice by a single replicative cycle of herpes simplex virus. J. Virol. **75:**83–89.
86. Reference deleted.
87. **Friedman, H. M., L. Wang, M. K. Pangburn, J. D. Lambris, and J. Lubinski.** 2000. Novel mechanism of antibody-independent complement neutralization of herpes simplex virus type 1. J. Immunol. **165:**4528–4536.
88. **Fuller, A. O., R. E. Santos, and P. G. Spear.** 1989. Neutralizing antibodies specific for glycoprotein H of herpes simplex virus permit viral attachment to cells but prevent penetration. J. Virol. **63:**3435–3443.
89. **Gallichan, W. S., R. N. Woolstencroft, T. Guarasci, M. J. McCluskie, H. L. Davis, and K. L. Rosenthal.** 2001. Intranasal immunization with CpG oligodeoxynucleotides as an adjuvant dramatically increases IgA and protec-

tion against herpes simplex virus-2 in the genital tract. J. Immunol. **166:** 3451–3457.
90. **Gangappa, S., S. P. Deshpande, and B. T. Rouse.** 2000. Bystander activation of CD4+ T cells accounts for herpetic ocular lesions. Investig. Ophthalmol. Visual Sci. **41:**453–459.
91. **Geiss, B. J., T. J. Smith, D. A. Leib, and L. A. Morrison.** 2000. Disruption of virion host shutoff activity improves the immunogenicity and protective capacity of a replication-incompetent herpes simplex virus type 1 vaccine strain. J. Virol. **74:**11137–11144.
92. **Ghebrekidan, H., U. Ruden, S. Cox, and M. Grandien.** 1999. Prevalence of herpes simplex virus types 1 and 2, cytomegalovirus, and varicella-zoster virus infections in Eritrea. J. Clin. Virol. **12:**53–64.
93. **Ghiasi, H., A. B. Nesburn, and S. L. Weschler.** 1996. Vaccination with a cocktail of seven recombinantly expressed HSV-1 glycoproteins protects against ocular HSV-1 challenge more efficiently than vaccination with any individual glycoprotein. Vaccine **14:**107–112.
94. **Gilden, D. H., R. Gesser, J. Smith, M. Wellish, J. J. Laguardia, R. J. Cohrs, and R. Mahalingam.** 2001. Presence of VZV and HSV-1 DNA in human nodose and celiac ganglia. Virus Genes **23:**145–147.
95. **Goldsmith, K., W. Chen, D. C. Johnson, and R. L. Hendricks.** 1998. Infected cell protein (ICP)47 enhances herpes simplex virus neurovirulence by blocking the CD8 T cell response. J. Exp. Med. **187:**341–348.
96. **Haarr, L., D. Shukla, E. Rodahl, M. C. Dal Canto, and P. G. Spear.** 2001. Transcription from the gene encoding the herpesvirus entry receptor nectin-1 (HveC) in nervous tissue of adult mouse. Virology **287:**301–309.
97. **Halford, W. P., B. M. Gebhardt, and D. J. J. Carr.** 1997. Acyclovir blocks cytokine gene expression in trigeminal ganglia latently infected with herpes simplex virus type 1. Virology **238:**53–63.
98. **Hall, M. J., and K. Katrak.** 1986. The quest for a herpes simplex virus vaccine: background and recent development. Vaccine **4:**138–150.
99. **Hashido, M., T. Kawana, Y. Matsunaga, and S. Inouye.** 1999. Changes in prevalence of herpes simplex virus type 1 and 2 antibodies from 1973 to 1993 in rural districts of Japan. Microbiol. Immunol. **43:**177–180.
100. **Haugen, T. H., B. Alden, S. Matthey, and D. Nicholson.** 1993. Restriction enzyme fragment length polymorphisms of amplified herpes simplex virus type-1 DNA provide epidemiologic information. Diagn. Microbiol. Infect. Dis. **17:**129–133.
101. **Heineman, T. C., M. L. Clements-Mann, G. A. Poland, R. M. Jacobson, A. E. Izu, D. Sakamoto, J. Eiden, G. A. Van Nest, and H. H. Hsu.** 1999. A randomized, controlled study in adults of the immunogenicity of a novel hepatitis B vaccine containing MF59 adjuvant. Vaccine **17:**2769–2778.
102. **Hemmi, H., T. Kaisho, O. Takeuchi, S. Sato, H. K. Sanjo, T. Hoshino, H. Horiuchi, K. Tomizawa, S. Takeda, and S. Akira.** 2002. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat. Immunol. **3:**196–200.
103. Reference deleted.
104. **Highlander, S. L., W. H. Cai, S. Person, M. Levine, and J. C. Glorioso.** 1988. Monoclonal antibodies define a domain on herpes simplex virus glycoprotein B involved in virus penetration. J. Virol. **62:**1881–1888.
105. **Highlander, S. L., S. L. Sutherland, P. J. Gage, D. C. Johnson, M. Levine, and J. C. Glorioso.** 1987. Neutralizing monoclonal antibodies specific for herpes simplex virus glycoprotein D inhibit virus penetration. J. Virol. **61:**3356–3364.
106. **Hill, A., P. Jugovic, I. York, G. Russ, J. Bennink, J. Yewdell, H. Ploegh, and D. Johnson.** 1995. Herpes simplex virus turns off the TAP to evade host immunity. Nature **375:**411–415.
107. **Hill, S., X. Zhang, M. E. G. Boursenell, J. G. Shields, P. Ricciardi-Castagnoli, and J. K. Hickling.** 1997. Generation of a primary immune response to a genetically inactivated (DISC) herpes simplex virus and wild type virus. Biochem. Soc. Trans. **25:**200S.
108. **Holland, D. J., M. Miranda-Saksena, R. A. Boadle, P. Armati, and A. L. Cunningham.** 1999. Anterograde transport of herpes simplex virus proteins in axons of peripheral fetal neurons: an immunoelectron microscopy study. J. Virol. **73:**8503–8511.
109. **Howland, W. W., L. E. Golitz, W. L. Weston, and J. C. Huff.** 1984. Erythema multiforme: clinical, histopathologic, and immunologic study. J. Am. Acad. Dermatol. **112:**387–390.
110. **Hsu, H., I. Solovyev, A. Colombero, R. Elliott, M. Kelley, and W. J. Boyle.** 1997. ATAR, a novel tumor necrosis factor receptor family member, signals through TRAF2 and TRAF5. J. Biol. Chem. **272:**13471–13474.
111. **Inatsuki, A., M. Yasukawa, and Y. Kobayashi.** 1991. The effect of human T cell leukemia type I infection on a herpes simplex virus-specific CD8+ cytotoxic T cell clone. Br. J. Hematol. **77:**311–314.
112. **Itzakhi, R. F., W. R. Lin, D. Shang, G. K. Wilcock, B. Faragher, and G. A. Jamieson.** 1997. Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. Lancet **349:**241–244.
113. **Jacobson, J. G., K. L. Ruffner, M. Kosz-Vnenchak, C. B. Hwang, K. K. Wobbe, D. M. Knipe, and D. M. Coen.** 1993. Herpes simplex thymidine kinase and specific stages of latency in murine trigeminal ganglia. J. Virol. **67:**6903–6908.
114. **Kalimo, K. O. K., I. A. Joronen, and V. K. Havu.** 1983. Cell-mediated immunity against herpes simplex virus envelope, capsid, excreted, and crude antigens. Infect. Immun. **39:**24–28.
115. **Kaul, R., F. A. Plummer, J. Kimani, T. Dong, P. Kiama, T. Rostron, E. Njagi, K. S. MacDonald, J. J. Bwayo, A. J. McMichael, and S. L. Rowland-Jones.** 2000. HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. J. Immunol. **164:**1602–1611.
116. **Keadle, T. L., N. Usui, K. A. Laycock, J. K. Miller, J. S. Pepose, and P. M. Stuart.** 2000. IL-1 and TNF-alpha are important factors in the pathogenesis of murine recurrent herpetic stromal keratitis. Investig. Ophthalmol. Visual Sci. **41:**96–102.
117. **Klenerman, P., and R. M. Zinkernagel.** 1998. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. Nature **394:**482–485.
118. **Koelle, D. M., H. Abbo, A. Peck, K. Ziegweid, and L. Corey.** 1994. Direct recovery of HSV-specific T lymphocyte clones from human recurrent HSV-2 lesions. J. Infect. Dis. **169:**956–961.
119. **Koelle, D. M., J. Benedetti, A. Langenberg, and L. Corey.** 1992. Asymptomatic reactivation of herpes simplex virus in women after the first episode of genital infection. Ann. Intern. Med. **116:**433–437.
120. **Koelle, D. M., H. Chen, M. A. Gavin, A. Wald, W. W. Kwok, and L. Corey.** 2001. CD8 CTL from genital herpes simplex lesions: recognition of viral tegument and immediate early proteins and lysis of infected cutaneous cells. J. Immunol. **166:**4049–4058.
121. **Koelle, D. M., L. Corey, R. L. Burke, R. J. Eisenberg, G. H. Cohen, R. Pichyangkura, and S. J. Triezenberg.** 1994. Antigenic specificity of human $CD4^+$ T cell clones recovered from recurrent genital HSV-2 lesions. J. Virol. **68:**2803–2810.
122. **Koelle, D. M., J. M. Frank, M. L. Johnson, and W. W. Kwok.** 1998. Recognition of herpes simplex virus type 2 tegument proteins by CD4 T cells infiltrating human genital herpes lesions. J. Virol. **72:**7476–7483.
123. **Koelle, D. M., C. M. Posavad, G. R. Barnum, M. L. Johnson, J. M. Frank, and L. Corey.** 1998. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. J. Clin. Investig. **101:**1500–1508.
124. **Koelle, D. M., S. N. Reymond, H. Chen, W. W. Kwok, C. McClurkan, T. Gyaltsong, E. W., Petersdorf, W. Rotkis, A. R. Talley, and D. A. Harrison.** 2000. Tegument-specific, virus-reactive CD4 T cells localize to the cornea in herpes simplex virus interstitial keratitis in humans. J. Virol. **74:**10930–10938.
125. **Koelle, D. M., M. Schomogyi, and L. Corey.** 2000. Recovery of antigen-specific T-cells from the uterine cervix of women with genital herpes simplex virus type 2 virus infection. J. Infect. Dis. **182:**662–670.
126. **Koelle, D. M., M. Schomogyi, C. McClurkan, S. N. Reymond, and H. B. Chen.** 2000. CD4 T-cell responses to herpes simplex virus type 2 major capsid protein VP5: comparison with responses to tegument and envelope glycoproteins. J. Virol. **74:**11422–11425.
127. **Koelle, D. M., M. A. Tigges, R. L. Burke, F. W. Symington, S. R. Riddell, H. Abbo, and L. Corey.** 1993. Herpes simplex virus infection of human fibroblasts and keratinocytes inhibits recognition by cloned CD8+ cytotoxic T lymphocytes. J. Clin. Investig. **91:**961–968.
128. **Kohl, S.** 1991. Role of antibody-dependent cellular cytotoxicity in defense against herpes simplex virus infections. Rev. Infect. Dis. **13:**108–114.
129. **Kokuba, H., L. Aurelian, and J. Burnett.** 1999. Herpes simplex virus associated erythema multiforme (HAEM) is mechanistically distinct from drug-induced erythema multiforme: interferon-gamma is expressed in HAEM lesions and tumor necrosis factor-alpha in drug-induced erythema multiforme lesions. J. Investig. Dermatol. **113:**808–815.
130. **Kokuba, H., S. Imafuku, J. W. Burnett, and L. Aurelian.** 1999. Longitudinal study of a patient with herpes-simplex-virus-associated erythema multiforme: viral gene expression and T cell repertoire usage. Dermatology **198:**233–242.
131. **Kokuba, H., S. Imafuku, S. Huang, L. Aurelian, and J. W. Burnett.** 1998. Erythema multiforme lesions are associated with expression of a herpes simplex virus (HSV) gene and qualitative alterations in the HSV-specific T-cell response. Br. J. Dermatol. **138:**952–964.
133. **Kramer, M. F., and D. M. Coen.** 1995. Quantification of transcripts from the ICP4 and thymidine kinase genes in mouse ganglia latently infected with herpes simplex virus. J. Virol. **69:**1389–1399.
134. **Kruse, M., O. Rosorius, F. Kratzer, G. Stelz, C. Kuhnt, G. Schuler, J. Hauber, and A. Steinkasserer.** 2000. Mature dendritic cells infected with herpes simplex virus type 1 exhibit inhibited T-cell stimulatory capacity. J. Virol. **74:**7127–7136.
135. **Kuklin, N. A., M. Daheshia, S. Chun, and B. T. Rouse.** 1998. Role of mucosal immunity in herpes simplex virus infection. J. Immunol. **160:**5998–6003.
136. **Kuklin, N., M. Daheshia, K. Karem, E. Manickan, and B. T. Rouse.** 1997. Induction of mucosal immunity against herpes simplex virus by plasmid DNA immunization. J. Virol. **71:**3138–3145.
137. **Kumaraguru, U., M. Gierynska, S. Norman, B. D. Bruce, and B. T. Rouse.** 2002. Immunization with chaperone-peptide complex includes low-avidity

cytotoxic T lymphocytes providing transient protection against herpes simplex virus infection. J. Virol. **76:**136–141.
138. **Kwok, W. W., J. A. Gebe, A. Liu, S. Agar, N. Ptacek, J. Hammer, D. M. Koelle, and G. T. Nepom.** 2001. Rapid epitope identification from complex class II-restricted T-cell antigens. Trends Immunol. **22:**583–588.
139. **Kwok, W. W., A. W. Liu, E. J. Novak, J. A. Gebe, S. N. Reymond, R. A. Ettinger, G. T. Nepom, and D. M. Koelle.** 2000. HLA-DQ tetramers identify epitope-specific T-cells in peripheral blood of herpes simplex virus-2-infected individuals: direct detection of immunodominant antigen responsive cells. J. Immunol. **164:**4244–4249.
140. **Kwon, B. S., K. B. Tan, J. Ni, K. O. Oh, Z. H. Lee, K. K. Kim, S. Wang, R. Gentz, G. L. Yu, J. Harrop, S. D. Lyn, C. Silverman, T. G. Porter, A. Truneh, and P. R. Young.** 1997. A newly identified member of the tumor necrosis factor receptor superfamily with a wide tissue distribution and involvement in lymphocyte activation. J. Biol. Chem. **272:**14272–14276.
141. **LaCasse, R. A., K. Follis, M. Trahey, J. D. Scarborough, D. R. Littman, and J. H. Nunberg.** 1999. Fusion-competent vaccines: broad neutralization of primary isolates of HIV. Science **283:**357–362.
142. **Lakeman, A. D., A. J. Nahmias, and R. J. Whitley.** 1986. Analysis of DNA from recurrent genital herpes simplex isolates by restriction endonuclease digestion. Sex. Transm. Dis. **13:**61–66.
143. **Langenberg, A. G., R. L. Burke, S. F. Adair, R. Sekulovich, M. Tigges, C. L. Dekker, and L. Corey.** 1995. A recombinant glycoprotein vaccine for herpes simplex virus type 2: safety and immunogenicity. Ann. Intern. Med. **122:**889–898.
144. **Langenberg, A. G. M., L. Corey, R. L. Ashley, W. P. Leong, and S. E. Straus.** 2000. A prospective study of new infections with herpes simplex virus type 1 and type 2. N. Engl. J. Med. **341:**1432–1438.
145. **Leib, D. A., M. A. Machalek, B. R. Williams, R. H. Silverman, and H. W. Virgin.** 2000. Specific phenotypic restoration of an attenuated virus by knockout of a host resistance gene. Proc. Natl. Acad. Sci. USA **97:**6097–6101.
146. **Lekstrom-Himes, J. A., L. Pesnicak, and S. E. Straus.** 1998. The quantity of latent viral DNA correlates with the relative rates at which herpes simplex virus types 1 and 2 cause recurrent genital herpes outbreaks. J. Virol. **72:**2760–2764.
147. **Lemak, M. A., M. Duvic, and S. F. Bean.** 1986. Oral acyclovir for the prevention of herpes-associated erythema multiforme. J. Am. Acad. Dermatol. **15:**50–54.
149. **Liu, T., K. M. Khanna, B. N. Carriere, and R. L. Hendricks.** 2001. Gamma interferon can prevent herpes simplex virus type 1 reactivation from latency in sensory neurons. J. Virol. **75:**11178–11184.
150. **Liu, T., K. M. Khanna, X. Chen, D. J. Fink, and R. L. Hendricks.** 2000. CD8(+) T cells can block herpes simplex virus type 1 (HSV-1) reactivation from latency in sensory neurons. J. Exp. Med. **191:**1459–1466.
151. **Liu, Y. J.** 2001. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell **106:**259–262.
152. **Lorenzo, M. E., H. L. Ploegh, and R. S. Tirabassi.** 2001. Viral immune evasion strategies and the underlying cell biology. Semin. Immunol. **13:**1–19.
153. **Lu, P., F. E. Jones, H. A. Saffran, and J. R. Smiley.** 2001. Herpes simplex virus virion host shutoff protein requires a mammalian factor for efficient in vitro endoribonuclease activity. J. Virol. **75:**1172–1185.
154. **Lubinski, J., L. Wang, D. Mastellos, A. Sahu, J. H. Lambris, and H. M. Friedman.** 1999. In vivo role of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. J. Exp. Med. **190:**1637–1646.
155. **Maccario, R., P. Comoli, E. Percivalle, D. Montagna, F. Locatelli, and G. Gerna.** 1995. Herpes simplex virus-specific human cytotoxic T-cell colonies expressing either gamma-delta or alpha-beta T-cell receptor: role of accessory molecules on HLA-unrestricted killing of virus-infected targets. Immunology **85:**49–56.
156. **Maccario, R., M. G. Revello, P. Comoli, D. Montagna, F. Locatelli, and G. Gerna.** 1993. HLA-unrestricted killing of HSV-1-infected mononuclear cells. J. Immunol. **150:**1437–1445.
157. **Malmgaard, L., S. R. Paludan, S. C. Mogensen, and S. Ellerman-Eriksen.** 2000. Herpes simplex virus type 2 induces secretion of IL-12 by macrophages through a mechanism involving NF-kappaB. J. Gen. Virol. **81:**3011–3020.
158. **Malmstrom, M., H. Ruokonene, Y. T. Kontinnen, V. Bergoth, M. Segerberg-Konttinen, J. Hietanen, D. Nordstrom, and M. Haapala.** 1990. Herpes simplex virus antigens and inflammatory cells in oral lesions in recurrent erythema multiforme. Immunoperoxidase and autoradiographic studies. Acta Dermato-Venereol. **70:**405–410.
159. **Manickan, E., M. Francotte, N. Kuklin, M. Dewerchin, C. Molitor, D. Gheysen, M. Slaoui, and B. T. Rouse.** 1995. Vaccination with recombinant vaccinia viruses expressing ICP27 induces protecting immunity against herpes simplex virus through $CD4^+$ $Th1^+$ T cells. J. Virol. **69:**4711–4716.
160. **Markert, J. M., M. D. Medlock, S. D. Rabkin, T. Todo, W. D. Hunter, C. A. Palmer, F. Feigenbaum, C. Tornatore, F. Tufaro, and R. L. Martuza.** 2000. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther. **7:**867–874.
161. **Marrack, P., J. Kappler, and T. Mitchell.** 1999. Type I interferons keep activated T cells alive. J. Exp. Med. **189:**521–530.
162. **Marsters, S. A., T. M. Ayers, M. Skubatch, C. L. Gray, M. Rothe, and A. Ashkenazi.** 1997. Herpesvirus entry mediator, a member of the tumor necrosis factor receptor (TNFR) family, interacts with members of the TNFR-associated receptor family and activates the transcription factors NF-kappa-B and AP-1. J. Biol. Chem. **272:**14029–14032.
163. **Martin, S., B. Moss, P. W. Berman, L. A. Laskey, and B. T. Rouse.** 1987. Mechanisms of antiviral immunity induced by a vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D: cytotoxic T cells. J. Virol. **61:**726–734.
164. **Martinez, W. M., and P. G. Spear.** 2001. Structural features of nectin-2 (hveb) required for herpes simplex virus entry. J. Virol. **75:**11185–11195.
165. **Martz, E., and S. R. Gamble.** 1992. How do CTL control virus infection? Evidence for prelytic halt of herpes simplex. Virus Immun. **5:**81–91.
166. **Mastrolorenzo, A., L. Tiradritti, L. Salimberi, and G. Zuccati.** 1995. Multicentre clinical trial with herpes simplex virus vaccine in recurrent herpes infection. Int. J. STD AIDS **6:**431–435.
167. **Mata, M., M. Zhang, X. Hu, and D. J. Fink.** 2001. HveC (nectin-1) is expressed at high levels in sensory neurons, but not in motor neurons, of the rat peripheral nervous system. J. Neurovirol. **7:**476–480.
168. **Mauri, D. N., R. Ebner, R. I. Montgomery, K. D. Kochel, T. C. Cheung, G-L. Yu, S. Ruben, M. Murphy, R. J. Eisenberg, G. H. Cohen, P. G. Spear, and C. F. Ware.** 1998. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. Immunity **8:**21–30.
169. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.
170. **McGeoch, D. J., A. Dolan, S. Donald, and D. H. Brauer.** 1986. Complete DNA sequence of the short repeat region in the genome of herpes simplex virus type 1. Nucleic Acids Res. **14:**1727–1745.
171. **McLean, C. S., M. Erturk, R. Jennings, D. N. Challanain, A. C. Minson, I. Duncan, M. E. Boursnell, and S. C. Inglis.** 1994. Protective vaccination against primary and recurrent disease caused by herpes simplex virus (HSV) type 2 using a genetically disabled HSV-1. J. Infect. Dis. **170:**1110–1119.
172. **McLean, C. S., D. Ni Challanian, I. Duncan, M. E. Boursnell, R. Jennings, and S. C. Inglis.** 1996. Induction of a protective immune response by mucosal vaccination with a DISC HSV-1 vaccine. Vaccine **14:**987–992.
173. **Meignier, B., R. Longnecker, and B. Roizman.** 1988. In vivo behavior of genetically engineered herpes simplex viruses R7017 and R7020: construction and evaluation in rodents. J. Infect. Dis. **158:**602–614.
174. **Meignier, B., B. Martin, R. J. Whitley, and B. Roizman.** 1990. In vivo behavior of genetically engineered herpes simplex viruses R7017 and R7020. II. Studies in immunocompetent and immunocompromised owl monkeys (*Aotus trivirgatus*). J. Infect. Dis. **162:**313–321.
175. **Mertz, G. J., R. Ashley, R. L. Burke, J. Benedetti, C. Critchlow, C. C. Jones, and L. Corey.** 1990. Double-blind, placebo-controlled trial of a herpes simplex virus type 2 glycoprotein vaccine in persons at high risk for genital herpes infection. J. Infect. Dis. **161:**653–660.
176. **Mertz, G. J., J. Benedetti, R. Ashley, S. Selke, and L. Corey.** 1992. Risk factors for the sexual transmission of genital herpes. Ann. Intern. Med. **116:**197–202.
177. **Mertz, G. J., G. Peterman, R. Ashley, J. L. Jourden, D. Salter, L. Morrison, A. McLean, and L. Corey.** 1984. Herpes simplex virus type-2 glycoprotein-subunit vaccine: tolerance and humoral and cellular responses in humans. J. Infect. Dis. **150:**242–249.
178. **Mertz, G. J., O. Schmidt, J. L. Jourden, M. E. Guinan, M. L. Remington, A. Fahnlander, C. Winter, K. K. Holmes, and L. Corey.** 1985. Frequency of acquisition of first-episode genital infection with herpes simplex virus from asymptomatic and symptomatic source contacts. Sex. Transm. Dis. **12:**33–39.
179. **Mester, J. C., S. L. Highlander, A. P. Osmand, J. C. Glorioso, and B. T. Rouse.** 1990. Herpes simplex virus type 1-specific immunity induced by peptides corresponding to an antigenic site of glyoprotein B. J. Virol. **64:**5277–5283.
180. **Miegnier B.** 1985. Vaccination against herpes simplex virus infections, p. 265–296. *In* B. Roizman and C. Lopez (ed.), The herpesvirus. Immunology and prophylaxis of human herpesvirus infection, vol. 4. Plenum Press, New York, N.Y.
181. **Mikloska, A., and A. L. Cunningham.** 1998. Herpes simplex virus type 1 glycoproteins gB, gC, and gD are major targets for CD4 T-lymphocyte cytotoxicity in HLA-DR expressing human epidermal keratinocytes. J. Gen. Virol. **79:**353–361.
182. **Mikloska, A., A. M. Kesson, M. E. T. Penfold, and A. L. Cunningham.** 1996. Herpes simplex virus protein targets for CD4 and CD8 lymphocyte cytotoxicity in cultured epidermal keratinocytes treated with interferon-gamma. J. Infect. Dis. **173:**7–17.
183. **Mikloska, Z., L. Bosnjak, and A. L. Cunningham.** 2001. Immature mono-

cyte-derived dendritic cells are productively infected with herpes simplex virus type 1. J. Virol. **75:**5958–5964.
184. **Mikloska, Z., and A. L. Cunningham.** 2001. Alpha and gamma interferons inhibit herpes simplex virus type 1 infection and spread in epidermal cells after axonal transmission. J. Virol. **75:**11821–11826.
185. **Mikloska, Z., V. A. Danis, S. Adams, A. R. Lloyd, D. L. Adrian, and A. L. Cunningham.** 1998. In vivo production of cytokines and beta (C-C) chemokines in human recurrent herpes simplex lesions-do herpes simplex virus-infected keratinocytes contribute to their production? J. Infect. Dis. **177:**827–838.
186. **Mikloska, Z., M. Ruckholdt, I. Ghadiminejad, H. Dunckley, M. Denis, and A. L. Cunningham.** 2000. Monophosphosphoryl lipid A and QS21 increase CD8 T lymphocyte cytotoxicity to herpes simplex virus-2 infected cell proteins 4 and 27 through IFN-gamma and IL-12 production. J. Immunol. **164:**5167–5176.
187. **Mikloska, Z., P. P. Sanna, and A. L. Cunningam.** 1999. Neutralizing antibodies inhibit axonal spread of herpes simplex virus type 1 to epidermal cells in vitro. J. Virol. **73:**5934–5944.
188. **Milligan, G. N., D. I. Bernstein, and N. Bourne.** 1998. T lymphocytes are required for protection of the vaginal mucosae and sensory ganglia of immune mice against reinfection with herpes simplex virus type 2. J. Immunol. **160:**6093–6100.
189. **Miranda-Saksena, M., P. Armati, R. A. Boadle, D. J. Holland, and A. L. Cuningham.** 2000. Anterograde transport of herpes simplex virus type 1 in cultured, dissociated human and rat dorsal root ganglion neurons. J. Virol. **74:**1827–1839.
190. **Miura, S., C. C. Smith, J. W. Burnett, and L. Aurelian.** 1992. Detection of viral DNA within skin of healed recurrent herpes simplex virus infection and erythema multiforme lesions. J. Investig. Dermatol. **98:**68–72.
191. **Montgomery, R. L., M. S. Warner, B. J. Lum, and P. G. Spear.** 1996. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell **87:**427–436.
192. **Morrison, L. A., and D. M. Knipe.** 1996. Mechanisms of immunization with a replication-defective mutant of herpes simplex virus 1. Virology **220:**402–413.
193. **Nagashunmugam, T., J. Lubinski, L. Wang, L. T. Goldstein, B. S. Weeks, P. Sundaresan, E. H. Kang, G. Dubin, and H. M. Friedman.** 1998. In vivo immune evasion mediated by the herpes simplex virus type 1 immunoglobulin G Fc receptor. J. Virol. **72:**5351–5359.
194. **Nass, P. H., K. L. Elkins, and J. P. Weir.** 1998. Antibody response and protective capacity of plasmid vaccines expressing three different herpes simplex virus glycoproteins. J. Infect. Dis. **178:**611–617.
195. **Nguyen, L. H., D. M. Knipe, and R. W. Finberg.** 1992. Replication-defective mutants of HSV induce cellular immunity and protect against lethal infection. J. Virol. **66:**7067–7072.
196. **Orton, P. W., J. C. Huff, M. G. Tonnesen, and W. L. Weston.** 1984. Detection of herpes simplex viral antigen in skin lesions of erythema multiforme. Ann. Intern. Med. **101:**48–50.
197. **Ott, G., G. L. Barchfeld, D. Chernoff, R. Radhakrishnan, P. van Hoogevest, and G Van Nest.** 1995. MF59: Design and evaluation of a safe and potent adjuvant for human vaccines, p. 277–296. *In* M. F. Powell, and M. J. Newman (ed.), Vaccine design: the subunit and adjuvant approach. Plenum Press, New York, N.Y.
198. **Ott, G., G. Van Nest, and R. L. Burke.** 1991. The use of muramyl peptides as vaccine adjuvants, p. 89–114. *In* E. Koff (ed.), Vaccine research: a series of advances, vol. 1. Marcel Dekker, New York, N.Y.
199. **Overall, J. C., S. L. Spruance, and J. A. Green.** 1981. Viral-induced leukocyte interferon in vesicle fluid from lesions of recurrent herpes labialis. J. Infect. Dis. **143:**543–547.
200. **Paludan, S. R.** 2001. Requirements for the induction of interleukin-6 by herpes simplex virus-infected leukocytes. J. Virol. **75:**8008–8015.
201. **Paludan, S. R., S. Ellerman-Eriksen, V. Kruys, and S. C. Mogensen.** 2001. Expression of TNF-alpha by herpes simplex virus-infected macrophages is regulated by a dual mechanism: transcriptional regulation by NF-kappa-B and activating transcription factor 2/jun and translational regulation through the AU-rich region of the 3′ untranslated region. J. Immunol. **167:**2202–2208.
202. **Paludan, S. R., and S. C. Mogensen.** 2001. Virus-cell interactions regulating induction of tumor necrosis factor alpha production in macrophages infected with herpes simplex virus. J. Virol. **75:**10170–10178.
203. **Panoutsakopoulou, V., M. E. Sanchirico, K. M. Huster, M. Jansson, F. Granucci, D. J. Shim, K. W. Wucherpfennig, and H. Cantor.** 2001. Analysis of the relationship between viral infection and autoimmune disease. Immunity **15:**137–147.
204. **Paquet, P., and G. E. Pierard.** 1997. Erythema multiforme and toxic epidermal necrolysis: a comparative study. Am. J. Dermatopathol. **19:**127–132.
205. **Parr, E. L., and M. B. Parr.** 1998. Immunoglobulin G, plasma cells, and lymphocytes in the murine vagina after vaginal or parenteral immunization with attenuated herpes simplex virus type 2. J. Virol. **72:**5137–5145.
206. **Parr, M. B., and E. L. Parr.** 1998. Mucosal immunity to herpes simplex virus type 2 infection in the mouse is impaired by in vivo depletion of T lymphocytes. J. Virol. **72:**2677–2685.
207. **Pass, R. F., A. M. Duliege, S. Bopanna, R. Sekulovich, S. Percell, W. Britt, and R. L. Burke.** 1999. A subunit cytomegalovirus vaccine based on recombinant envelope glycoprotein B and a new adjuvant. J. Infect. Dis. **180:**970–975.
208. **Peng, T., M. Ponce-de-Leon, H. Jiang, G. Dubin, J. M. Lubinski, R. J. Eisenberg, and G. H. Cohen.** 1998. The gH-gL complex of herpes simplex virus (HSV) stimulates neutralizing antibody and protects mice against HSV type 1 challenge. J. Virol. **72:**65–72.
209. **Pereira, R. A., and A. Simmons.** 1999. Cell surface expression of H2 antigens on primary sensory neurons in response to acute but not latent herpes simplex virus infection in vivo. J. Virol. **73:**6484–6489.
210. **Pereira, R. A., M. M. Simon, and A. Simmons.** 2000. Granzyme A, a noncytolytic component of $CD8^+$ cell granules, restricts the spread of herpes simplex virus in the peripheral nervous systems of experimentally infected mice. J. Virol. **74:**1029–1032.
211. **Periera, R. A., A. Scalzo, and A. Simmons.** 2001. Cutting edge: a NK complex-linked locus governs acute versus latent herpes simplex virus infection of neurons. J. Immunol. **166:**5869–5873.
212. **Posavad, C. M., S. Barcy, M. L. Huang, D. M. Koelle, S. J. Polyak, and L. Corey.** 2000. Long-term persistence of herpes simplex virus-specific CD8+ CTL clones derived from genital lesions. J. Immunol. **165:**1146–1152.
213. **Posavad, C. M., D. M. Koelle, and L. Corey.** 1996. High frequency of $CD8^+$ cytotoxic T-lymphocyte precursors specific for herpes simplex viruses in persons with genital herpes. J. Virol. **70:**8165–8168.
214. **Posavad, C. M., D. M. Koelle, M. F. Shaughnessy, and L. Corey.** 1997. Severe genital herpes infections in HIV-infected individuals with impaired HSV-specific CD8+ cytotoxic T lymphocyte responses. Proc. Natl. Acad. Sci. USA **94:**10289–10294.
215. **Raftery, M. J., C. K. Behrens, A. Muller, A. Krammer, H. Walczak, and G. Schonrich.** 1999. Herpes simplex virus type 1 infection of activated cytotoxic T cells: induction of fratricide as a mechanism of viral immune evasion. J. Exp. Med. **190:**1103–1114.
216. **Riddell, S. R, M. Elliott, D. A. Lewinsohn, M. J. Gilbert, L. Wilson, S. A. Manley, S. D. Lupton, R. W. Overall, T. C. Reynolds, L. Corey, and P. D. Greenberg.** 1996. T-cell mediated rejection of gene-modified HIV-specific cytotoxic T lymphocytes in HIV-infected patients. Nat. Med. **2:**216–223.
217. **Rogge, L., L. Barberis-Maino, M. Biffi, N. Passini, D. H. Presky, U. Gubler, and F. Sinigaglia.** 1997. Selective expression of an interleukin-12 receptor component by human T helper 1 cells. J. Exp. Med. **185:**825–831.
218. **Roizman, B., and D. M. Knipe.** 2001. Herpes simplex viruses and their replication, p. 2399–2459. *In* D. M. Knipe, P. M. Howley, and D. E. Griffin (ed.), Fields virology, 4th ed., vol. 2. Lippincott, Philadelphia, Pa.
219. **Roizman, B., and P. E. Pellett.** The family herpesviridae: a brief introduction, p. 2381–2397. *In* D. M. Knipe, P. M. Howley, and D. E. Griffin (ed.), Fields virology, 4th ed., vol. 2. Lippincott, Philadelphia, Pa.
220. **Rosenthal, K. S., M. Mao, W. I. Horne, C. Wright, and D. Zimmerman.** 1999. Immunization with a LEAPS heteroconjugate containing a CTL epitope and a peptide from beta-2-microglobulin elicits a protective and DTH response to herpes simplex virus type 1. Vaccine **17:**535–542.
221. **Sakaoka, H., T. Aomori, T. Gouro, and Y. Kumamoto.** 1995. Demonstration of either endogenous recurrence or exogenous reinfection by restriction endonuclease cleavage analysis of herpes simplex virus from patients with recrudescent genital herpes. J. Med. Virol. **46:**387–396.
222. **Salio, M., M. Cella, M. Suter, and A. Lanzavecchia.** 1999. Inhibition of dendritic cell maturation by herpes simplex virus. Eur. J. Immunol. **29:**3245–3253.
223. **Sanchez-Pescador, L., P. Paz, D. Navarro, L. Pereira, and S. Kohl.** 1992. Epitopes of herpes simplex virus type 1 glycoprotein B that bind type-common neutralizing antibodies elicit type-specific antibody-dependent cellular cytotoxicity. J. Infect. Dis. **166:**623–627.
224. **Sandberg, K., P. Matsson, and G. V. Alm.** 1990. A distinct population of nonphagocytic and low level CD4+ null lymphocytes produce IFN-α after stimulation by herpes simplex virus-infected cells. J. Immunol. **145:**1015–1020.
225. **Sarisky, R. T., T. T. Nguyen, K. E. Duffy, R. J. Wittrock, and J. J. Leary.** 2000. Difference in incidence of spontaneous mutations between herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. **44:**1524–1529.
226. **Sarrias, M. R., J. C. Whitbeck, I. Rooney, L. Spruce, B. K. Kay, R. I. Montgomery, P. G. Spear, C. F. Ware, R. J. Eisenberg, R. J. Cohen, and J. D. Lambris.** 1999. Inhibition of herpes simplex virus gD and lymphotoxin-a binding to HveA by peptide antagonists. J. Virol. **73:**5681–5687.
227. **Sarrias, M. R., J. C. Whitbeck, I. Rooney, C. F. Ware, R. J. Eisenberg, G. H. Cohen, and J. D. Lambris.** 2000. The three HveA receptor ligands, gD, LT-alpha and LIGHT bind to distinct sites on HveA. Mol. Immunol. **37:**665–673.
228. **Sawtell, N. M.** 1998. The probability of in vivo reactivation of herpes simplex virus type 1 increases with the number of latently infected neurons in the ganglia. J. Virol. **72:**6888–6892.
229. **Sawtell, N. M., R. L. Thompson, L. R. Stanberry, and D. I. Bernstein.** 2001. Early intervention with high-dose acyclovir treatment during primary herpes simplex virus infection reduces latency and subsequent reactivation in the nervous system in vivo. J. Infect. Dis. **184:**964–971.

230. **Schmid, D. S., M. L. Thieme, H. E. Gary, and W. C. Reeves.** 1997. Characterization of T cell responses to herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) using a TNF-beta ELISpot cytokine assay. Arch. Virol. **142:**1659–1671.
231. **Scognamiglio, P., D. Accapezzato, M. A. Casciaro, A. Cacciani, M. Artini, G. Bruno, M. L. Chircu, J. Sidney, S. Southwood, S. Abrignani, A. Sette, and V. Barnaba.** 1999. Presence of effector CD8+ T cells in hepatitis C virus-exposed healthy seronegative donors. J. Immunol. **162:**6681–6689.
232. **Sellati, T. J., S. L. Waldrop, J. C. Salazar, P. R. Bergstresser, L. J. Picker, and J. D. Radolf.** 2001. The cutaneous response in humans to Treponema pallidum lipoprotein analogues involves cellular elements of both innate and adaptive immunity. J. Immunol. **166:**4131–4140.
233. **Shaikh, R. B., S. Santee, S. W. Granger, K. Butrovich, T. Cheung, M. Kronenberg, H. Cheroutre, and C. F. Ware.** 2001. Constitutive expression of LIGHT on T cells leads to lymphocyte activation, inflammation, and tissue destruction. J. Immunol. **167:**6330–6337.
234. **Shukla, D., and P. G. Spear.** 2001. Herpesviruses and heparin sulfate: an intimate relationship in aid of viral entry. J. Clin. Investig. **108:**503–510.
235. **Siegal, F. P., N. Kadowaki, M. Shodell, P. A. Fitzgerald-Bocarsly, K. Shah, S. Ho, S. Antonenko, and Y.-J. Liu.** 1999. The nature of the principal type 1 interferon-producing cells in human blood. Science **284:**1835–1837.
236. **Simmons, A., and D. C. Tscharke.** 1992. Anti-CD8 impairs clearance of herpes simplex virus from the nervous system: implications for the fate of virally infected neurons. J. Exp. Med. **175:**1337–1344.
237. **Sin, J. L., J. J. Kim, R. L. Arnold, K. E. Shroff, D. McCallus, C. Pachuk, S. P. McElhiney, M. W. Wolf, S. J. Pompa-de Bruin, T. J. Higgins, R. B. Ciccarelli, and D. B. Weiner.** 1999. IL-12 gene as a DNA vaccine adjuvant in a herpes mouse model: IL-12 enhances TH1-type CD4+ T cell-mediated protective immunity against herpes simplex virus-2 challenge. J. Immunol. **162:** 2912–2921.
238. **Sin, J. L., J. J. Kim, J. D. Boyer, R. B. Ciccarelli, T. J. Higgins, and D. B. Weiner.** 1999. In vivo modulation of vaccine-induced immune responses toward a Th1 phenotype increases potency and vaccine effectiveness in a herpes simplex type 2 mouse model. J. Virol. **73:**501–509.
239. **Sin, J. I., J. J. Kim, K. E. Ugen, R. B. Ciccarelli, T. J. Higgins, and D. B. Weiner.** 1998. Enhancement of protective humoral (Th2) and cell-mediated (Th1) immune responses against herpes simplex virus-2 through co-delivery of granulocyte-macrophage colony-stimulating factor expression cassettes. Eur. J. Immunol. **28:**3530–3540.
240. **Sin, J.-I., and D. B. Weiner.** 2000. Improving DNA vaccines targeting viral infection. Intervirology **43:**233–246.
241. **Singh, M., J. R. Carlson, M. Briones, M. Ugozzoli, J. Kazzaz, J. Barackman, G. Ott, and D. O'Hagan.** 1998. A comparison of biodegradable bioparticles and MF59 as systemic adjuvants for recombinant gD from HSV-2. Vaccine **16:**1822–1827.
242. **Skinner, G. R. B., J. A. Davies, S. Dundarov, and P. Andonov.** 2000. Prevention of herpes genitalis by the "Bulgarian" vaccine EHSV-2V(PRK): preliminary clinical evidence. Croat. Med. J. **41:**378–383.
243. **Skinner, G. R. B., M. E. Turyk, C. A. Benson, G. D. Wilbanks, P. Heseltine, J. Galpin, R. Kaufman, L. Goldberg, C. E. Hartley, and A. Buchan.** 1997. The efficacy and safety of Skinner herpes simplex vaccine towards modulation of herpes genitalis; report of a prospective double-blind placebo-controlled trail. Med. Microbiol. Immunol. **186:**31–36.
244. **Smith, C. C., and L. Aurelian.** 1997. The large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) is associated with the virion tegument and has PK activity. Virology **234:**235–242.
245. **Sobel, R. A., A. B. Collins, R. B. Colvin, and A. K. Bhan.** 1986. The in situ cellular immune response in acute herpes simplex encephalitis. Am. J. Pathol. **125:**332–338.
246. **Spatz, M., H. M. Wolf, V. Thon, J. M. Gampfer, and M. M. Eibl.** 2000. Immune response to the herpes simplex type 1 regulatory proteins ICP8 and VP16 in infected persons. J. Med. Virol. **62:**29–36.
247. **Spear, P. G.** 2001. A first step towards understanding membrane fusion induced by herpes simplex virus. Mol. Cell **8:**2–4.
248. **Spear, P. G., R. J. Eisenberg, and G. H. Cohen.** 2000. Three classes of surface receptors for alphaherpesvirus entry. Virology **275:**1–8.
249. **Speck, P., and A. Simmons.** 1998. Precipitous clearance of herpes simplex virus antigens from the peripheral nervous systems of experimentally infected C57BL/10 mice. J. Gen. Virol. **79:**561–564.
250. **Spector, F. C., E. R. Kern, J. Palmer, R. Kaiwar, T. A. Cha, P. Brown, and R. R. Spaete.** 1998. Evaluation of a live attenuated recombinant virus RAV 9395 as a herpes simplex virus type 2 vaccine in guinea pigs. J. Infect. Dis. **177:**1143–1154.
251. **Spruance, S. L., T. G. Evans, M. B. McKeough, L. Thai, B. A. Araneo, R. A. Daynes, E. M. Mishkin, and A. S. Abramovitz.** 1995. Th1/Th2-like immunity and resistance to herpes simplex labialis. Antiviral Res. **28:**39–55.
252. Reference deleted.
253. **Spruance, S. L., S. K. Tyring, M. H. Smith, and T. C. Meng.** 2001. Application of a topical immune response modifier, resiquimod gel, to modify the recurrence rate of recurrent genital herpes: a pilot study. J. Infect. Dis. **184:**196–200.
254. **Stanberry, L., A. Cunningham, G. Mertz, A. Mindel, B. Peters, M. Reitano, S. Sacks, A. Wald, S. Wassilew, and P. Woolley.** 1999. New developments in the epidemiology, natural history and management of genital herpes. Antiviral Res. **42:**1–14.
255. **Stanberry, L. R., A. L. Cunningham, A. Mindel, L. L. Scott, S. L. Spruance, F. Y. Aoki, and C. J. Lacey.** 2000. Prospects for control of herpes simplex virus disease through immunization. Clin. Infect. Dis. **30:**549–566.
256. **Stanberry, L. R., S. A. Floyd-Reising, B. L. Connelly, S. J. Alter, M. J. R. Gilchrist, C. Rubio, and M. G. Myers.** 1994. Herpes simplex viremia: report of eight pediatric cases and review of the literature. Clin. Infect. Dis. **18:**401–407.
257. **Stanberry, L. R., S. L. Spruance, A. L. Cunningham, D. I. Bernstein, A. Mindel, S. Sacks, S. Tyring, F. Y. Aoki, M. Slaoui, M. Denis, P. Vandepapeliere, and G. Dubin.** 2002. Glycoprotein D adjuvant vaccine to prevent genital herpes. N. Engl. J. Med. **21:**1652–1661.
258. **Straus, S. E., L. Corey, R. L. Burke, B. Savarese, G. Barnum, P. R. Krause, R. G. Kost, J. L. Meier, R. Sekulovich, S. F. Adair, and C. L. Dekker.** 1994. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. Lancet **343:**1460–1463.
259. **Straus, S. E., B. Savarese, M. Tigges, A. G. Freifeld, P. R. Krause, D. M. Margolis, J. L. Meier, D. P. Paar, S. F. Adair, D. Dina, C. Dekker, and R. L. Burke.** 1993. Induction and enhancement of immune responses to herpes simplex virus type 2 in humans by use of a recombinant glycoprotein D vaccine. J. Infect. Dis. **167:**1045–1052.
260. **Straus, S. E., A. Wald, R. G. Kost, R. McKenzie, A. G. M., Langenberg, P. Hohman, J. Lekstrom, E. Cox, M. Nakamura, R. Sekulovich, A. Izu, C. Dekker, and L. Corey.** 1997. Immunotherapy of recurrent genital herpes with recombinant herpes simplex virua type 2 glycoproteins B and D: results of a placebo-controlled vaccine trial. J. Infect. Dis. **176:**1129–1134.
261. **Streilein, J. W., M. R. Dana, and B. R. Ksander.** 1997. Immunity causing blindness: five different paths to herpes stromal keratitis. Immun. Today **18:**443–449.
263. **Struyf, F., C. M. Posavad, E. Keyearts, M. Van Ranst, L. Corey, and P. G. Spear.** 2001. Search for polymorphisms in the genes for herpesvirus entry mediator, nectin-1, and nectin-2 in immune seronegative individuals. J. Infect. Dis. **185:**36–44.
264. **Sucato, G., A. Wald, E. Wakabayashi, J. Vieira, and L. Corey.** 1998. Evidence of latency and reactivation of both herpes simplex virus (HSV)-1 and HSV-2 in the genital region. J. Infect. Dis. **177:**1069–1072.
265. **Suzuki, K., D. Hu, T. Bustos, J. Zlotogora, A. Richieri-Costa, J. A. Helms, and R. A. Spritz.** 2000. Mutations in PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. Nat. Genet. **4:**427–430.
266. **Suzutani, T., M. Nagamine, T. Shibaki, M. Ogasawara, I. Yoshida, T. Daikoku, Y. Nishiyama, and M. Azuma.** 2000. The role of the UL41 gene of herpes simplex virus type 1 in evasion of non-specific host defense mechanisms during primary infection. J. Gen. Virol. **81:**1763–1771.
267. **Swetter, S. M., E. L. Hill, E. L. Kern, D. M. Koelle, C. M. Posavad, and S. Safrin.** 1998. Chronic vulvar ulceration in an immunocompetent woman due to acyclovir-resistant, thymidine-kinase-deficient herpes simplex virus. J. Infect. Dis. **177:**543–550.
268. **Tabi, Z., M. Moutaftsi, and L. K. Borysiewicz.** 2000. Human cytomegalovirus pp65- and immediate early 1 antigen-specific HLA class I-restricted cytotoxic T cell responses induced by cross-presentation of viral antigens. J. Immunol. **166:**5695–5703.
269. **Terhune, S. S., K. T. Coleman, R. Sekulovich, R. L. Burke, and P. G. Spear.** 1998. Limited variability of glycoprotein gene sequences and neutralizing targets in herpes simplex virus type 2 isolates and stability on passage in cell culture. J. Infect. Dis. **178:**8–15.
270. **Thoelen, S., N. De Clercq, and N. Tornieporth.** 2001. A prophylactic hepatitis B vaccine with a novel adjuvant system. Vaccine **19:**2400–2403.
271. **Tigges, M. A., D. M. Koelle, K. Hartog, R. E. Sekulovich, L. Corey, and R. L. Burke.** 1992. Human $CD8^+$ herpes simplex virus-specific cytotoxic T lymphocyte clones recognize diverse virion protein antigens. J. Virol. **66:** 1622–1634.
272. **Tigges, M. A., S. Leng, D. C. Johnson, and R. L. Burke.** 1996. Human herpes simplex (HSV)-specific CD8+ CTL clones recognize HSV-2-infected fibroblasts after treatment with IFN-gamma or when virion host shutoff functions are disabled. J. Immunol. **156:**3901–3910.
273. **Tomazin, R., N. E. van Schoot, K. Goldsmith, P. Jugovic, P. Sempe, K. Fruh, and D. C. Johnson.** 1998. Herpes simplex virus type 2 ICP47 inhibits human TAP but not mouse TAP. J. Virol. **72:**2560–2563.
274. **Torseth, J. W., G. H. Cohen, R. J. Eisenberg, P. W. Berman, L. A. Lasky, C. P. Cerini, C. J. Heilman, S. Kerwar, and T. C. Merigan.** 1987. Native and recombinant herpes simplex virus type 1 envelope proteins induce human immune T-lymphocyte responses. J. Virol. **61:**1532–1539.
275. **Torseth, J. W., and T. C. Merigan.** 1986. Significance of local gamma interferon in recurrent herpes simplex infection. J. Infect. Dis. **153:**979–983.
276. **Tortorella, D., B. E. Gewurz, M. H. Furman, D. J. Schust, and H. L. Ploegh.** 2000. Viral subversion of the immune system. Ann. Rev. Immunol. **18:**861–926.
277. **Valensi, J. M., J. R. Carlson, and G. Van Nest.** 1994. Systemic cytokine

profiles in Balb/c mice immunized with trivalent influenza vaccine containing MF59 oil emulsion and other advanced adjuvants. J. Immunol. **153:** 4029–4039.
278. **Van Voorhis, W. C., L. K. Barrett, D. M. Koelle J. M. Nasio, F. A. Plummer, and S. A. Lukehart.** 1996. Primary and secondary syphilis lesions contain mRNA for Th1 cytokines and activated cytolytic T cells. J. Infect. Dis. **173:**491–495.
279. **Vasilakos, J. P., R. M. A. Smith, S. J. Gibson, J. M. Lindh, L. K. Pederson, M. J. Reiter, M. H. Smith, and M. A. Tomai.** 2000. Adjuvant activities of immune response modifier R-848: comparison with CpG ODN. Cell. Immunol. **204:**64–74.
280. **Verjans, G. M., M. E. M. Dings, J. McLauchlan, A. van der Kooi, P. Hoogerhout, H. F. Brugghe, H. A. M. Timmermans, G. S. Baarsma, and A. D. M. E. Osterhaus.** 2000. Intraocular T cells of patients with herpes simplex (HSV)-induced acute retinal necrosis recognize HSV tegument proteins VP11/12 and VP13/14. J. Infect. Dis. **182:**923–927.
281. **Verjans, G. M. G. M., L. Remeijer, C. M. Mooy, and A. D. M. E. Osterhaus.** 2000. Herpes simplex virus specific T-cells infiltrate the cornea of patients with herpes stromal keratitis: no evidence for intra-corneal autoreactive T-cells in human HSK. Investig. Ophthalmol. Visual Sci. **41:**2607–2612.
282. **Verjans, G. M. G. M., L. Remeijer, R. S. van Binnendijk, J. G. C. Cornelissen, H. J. Volker-Dieben, S. G. Baarsma, and A. D. M. E. Osterhaus.** 1998. Identification and characterization of herpes simplex virus-specific CD4+ T cells in corneas of herpetic stromal keratitis patients. J. Infect. Dis. **177:**484–488.
283. **Wachsman, M., M. Kulka, C. C. Smith, and L. Aurelian.** 2001. A growth and latency compromised herpes simplex virus type 2 mutant (ICP10DeltaPK) has prophylactic and therapeutic protective activity in guinea pigs. Vaccine **19:**1879–1890.
284. **Wagner, H. U., E. Van Dyck, E. Roggen, A. J. Nunn, A. Kamali, D. S. Schmid, J. G. Dobbins, and D. W. Mulder.** 1994. Seroprevalence and incidence of sexually transmitted diseases in a rural Ugandan population. Int. J. STD AIDS **5:**332–337.
285. **Wald, A., J. Zeh, S. Selke, R. Ashley, and L. Corey.** 1995. Virologic characteristics of subclinical and symptomatic genital herpes infections. N. Engl. J. Med. **333:**770–775.
286. **Weinstein, D. L., D. G. Walker, H. Akiyama, and P. L. McGeer.** 1990. Herpes simplex virus type I infection of the CNS induces major histocompatibility complex antigen expression on rat microglia. J. Neurosci. Res. **26:**55–65.
287. **Westra, D. F., H. B. Kuiperij, G. W. Welling, A. J. Scheffer, T. H. The, and S. Welling-Wester.** 1999. Domains of glycoprotein H of herpes simplex virus type 1 involved in complex formation with glycoprotein L. Virology **261:** 96–105.
288. **Westra, D. F., G. M. G. M. Verjans, A. D. Osterhaus, A. van Kooij, G. W. Welling, A. J. Scheffer, T. H. The, and S. Welling-Wester.** 2000. Natural infection with herpes simplex virus type 1 (HSV-1) induces humoral and T cell responses to the HSV-1 glycoprotein H:L complex. J. Gen. Virol. **81:**2011–2015.
289. **Whitbeck, J. C., S. A. Connolly, S. H. Willis, W. Hou, C. Krummenacher, M. Ponce de Leon, H. Lou, I. Baribuad, R. J. Eisenberg, and G. H. Cohen.** 2001. Localization of the gD-binding region of the human herpes simplex virus receptor. HveA. J. Virol. **75:**171–180.
290. **Whitbeck, J. C., M. I. Muggeridge, A. H. Rux, W. Hou, C. Krummenacher, H. Lou, A. van Geelen, R. J. Eisenberg, and G. H. Cohen.** 1999. The major neutralizing antigenic site on herpes simplex virus glycoprotein D overlaps a receptor-binding domain. J. Virol. **73:**9879–9890.
291. **Whitley, R. J.** 1997. Herpes simplex virus vaccines, p. 727–747. *In* M. M. Levine, G. C. Woodrow, J. B. Kaper, and G. S. Cobon (ed), New generation vaccines, 2nd ed Marcel Dekker. New York, NY.
292. **Whitley, R. J.** 2001. Herpes simplex viruses, p. 2461–2509. *In* D. M. Knipe, P. M. Howley, and D. E. Griffin, (ed.), Fields virology 4th ed., vol. 2. Lippincott, Philadelphia, Pa.
293. Reference deleted.
294. **Yasukawa, M., A. Inatsuki, and Y. Kobayashi.** 1988. Helper activity in antigen-specific antibody production mediated by CD4+ human cytotoxic T cell clones directed against herpes simplex virus. J. Immunol. **140:**3419–3425.
295. **Yasukawa, M., and Y. Kobayashi.** 1985. Inhibition of herpes simplex virus replication in vitro by human cytotoxic T cell clones and natural killer cell clones. J. Gen. Virol. **66:**2225–2229.
296. **Yasukawa, M., H. Ohminami, Y. Yakushijin, J. Arai, A. Hasegawa, Y. Ishida, and S. Fujita.** 1999. Fas-independent cytotoxicity mediated by CD4+ CTL directed against herpes simplex virus-infected cells. J. Immunol. **162:**6100–6106.
297. **York, I. A., C. Roop, D. W. Andrews, S. R. Riddell, F. L. Graham, and D. C. Johnson.** 1994. A cytosolic herpes simplex protein inhibits antigen presentation to CD8+ T lymphocytes. Cell **77:**525–535.
298. **Yu, Z., K. L. Karem, S. Kanangat, E. Manickan, and B. T. Rouse.** 1998. Protection by minigenes: a novel approach of DNA vaccines. Vaccine **16:**1660–1667.
299. **Zarling, J. M., P. A. Moran, L. Brewer, R. Ashley, and L. Corey.** 1988. Herpes simplex virus (HSV)-specific proliferative and cytotoxic T-cell responses in humans immunized with an HSV type 2 glycoprotein subunit vaccine. J. Virol. **62:**4481–4485.
300. **Zarling, J. M., P. A. Moran, R. L. Burke, C. Pachl, P. W. Berman, and L. A. Lasky.** 1986. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. IV. Recognition and activation by cloned glycoproteins gB and gD-1. J. Immunol. **136:**4669–4673.
301. **Zhao, Z. S., F. Granucci, L. Yeh, P. A. Schaffer, and H. Cantor.** 1998. Molecular mimicry by herpes simplex virus type 1: autoimmune disease after viral infection. Science **279:**1344–1347.
302. **Zheng, B., F. L. Graham, D. J. Johnson, T. Hanke, M. R. McDermott, and L. Prevec.** 1993. Immunogenicity in mice of tandem repeats of an epitope from herpes simplex gD protein when expressed by recombinant adenovirus vectors. Vaccine **11:**1191–1198.