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CD4 T-Cell Responses to Herpes Simplex Virus Type 2 Major Capsid Protein VP5: Comparison with Responses to Tegument and Envelope Glycoproteins

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We used CD4 lymphocyte clones from herpes simplex virus type 2 (HSV-2) lesions or the cervix and molecular libraries of HSV-2 DNA to define HSV-2 major capsid protein VP5 and glycoprotein E (gE) as T-cell antigens. Responses to eight HSV-2 glycoprotein, tegument, nonstructural, or capsid antigens were compared in 19 donors. Recognition of VP5 and tegument VP22 were similar to that of gB2 and gD2, currently under study as vaccines. These prevalence data suggest that HSV capsid and tegument proteins may also be candidate vaccine antigens.

CD4 T-cell responses to herpes simplex virus (HSV) may have several functional roles, including secretion of cytokines with antiviral and immunostimulatory activity, cytotoxic T-lymphocyte (CTL) activity, inhibition of viral replication, and B-cell help (37–39). CD4 reactivity with glycoproteins B (gB) and D is therefore one rationale for their use as HSV vaccines (40, 41). Responses to gB and gD are prevalent in peripheral blood mononuclear cells (PBMC) (31, 41), but few lesion-derived CD4 clones recognize these proteins (13). Recognition of gB or gD occurs in about 40 to 50% of bulk skin-infiltrating lymphocyte cell lines expanded from recurrent genital HSV type 2 (HSV-2) lesions (15).

The spectrum of HSV T-cell antigens is expanding. Tegument protein U L48 (VP16) contains at least eight CD4 T-cell epitopes (14, 16). Tegument proteins U L21 and U L49 (VP22), nonstructural protein U L50 (dUTPase), and gC2 are antigens for HSV-2 lesion-derived CD4 T-cell clones (13, 14). Type-common epitopes in U L21 and U L49 are recognized by potentially pathogenic local CD4 T cells in herpes simplex keratitis (15a). CD4 clones from HSV-1 retinitis are stimulated by tegument proteins U L46 and U L47 (33), and gH and gL of HSV-1 are also antigenic for PBMC (36). We now add the first virion capsid protein and an additional glycoprotein to the set of known HSV T-cell antigens. We have also begun to compare the prevalence of CD4 responses to specific HSV-2 proteins in PBMC.

T-cell clone ESL2.2 (105/well), derived from a recurrent HSV-2 lesion and expanded as previously described (14), is CD4+ (13) and reacts with a 1:100 dilution of UV-treated sonicates of HSV-2 333 (11) (Table 1) but not with HSV-1 E115 (31). Autologous irradiated PBMC (105/well) were used as antigen-presenting cells (APC) in triplicate proliferation assays (12). To make genetic libraries, HSV-2 HG52 DNA have been previously described (16). Libraries were made with plasmids BB40 and HindIII “l,” containing most of HSV-2 HG52 U L45 (provided by A. Davison), by using the same restriction endonucleases and expression vectors described above. Library pUEX1-U L45-SmaI-AluI and derivative clone U L19 were positive (Table 1). U L19 contains an AluI fragment with HSV-2 nucleotides 37,341 to 36,616 encoding amino acids 1078 to 1319 of U L19 (5), which were in frame with β-galactosidase. The U L19 gene encodes the major capsid protein VP5. A Smal fragment (150,831 to 150,596) from within ICP4, in reverse orientation, followed the U L19 fragment in A7AA47. Reactivity with U L19 was confirmed by expressing full-length PCR-amplified U L19 (15a).

An HSV-2 type-specific CD4+ clone (EA.17) from the cervix of a subject with recurrent genital herpes was studied in a similar fashion. Clone derivation, documentation of CTL activity, and mapping of the epitope to the U L19 region of HSV-2 DNA have been previously described (16). Libraries were made with plasmids BB40 and HindIII “l,” containing most of HSV-2 HG52 U L45 (provided by A. Davison), by using the same restriction endonucleases and expression vectors described above. Library pUEXI-U L46-SmaI-AluI and derivative clone U L19 were positive (Table 1). U L19 contains an AluI fragment with HSV-2 nucleotides 143,798 to 144,662, predicted to encode a short region (143,798 to 143,843) of nonsense peptide 5′ to the viral ATG, which is followed in frame by amino acids 1 to 259 of U L8 (gE).

These results show for the first time that the clonal human HSV-specific cellular immune responses include cells reactive with a viral capsid protein. Earlier observations of human PBMC proliferative responses to whole HSV capsid preparations (10, 19) could be due to reactivity with VP5 alone or in combination with other capsid proteins. T-cell reactivity with gE in humans has not been reported; mice develop probable CD4 responses against the HSV-1 homologue (8). Having previously defined several other CD4 antigens using lesion-derived cells, we next compared reactivity with these new antigens to that of previously known proteins.

Full-length open reading frames for U L19, U L21, U L49, U L50, and U L8 were each amplified by PCR from genomic HSV-2 HG52 DNA or derivative plasmids, as described elsewhere (15a), by using a proofreading DNA polymerase. Protein expression required the pcDNA3.1/His series (Invitrogen),
TABLE 1. Specificity and HLA restriction of lesion- and cervix-derived CD4+ T-cell clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>Source</th>
<th>Whole HSV-1 protein</th>
<th>Whole HSV-2 protein</th>
<th>pUEX1-based library</th>
<th>pUEX2-based library</th>
<th>pUEX3-based library</th>
<th>Active library clone</th>
<th>Predicted HSV-2 protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESL2.2</td>
<td>Recurrent day 3 genital HSV-2 lesion</td>
<td>0</td>
<td>66,905</td>
<td>17</td>
<td>192</td>
<td>1,092</td>
<td>94,760</td>
<td>U19 amino acids</td>
</tr>
<tr>
<td>EA.17</td>
<td>Cervix</td>
<td>3,298</td>
<td>53,283</td>
<td>5,860</td>
<td>272</td>
<td>13</td>
<td>15,686</td>
<td>U8 amino acids 1–259</td>
</tr>
</tbody>
</table>

* Results are Δcpm from triplicate [3H]thymidine incorporation assays.

** DNA used in library was from full-length HSV-2 HG52 for ESL2.2 and from HG52 plasmids covering U5 for EA.17.

except that of U149, which used pEGFP-C1 (Clontech). Proteins were tested as sonicates of transiently transfected (Fugene-6; Boehringer Mannheim) Cos-7 cells. Each (except U58, for which APC were unavailable) was highly antigenic for the index T-cell clone at 1:100 (15a) and was used at this dilution. Purified gB2 and gD2 (missing the signal and transmembrane regions) and U148 (provided by R. L. Burke and M. A. Tiggas) were used at 1 μg/ml as described previously (15). This dose was previously found to be optimal for stimulating HSV anti-
gen-specific CD4 T-cell clones.

Responder cell lines were Ficoll-purified PBMC (2 × 10^6 in 24-well plates in T-cell medium [13]) stimulated for 12 to 14 days with whole HSV-2 antigen with growth supported by interleukin 2 (Hemagen) (32 U/ml) from day 5. Donors were 19 HSV-2-infected, HIV-uninfected adults with symptomatic genital herpes caused by HSV-2 (specimens were kindly provided by A. Wald and the clinical staff at the Virology Research Clinic, Seattle, Wash.). Responder cells (10^5/well) were incubated in triplicate 3-day proliferation assays. Positive control was whole HSV-2 333 antigen; negative controls were media, mock virus, and sonicates of Cos-7 cells transfected with an empty vector. Results are expressed in counts per minute (cpm) as Δcpm = mean experimental cpm – mean control cpm.

All donors had a positive response to whole HSV-2 antigen (mean Δcpm, 35,374; range 11,831 to 56,488) (Fig. 1). None had a significant response to mock Vero cell preparation in comparison to media (mean Δcpm, 132; range, 328 to 518) or to an empty vector in comparison to media. We set the Δcpm criterion for a positive response to an HSV-2 antigen at 2,000. Responses to glycoproteins ranged from 58% for gE2 (11 of 19) and 68% for truncated gD2 (13 of 19) to 84% for truncated gB2 (16 of 19). Among the tegument proteins, responses to U149 (14 of 19 [74%]) were more prevalent than responses to VP16 (U148) (6 of 17 [35%]) or U141 (8 of 19 [42%]). For the nonstructural protein U50 (dUTPase), responses were seen in 5 of 19 persons (26%); while for major capsid protein U149, responses were seen in 10 of 12 persons (83%). The diversity of the proliferative response to HSV varied considerably. The median number of antigens recognized was four, with a range of one to eight (Fig. 2). We sought to determine if the diversity of the response as measured in the HSV-2-specific line correlated with the proliferative response in fresh PBMC. PBMC from our panel of donors were stimulated for 5 days with whole HSV-2 and net [3H]thymidine incorporation in comparison to mock Vero antigen measured in triplicate. The Spearman rank correlation coefficient (Instat; Graphpad Software) is 0.48, which has a two-tailed P value of 0.044 for the relationship between the magnitude of the proliferative response among fresh PBMC and the diversity of the response.

Both subjective and objective spectrums of infection and disease severity exist for genital HSV-2 infection. Only a mi-
nority of persons with HSV-2 infection are aware of this (3), and even after patient education and repeated examinations correlated with viral cultures, some individuals do not have recognizable lesions (6, 18, 35). The rate of HSV-2 shedding from the anogenital tract varies up to 10-fold among immuno-
competent women, as measured by serial specimens from multiple anatomic sites and sensitive PCR testing (34). Higher shedding is likely to result in an increased risk of transmission of this medically important virus. Little is known concerning the viral, host immune, or other host correlates of either objective viral shedding rates or subjective disease severity. It is recognized that CD4 responses are required to prime and maintain many CD8 responses (9, 20) as well as antibody responses and are important in genital tract defense against HSV-2 in animals (21). Local and systemic gamma interferon, a product of several lymphocyte subsets, may be correlated with disease severity in humans (4, 32). CD4 responder cells are present early in herpetic lesions and contribute to local CTL activity (15). Different HSV antigens administered in an identical fashion (likely to elicit mainly CD4 responses) may elicit unique cytokine profiles in animals after viral challenge (7). Animal models have also shown the importance of CD8 T cells in ganglionic control of HSV (23, 24, 28, 29), and CD8 responses were correlated with disease severity in human immuno
deficiency virus- and HSV-2-coinfected humans (25). The specificity, breadth, evolution, and magnitude of both CD4 and CD8 responses to HSV are therefore of interest.
Our results indicate that major capsid protein VP5 and tegument protein VP22 frequently elicit CD4 responses in PBMC. Reactivity with VP5 is perhaps not surprising, as this protein is quite abundant in virions (27), is long, might therefore contain many HLA class II binding motifs (27), and elicits a strong antibody response (1). We used HSV-chemiluminescent cell lines as responders for this initial screen, as fresh PBMC were found to proliferate to the unpurified mock antigen preparations made with empty vectors. Possibly, responses to some HSV proteins may have been differentially amplified or lost during this cycle of enrichment of HSV-specific cells. Future studies will use more highly purified antigens suitable for direct interrogation of enrichment of HSV-specific cells. Future studies will use more highly purified antigens suitable for direct interrogation of panels of PBMC in proliferation and cytokine stimulation formats. In this way, the possible correlations between CD4 responses and the severity of infection or disease can be rigorously explored in defined patient populations.

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