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Analysis of human tonsil and cancer DNAs and RNAs for DNA sequences of group C (serotypes 1, 2, 5, and 6) human adenoviruses

(human cancer/latent tonsil infections/molecular hybridization/Southern blot technique)

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ABSTRACT Group C human adenoviruses (Ads) of serotypes 1, 2, 5, and 6 infect most children and commonly cause latent infections of lymphoid tissues. Ads transform cells into a malignant-like phenotype; the oncogenic genetic information is in the left 8% of the viral genome, in the *Hin*dIII-G DNA fragment. We have investigated the molecular basis for group C Ad latent infections in human tonsils as well as whether these viruses are linked to human cancer. Tonsil or cancer DNAs and RNAs were assayed for Ad sequences by liquid-phase saturation-hybridization with *in vitro*-labeled Ad5 *Hin*dIII-G fragment. About 25% of the 52 tonsils analyzed contained DNA or RNA sequences specific to *Hin*dIII-G, indicating that Ad transforming sequences are expressed as RNA in tonsils. Southern blotting analysis of four tonsil DNAs revealed multiple copies of the complete Ad genome in a free state and provided evidence for an unusual form of the Ad genome, possibly Ad DNA integrated into cellular DNA. In assays of human cancers, no Ad sequences were detected in DNAs from 26 squamous cell carcinomas (Cas), 3 adenocarcinomas, 4 oat cell Cas, 5 stomach Cas, 5 small intestine Cas, 15 colon Cas, 6 rectum Cas, 5 Hodgkin and 6 non-Hodgkin lymphomas, and 2 breast Cas. Reconstruction experiments indicated that the *Hin*dIII-G probe could detect 1 copy per cell of 0.2–0.3% of the viral genome. No *Hin*dIII-G-specific sequences were detected in RNAs from 21 squamous cell Cas, 3 oat cell Cas, 2 stomach Cas, or 18 colon Cas. In six other experiments using the complete Ad2 genome as probe, no Ad sequences were found in DNAs from 6 lung Cas, 12 normal lung tissues, 33 gastrointestinal Cas, 19 normal gastrointestinal tissues, 6 Hodgkin lymphomas, 3 breast Cas, or 4 kidney Cas, at a sensitivity of about 1 copy per tumor cell of 5–10% of the Ad2 genome. All Ad-induced cancer cells should contain at least 1 copy of 1–6% of the viral genome, the minimal size of the transforming region, and probably should contain multiple copies of more of the genome. Therefore, our data are definitive evidence against group C Ads being the cause of the cancers tested, which represent about 50% of the cancer incidence in the United States. Of additional interest, we did not detect Ad2 sequences in RNAs from 7 human placentas, 12 normal lungs, or 19 normal gastrointestinal tissues (nor in 44 cancer or 23 tonsil RNAs). Thus, we did not confirm a recent report of the presence of Ad2 RNA in RNAs from human placentas; the possibility that a small population of cells in placenta expresses group C "related" sequences is not ruled out.

The 31 recognized human adenovirus (Ad) serotypes (Ad1-31) form five DNA genome homology groups, A–E (1). The groups differ in properties, including transforming gene homology (2), epidemiology and pathogenicity, host range, size of virion proteins, and immunological specificity (see refs. 3–10). Group C types (Ad1, -2, -5, and -6) are the most common and infect the majority of young children, usually in respiratory, gastrointestinal, and lymphoid tissues. These infections have a tendency to become latent, especially in lymphoid tissue.

Ads induce tumors in animals or transform cultured cells or do both. The genetic information for cell transformation is located in the left 8% of the group C Ad genome, as indicated by transformation of cells by transfection with *Hin*dIII-G fragment (left 8% of genome) (11) and the persistence of these sequences in Ad-transformed cells (12, 13). Many transformed cells contain multiple copies of variable portions of the remainder of the genome (12). There is no evidence that Ads transform cells by a "hit-and-run" mechanism, whereby the viral genome would be absent from the transformed cell.

Considering the ubiquity and oncogenicity of group C Ads, an important question is whether they cause human cancer. Another important and possibly related problem is the molecular basis for Ad latent infections. To address these questions, we assayed DNAs and RNAs from human cancers and normal human tonsils for Ad sequences by using sensitive liquid-phase molecular hybridizations with two *in vitro* labeled probes, Ad5 *Hin*dIII-G transforming fragment and the complete Ad2 genome. Tonsil DNAs were also examined by using the Southern blot technique.

MATERIALS AND METHODS

Human tumors were obtained from J. Gruber and I. Sekely (Office of Program Resources and Logistics, National Cancer Institute, E. Harrison* (Mayo Clinic), M. Gardner (University of Southern California), and H. Pinkerton* (St. Louis University). Tonsils were obtained from J. Blair (St. Louis University). Tissue DNAs and RNAs were extracted for liquid hybridization as described (14). High molecular weight tonsil DNAs were extracted by a modification of procedures described by Graham (15) and Blin and Stafford (16). Briefly, tissues were frozen and then pulverized in dry ice, treated with proteinase K (100 μg/ml) and 4% Sarkosyl at 50°C, extracted with phenol/chloroform, dialyzed, treated with RNase, extracted with chloroform, and dialyzed extensively.

Standard procedures were used for growth of virus in suspension culture, plaque assay of virus, protease-assisted extraction of viral DNA (4, 14, 17), and preparation of the Ad5 *Hin*dIII-G fragment (4, 18). Ad2 DNA and Ad5 *Hin*dIII-G fragment DNA were labeled *in vitro* by "nick translation" as described (4, 19). The specific radioactivities of the probes are given in the legends. Such *in vitro*-labeled Ad DNA probes have been extensively characterized (4, 19); they are uniformly labeled and reassociate to completion with expected kinetics. Ad2 and Ad5 *Hin*dIII-G DNAs were used interchangeably because these DNAs are indistinguishable by hybridization (1, 2).

Hybridizations were conducted in mixtures (50 μl) containing 6 mg of DNA (tumor, transformed cell, or calf thymus) per ml, 500 or 1000 cpm of probe DNA, 0.72 M NaCl, 10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) at pH 6.7, 1 mM EDTA, and 0.05% sodium dodecyl sulfate. Hybridization was

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Abbreviations: Ad1-31, human adenovirus serotypes 1–31; gastrointestinal, GI.

* Deceased.

estimated by batchwise chromatography on hydroxylapatite (4). In all analyses, reconstruction experiments were done to estimate sensitivity; these reaction mixtures contained 0.2 or 1 copy of unlabeled Ad2 DNA per cell and 6 mg of calf thymus DNA per ml. As another positive control, DNAs or RNAs from two Ad-transformed rat cell lines were also always assayed. One line, 8617, contains the left and right portions of the genome (12), and the other line, 5RK clone I, contains 70% of *Hin*dIII-G DNA and synthesizes RNA specific to 40–50% of the G fragment (18).

Southern blot analysis was performed basically as described by Southern (20) with minor modifications (4).

RESULTS

Analysis of Ad Sequences in Human Tonsils. We have analyzed the state of the Ad genome in normal human tonsils, as a means to elucidate group C Ad latent infections at the molecular level and to provide a rationale for studies of the Ad–cancer relationship. When assayed by liquid hybridization with the *Hin*dIII-G probe, 5 of 20 tonsil DNAs gave 82–97% hybridization; RNA from 3 of these tonsils gave 29–32% hybridization (Table 1). Of an additional 20 tonsil RNAs assayed, 4 gave 34–41% hybridization. RNA from Ad2 early infected cells or Ad2-transformed cells hybridized 30–40% of *Hin*dIII-G fragment, the same as tonsils. Five of 12 tonsil DNAs assayed by Southern blotting were also found to contain Ad DNA (see below). We conclude that about 25% of tonsils (14 of 52) contain Ad DNA and that some or all of these express as RNA the Ad-transforming sequences.

To identify the Ad serotype and to obtain information on the physical state of the Ad genome in tonsils—e.g., free DNA or integrated—we analyzed tonsil DNAs by Southern blotting using endonuclease *Eco*RI. Typical data are shown in Fig. 1 and demonstrate that tonsils contain >20 copies of the complete Ad genome per cell (i.e., the tonsil DNAs contain more Ad DNA than do the Ad reconstructions which were at 20 copies per cell).

Table 1. Analysis of normal human tonsil DNAs and RNAs for group C Ad transforming sequences

Tonsil sample	% hybridization DNA	RNA
M023H	85.1	28.7
M023I	92.9	30.8
M023N	82.0	32.0
G034Q	97.2	ND
G034R	92.4	ND
G077K	ND	34.2
M007X	ND	40.6
M008J	ND	35.7
M008M	ND	36.5
7 tonsils	0	0
8 tonsils	0	ND
16 tonsils	ND	0

In vitro ^{32}P-labeled Ad5 *Hin*dIII-G fragment (10^8 cpm/μg) was hybridized with tonsil DNA (6 mg/ml) to an equivalent C_0t (product of the initial DNA concentration, as moles of nucleotides per liter, and the hybridization time in seconds, with the rate corrected for an equivalent Na^+ concentration of 0.18 M) of 15,000 or with tonsil RNA (5 mg/ml) to an equivalent C_rt of 15,000. Background due to self-hybridization of the probe (12–15%) was subtracted. With the DNA samples, the values were normalized to maximal hybridization of the probe with excess homologous DNA (83–93%). In reconstructions, 0.2 copy of Ad2 DNA per cell gave 36.2% hybridization, 1 copy gave 68.5%, and 8617 cell DNA gave 96.1%. RNA from Ad2 late infected KB cells and from 8617 cells gave 44.1 and 37.9% hybridization, respectively. ND, not done.

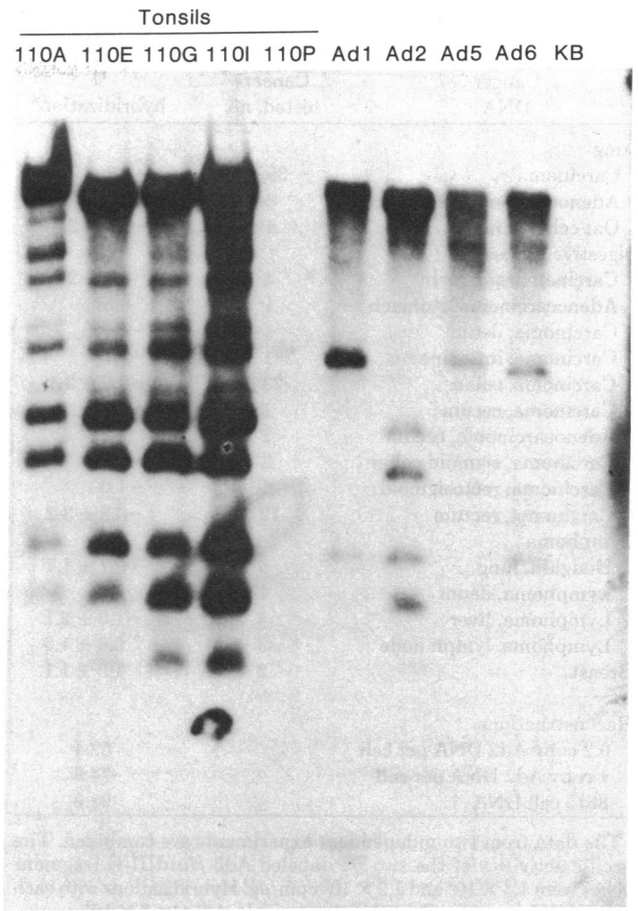

FIG. 1 Detection of fragments of DNA that contain Ad2-specific sequences after digestion of human tonsil DNA with *Eco*RI. Five preparations of human tonsil DNA, human KB cell DNA, and 20 copy equivalents of Ad1, 2, 5, and 6 DNA mixed with KB DNA were digested with *Eco*RI, electrophoresed on 0.8% agarose gels, transferred to nitrocellulose paper, annealed with 2×10^6 cpm of *in vitro*-labeled Ad2 [^{32}P]DNA (complete genome; specific activity $1–2 \times 10^8$ cpm/μg), and autoradiographed. Four of the five tonsil blots contained all six *Eco*RI Ad2 DNA fragments as well as additional fragments of unknown nature. One tonsil DNA (110P) and KB cell DNA had no detectable Ad2 DNA sequences.

Each positive tonsil DNA showed the six *Eco*RI fragments diagnostic of Ad2 (*Eco*RI cleaves Ad2 DNA into six fragments, Ad6 DNA into four fragments, and Ad5 and Ad1 DNAs into three fragments). All tonsils also showed restriction fragments that differed from any of the fragments of the four group C Ads; these may represent an unusual form of the Ad genome or possibly Ad sequences integrated into tonsil DNA.

Analysis of Human Cancer DNAs or RNAs for Ad Sequences. Most cancers analyzed were of the lung or gastrointestinal tract because group C Ads infect tissues at these sites and because these are the most common types of cancers in the United States. We also examined lymphomas because Ads are latent in lymphoid tissues. Table 2 summarizes two experiments, with two different Ad5 *Hin*dIII-G probes, in which 111 cancer DNAs were assayed. Only the mean reassociation values for each tumor type are given; all hybridization values are within 2–4% of the mean value of the probe reassociation in the presence of calf thymus DNA (10–11%). No Ad sequences were detected in any cancer DNAs. In reconstructions, 0.2 copy of Ad2 DNA per cell gave 52.4% above background, and 1 copy per cell gave 74.9%. Thus, the sensitivity of these tests was about 0.05 copy per cell of Ad DNA; 8617 cell DNA gave 91.0% hybridization.

Table 2. Summary of analyses of human cancer DNAs for group C Ad *Hin*dIII-G transforming fragment-specific DNA sequences

Cancer DNA	Cancers tested, no.	% hybridization*
Lung		
Carcinoma†	39	−0.8 ± 3.7
Adenocarcinoma	6	−0.9 ± 3.2
Oat cell carcinoma	4	−1.0 ± 2.3
Digestive system		
Carcinoma, stomach	4	0.7 ± 2.1
Adenocarcinoma, stomach	1	0.5
Carcinoma, ileum	1	−4.4
Carcinoma, intestine	1	−2.7
Carcinoma, colon	23	−0.6 ± 3.4
Carcinoma, cecum	1	3.8
Adenocarcinoma, cecum	2	1.2
Carcinoma, sigmoid colon	2	4.6
Carcinoma, rectosigmoid	1	−1.0
Carcinoma, rectum	12	−0.8 ± 3.2
Lymphoma		
Hodgkin, lung	5	−0.7 ± 1.7
Lymphoma, ileum	1	2.4
Lymphoma, liver	3	−1.0 ± 2.1
Lymphoma, lymph node	3	0.6 ± 1.0
Breast	2	1.0 ± 1.1
Reconstructions		
0.2 copy Ad2 DNA per cell		52.4
1 copy Ad2 DNA per cell		74.9
8617 cell DNA		91.0

The data from two independent experiments are combined. The specific activities of the two ^{32}P-labeled Ad5 *Hin*dIII-G fragment probes were 1.3×10^8 and 1.2×10^8 cpm/μg. Hybridizations with each cancer DNA were conducted to an equivalent C_0t of 2×10^4.

* Shown as mean ± SD. The self-hybridizing backgrounds of the probes (10–11%) have been substracted; the values are normalized to maximal hybridization in the presence of 267 copies of Ad2 DNA per cell (96%).

† Carcinomas of the lung are mainly squamous cell carcinomas.

Table 3. Summary of analyses of human lung and gastrointestinal system cancer RNAs for group C Ad *Hin*dIII-G transforming fragment-specific sequences

RNA*	Cancers tested, no.	% hybridization†
Lung		
Carcinoma	21	−0.2 ± 0.8
Oat cell carcinoma	3	−0.6 ± 0.8
Digestive system		
Carcinoma, stomach	1	−0.1
Adenocarcinoma, stomach	1	1.0
Carcinoma, colon	17	0.6 ± 1.4
Adenocarcinoma, colon	1	1.2
Positive controls:		
Ad2 late infected KB cell		43.2
8617 cell cytoplasmic		41.5
5RK cell cytoplasmic		21.0

Hybridizations were conducted with 5 mg of cancer or cell RNA per ml to an equivalent C_rt of 10^4. The specific activity of the Ad5 *Hin*dIII-G fragment probe was 1.0×10^8 cpm/μg.

* The DNAs of eight of these cancers were also assayed for G-fragment-specific sequences; the results are included in Table 2.

† Shown as mean ± SD. The self-hybridizing background of the probe (7.5%) was subtracted.

Cancer RNAs were assayed because Ad sequences might be amplified in RNA. No Ad sequences were detected in 24 lung or 20 gastrointestinal cancer RNAs, with *Hin*dIII-G as probe (Table 3) (8 of the lung and 8 of the gastrointestinal cancers that were analyzed for RNA were also analyzed for DNA). In the same experiment, 8617 and 5RK cell cytoplasmic RNAs gave 41.5 and 21.0% hybridization, respectively, and Ad2-infected cell RNA (late stage of infection) gave 43.2% hybridization.

In initial stages of our studies, we screened cancer DNAs by using the complete Ad2 DNA genome as probe. In six independent experiments, no Ad sequences were detected in 52 cancer DNAs (34 of these DNAs were also tested with the *Hin*dIII-G fragment probe); 12 normal lung and 19 normal gastrointestinal DNAs were also negative (Table 4). The reconstruction hybridization control values for the six experiments are presented in Table 5. DNA from 8617 cells gave 22–26% hybridization. One copy of Ad2 DNA per cell gave 39–54% hybridization, and in one experiment 0.2 copy of Ad2 DNA per cell gave 17%. Therefore, we should have detected Ad DNA sequences in tumor DNAs containing about 0.1 copy of the viral genome per cell.

Analysis of Human Placenta RNAs for Ad Sequences. It has been reported (21) that 10 of 10 normal human placentas contained RNA that hybridized with Ad2 DNA, including the transforming region. The assay procedures used were hybridization of ^{125}I-labeled tissue RNA with Southern transfers (20) of restricted Ad2 DNA or with Ad2 DNA simply spotted onto nitrocellulose paper, as well as *in situ* hybridization of tissue RNA with ^{3}H-labeled Ad2 DNA. As presented in Tables 1 and 3, we did not detect group C Ad transforming sequences in RNAs from 23 tonsils or 44 cancers of human origin when we used our sensitive and well-characterized liquid hybridization procedure. To test whether Ad sequences might be restricted to placentas, we analyzed RNA from four full-term and three fetal human placentas by using either the Ad5 *Hin*dIII-G fragment or the complete Ad2 genome as probe; no Ad sequences were detected (Table 6). DNAs from these same placentas were also negative for Ad2 sequences (data not shown). Based upon our reconstructions with Ad2 DNA, we estimate that we could detect 1 copy of G-fragment sequences per 10–20 cells.

DISCUSSION

Group C Ads readily form latent infections of lymphoid tissues, especially tonsils. Ads usually cannot be isolated directly from tonsils, and it is necessary to culture the tonsils for several weeks. These cultivation studies suggest that about 1 in 10^7 tonsil cells contained virus (22). Our blotting studies demonstrate that normal human tonsils contain >20 copies of the apparently complete Ad genome per cell. Therefore, although it requires lengthy *in vitro* incubation of tonsil tissue cells to isolate Ads, these cells in fact contain on the average many copies of the Ad genome per cell and transcribe at least some (left 8% of genome) of the Ad genome into RNA. Perhaps antiviral antibodies in the tonsil tissues limit the spread of virus and account for persistent infections and the difficulty of direct isolation of Ads from tonsils.

All tonsils that were positive by blotting contained whole Ad2 DNA molecules, as indicated by the presence of six *Eco*RI fragments. However, other fragments were also observed that did not comigrate with fragments of Ad2 or of Ad1, -5, or -6. These atypical fragments could represent unusual recombinants between Ad2 and another group C Ad serotype or perhaps Ad sequences integrated into cellular DNA. This latter possibility, together with the demonstration that Ad transforming sequences are transcribed in tonsils, strengthens the possibility that the Ads could be involved in human cancer.

Table 4. Summary of analyses of human cancer and normal tissue DNAs for group C Ad DNA sequences, with the complete Ad2 genome as probe

DNA	No. tested*	% hybridization†
Normal tissues:		
Lung	12 (0)	−0.2 ± 1.5
Digestive system		
Colon	18 (0)	1.3 ± 0.8
Rectum	1 (0)	−3.1
Cancers:		
Lung		
Carcinoma	6 (5)	0.1 ± 0.5
Digestive system		
Carcinoma, stomach	4 (4)	0.7 ± 1.1
Adenocarcinoma, stomach	1 (1)	−0.1
Carcinoma, colon	13 (7)	0.4 ± 2.1
Adenocarcinoma, colon	2 (0)	−1.0
Adenocarcinoma, cecum	3 (1)	0.6 ± 1.6
Adenocarcinoma, cecum	1 (1)	2.3
Carcinoma, sigmoid colon	2 (2)	1.0
Carcinoma, rectosigmoid	1 (1)	−0.8
Carcinoma, rectum	5 (4)	−0.2 ± 2.2
Lymphoma		
Hodgkin, lung	5 (5)	−0.1 ± 0.8
Hodgkin, spleen	1 (0)	−0.9
Ileum, lymphoma	1 (1)	0.8
Kidney		
Hypernephroma	3 (0)	−0.7 ± 0.6
Carcinoma	1 (0)	−1.3
Breast	3 (2)	0.0 ± 0.7

These are the combined data from six separate experiments using six distinct Ad2 complete genome probes. Table 5 gives the probe and hybridization details as well as the reconstruction controls done simultaneously with the tumor DNA analyses.

* Numbers in parentheses indicate the number of tumor DNAs also assayed by using the *Hin*dIII-G fragment probe.

† Shown as mean ± SD. The self-hybridizing background of the probe has been subtracted, and the values normalized to maximal hybridization in the presence of 267 copies of Ad2 DNA per cell.

In studies of cancer tissue, with Ad5 *Hin*dIII-G fragment as probe, no Ad transforming sequences were detected in DNAs from 49 lung and 48 gastrointestinal cancers or in RNAs from 24 lung and 20 gastrointestinal cancers (both DNAs and RNAs were tested from 8 each of the lung and GI cancers, giving a total of 65 and 60 distinct lung and gastrointestinal cancers examined). The probes hybridized with DNAs and RNAs from Ad2- or Ad5-transformed cells, proving that they could detect integrated viral sequences. Reconstruction experiments indicated that the probes could detect about 0.05 copy of the *Hin*dIII-G fragment per tumor cell (stated another way, the probes could detect 1 copy of about 0.5% of the viral genome per cell). Because the group C Ad transforming region is >0.5%, our data argue strongly against group C Ads as etiological agents in 98% of lung or colorectal cancers. An additional 1 lung and 11 gastrointestinal cancer DNAs were also found to be negative with the complete genome as probe. These findings are significant, considering that almost everyone has been infected by these viruses and that they infect tissues of both the respiratory and digestive tracts.

Table 6. Analysis of human placenta RNA for group C Ad transforming sequences

RNA sample*	% hybridization: Ad5 *Hin*dIII-G fragment	% hybridization: Ad2 DNA
Full-term placenta	16.4	11.3
Full-term placenta	16.2	12.6
Full-term placenta	16.6	12.5
Full-term placenta	16.7	13.0
Fetal placenta	18.1	11.1
Fetal placenta	17.6	11.0
Fetal placenta	17.6	12.3
Controls:		
Yeast	15.8	10.8
Ad2 "late" infected KB cells	58.4	56.2

In vitro ^{32}P-labeled Ad5 *Hin*dIII-G fragment (6.1×10^7 cpm/μg) or Ad2 DNA (1.6×10^8 cpm/μg) was hybridized with RNAs to an equivalent C_rt of 17,000. Each 50-μl reaction mixture contained 1000 cpm of probe and 5 mg of placenta or yeast RNA per ml or 1 mg of Ad2 late infected KB cell RNA and 4 mg of yeast RNA per ml. The data have not been corrected for self-hybridization of the probe or normalized.

* The fetal placenta samples represented 2- to 3-month fetuses and included all the tissues of the fetus.

Group C Ads readily become latent in lymphoid tissues, and Ad transforming sequences are expressed as RNA. We assayed DNAs from five Hodgkin and seven non-Hodgkin lymphomas by using the ^{32}P-labeled *Hin*dIII-G probe. The same five Hodgkin and one non-Hodgkin DNA, plus one additional non-Hodgkin DNA were also examined by using the entire genome as probe. No Ad sequences were detected. Thus, these viruses are not obligatory causes of lymphoid cancer.

In conclusion, although group C Ads are ubiquitous and have oncogenic properties, there is no evidence that they cause human cancer. However, the cancers we have tested account for only about 50% of the types found in the United States (23). Additional studies are required to evaluate whether they may be a major cause of a cancer type that we have not examined or whether they may be a rare cause of lung, gastrointestinal, or lymphoid cancer. For example, in view of the latency and

Table 5. Reconstruction hybridization controls for the tissue DNA analyses summarized in Table 4

Exp.	Probe specific activity, cpm/μg	Equivalent C_0t	Self-hybridizing background U	Self-hybridizing background C	0.2 copy Ad2 DNA U	0.2 copy Ad2 DNA C	1 copy Ad2 DNA U	1 copy Ad2 DNA C	267 copies Ad2 DNA U	267 copies Ad2 DNA C	8617 cell DNA U	8617 cell DNA C
1	1.1×10^8 (^{32}P)	3.0×10^4	21.7	0	ND	ND	68.1	49.4	94.0	100	ND	ND
2	1.5×10^8 (^{32}P)	3.0×10^4	11.2	0	ND	ND	75.5	68.6	93.7	100	35.4	25.8
3	5.0×10^8 (^{32}P)	2.5×10^3	7.6	0	ND	ND	43.1	39.1	90.7	100	31.0	25.8
4	2.1×10^8 (^{32}P)	2.5×10^3	7.3	0	ND	ND	46.1	45.2	85.8	100	26.2	22.0
5	1.1×10^8 (^{32}P)	2.7×10^3	9.8	0	ND	ND	50.3	44.5	91.1	100	30.1	22.3
6	8.0×10^6 (^{3}H)	2.0×10^3	16.7	0	32.6	16.6	68.8	54.4	95.7	100	39.9	24.2

* U and C designate uncorrected and corrected hybridization values, respectively. Hybridization values were corrected by subtracting the self-hybridization background of the probe and normalizing to maximal possible hybridization with 267 copies of Ad2 DNA. ND, not done.

expression of the Ad2 transforming region in human tonsils, it is important to analyze tumors of the buccal cavity and pharynx, which cause more than 20,000 deaths in the United States each year.

Our *Hin*dIII-G fragment probe hybridizes only 5–10% with DNAs of human Ad serotypes outside of group C (2), so these data are not applicable to non-group C Ads. In other studies similar to those reported here, we were unable to detect sequences of group A (24), group B (25), or group E (26) Ads or of human BK virus (27) in human cancer DNAs. Therefore, we have found no evidence as yet that Ads or papovaviruses are involved in human cancer.

Jones *et al.* have reported (21) that RNAs from 10 of 10 human placentas, 1 adult human liver, and several gorilla tissues contain sequences homologous to the four Ad2 DNA regions including the transforming region. Because these were normal tissues with no history of Ad2 infection, this finding, if true, would have profound implications. The methods used by Jones *et al.* included (*i*) hybridization of ^{125}I-labeled tissue RNA with Ad2 DNA immobilized on nitrocellulose filters and (*ii*) *in situ* hybridization with ^{3}H-labeled Ad2 DNA. These methods are not in common use, and it is difficult to evaluate their reliability and sensitivity. By our sensitive and well-characterized liquid hybridizations using either Ad5 *Hin*dIII-G or Ad2 complete genome as ^{32}P-labeled probe, we did not detect Ad sequences in human RNAs or DNAs from 4 full-term or 3 fetal placentas, 44 malignant tissues, or 23 tonsils. On the basis of our reconstruction experiments, we conclude that human placentas or other tissues do not contain Ad-specific RNA or DNA sequences or, if they do contain them, they are present at less than 1 copy per 10–20 cells. However, it is conceivable that human cells contain DNA sequences only partially related to Ad DNA that were not detected under our relatively stringent hybridization conditions.

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