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Group C Adenovirus DNA Sequences in Human Lymphoid Cells

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Human peripheral blood lymphocytes from healthy adults, cord blood lymphocytes, and lymphoblastoid cell lines were screened by hybridization for the presence of group C adenovirus DNA sequences. In 13 of 17 peripheral blood lymphocyte samples from adults, 1 of 10 cord blood samples, and seven of seven lymphoblastoid cell lines tested, results were positive for Group C adenovirus DNA (adenovirus 1 [Ad1], Ad2, Ad5, or Ad6). About 1 to 2% of the lymphocytes carried 50 to 100 viral genome copies per positive cell, as estimated by in situ hybridization. Infectious virus representing all members of group C were recovered, but cultivation in the presence of adenovirus antibody did not cure the cells of free viral genomes. Viral DNA was found in B, T, and N cells but only in 1 of 10 cord blood samples. The results suggest that group C adenovirus infections in childhood result in the persistence of the viral genome in circulating lymphocytes.

Adenoviruses are commonly isolated from human tonsils and adenoids. Members of group C (adenovirus 1 [Ad1], Ad2, Ad5, and Ad6) are the most frequent isolates (2, 8, 18, 23, 26). Although these viruses fail to grow in resting lymphocytes, lectin-stimulated or Burkitt's lymphoma cells support some virus growth (9, 10, 11, 24). The few reports which have appeared to date on this subject involved cocultivation of explanted lymphoid tissue or lymphocytes with continuous lines of human cells which support virus growth. This has led to amplification of the adenovirus, permitting identification by immunological methods. Green et al. (13) detected group C adenovirus sequences by molecular hybridization in 25% of 52 tonsils tested. A direct demonstration of these viruses in lymphocytes without culture has not been reported. We undertook this approach, using DNA-DNA hybridization with a cloned Ad2 probe to determine the prevalence of these viruses and the state of the viral genome in peripheral blood lymphocytes (PBL).

In general, the lymphocytes were purified from 10 to 15 ml of heparinized blood, and total DNA was extracted from nuclei by conventional methods. Samples of this DNA were digested to completion with restriction nucleases *Sal*I and *Bam*HI and occasionally *Eco*RI and *Xho*I. The DNA fragments were electrophoresed in agarose, transferred onto nitrocellulose membranes, and hybridized with a cloned Ad2 probe labeled with ^{32}P. Of 17 samples examined this way, 13 were positive for adenovirus DNA. All of the four adenovirus workers tested were positive. Negative controls consisted of DNA from HEp2 and HeLa cells; positive controls consisted of Ad2 genomic DNA and DNA from an Ad2-transformed rat cell line (F4). Figure 1 shows some examples of the types of result obtained. Samples from most individuals contained free viral DNA of the complete viral genome. In addition to free viral molecules, hybridization to high-molecular-weight bands which did not comigrate with marker viral DNA bands suggests the presence of integrated viral sequences. A fragment of 6,600 base pairs appeared to be common to several samples digested with *Sal*I (Fig. 1). The nature of this band is not known and is currently under investigation. The stability of the band pattern was examined in samples from several donors over a period of 2 years. No detectable variation was observed. To obtain an estimate of the proportion of lymphocytes which carried viral DNA, in situ hybridization was performed on cell smears (14). Hybridization was found to be restricted to between 1 and 2% of cells (Fig. 2).

The distinctness and intensity of the Southern patterns suggested (i) the presence of a unique serotype of virus and (ii) the presence, on the average, of approximately 50 to 100 copies of viral DNA per carrier lymphocyte. Taken together,

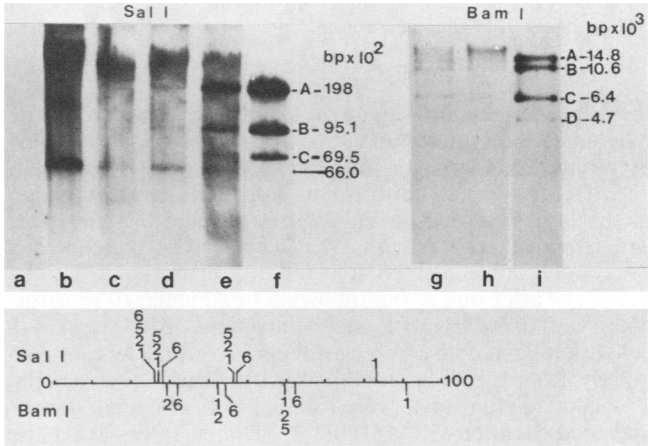

FIG. 1. Southern blots of adult PBL DNA hybridized with Ad2 DNA. PBL were obtained from long-time adenovirus workers, donors 2 and 4 (lanes b and c) and from unrelated healthy subjects from the general population donors 1 (lanes d and g) and 3 (lanes d). The sizes of restriction fragments are given in base pairs to the right of the Ad2 marker lanes (lanes f and i). *Sal*I and *Bam*HI (Bam I) restriction maps of Ad1, Ad2, Ad5, and Ad6 are given at the bottom of the figure for reference. High-molecular-weight DNA was extracted from the nuclei of PBL from 10 ml of fresh heparinized blood, digested to completion with *Sal*I or *Bam*HI and electrophoresed in a 1% agarose slab gel. The DNA was blotted onto nitrocellulose and hybridized to a ^{32}P-labeled Ad2 probe (specific activity, 4×10^8 cpm/μg of DNA) as described previously (5). The probe consisted of an equimolar mixture of pBR322 plasmids containing the 10 Ad2 *Hin*dIII fragments.

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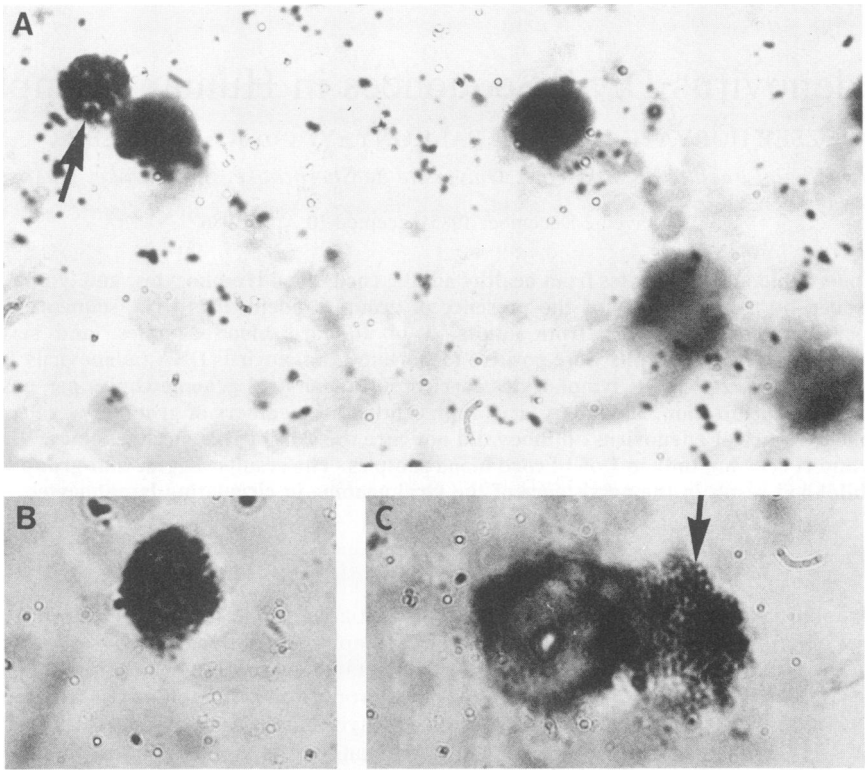

FIG. 2. Detection of adenovirus DNA sequences by in situ hybridization in PBL. (A) Freshly isolated PBL from donor 1. One of the cells is labeled (arrow). (B) Labeled phytohemagglutinin blast of cells from donor 1. (C) Labeled and unlabeled phytohemagglutinin blasts of cells from donor 3. Magnification, ×3,300. The cells were fixed with methanol-acetic acid (3:1) on microscope slides (acid cleaned, treated with Denhardt solution). The samples were digested with pronase, denatured in boiling water 10 s, and dehydrated. Hybridization (14) was carried out with cloned adenovirus DNA labeled with ^{35}S-labeled dATP (A and B), or [^{3}H]dATP (C) (specific activity, 2×10^8 to 4×10^8 cpm/μg of DNA). After extensive washing, the samples were dehydrated and coated with NTB-2 (Kodak) emulsion and developed after 2 (B and C) or 4 weeks (A). The slides were stained with Giemsa, and 2,000 cells were examined to determine the proportion showing a silver grain count higher than that of the background.

these data suggest that adenovirus infection may result in the fixation of the viral DNA within one lymphocyte, followed by proliferation, giving rise to the uniform results observed.

Attempts were made to isolate infectious virus by fusion of the lymphoid cells with Ad5-transformed 293 cells (12) and by cocultivation with HEp-2 cells. The lymphocytes were either used immediately after preparation from the donors or after one of the following treatments: (i) transformation of B cells with Epstein-Barr virus (EBV), or (ii) T cells stimulated with soluble proteins from Ad1, Ad2, or Ad5 (4). By these methods, infectious adenoviruses representing all members of group C were isolated from two adult donors and studied intensively (Table 1). Donor 1 was from the general population, and donor 2 was an adenovirus worker. During the virus rescue experiments, cytopathic effect was observed only after several blind passages, which suggests that production of infectious virus was rather a rare event. There was no cytopathic effect on Hep-2 or 293 cells infected with soluble proteins used for stimulation of antigen-specific lymphocytes even after several passages. Cocultivation of phytohemagglutinin-stimulated lymphocytes from donor 1 in the presence of Ad2 antibodies for 30 days failed to cure the cells of viral DNA.

Attempts were made to determine which cell population is responsible for carrying the viral DNA. T cells and non-T populations were separated by the erythrocyte rosette method, and prepared DNAs from three samples were analyzed separately. Both cell populations were found to be positive in each case. The B-cell lines transformed by EBV and the antigen-specific cell lines were also found to be positive (Fig. 3, lanes a, e, and f). Besides the genuine *Eco*RI bands of Ad2, additional bands appeared on Southern blots (Fig. 3, lanes b and c). We could not determine whether they originated from another serotype or from integrated sequences.

Because 13 of 17 adults contained adenovirus DNA in their lymphocytes, we also screened the lymphocytes from 10 separate cord bloods from normal delivery of healthy pregnancies. Only 1 of the 10 samples was positive. This positive cord blood gave a Southern blot pattern similar to an adult pattern, including the high-molecular-weight band, suggesting integrated sequences. Therefore, we suggest that in most cases the viral genomes are not vertically transmitted but are probably acquired during a childhood infection.

To obtain a continuous and convenient source of lymphocytes for studies on the association of adenoviral DNA with lymphocytes, we screened by DNA-DNA hybridization the following (mostly lymphoblastoid) cell lines of hematologic origin for the presence of adenoviral DNA: Raji; MC-1, both Burkitt's lymphoma cells; U-698M from a B-cell lymphosarcoma; 4672, from a B-cell myeloma line; 1301, from an N-cell acute lymphocytic leukemia; K-562 erythroleukemia; B95-8 from an EBV-transformed monkey B-cell line. Surprisingly, all seven cell lines hybridized with

TABLE 1. Isolation of adenoviruses from human lymphocytes

Donor, cell type	Treatment	Virus isolated[a]
Donor 1		
PBL⁻	Fusion with 293 cells[b]	2
PBL	Cocultivation with HEp-2 cells[c]	1, 2, 5
T cells	Stimulation with PHA and IL_2[c]	1, 5
B line	Transformation with EBV, clone 1[c,d]	5
B line	Transformation with EBV, clone 2[c,d]	2
B line	Transformation with EBV, clone 3[c,d]	5
Cell line	Stimulation with Ad1 protein and IL_2[c,e]	1, 2
Cell line	Stimulation with Ad2 protein and IL_2[c,e]	2, 5
Donor 2		
PBL	Fusion with 293 cells[b]	2
PBL	Cocultivation with HEp-2 cells[c]	1, 2, 5, 6
Cell line	Stimulation with Ad1 protein and IL_2[c,e]	1, 5
Cell line	Stimulation with Ad5 protein and IL_2[c,e]	5

[a] Identification of the isolated viruses was based on restriction endonuclease digestion of DNA from purified virions. In some cases the viruses were isolated by plaque assay, and separate plaques were analyzed.

[b] Fusion with 293 cells was followed by three to five blind passages in HEp-2 cells.

[c] Cocultivation with HEp-2 cells was for 5 days and was followed by blind pasages as described in footnote *b*. PHA, Phytohemagglutinin; IL_2, interleukin 2.

[d] EBV was obtained from the culture medium of B95-8 cells.

[e] Soluble viral proteins were collected from the top portion of CsCl gradients during virus purification, dialyzed against distilled water, and digested with micrococcal nuclease. Lymphocytes were cultured in the presence of these antigens for 10 days, then for a further 20 days in the presence of both antigen and interleukin 2 as described previously (4).

Ad2 DNA, giving the *Bam*HI restriction pattern typical of free genomic DNA of Ad1, Ad2, and Ad6, which are indistinguishable by this enzyme (Fig. 4). On the basis of the restriction pattern, only Ad5 can be ruled out. The hybridization intensity, compared with the 16-copy F4 cell line, suggests approximately one viral DNA copy per cell. The monkey cells probably acquired the human adenovirus genome via transformation with an adenovirus-contaminated EBV preparation, although adenovirus-related RNA was detected from gorilla tissues by Jones et al. (19). These results confirm B cells and add N cells to the types of lymphocytes capable of carrying a cryptic adenoviral genome. They also raise questions about the possible interactions between these DNA viruses. Such a question has already been posed for the interaction between EBV and murine leukemia virus in terms of a possible mechanism of activation of cellular oncogenes in human B-lymphoid cells by interaction with a ubiquitous DNA virus (20). The present results show that group C human adenovirus DNA is acquired during childhood and is capable of persisting in free or integrated form in peripheral blood B, T, or N lymphocytes in most adults. Adenoviruses have been shown to be immunosuppressive in vitro. Infection of human PBL by adenoviruses causes (i) a 10-fold reduction in the efficiency of phytohemagglutinin stimulation of PBL, (ii) a reduction in the so-called active T cells (sheep erythrocyte rosette-forming capacity), and (iii) the production of soluble factors which decrease the phagocytic activity of polymorphonuclear granulocytes (15; unpublished data). The amount of sialic acid decreased on the surface of PBL infected with Ad5 in vitro (J. Horvath and I. Budavary, unpublished results). The Ad2-encoded E3 19,000-molecular-weight protein (and probably all of those encoded by group C

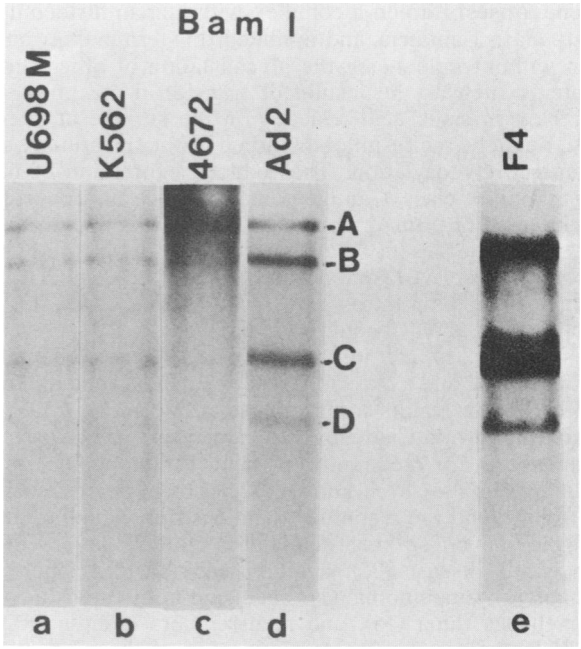

FIG. 4. Human lymphoblastoid cell lines contain group C adenovirus DNA. Southern blot hybridization of *Bam*HI-digested DNA (Bam I) from a B-cell lymphosarcoma (U698M), a B-cell myeloma (4672), and an erythroleukemia line (K562). Lane d, Ad2 marker DNA (fragments are indicated to the right of the lane); lane e, an Ad2-transformed rat cell line which contains 16 copies of nearly the entire Ad2 genome.

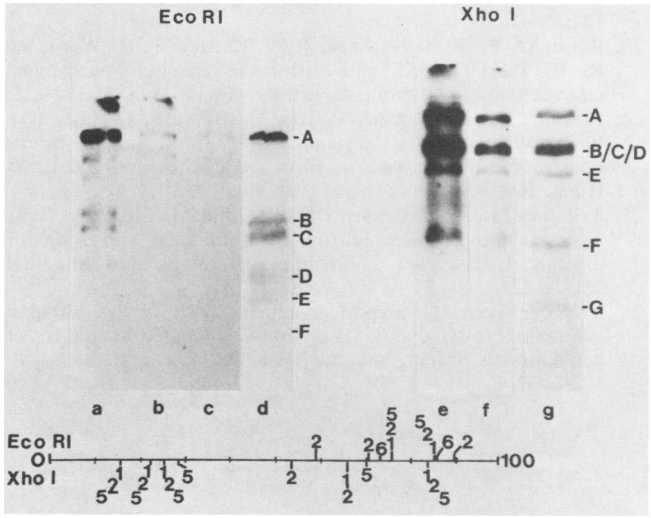

FIG. 3. Southern blots of DNA of a lymphoblastoid cell line and lymphoid cells digested with *Eco*RI or *Xho*I. Lanes: a, e, and f, a lymphoblastoid cell line originating from PBL from donor 2 after transformation by soluble antigens of AD1 (see details in Table 1); b and c, T cells and non-T cells, respectively, from donor 1 (separated by the sheep erythrocyte rosette method and Ficoll-Hypaque centrifugation); d and g, Ad2 marker digested with *Eco*RI and *Xho*I, respectively. (Lane e is the same as lane f but is overexposed to render visible two additional bands in the high-molecular-weight region.) *Eco*RI and *Xho*I restriction maps are given for Ad1, Ad2, Ad5, and Ad6 and Ad1, Ad2, and Ad5, respectively. Methods used were as described in the legend to Fig. 1.

adenoviruses) formed a complex with human histocompatibility class 1 antigens and inhibited this terminal glycosylation without influencing the glycosylation of other surface proteins such as the insulin or transferrin receptors. The decrease in sialic acid residues on the surface of infected PBL might be the result of disturbed histocompatibility class 1 antigen glycosylation. The reduced expression of histocompatibility class 1 antigens may provide an opportunity for infected or transformed cells to escape from cytotoxic T cells (6). The present results suggest that the in situ hybridization between Ad2 and normal human RNA or RNA from human brain tumors, as detected by Jones et al. (19) and Ibelgaufts et al. (17), could have been due to PBL present in these tissues, in addition to the limited sequence homology between Ad2 and a class of transcribed eucaryotic repetitive DNA (3). Our results confirm those of Green et al. (13), who found that group C adenovirus sequences, particularly Ad2 sequences, are frequently present in lymphoid tissues. Adenoviruses are well known as causes of acute infections of the eye and the respiratory and gastrointestinal systems, but they have also been detected in patients suffering from Hodgkin's disease (25), acquired immune deficiency syndrome (7), pneumonia (1), inclusion-body myositis (21), bronchial asthma (16), and recurrent oral aphthae (22) as well. However, the exact relationship of these apparently ubiquitous viruses to these latter human diseases remains to be determined. Caution should be exercised in the interpretation of isolation of adenoviruses from such diverse sources as they may derive from lymphoid cells present in these tissues.

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