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NOTES

Nonpermissivity of Human Peripheral Blood Lymphocytes to Adenovirus Type 2 Infection

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The capacity of freshly explanted human peripheral blood lymphocytes (PBL) to support the replication of human adenovirus type 2 (Ad2) was investigated. Unlike other types of human cells, PBL were found to be highly nonpermissive. Ad2 adsorbed 30 to 40% of both T and non-T cells. Virus uncoating was very slow and inefficient, resulting in a 40-fold reduction compared with HEp-2 cells. On a population basis, viral DNA synthesis was reduced 460-fold and infectious virus production was reduced 10^6-fold. Only 0.35% of PBL produced infectious centers, yielding 0.8 PFU per infected cell. Phytohemagglutinin stimulation increased DNA synthesis 23-fold, infectious centers 11-fold, and virus yield 14-fold. We conclude that resting human PBL are highly nonpermissive to Ad2 infection and that phytohemagglutinin can only marginally lift this nonpermissiveness.

Adenovirus infection in most cell cultures of human origin leads to lytic infection, resulting in production of infectious virus. In nonhuman cells, replication is generally incomplete, resulting in restricted virus growth, or infectious virus is not produced at all. Virus growth restriction generally affects the efficiency of viral DNA replication and late gene expression (11, 17, 18, 24, 38, 39). Semipermissive and nonpermissive infections can potentially result in transformation of host cells (16).

Adenoviruses have frequently been reported in human lymphoid tissue (1, 12, 22, 32, 40). We have reported the presence of adenovirus DNA sequences in peripheral blood lymphocytes (PBL) (21). Rescue of infectious viruses has required the use of indicator cells, and this method has not always been successful (1, 21, 25). It appears that, though PBL frequently carry adenoviruses, they are not permissive for virus replication. There were preliminary data that in vitro adenovirus can multiply in phytohemagglutinin (PHA)-stimulated lymphoid cells and at very low yield in long-term lymphocyte cultures from tonsils (2, 26, 36). The availability of new, specific methods has made it possible to study the interaction of these viruses and PBL in more detail. In this study, we examined the nature of the block to replication of adenovirus type 2 (Ad2) in resting and stimulated PBL.

Lymphocytes were purified from heparinized venous blood of healthy donors by Ficoll-Hypaque centrifugation (4) and depletion of adherent cells. The attachment assays were done with PBL from adults. In other experiments, PBL from adult donors and cord blood were used, and the results were discussed together because cells of both origins gave the same results. Lymphocyte subpopulations of adult and cord blood apparently differ only in that the latter contains fewer T-suppressor phenotypes (29). Part of the cell suspension was cultured in RPMI 1640 medium (GIBCO Laboratories) supplemented with 20% autologous serum for 24 h at 37°C in 5% CO_2 with or without 0.5 μg of PHA (Phytohemagglutinin P; Difco Laboratories). T and non-T lymphocytes were separated from the rest of the cells by the sheep erythrocyte rosette method (23) and Ficoll-Hypaque gradient centrifugation.

Fluorescein isothiocyanate-labeled Ad2 particles enabled us to visualize attachment of the virus on the surface of PBL and HEp-2 cells. High multiplicity (3×10^5 virions per cell) and a 3-h incubation period were used to attain saturation of cellular receptors, and a temperature of 0°C was used to minimize capping (34, 35). After incubation, the cells were washed and fixed in buffered 0.015% glutaraldehyde, and smears were made on microscope slides. The proportion of mononuclear cells which contained virus receptors was 35.5% when they were studied immediately after separation from the blood. When the attachment experiment was done on cells cultured for 24 h, 26.5% were positive; PHA treatment increased the proportion to 33%. Study of T and non-T cells revealed that both cell populations can bind fluorescein isothiocyanate-labeled Ad2 virions (39 and 25%, respectively).

Measuring the attachment of radioactively labeled virions enabled us to estimate the number of virion-binding sites. The average number of Ad2 receptors was 780 per cell in unstimulated lymphocytes and increased to 980 per cell after PHA pretreatment for 24 h. In comparison, HEp-2 cells had 7,000 receptors per cell. Since these are averages in a population of PBL in which approximately 70% of the cells exhibited no fluorescence in the foregoing experiments, these measurements must be viewed as underestimates. If allowance for the difference in cell size is made (the surface of a lymphocyte is about one-third that of a HEp-2 cell), the receptor density may be comparable between HEp-2 cells and the population of PBL which expresses receptors for the virus. We conclude that adenovirus adsorption was not impaired in PBL.

Virus uncoating was studied by measuring the amount of

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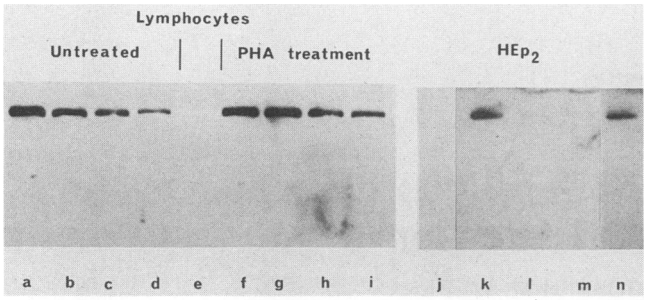

FIG. 1. Virus uncoating. Micrococcal nuclease digestion of HEp-2 and lymphoid cell DNAs. The cells were infected with 10 PFU of Ad2 per cell, and the cell lysates were digested with 3,000 U of micrococcal nuclease before infection (lanes e and j) and 2 h (lanes a, f, and k), 4 h (lanes b, g, and l), 8 h (lanes c, h, and m) and 16 h (lanes d, i, and n) p.i. The nitrocellulose blot from the 1.4% gel was hybridized with cloned adenovirus-specific probe representing the whole viral genome. The exposure time for HEp-2 samples was 10 times shorter than that for the lymphoid cells.

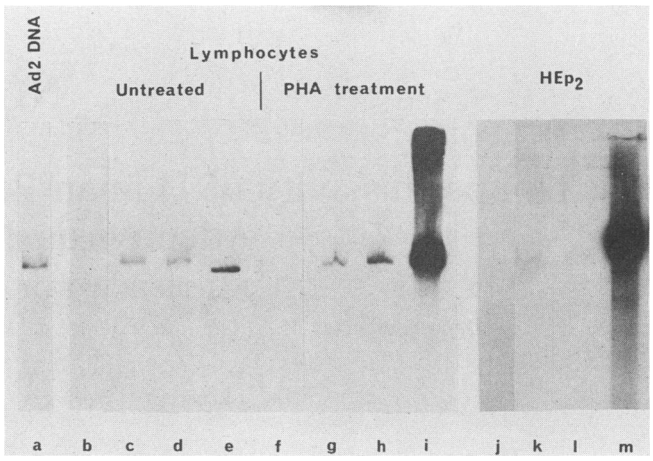

FIG. 2. Synthesis of high-molecular-weight DNA detected by Southern hybridization. Serial samples were taken from control (lanes b, f, and j) and infected cultures at 3 h (lanes c, g, and k), 6 h (lanes d, h, and l), and 24 h (lanes e, i, and m) p.i. The exposure was 10 times shorter than that of lymphoid cells in the case of HEp-2 cell DNA autoradiography.

viral DNA protected within capsids after micrococcal nuclease digestion of lysed cells (8). The band in the high-molecular-weight region represents the virion DNA protected against digestion by the capsid (Fig. 1). At 2 h postinfection (p.i.), the intensity of this band reached a maximal level in HEp-2 cells (Fig. 1, lane k) and then rapidly decreased, showing almost complete decapsidation (Fig. 1, lane l). Newly assembled virions appeared at 16 h (Fig. 1, lane n). The lymphoid cells contained comparable amounts of encapsidated DNA at 2 h p.i. (Fig. 1, lanes a and f), but unlike in HEp-2 cells, the DNA subsequently did not become accessible to micrococcal nuclease, indicating that the uncoating process was very slow and ineffective. PHA stimulation of lymphocytes had no detectable effect on uncoating (Fig. 1, lanes f to i).

Similar results were obtained when isolated nuclei were analyzed (data not shown). This suggests that these viruses were probably attached to the exterior surface of the nucleus and that, in the case of measurements of whole-cell lysates, the observed protected DNA was not cell surface virus. These results clearly demonstrate that PBL have a major block in both the rate and efficiency of virus uncoating. In comparison with HEp-2 cells, uncoating in PBL was down approximately 40-fold.

To determine whether the small amount of virus uncoated in PBL could establish an infection, we compared the efficiency of viral DNA synthesis in resting or stimulated PBL with that in HEp-2 cells. To obtain quantitative results, the method of Eckhardt (10), as modified by Gardella et al. (19), was used. The method yields semiquantitative results because it does not require preparation and manipulation of the cells. Whole cells were washed once in phosphate-buffered saline and loaded onto an agarose gel, which consisted of a lysing part with 2% sodium dodecyl sulfate and pronase (1 mg/ml) and a separating part. After electrophoresis and partial depurination with 0.25 M HCl, the DNA was denatured, neutralized, and transferred to a nitrocellulose membrane. Ad2 DNA was detected by hybridization. There was only a threefold increase in Ad2 DNA in nonstimulated mononuclear cells after 24 h, whereas in PHA-stimulated lymphocytes the increase was about 20-fold (Fig. 2). The same number of HEp-2 cells (10^6 per well) produced 23 times more DNA than did PHA-stimulated lymphocytes (Fig. 2, lane m). Therefore, DNA synthesis was approximately 460-fold lower in resting PBL as compared with permissive HEp-2 cells.

Capsid proteins could not be detected in PBL by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [^{35}S] methionine-labeled cells, even at 5 days p.i.

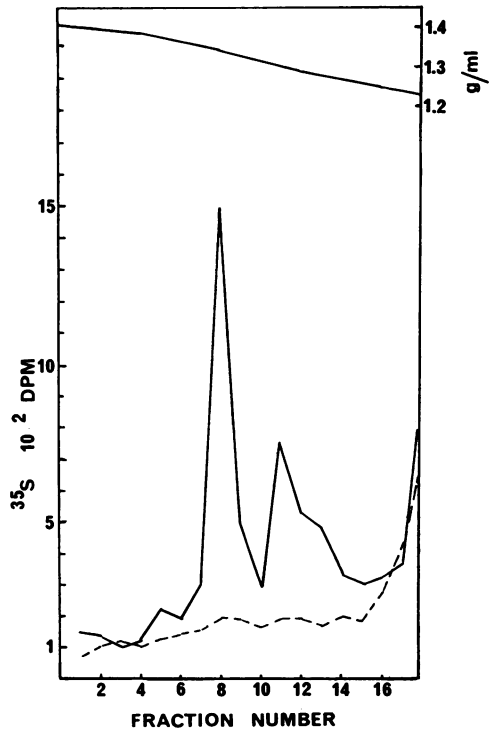

FIG. 3. Synthesis of new virions. PBL with (———) or without (– – –) PHA pretreatment were infected with 10 PFU of Ad2 per cell and at 20 h p.i. they were labeled with [^{35}S]methionine. The cells were frozen and thawed three times at 120 h p.i., the cell debris was removed, and the supernatant was layered on a preformed CsCl gradient and centrifuged at 200,000 × *g* for 1 h. Radioactivity was measured in 10-drop fractions collected by puncturing the tubes at the bottom.

Virus assembly was measured by CsCl density gradient centrifugation of metabolically labeled new virions from infected PBL with or without PHA treatment. Preliminary experiments showed that virus assembly in PBL could only be detected after PHA stimulation and at 5 days p.i. Figure 3 shows a typical gradient profile. No virus could be detected in resting PBL. PBL stimulated for 24 h before infection produced a detectable viral peak at 1.34 g/ml, corresponding to complete virions and a smaller peak at density 1.27 g/ml, corresponding to empty shells.

An infectious-center assay was performed to determine the proportion of infected lymphocytes capable of producing infectious virus. PBL were infected with Ad2, and samples were taken every 24 h and tested on HEp-2 monolayers by counting the resulting plaques. Infected HEp-2 cells, used as a control, were 14% positive after 24 h and 80% positive after 48 h p.i. Both unstimulated and PHA-treated lymphocytes contained infectious virus, and the proportion of virus-carrying cells rose steadily until day 5 p.i. (Fig. 4). At this point, about 0.35% of unstimulated and 4% of PHA-stimulated cells produced infectious centers.

To study infectious virus production, samples were taken from infected cells every 24 h and the cells were lysed by repeated freeze-thaw cycles. The yield of infectious virus was determined on HEp-2 cells by plaque assay (Fig. 5). Unstimulated PBL produced so little virus that it was difficult to measure (3×10^3 PFU/10^6 cells).

PHA stimulation resulted in a significant increase in virus production to 4.4×10^5 PFU/10^6 cells. In comparison, HEp-2 cells produced 2.6×10^9 PFU/10^6 cells. This agrees well with our long experience of growing Ad2 in HEp-2 cells. The slow growth of the virus in PBL renders interpretation difficult in terms of one-step growth. Taking samples beyond day 5 may risk superimposition of a second cycle of infection on the first because of possible infection of uninfected PBL.

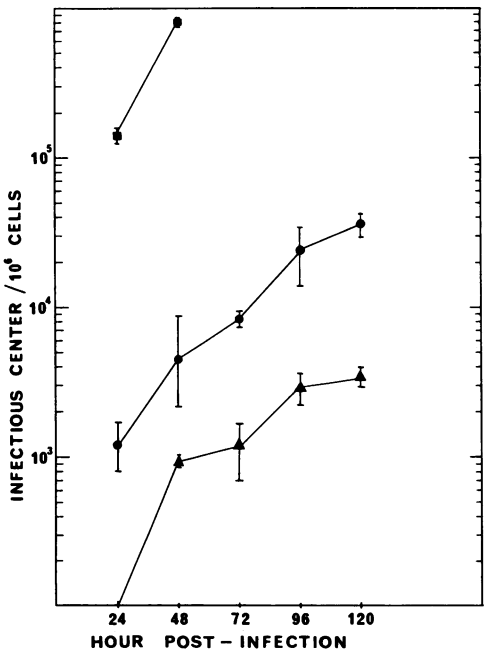

FIG. 4. Infectious-center assay. At different intervals p.i., serial cell samples were taken from Hep-2 cells (■) and PBL with (●) or without (▲) PHA pretreatment, washed in phosphate-buffered saline, and then seeded over HEp-2 monolayers. The cultures were overlaid with agar, and plaques were counted after 12 days.

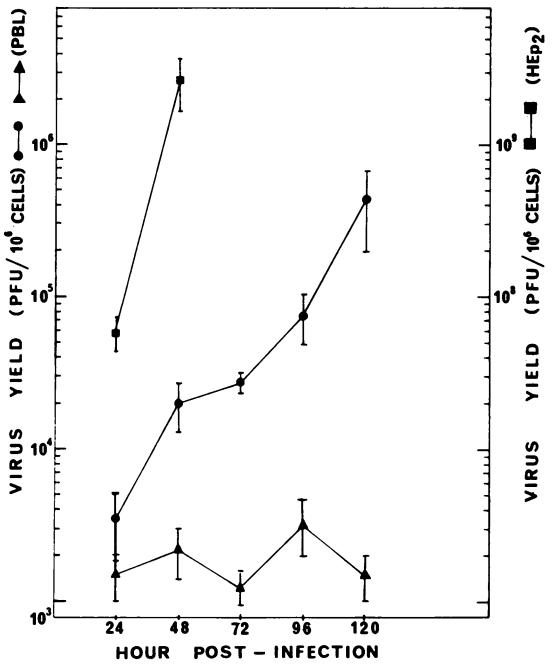

FIG. 5. Plaque assay of infectious virus produced by HEp-2 cells (■) and PBL with (●) or without (▲) PHA pretreatment. From serial samples, the cells were lysed by multiple freeze-thaw cycles, the cell debris was removed, and HEp-2 cells were infected with appropriate dilutions of the supernatant. Plaques were counted after 12 to 14 days.

From the data in Fig. 4 and 5, it is possible to calculate the mean number of PFU produced per infected cell. HEp-2 cells produced 3.7×10^3 PFU per cell, compared with 0.8 PFU per cell for resting PBL and 11 PFU per cell for PHA-treated PBL.

When e compared the proportion of lymphocytes (30 to 40%) wh ch could bind virions with that of cells which actually produced infectious viruses, we found striking differences. The infectious-center assay showed that the proportion of the unstimulated lymphoid cell population able to produce infectious virus was less than 1%. PHA pretreatment increased the proportion of productive cells to 4%. The prolonged time course of apparent virus production can be explained by a longer eclipse period and also a gradual increase in the number of cells capable of producing infectious virus. When the results were considered together, major impediments to PBL permissivity were encountered in uncoating, viral DNA synthesis, and infectious-virus assembly. When PBL were compared with permissive HEp-2 cells, DNA synthesis accounted for a 460-fold reduction, whereas virus assembly accounted for a 10^6-fold reduction. It appears that virus production in lymphoid cells requires continuously dividing cells or cells actively engaged in DNA and protein synthesis.

To our knowledge, this is the first detailed study of adenovirus permissivity of human PBL. Previous studies had been confined to lymphoblastoid cell lines. Adenovirus infection was studied in in vitro Epstein-Barr virus-transformed lymphoid cultures, in Burkitt's lymphoma cell lines, and in the T-lymphoblast line Molt-4 as well (2, 13–15). All of these cells could produce a low level of infectious adenovirus. Soon after this paper was submitted for publication, Lavery et al. (28) reported that Ad2 and Ad5 infection of

several established T- and B-lymphoblastoid cell lines is followed by viral DNA, RNA, and protein synthesis, as well as infectious-virus production. The efficiency, however, was different and characteristic for each cell line, some of them approaching the efficiency of HeLa cells. Faucon et al. (15) reported virions which differed from input virus in density, DNA content, and restriction enzyme pattern. Cell growth and viability were largely unaltered. In Epstein-Barr virus-negative Jijoye cells, adenovirus infection caused an increase in the production of Epstein-Barr virus antigen, showing an interesting interaction between two DNA tumor viruses (13).

Slowly progressing adenovirus infection does not seem to interfere with the vital functions of cells. However, specific functions could be disturbed. Adenovirus infection of PBL in vitro disturbs the mitogen response and inhibits T-cell rosette formation with sheep erythrocytes (20). Viral persistence in lymphocytes could, perhaps, involve downregulation of the major histocompatibility class I antigen by the viral E1a (9, 37) or E3 gene (5, 33), as documented in tissue cultures of nonlymphoid origin. It is also a possibility that adenovirus-carrying lymphocytes host another virus infection, such as human immunodeficiency virus. Possible interactions between DNA viruses and retroviruses have already been reported. Epstein-Barr virus enhanced the expression of murine leukemia virus proteins in human lymphoma lines (27). Herpes simplex virus infection increased gene expression directed by the human immunodeficiency virus long terminal repeat (31). It is not known whether frequent isolation of an otherwise rare adenovirus serotype from acquired immunodeficiency syndrome patients (7) is indicative of some type of interaction between these viruses. Such interactions might be facilitated by a compromised cell-mediated immunity system, a frequently reported concomitant of serious adenovirus infections (3, 6, 30).

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