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# Genomic Variation of Adenovirus Type 5 Isolates Recovered from Bone Marrow Transplant Recipients

DOUGLAS H. WEBB,[1] ANTHONY F. SHIELDS,[2,3] AND KENNETH H. FIFE[1,4]*

*Department of Medicine[1] and Department of Microbiology and Immunology,[4] Indiana University School of Medicine, Indianapolis, Indiana 46223, and the Fred Hutchinson Cancer Research Center[2] and Department of Medicine,[3] University of Washington, Seattle, Washington 98105*

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**We characterized the genomic variation of adenovirus type 5 isolates recovered from bone marrow transplant recipients in Seattle between 1976 and 1982. By restriction endonuclease analysis, we identified three new adenovirus genomic variants, each associated with a single invasive adenovirus infection. In addition, we were able to obtain suggestive evidence for a nosocomial spread of a particular group of isolates within this population. This study demonstrates that the technique of restriction endonuclease analysis is an important epidemiological tool for investigating viral infections.**

A systematic review of adenovirus infections occurring after bone marrow transplantation in Seattle over a 6-year period showed an overall incidence of adenovirus infection of at least 4.9% (8). The distribution of adenovirus types (or species) found in these patients was distinct from that found in community surveys, showing a higher proportion of adenovirus type 5 (Ad5) (subgenus C) and a group of the closely related Ad11, Ad34, and Ad35 (subgenus B) in the bone marrow transplant patients. It was felt that the source of infection in most cases was endogenous viral reactivation as a result of posttransplant immunosuppression.

We now report the molecular characterization (as determined by restriction endonuclease analysis) of the Ad5 isolates obtained from these marrow transplant patients and relate these findings to the clinical illnesses present.

## MATERIALS AND METHODS

The population of patients studied, as well as the methods and materials used for pathological and virological analysis, has previously been described (8). Viral stocks were frozen at −70°C. DNA analysis was performed as previously described (5). Briefly, virus was grown in 1-cm$^2$ wells of confluent HeLa cells until the cytopathic effect was extensive. Viral DNA was extracted from the infected cells by a modification (9) of the Hirt procedure (6). The DNA was concentrated by ethanol precipitation and then digested with the restriction endonuclease *Eco*RI, *Hpa*I, *Xho*I, *Hin*dIII, *Kpn*I, or *Sma*I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) under conditions specified by the supplier. After digestion, the DNA was loaded onto a 0.7% agarose gel and run at 80 V for 75 min in a 50 mM Tris-borate-EDTA (pH 8) buffer system. Bands were visualized by being stained with 0.25 μg of ethidium bromide per ml and inspected under UV light. Stained gels were photographed on Polaroid type 57 film through a Wratten 23A filter.

## RESULTS

DNA analysis by restriction endonuclease digestion in conjunction with agarose gel electrophoresis was performed on isolates from 14 of the 18 patients identified as having Ad5infections (8). Among the other four patients not included in the analysis, three had been typed initially by neutralization and were no longer available for DNA analysis, and the remaining patient had two distinct types (Ad2 and Ad5) that were identified from the same specimen.

Type determination was initially made by microneutralization analysis as previously described (5, 8) and confirmed by the restriction endonuclease cleavage patterns generated by *Sma*I digestion. All 14 isolates showed *Sma*I digestion patterns identical to that of the Ad5 prototype (Table 1). Five isolates showed digestion patterns similar to that of prototype Ad5 for each of four additional restriction endonucleases (*Eco*RI, *Hpa*I, *Hin*dIII, and *Kpn*I). The seven isolates of group 2, designated the major variant group, all showed an aberrant cleavage pattern for each of these latter four enzymes. Two other isolates differed in cleavage patterns from both those of the prototype and major variant and were different from each other (Table 1; Fig. 1).

Also summarized in Table 1 is the cleavage pattern of a previously described genomic variant isolated in Prague, Czechoslovakia, in the autumn and winter season of 1978 and 1979 and designated Ad5a by Bruckova et al. (3). None of our isolates had a cleavage pattern identical to that reported for this variant.

Cleavage maps were constructed for each of the three new adenovirus genomic variants (Fig. 2), which are designated subtypes Ad5b, Ad5c, and Ad5d. Loss of a cleavage site was inferred by the loss of two fragments known to be adjacent in the prototype, coupled with the appearance of a new fragment with an apparent molecular weight equal to the sum of the missing fragments. New cleavage sites were mapped by redigesting overlapping fragments from the region where a new site was located. The locations of added or deleted sites were confirmed by performing double digests with enzymes *Hpa*I, *Kpn*I, *Hin*dIII, and *Sma*I individually and in various combinations.

The clinical courses of the 14 patients from whom the isolates were obtained were reviewed and compared with the particular genome type of Ad5 isolated. Three patients (unique patient numbers 1405, 1571, and 1624 [8]) had invasive adenovirus infections, and the virus isolated from each showed one of the variant patterns described. All three patients died from pneumonia; the patients from whom Ad5c

* Corresponding author.

TABLE 1. Summary of restriction endonuclease cleavage patterns

| Enzyme | Cleavage pattern for adenovirus group (no. of patients)[a] | | | | |
|---|---|---|---|---|---|
| | Ad5 (5) | Ad5b (7) | Ad5c (1) | Ad5d (1) | Ad5a[b] |
| *Sma*I | P | P | P | P | P |
| *Eco*RI | P | A | P | A | P |
| *Hpa*I | P | A | B | A | A |
| *Xho*I | P | P | P | P | P |
| *Hin*dIII | P | A | A | A | A |
| *Kpn*I | P | A | A | B | P |

[a] P, Prototype pattern; A, major variant pattern; B, second distinct variant pattern.
[b] As described by Bruckova et al. (3).

and Ad5d were isolated (1571 and 1624) yielded autopsy findings by routine histology, immunohistology, and culture of lung tissue suggesting adenovirus pneumonia. These two patients also manifested graft-versus-host disease. The third patient, whose adenovirus isolate was among the major variant group (Ad5b), also died from pneumonia, but lung tissue was not obtained, and no autopsy was performed. Adenovirus was grown from a duodenal aspirate, and the patient was shown to have gastric erosions thought to represent adenovirus infection in the gastrointestinal tract. Whether adenovirus contributed to the pneumonia and subsequent death of the patient is uncertain.

All patients in the study received similar types of immunosuppressive drugs, although several different protocols were used. Levels of immunosuppression among patients with invasive adenovirus infections appeared comparable to those of patients without invasive adenovirus infections.

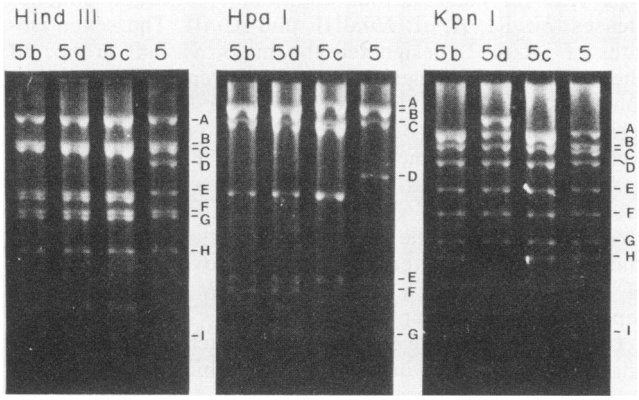


FIG. 1. *Hpa*I, *Kpn*I, and *Hin*dIII digestion patterns for the three new Ad5 genomic variants. The lanes are labeled with the subtype designations proposed in the text. The far right lane in each restriction endonuclease group shows prototype Ad5 (labeled 5), and the letters to the right of the gels refer to the fragment designations for prototype Ad5 (for comparisons between prototype and variants). The *Hin*dIII digests show a loss of the D fragment in Ad5b, Ad5c, and Ad5d (due to the presence of an additional cleavage site); the *Hpa*I digests also show a loss of the D fragment in the three genomic variants (due to an additional cleavage site) and displacement of the B fragment downward in Ad5c due to a change in the cleavage site between the B and F fragments (producing a smaller-molecular-weight B fragment); the *Kpn*I digests show the displacement of the C fragment upward (higher molecular weight) in Ad5b, Ad5c, and Ad5d due to loss of the cleavage site between the C and I fragments. In addition, Ad5d shows an A fragment of higher molecular weight due to loss of a cleavage site between the A and H fragments.

Also, there appeared to be essentially no difference in the degree of immunosuppression between the five patients with prototype Ad5 isolated and the nine patients with Ad5 genomic variants isolated.

All five patients whose isolates had enzyme patterns similar to that of prototype Ad5 had adenovirus that was first isolated over a 15-day period in late February and early March 1982. These five patients were all hospitalized at the Fred Hutchinson Cancer Research Center for various periods between December 1981 and April 1982. At no time during the 5-month period were all five patients hospitalized at the same time, but before and during the 15-day period, all were seen by many of the same staff members. Three of these patients had viruses that were isolated during hospitalization, and the other two were tested as outpatients. The timing of the isolates and hospitalizations is shown in Fig. 3.

## DISCUSSION

We have identified nine human adenovirus isolates from bone marrow transplant recipients which have neutralization antibody patterns typical of Ad5 but whose restriction endonuclease cleavage patterns are heterogeneous and distinct from those of prototype Ad5. These isolates fall into one major and two minor groups that differ from the isolates of prototype Ad5 with respect to the patterns of three or more enzymes in different regions of the genome; the nine isolates also appear distinct from Ad5a. We propose that these new isolates be designated genome types 5b, 5c, and 5d as defined by the maps shown in Fig. 2.

Ad5a was linked to an outbreak of respiratory disease in Prague, Czechoslovakia, in 1978-1979 (3) and was distinctive in that it was associated with a relatively high percentage of lower respiratory tract infections, mostly in the form of

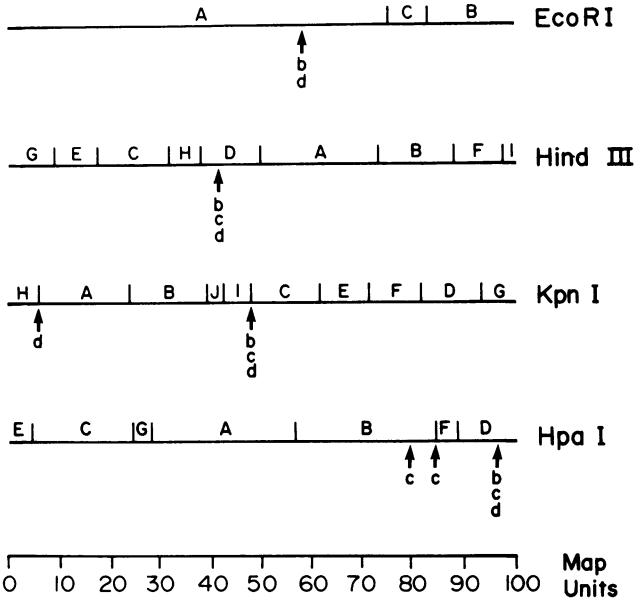


FIG. 2. Cleavage maps for the new adenovirus genome types for the restriction endonucleases *Eco*RI, *Hin*dIII, *Kpn*I, and *Hpa*I. The prototype maps and fragment designations are shown for Ad5 above each line as summarized by Broker (1). The arrows show the positions of difference in the cleavage sites for the genome types. Arrows at an existing site imply deletion of the site in the genome type; arrows away from an existing cleavage site imply a new site. The letters at the arrows refer to the genome type(s) having that specific change.

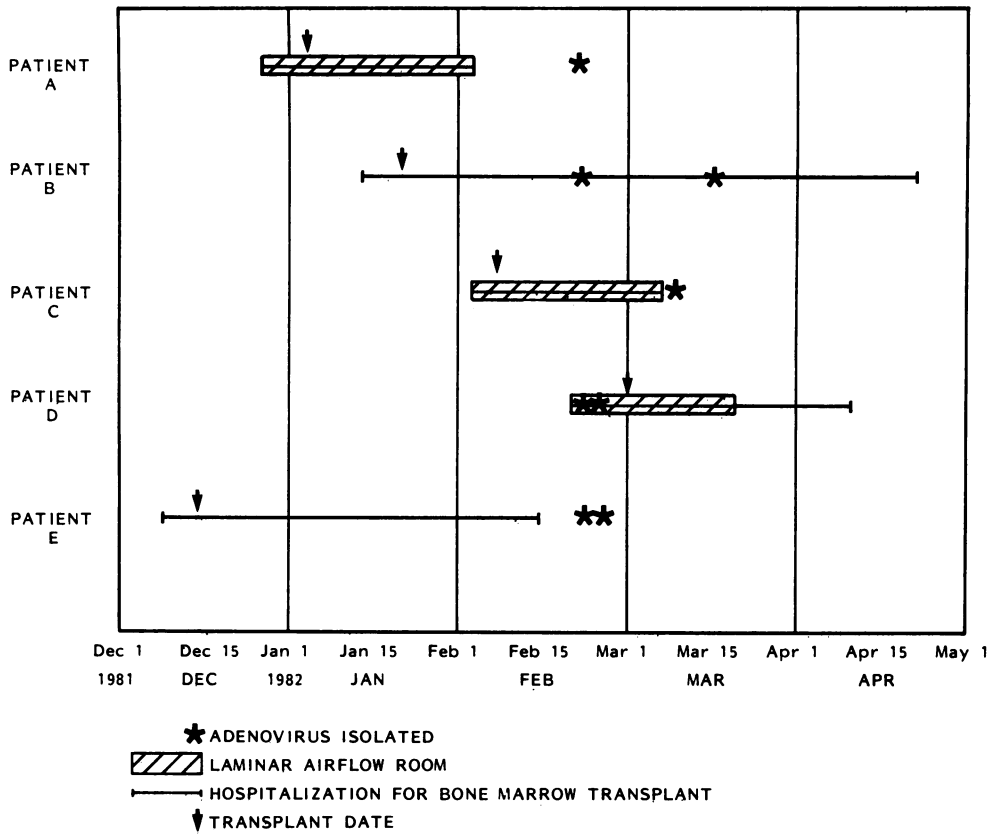


FIG. 3. Summary of possible nosocomial outbreak of prototype Ad5 infection. The timing of the adenovirus isolation is shown in relation to time of hospitalization, use of rooms with laminar airflow, and time of bone marrow transplantation. Patients A and C spent their entire hospital stays in laminar airflow rooms.

bronchopenumonia, and occurred primarily in younger patients (mean age, 4.5 years). The restriction endonuclease analysis of the Ad5a isolates differed from that of the Ad5 prototype with respect to three restriction endonucleases. *Bam*HI yielded two additional cleavage sites, whereas *Hin*dIII and *Hpa*I each yielded one extra site. The Ad5a digestion patterns for *Hin*dIII appeared identical to those for our new variants Ad5b and Ad5d but distinct from Ad5c. The Ad5a *Hpa*I digestion pattern appeared identical to those for Ad5b, Ad5c, and Ad5d. The *Bam*HI pattern for Ad5a was distinct from those of our new variants.

There is no well-established definition of a genome type or variant of adenovirus. We previously elected to use the criterion (as for the Ad1 and Ad2 genomic variants previously described [4]) that "changes in the cleavage patterns must be demonstrable for two or more restriction endonuclease cleavage sites that are separated by more than 1% of the genome" (4). The previously described variant (Ad5a) for Ad5 fulfills this criterion, as do our three newly described variants when compared with prototype Ad5, although Ad5d differs from type Ad5b only by the addition of one *Kpn*I cleavage site (Fig. 2) and thus could be considered as a minor variant of Ad5b.

The identification of genomic variants of adenovirus is important for two reasons. First, the DNA cleavage patterns can be used as strain markers in epidemiological studies, as previously described by Wadell et al. (12, 13) for Ad7b, and second, some genomic variants seem to be associated with enhanced virulence. Some cleavage site alterations among our isolates may, in fact, be markers for virulence, as suggested by the unique cleavage site addition in our Ad5d variant and two distinctive cleavage site additions in our Ad5c variant. Both variants were associated with an invasive adenovirus infection. Host susceptibility to invasive adenovirus infection clearly seems to play a major role as well, and larger surveillance studies within specific populations, with restriction endonuclease analysis of viral genomes, are needed to be certain that these variants are consistently associated with invasive infection.

Most of the patients with Ad5 infections were seropositive before the transplantation (as shown by 12 of 15 available frozen sera), suggesting viral reactivation as the major source of infection (D. T. Purtillo, R. White, A. Filiprovich, J. Kersey, and L. Zelkowitz, Letter, N. Engl. J. Med. **312**:1707–1708, 1985), although susceptibility to exogenous infection may have been altered by posttransplant immunosuppression. That all of the Ad5 prototype isolates were cultured within a 15-day period suggests a common source for this particular group of isolates. Possibilities include nosocomial spread by hospital patients or staff or contamination in the laboratory. The last possibility seems unlikely because three of the patients had two isolates, which were obtained at different times. Furthermore, one of the patients from Swedish Hospital (the other hospital involved in this study) had a different Ad5 genomic variant that was isolated during this same period and cultured in the same lab. Spread of the virus on the ward seems most likely, but even this is somewhat hard to reconcile. Patients A, B, and E were on the ward together in late January and early February. Patients B and E were in adjacent (non-laminar

airflow) rooms, but patient A was in laminar airflow throughout his hospitalization. Also, patients A and E had viruses that were isolated 1 to 3 weeks after discharge. Both patients C and D were in rooms with laminar airflow during most of their hospitalization and had viruses that were isolated while these patients were in the hospital. The most likely common route of infection was respiratory spread by a staff member, although there is no direct evidence for this mode of transmission. We have no clinical or laboratory data on hospital staff illness during the time of the prototype adenovirus isolations from our bone marrow transplant patients to indicate a similar outbreak among hospital staff. Nosocomial spread of Ad5 has not been previously reported, and the evidence for nosocomial spread among our cluster of patients is only suggestive.

Nosocomial spread of Ad7 has been reported by several investigators (2, 7, 10, 11, 14). The most recent and best documented study was by Straube and colleagues (11), who reported an outbreak of Ad7b in a hospital for children in San Diego, California, in 1980. Four of six patients infected with Ad7b died, and all had underlying respiratory disease. All 11 hospital employees from whom Ad7 was cultured were nurses working in a unit with an infected patient. A large number of employees developed clinical illness, including all 11 with positive cultures.

In conclusion, we have characterized three new Ad5 genomic variants recovered from bone marrow transplant recipients. Each of these variants was associated with an invasive adenovirus infection, and it is postulated that these variations in the Ad5 genome may be related to virulence, at least in an immunocompromised host.

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