Depressed Specific Cell-Mediated Immunity to Herpes Simplex Virus Type 1 in Patients with Recurrent Herpes Labialis

Y. H. THONG,1 MONROE M. VINCENT, SALLY A. HENSEN, DAVID A. FUCCILLO, MAREK ROLA-PLESZCZYNSKI, AND JÖSEPH A. BELLANTI*

Department of Pediatrics and Microbiology, Georgetown University School of Medicine, Washington, D.C. 20007*, Microbiological Associates, Inc., and Infectious Disease Branch, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20014

Received for publication 24 March 1975

Cell-mediated immunity to herpes simplex virus type 1 (HSV-1) was assessed by a lymphocytotoxicity 45Cr-release microassay procedure, using the MA-160 human prostatic adenoma cell line chronically infected with HSV-1 and its parent cell line as control. The specific immune release mean ± standard deviation for nine asymptomatic patients with recurrent HSV-1 infections was 13.7 ± 8.1%, compared to 28.9 ± 8.4% in seven normal seropositive controls (P < 0.01). In four additional patients studied serially, the cell-mediated immunity was significantly increased during the recrudescence of herpetic infection, with a mean specific immune release value of 51.7 ± 27.8%, compared to 8.7 ± 1.5% during the convalescent period 2 to 10 weeks later (P < 0.05). These findings suggest that patients with recurrent HSV-1 infections have vigorous cellular immune responses during the acute phase but a specific impairment of cell-mediated immunity during the quiescent period, which may in part account for their susceptibility to recurrent herpetic infections.

Approximately one-third of the individuals who undergo primary infection with herpes simplex virus (HSV) subsequently develop recurrent herpetic infections (15). The ability of herpesviruses to persist in host cells may in part account for this phenomenon (22, 26, 27). Immunological mechanisms may also play a role in these latent viral infections by modification of the frequency and severity of recurrences or restriction of spread of virus within the infected host. Neutralizing antibodies are effective neither in preventing recurrent HSV infections (6) nor in curtailing cell-to-cell propagation of virus in tissue culture (14). The role of cell-mediated immunity in these infections is supported by the demonstration of protective immunity afforded animals after the adoptive transfer of immune lymphocytes (9, 10) and by the enhancement of infection after immunosuppression by drugs or neonatal thymectomy (19, 23, 36). Further evidence is provided by the clinical observations that patients with compromised cell-mediated immunity are more prone to develop severe and sometimes fatal HSV infections (16, 18, 20, 21). In the present studies, specific cell-mediated immunity to HSV type 1 (HSV-1) was measured in patients with recurrent herpes labialis using a 45Cr-release microassay procedure (31).

MATERIALS AND METHODS

The study population consisted of 13 adults with recurrent herpes labialis who were otherwise free from disease. Nine of the subjects were studied during their asymptomatic phase, and four were studied during recrudescent infection. Healthy individuals without recurrent HSV infections served as controls.

Serum antibody to HSV-1 was measured by a micro-indirect hemagglutination technique developed by Fuccillo et al. (12). A 45Cr-release microassay procedure for measurement of cell-mediated immunity to HSV-1 was performed as previously described (31). Target cells used in these experiments consisted of the MA-160 human prostatic adenoma cell line (11, 25) persistently infected with HSV-1, which was kindly supplied by Andre J. Nahmias. The development of this HSV-1-infected subline will be described elsewhere (M. M. Vincent, D. A. Fuccillo, S. A. Hensen, et al., submitted for publication) but it will be noted here that the virus-host cell relationship is one of noncytolytic, nonproductive infection. Under the conditions of these experiments, indirect fluorescent antibody staining showed that 60% of the MA-72064 cells had diffuse intracytoplasmic fluorescence using fixed

1Present address: Department of Pediatrics, University of Malaya Medical Center, Kuala Lumpur, Malaysia.

* Corresponding author.

INFECTION AND IMMUNITY, July 1975, p. 76-80
Copyright © 1975 American Society for Microbiology
Vol. 12, No. 1
Printed in U.S.A.

76
preparations. The parent MA-160 cell line, employed as control target cells, did not reveal specific fluorescence.

A concentration of $2 \times 10^6$ infected or control target cells were labeled with 100 $\mu$Ci of sodium $^{51}$Cr-chromate, incubated for 1 hour at 37 C, washed thrice, and resuspended in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 U of penicillin per ml, and 100 $\mu$g of streptomycin per ml. Purified suspensions of lymphocytes were prepared from 10 ml of heparinized blood by centrifugation on a Hypaque-Ficoll gradient (34), washed, and resuspended in RPMI 1640 medium.

Test cultures consisted of $5 \times 10^6$ lymphocytes and $5 \times 10^5$ target cells in 0.2 ml of medium, giving an attacker-to-target cell ratio of 100:1. Additional controls consisted of target cells without lymphocytes. All experiments were performed in triplicate. The cultures were incubated at 37 C in Falcon Plastics Microtest II 3040 tissue culture plates placed on a rocker platform in a humidified atmosphere containing 5% carbon dioxide.

The cultures were harvested at two time periods, 18 and 24 h, by an apparatus which separated the medium containing the released $^{51}$Cr from the reacting cells for counting in a Packard Tri-Carb liquid scintillation spectrometer. The percentage of $^{51}$Cr-release for each cell line was calculated as follows: percentage of release = counts per minute of $^{51}$Cr released from target cells and lymphocytes during incubation - counts per minute of $^{51}$Cr released from target cells alone during incubation/total $^{51}$Cr releasable - $^{51}$Cr released at 0 time $\times 100$. The specific immune release was obtained by subtraction of the percentage of release for HSV-1-infected cells from that for control cells. The highest specific immune release for time periods of either 18 or 24 h was used as the index of cell-mediated immunity to HSV-1.

RESULTS

The results of micro-indirect hemagglutination serum antibody titers and specific cell-mediated immunity to HSV-1 for nine asymptomatic patients with recurrent herpes labialis and 14 control subjects are presented in Table 1 and Fig. 1. Seven of the control subjects had no detectable serum antibody to HSV-1 and failed to demonstrate lymphocytotoxicity. In seven additional controls with positive HSV-1 antibody titers (geometric mean titer, 1:43), the mean (± standard deviation) specific immune release was 28.9 ± 8.4% (Table 1). In contrast, the nine subjects with recurrent herpes labialis studied during the quiescent period had significantly higher antibody titers than seropositive controls (geometric mean titer, 1:293); however, in this group, a significantly diminished cell-mediated immunity to HSV-1 was observed, with a mean (± standard deviation) specific immune release value of $13.7 \pm 8.1\%$ ($t = 3.65; P < 0.01$).

### Table 1. Comparison of humoral and cell-mediated immunity to HSV-1 between asymptomatic subjects with recurrent herpes labialis and controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. studied</th>
<th>HSV-1 indirect hemagglutination titer</th>
<th>Mean (± SD)* specific immune release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With recurrent herpes labialis</td>
<td>9</td>
<td>9 positive (1:293)</td>
<td>$13.7 \pm 8.1%$</td>
</tr>
<tr>
<td>Without recurrent herpes labialis</td>
<td>14</td>
<td>7 positive (1:43)</td>
<td>$28.9 \pm 8.4%$</td>
</tr>
</tbody>
</table>

* Geometric mean titers in parentheses.
* SD, Standard deviation.
* Significant difference from seropositive controls ($P < 0.01$).

![Fig. 1. Correlation between humoral and cell-mediated immunity to HSV-1 in asymptomatic subjects with recurrent herpes labialis and controls.](http://iai.asm.org/)

Humoral and cell-mediated immunity to HSV-1 was studied in four additional patients during and after the recrudescence of herpes labialis (Table 2). In each of the four subjects, no significant changes in antibody titers were observed after recurrent infection. In contrast,
the specific immune release was significantly elevated during the acute phase of recrudescent infection with a mean (± standard deviation) value of 51.7 ± 27.8% and fell to a value of 8.7 ± 1.5% during the convalescent period 2 to 10 weeks later (t = 3.09; P < 0.05). In one of these patients (no. 3) an elevated specific immune release was detected 6 days prior to the onset of clinically apparent herpetic lesions.

**DISCUSSION**

The recent isolation of HSV from human trigeminal ganglion by co-cultivation with susceptible cells (2, 3) and supportive studies in experimental animals (24, 32) indicate that the virus persists in neuronal cells in a noncytolytic and nonproductive form. Although a variety of physical, emotional, and hormonal factors have been associated with the recrudescence of herpes labialis (26, 27, 33), the precise mechanism of activation of HSV from a latent to a productive infection remains unclear. Cook and Stevens (8) and Mergan (17) have proposed that an intra-axonal propagation of HSV during recrudescence may account for the occurrence of lesions at the original sites.

Host resistance to viral infections depend upon the complex interplay of nonspecific and specific immunological events such as phagocytosis, interferon (1), cell-mediated immunity (13), and secretory (4) and circulating antibody responses. Based on data derived from animal studies (9, 10, 19, 23, 36), as well as clinical observations (16, 18, 20, 21), cell-mediated immunity is believed to be of primary importance in host defense against viruses such as HSV (17). In the present studies, a specific reduction of cell-mediated immunity to HSV-1 has been demonstrated in patients with recurrent herpes labialis during the quiescent period between recurrences. This may in part account for the propensity of these individuals to develop recurrent herpetic infections, presumably due to a decreased capacity of lymphocyte-mediated immune responses to control the latency of HSV infection. However, the ability of these individuals to respond with an enhanced specific cell-mediated immunity was demonstrated by the increased levels of specific immune release in all four patients studied during recrudescent herpetic infection. The biological significance of this is not clear, but it is reasonable to assume that the enhanced cell-mediated immunity may prevent the dissemination of virus to other tissues. This interpretation is supported by the clinical observation that individuals with recurrent herpes labialis who are otherwise free of underlying disease tend to have lesions that are self-limiting, whereas patients with impaired cell-mediated immunity such as primary immunodeficiencies or lymphomas, or who are on immunosuppressive therapy, tend to have HSV infections which are more severe and sometimes life-threatening (16, 18, 20, 21).

In contrast to the results of the present studies are the findings of Wilton et al. (35) and Russell (28) who showed a normal lymphoproliferative response to HSV-1 antigen in patients with recurrent herpes labialis. In addition, Russell et al. (29) found evidence of lymphocyte-mediated cytotoxicity in patients with recurrent labial herpes infections; whether the cytotoxicity in these patients was decreased compared to that of normal HSV-1 immune individuals without recurrence was not studied. When such a control HSV-immune population was included, Wilton et al. (35) showed a depression of macrophage migration inhibitory factor production and a decreased lymphocytotoxicity, as
measured by release of \(^{31}\text{Cr}\) from virus-free chick erythrocytes as an indicator system. The discrepancies between lymphoproliferative responses to HSV-1 and lymphocyte-mediated cytotoxicity to HSV-infected cells may be explained by differences in populations of lymphocytes involved in these two assays.

In the case of infection with "slow" or latent viruses, cell-mediated immune responses may contribute paradoxically to the pathogenesis of disease. This is exemplified by the enhanced mortality seen in adult mice after infection with lymphochytic choriomeningitis virus, as contrasted with lack of disease seen after infection of neonatally thymectomized or immunosuppressed adult mice (7). Further definition of immunological events associated with infections by HSV may provide a better understanding of the pathogenesis of slow virus disease (5) and may lead to better means for their clinical management.

ACKNOWLEDGMENTS

This work was supported in part by the U.S. Army Research and Development Command under a research contract (DA-49-193-MD-2633) and by Microbiological Associates, Inc. M. R.P. is the recipient of a Medical Research Council of Canada fellowship training grant.

We are indebted to Diane Hargrave, Laura Myers, Mamie Barr, Anne Hooke, Judith Walcher, Jane Hurd, and Val Abbassi for assistance.

ADDEDUM IN PROOF

Since submission of this paper for publication, there has appeared a recent study by Steele et al. (J. Infect. Dis. 131:528, 1975) which demonstrates enhanced lymphocyte blastogenesis but a decreased specific lymphocytotoxicity in patients with recurring herpes labialis.

LITERATURE CITED