INFECTION AND IMMUNITY, Nov. 1982, p. 795–797
0019-9567/82/110795-03$02.00/0

Vol. 38, No. 2

Herpes Simplex Virus Infection of Human T-Cell Subpopulations

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Received 5 April 1982/Accepted 14 July 1982

The ability of herpes simplex virus type 1 to productively infect human T-cell subpopulations was examined. Unstimulated helper/inducer (T4+) and cytotoxic/suppressor (T8+) lymphocytes limited herpes simplex virus replication as effectively as unseparated peripheral blood T cells (T3+). Phytohemagglutinin stimulation before infection resulted in equivalently productive herpes simplex virus infections in the three cell fractions.

The interaction of herpes simplex virus (HSV) with human lymphocytes is complex. Cellular immune mechanisms are important in controlling herpetic infection (1, 12), and there is evidence that HSV in turn can functionally affect lymphocytes (9, 10). In addition, these cells may be sites of growth and carriage of the virus in vivo (3, 8). Previous studies have demonstrated that HSV replicates preferentially in stimulated T cells (6, 11, 14). We have utilized monoclonal antibodies directed at specific T-cell surface antigens to determine whether there is a difference in the ability of T-cell subpopulations to support HSV replication either before or after mitogen stimulation. The T-cell surface antigens OKT3, OKT4, and OKT8 will be used to define peripheral blood T cells (T3+), helper/inducer (T4+), and cytotoxic/suppressor (T8+) populations, respectively (13).

Venous blood was obtained from five healthy adult volunteers. Three were seropositive and two seronegative for HSV as determined by enzyme-linked immunosorbent assay (courtesy of W. P. Corbett, Massachusetts General Hospital). The blood was centrifuged on Ficoll-Hypaque gradients (2), and the mononuclear cell fraction was collected and washed with TC 199 medium. After lysis of the red blood cells with NH_4Cl buffer, the mononuclear cells were washed again, and T cell enrichment was performed as follows. Each suspension of 40×10^6 mononuclear cells (in 12 ml) was mixed with 18 ml of a 0.5% solution of neuraminidase-treated sheep erythrocytes and 6 ml of 5% human serum albumin. The mixture was pelleted and incubated for 2 h at 4°C. After gentle suspension of the pellet and centrifugation on a Ficoll-Hypaque gradient, the T-cell-enriched fraction was collected and treated with NH_4Cl buffer for 5 to 10 min at 4°C to remove red blood cells.

Portions of the T-cell-enriched population (>95% T3+) were then subjected to complement-dependent antibody lysis to isolate T4+- and T8+-enriched lymphocyte populations, as follows. Each suspension of 20×10^6 T-cell-enriched cells was incubated with 5 μg of OKT8 or OKT4 antibodies in mouse ascitic fluid (kindly provided by Gideon Goldstein, Ortho Pharmaceutical Corp., Raritan, N.J.), respectively, in a final volume of 0.5 ml for 30 min on ice. A 2-ml portion of rabbit complement (1:4 dilution; Pel Freez, Rogers, Ark.) was added, and the mixture was incubated for 1 h at 37°C. The cells were washed and viability was determined by trypan blue dye exclusion. The lysis procedures resulted in >95% depletion of OKT8+ and OKT4+ cells, respectively, as judged by indirect immunofluorescence staining with the respective monoclonal antibody reagents.

The T3+, T4+, and T8+ cells were each separated into two fractions. One was placed in culture for 3 days in TC 199 medium with 250 U of penicillin per ml, 250 μg of streptomycin per ml, 2 mM L-glutamine, and 10% AB+ human serum in the presence of phytohemagglutinin (50 μg/ml) (Difco Laboratories, Detroit, Mich.). The other was infected with HSV type 1, strain KOS (kindly provided by P. Schaffer and D. Coen, Sidney Farber Cancer Institute). Between 10×10^6 and 20×10^6 cells were pelleted and suspended in 1 ml of virus preparation diluted to achieve a multiplicity of infection of 1 PFU per cell. An equal number of control cells was mock infected with virus-free medium. After a 1-h incubation on a shaker apparatus at 37°C, the

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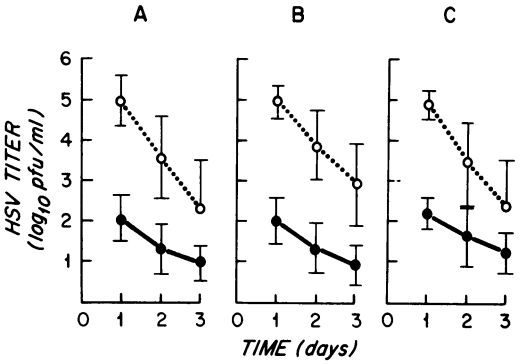

FIG. 1. HSV titer (log_{10} PFU/ml) in unstimulated T3+, T4+, and T8+ cell cultures (A, B, and C, respectively) on days 1, 2, and 3 after infection with HSV type 1 (●). The thermal inactivation curve of cell-free HSV is also shown (○). Mean of five experiments ± standard error of the mean (vertical lines).

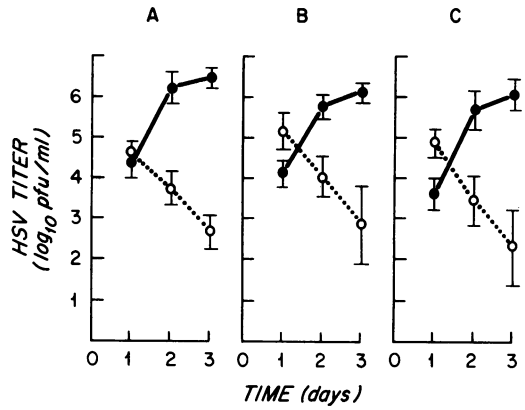

FIG. 2. HSV titer (log_{10} PFU/ml) in phytohemagglutinin-stimulated T3+, T4+, T8+ cell cultures (A, B, and C, respectively) on days 1, 2, and 3 following infection with HSV type 1 (●). The thermal inactivation curve of cell free HSV is also shown (○). Mean of 5 experiments ± standard error of the mean (vertical lines).

cells were washed three times with TC 199 medium and then placed in culture at 2.5×10^5 cells/ml of TC 199 medium with 10% AB+ human serum at 37°C in a 95% air–5% CO_2 atmosphere. The virus inoculum for each of the three cell fractions was incubated in parallel with the cell cultures to determine the thermal inactivation curve of cell-free HSV.

On days 1, 2, and 3 after infection, an aliquot of cell suspension was subjected to sonication in a model DF-101 Raytheon sonic oscillator, diluted serially (10-fold) in Eagle minimal essential medium and inoculated, in duplicate 0.1-ml amounts, onto Vero cell monolayers in 35-mm 6-well plates (Costar, Cambridge, Mass.). After a 1-h adsorption, the plates were overlaid with 0.5% agarose (Sigma Chemical Co., St. Louis, Mo.) in minimal essential medium supplemented with penicillin, streptomycin, L-glutamine, and 5% fetal calf serum. Plates were incubated for 4 days, stained with neutral red, and reincubated. Plaques were counted on day 5.

Cells which had been incubated with phytohemagglutinin for 3 days were harvested, washed twice with TC 199 medium, and then infected, cultured, and assayed as described.

The results demonstrated that HSV was unable to replicate in unstimulated peripheral blood T cells (T3+), confirming previous reports (6, 11, 14). When separated into the major T-cell subsets, the results were similar. Virus titer progressively declined from means (± standard error of the mean) of 2.09 (±0.54), 2.03 (±0.56), and 2.20 (±0.36) log_{10} PFU/ml to 0.98 (±0.40), 0.94 (±0.47), and 1.24 (±0.48) log_{10} PFU/ml on days 1 to 3 for the T3+, T4+, and T8+ cell populations, respectively (Fig. 1). The differences in virus titer observed on day 1 between unstimulated T-cell cultures and the thermal inactivation curve of cell-free HSV may reflect limitation of the available number of infectious virions by early events in virus-host cell interaction or removal of unadsorbed virus during initial washing.

In contrast, T3+, T4+, and T8+ cells which had been stimulated for 3 days with PHA before infection all actively replicated HSV to an equivalent degree (Fig. 2). Mean titer (± standard error of the mean) increased from 4.80 (±0.37), 4.15 (±0.31), and 3.63 (±0.36) log_{10} PFU/ml to 6.50 (±0.22), 6.17 (±0.20), and 6.11 (±0.35) log_{10} PFU/ml on days 1 to 3 for T3+, T4+, and T8+ cell populations, respectively. Mean virus titer of stimulated T3+, T4+, and T8+ cells was significantly higher than the respective unstimulated fractions on each of the 3 days after infection ($P < 0.05$, 0.01, and 0.01 for days 1, 2, and 3, respectively for each cell fraction; paired t test). No differences were observed when results obtained from HSV seropositive and seronegative individuals were compared.

These findings are similar to results reported for measles virus, which has been found to replicate in stimulated T mu and T gamma lymphocyte subsets (5). Such data have not been reported previously for HSV and extend previously published work on the HSV infectibility of T cells in two ways: (i) neither of the two major T-cell subsets now defined is permissive for HSV replication in the absence of stimulation; (ii) after mitogen stimulation, virus replicates to similar degrees in both helper (T4+) and suppressor (T8+) lymphocytes.

With regard to replication, therefore, no differences can be detected between the interaction

of HSV with T4+- and T8+-enriched lymphocyte populations. HSV–T-cell interactions are complex, however, and the ability of virus to persist or replicate or both in subsets of these cells is but one aspect of these interactions. Host resistance mediated by T-cell subpopulations is another major consideration and, to date, this area has not been fully defined. HSV-specific cytotoxic T cells have been described in both humans and animals (7, 15), and in adoptive transfer studies in mice, both helper and cytotoxic cells have been shown to confer protection (4). A possible implication of the findings reported herein is that all T cells are potential targets of infection, carriage, or persistence of HSV in vivo, irrespective of differences in immune responses mediated by different T-cell subpopulations.

This work was supported by Public Health Service grant CA 12464 from the National Cancer Institute, National Research Service Award F32 AI 06227, and the Victor DeSola Memorial Fund.

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