Human Lymphoblastoid Cells as Hosts for Parvoviruses H-1 and Rat Virus

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A human T-cell line (Molt-4) was shown by viral hemagglutination and infectivity assays to support the replication of rat virus (RV) and H-1 virus. In addition, H-1 virus, but not RV, multiplied in two human B-cell lines, AV-1 and NC-37. The ability to bind radioactively labeled RV was demonstrated for each of the cell lines, but viral adsorption occurred to a greater degree with Molt-4 cells than with either AV-1 or NC-37 cells. After challenge with RV, virus-specific antigens were detected in cells of the B-cell lines by the indirect immunofluorescence technique. Infection of AV-1 or NC-37 cells by RV apparently results in an abortive cycle of virus replication. Differences among the three cell lines that might influence infection with H-1 virus or RV are discussed.

The nondefective parvoviruses, which include H-1 virus and rat virus (RV), infect a relatively restricted range of hosts. RV multiplies in either rat or hamster cells, whereas H-1 virus additionally infects human and simian cells (24). Parvovirus replication is dependent on the physiological state of the host cell and is most efficient when infection occurs during the S phase (19, 21, 23). Therefore, continuous lines of human lymphoblastoid cells are potentially good hosts for parvoviruses because some cells engaged in DNA synthesis are always present in these cultures.

The great majority of human lymphoblastoid cell lines of B-cell origin carry genetic material of the Epstein-Barr virus (EBV). Several herpesviruses, including EBV, can function as partial helpers for the replication of defective parvoviruses (the adeno-associated viruses) by allowing production of viral DNA and antigens, but not infectious viral progeny (3, 5, 6). The human B-cell lines AV-1 and NC-37 are, respectively, producer and nonproducer cultures for infectious EBV, and each elaborates EBV-specific antigen (9, 15). The human T-cell line Molt-4 shows no evidence of endogenous EBV genetic material, either by DNA hybridization or by immunofluorescence tests for viral antigens (20). In this study, these three cell lines were examined for their ability to support the replication of H-1 virus and RV.

MATERIALS AND METHODS

Cells. Cell lines AV-1 and NC-37, originally established from peripheral blood samples of normal, healthy adults, were obtained from Paul Gerber, Bureau of Biologics, National Institutes of Health, Bethesda, Md. The Molt-4 cell line, one of four derived from the peripheral blood of an acute lymphoblastic leukemia patient, was generously supplied by Patricia Reichelderfer, Bureau of Biologics, National Institutes of Health. The cultures were grown as suspensions in RPMI medium 1640 (Grand Island Biological Co., Grand Island, N.Y.), supplemented with 10% fetal bovine serum (Flow Laboratories, Inc., Rockville, Md.), 50 U of penicillin G, and 50 μg of streptomycin, and were incubated at 37°C in plastic flasks placed in an upright position. Every 3 to 4 days, cells from one half of each culture were harvested by centrifugation (165 × g for 8 min) and resuspended in a volume of fresh medium equal to that used previously.

Rat embryo cells for propagation and assay of the viruses were obtained as primary cultures from Microbiological Associates, Inc., Bethesda, Md. Monolayer cultures were grown in Eagle minimum essential medium prepared with Earle salts and 10% fetal bovine serum. Cells were subcultivated by trypsinization and used for study between passage level 5 and 20.

Viruses. H-1 virus and RV were obtained from the American Type Culture Collection, Rockville, Md. Stocks of virus were prepared in rat embryo cells, filter sterilized, and stored at -80°C. Virus of high purity for use in adsorption experiments was prepared by the method of Robinson and Hetrick (16), which included centrifugation in sucrose and CsCl gradients. RV was radioactively labeled with [methyl-3H]thymidine (1H-RV) by the method of Salzman and Jori (17).

Viral hemagglutinin was detected by a microtiter hemagglutination (HA) test, using equal volumes (0.05 ml) of the virus sample and of a 0.5% suspension of guinea pig erythrocytes. A micro-hemagglutination inhibition test for serological identification of the virus employed equal volumes (0.025 ml) of specific antiserum and virus (8 HA units) and 0.05 ml of guinea pig erythrocytes. Infectivity titerations of H-1 virus and RV were performed on monolayers of rat embryo cells in microtiter plates. The cultures were observed daily...
for 10 days for development of cytopathic effects, and the titers are expressed as the number of 50% tissue culture infective doses (TCID$_{50}$) per 0.1 ml of virus sample.

**Infection of B- and T-cell lines.** Viable cell counts of 3- to 4-day-old cultures of AV-1, Molt-4, and NC-37 cells were determined by the trypan blue dye exclusion method. From 5 x 10$^6$ to 1 x 10$^7$ viable cells were infected with virus at multiplicity of infection ranging from 1.0 to 10 TCID$_{50}$ per cell. After adsorption for 1 h at 37°C, the cells were washed and resuspended to 1 x 10$^6$ cells per ml. Cells and fluids of both infected and control cultures were sampled periodically for viral assay.

**Assay of viral receptors.** AV-1, Molt-4, and NC-37 cultures were examined for the presence of RV receptors by incubating 2 x 10$^5$ cells of each cell line with 0.25 ml of 3H-RV (40 HA units per 0.05 ml) for 1 h at 37°C. After adsorption, the cells were washed three times in phosphate-buffered saline and resuspended in 0.25 ml of phosphate-buffered saline. Standard volumes (0.23 ml) of each sample were placed in scintillation vials and shaken with 1.0 ml of NCS (Amersham/Searle, Arlington Heights, Ill.). On the next day, 10 ml of a toluene-based scintillation solution was added to each vial, and the samples were counted in a Nuclear-Chicago model Unilux II liquid scintillation counter. Control samples, including cells without 3H-RV, phosphate-buffered saline alone, and 3H-RV alone, were handled as described above.

**Immunofluorescence.** Antisera to purified preparations of H-1 virus and RV were prepared in rabbits by administering at weekly intervals three 1.0-ml intravenous inoculations of virus with an HA titer of 512/0.05 ml. Both serum lots had micro-hemagglutination inhibition titers of 640 and did not cross-react with the heterologous virus. Viral antigens in infected cells were detected by the indirect immunofluorescence staining technique.

Suspensions of washed cells were dropped onto ringed glass slides (Virgo Reagents, Electro-Nucleonics Laboratories, Inc., Bethesda, Md.), allowed to air dry, and fixed in acetone. The antisera to RV and H-1 virus and a fluorescein-conjugated immunoglobulin G fraction of goat anti-rabbit serum (Cappel Laboratories, Inc., Downington, Pa.) were used at a concentration of 1:16. The conjugated serum was diluted in a 0.01% solution of Evans blue dye used as a counterstain. From 800 to 1,200 cells were counted for each sample and scored for the appearance of immunofluorescence.

**RESULTS**

**Infection with H-1 virus.** All three lymphoblastoid cell lines produced infectious viral progeny after incubation with H-1 virus. Extracellular viral hemagglutinin was detected as early as 20 h postinfection (Fig. 1). We previously reported that infection of the cultures with a lower multiplicity of infection (0.001) than that employed here resulted in the appearance of cell-associated, but not extracellular, viral HA by 24 h postinfection (4). Infectivity titers (TCID$_{50}$/0.1 ml) for samples of extracellular virus taken at 0 and 72 h were as follows: AV-1, 10$^{2.6}$ and 10$^{6.1}$; Molt-4, 10$^{2.4}$ and 10$^{6.0}$; NC-37, 10$^{3.5}$ and 10$^{5.7}$. The infectivity detected at 0 h probably represents unadsorbed virus or virus that eluted from the cells after adsorption.

**Infection with RV.** The three cell lines were infected with an RV multiplicity of 3.0. After adsorption, the cells were treated with a dilution of anti-RV serum sufficient to neutralize five times the quantity of virus inoculum used to eliminate residual virus in the culture medium. Viral HA and infectivity titers of both cells and culture fluids are shown in Fig. 2. The absence of virus in the culture medium at 0 h indicated that antiserum treatment effectively neutralized the unadsorbed virus. RV productively infected the Molt-4 cell culture, but viral hemagglutinin

![Fig. 1. Extracellular viral hemagglutinin in cultures of AV-1 (●), Molt-4 (▲), and NC-37 (■) cells infected with H-1 virus at a multiplicity of 1.0.](http://jvi.asm.org/)

![Fig. 2. Hemagglutination and infectivity titers of cell-associated (open symbols) and extracellular (closed symbols) virus in cultures of AV-1 (○, ●), Molt-4 (▲, ▼), and NC-37 (■, □) cells infected with RV at a multiplicity of 3.0.](http://jvi.asm.org/)
and infectivity were detected in neither AV-1 nor NC-37 cells throughout the experiment.

**Receptors for RV on lymphoblastoid cells.** Due to the differential response of the B-cell cultures to infection by H-1 virus and RV, AV-1 and NC-37 cells were examined for the ability to adsorb \(^3\)H-RV. In preliminary adsorption experiments with Molt-4 cells and \(^3\)H-RV, up to 55% of the total sample radioactivity was cell associated after 1 h at 37°C. Results of an adsorption experiment in which all three lymphoblastoid cell cultures were incubated with \(^3\)H-RV are shown in Table 1. Radioactivity (disintegrations per minute) found in the culture medium after the adsorption period, in subsequent washings, and in the cell samples is expressed as a percentage of the total counts recovered from a given culture. The data show that after adsorption, the virus remained associated with cells of each of the cultures. However, the percentage of total sample disintegrations per minute recovered with the cells was three- to fourfold greater in the Molt-4 culture than in the AV-1 or NC-37 cultures. Other experiments gave similar results, suggesting that RV binds more efficiently to Molt-4 cells than it does to AV-1 or NC-37 cells.

**H-1 virus and RV immunofluorescence.** Cultures of AV-1, Molt-4, and NC-37 cells challenged with H-1 virus were assayed for viral antigen by the indirect fluorescent-antibody (FA) technique. The numbers of fluorescent cells observed in the cultures after adsorption with H-1 virus are expressed as percentages of the total number of cells counted per sample (Fig. 3). Virus-specific immunofluorescence was observed in Molt-4 cells as early as 3 h postinfection and involved over 10% of the cells in all three cultures by 10 h. On the basis of both FA data and titers of extracellular viral HA present (Fig. 1), an initial round of virus replication was completed in each of the cell cultures by 20 to 24 h postinfection.

A progression of FA staining patterns was noted in H-1 virus-infected lymphoblastoid cells. Initially, fluorescence occurred in small areas, or patches, in the cell cytoplasm, nucleus, or both. At 10 h postinfection, brilliant immunofluorescence stained entire cells. From 20 to 72 h postinfection, a variety of these staining patterns was observed, although most fluorescence involved nuclei or whole cells.

In a similar experiment, AV-1, Molt-4, and NC-37 cell lines challenged with RV at a multiplicity of 10.0 were assayed for virus-specific immunofluorescence. Although the multiplicity of RV was 10-fold that of H-1 virus used in the previous experiment, the numbers of fluorescing cells observed were at least 10 times less in the RV-infected cultures (Fig. 4). Up to 12 h postinfection, fewer than 1% of the cells in any of the three cultures were FA positive. The greatest percentage of fluorescing cells was noted at 24 h in the AV-1 and Molt-4 cultures and at 12 h postinfection in the NC-37 cell culture. At 24 h, 3.6% of the Molt-4 cells counted were immunofluorescent for RV-specific antigens. This percentage was twice that observed at any time in the RV-infected B-cell cultures and, generally,
FA-positive Molt-4 cells stained more intensely than did cells of the AV-1 and NC-37 cultures.

**DISCUSSION**

Many of the nondefective paroviruses appear to multiply most efficiently in rapidly growing cell lines (10, 12, 14, 21, 23). In the present study, H-1 virus was shown to replicate in three human lymphocyte lines, whereas RV was shown to multiply in a human lymphoblastoid T-cell line and in Molt-4, but not in either of two human B-cell lines also examined. We reported elsewhere that infection of Molt-4 cells with RV can result in the establishment of persistently infected cell cultures (Bass and Hetrick, J. Infect. Dis., in press).

Titration of viral HA and infectivity and immunofluorescence data of H-1 virus infection in AV-1, Molt-4, and NC-37 cells and of RV infection in Molt-4 cells indicate that the time course of virus replication in these cells is similar to that reported for other host cells. Studies of H-1 virus with human cells NB and SMH (2) and of RV with rat and hamster cells (8, 18, 22) indicated that the latent period of virus replication was 10 to 16 h and was followed by viral synthesis up to 24 to 30 h postinfection. The percentage of cells competent for viral replication during an initial infection cycle, as determined by immunofluorescent staining, was at least 10-fold greater with H-1 virus than with RV.

RV failed to productively infect B-cell lines AV-1 and NC-37, despite challenge with relatively high multiplicities of virus. Adsorption studies with radioactively labeled RV did indicate that the B-cell lines bound RV, although to a lesser extent than did Molt-4 cells. Receptors for RV on AV-1 and NC-37 cells may be fewer in number or less efficient in binding virus. Results of the immunofluorescence assays showed that few of the B cells synthesized RV antigen(s) in what appeared to be an abortive replicative cycle with the virus.

Endogenous EBV in AV-1 and NC-37 cells could possibly interfere with RV replication through induction of interferon, because both RV and H-1 virus have been reported to be highly sensitive to inhibition by interferon (7, 13). The NC-37 cell line is negative for spontaneous production of interferon (1); however, similar information is not available for the AV-1 and Molt-4 cell lines. The fact that H-1 virus replicated to high titers in these cells suggests that under the conditions of the present study AV-1 and Molt-4 cells did not produce inhibitory levels of interferon.

Several studies have suggested that human lymphoblastoid B-cell lines vary widely in their susceptibility to viral infection. Reports of Zajac et al. (25) and Henle et al. (11) show that these differences are independent of the degree to which the cell lines express endogenous EBV infection, their ability to produce autogenous interferon, and the extent to which the cells adsorb challenge virus. The responses of B-cell lines AV-1 and NC-37 to infection with H-1 virus and RV may indicate that differences in metabolic functions relating to viral syntheses exist between B- and T-cells.

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**LITERATURE CITED**