Cross-reactivity between Enteric Adenoviruses and Adenovirus Type 4: Analysis of Epitopes by Solid-phase Immune Electron Microscopy

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(Accepted 15 August 1983)

SUMMARY

The immunological relationships between the two newly discovered serotypes of enteric adenoviruses, Ad40 and Ad41, and antisera to human adenoviruses representing all subgroups were studied by solid-phase immune electron microscopy. A pronounced two-way cross-reaction was seen between Ad40 (subgroup F) and Ad41 (subgroup G). Furthermore, a distinct one-way cross-reaction was noted between adenovirus type 4 antiserum (subgroup E) and virions of Ad40.

Adenoviruses which were difficult to cultivate in cell cultures were first identified by electron microscopy in stools from children with acute gastroenteritis (Flewett et al., 1975). They are designated enteric adenoviruses (Retter et al., 1979) and can be detected by electron microscopy, immunological techniques, such as immunoelectroosmophoresis and enzyme-linked immunosorbent assay (ELISA) (Jacobsson et al., 1979; Johansson et al., 1980), and by analysis of the virion polypeptide and DNA restriction pattern (Uhnoo et al., 1983; Wadell et al., 1980). Serological analysis has revealed two distinct serotypes, Ad40 and Ad41 (de Jong et al., 1983). In spite of a pronounced difference at the DNA level, Ad40 and Ad41 cross-react in haemagglutination-inhibition tests (de Jong et al., 1983). No relationship to the 39 known human adenovirus species has been found either by neutralization tests or by haemagglutination-inhibition tests (de Jong et al., 1983).

A solid-phase immune electron microscopy (SPIEM) technique utilizing Protein A for capturing specific antibodies (Svensson & von Bonsdorff, 1982) was used to study the interaction between surface antigens of enteric adenoviruses and different antisera against human adenoviruses representing all subgroups.

Stool specimens from infants, positive for adenovirus by electron microscopy, were further tested by an enteric adenovirus-specific ELISA (Johansson et al., 1980). Virions from Ad40 and Ad41 were obtained by propagation in strain 293 cells, an adenovirus type 5-transformed human embryonic kidney cell line (Takiff et al., 1981). Hyperimmune sera against Ad40 and Ad41 were produced by injecting rabbits intramuscularly with 25 μg of purified virions in Freund’s complete adjuvant. An intravenous booster (10 μg) was given 5 weeks later. The rabbits were bled by heart puncture a week later. Hyperimmune sera against virions of Ad31 (subgroup A), Ad3, 7, 11, 16, 21 (subgroup B), Ad1, 2, 5, 6 (subgroup C), Ad19 (subgroup D), and Ad4 (subgroup E) were used. All antisera had complement fixation titres of 128 to 512.

SPIEM was performed as described by Svensson & von Bonsdorff (1982). The optimal concentrations of Protein A and antisera were determined by box titrations. The highest virus-trapping efficiency was achieved with a Protein A concentration of 50 μg/ml and 1:100 serum dilution. Normal rabbit serum was used as a serum control at a 1:100 dilution. Briefly, carbon-Formvar-coated grids (400-mesh) were floated for 20 min on 10 μl (50 μg/ml) drops of Protein A (Pharmacia) and then drained. Thereafter, the grids were floated on 20 μl drops of diluted...
antiserum for 20 min. The antibody-coated grids were washed with 10 drops of phosphate-buffered saline (PBS), drained and finally floated for 1 h at room temperature on 20 µl virus suspension (diluted to a suitable concentration after preliminary experiments). Before staining with 3% phosphotungstic acid (pH 7) the grids were washed with 10 drops of PBS and 10 drops of distilled water. The result was read by counting the number of virions for 5 min in the electron microscope (Philips 300) at a magnification of 28000 ×. Mean values from four grids are presented.

An extensive reciprocal cross-reaction was seen between adenovirus types 40 and 41 (Fig. 1). This observation was confirmed by examination with three different antisera to each virus type. Ad40 and Ad41 virions showed no heterotypic reaction with antisera to representatives of subgroup A (Ad31), subgroup B (Ad3, 7, 11, 16, 21), subgroup C (Ad1, 2, 5, 6) or subgroup D (Ad19). This is in agreement with results reported for haemagglutination-inhibition and neutralization tests (de Jong et al., 1983).

Interestingly, Ad40 virions gave a distinct reaction by SPIEM with Ad4 antiserum, which is the only member of subgroup E (Fig. 1, 2). This reaction was not seen with Ad41 virions (Fig. 1). This observation was confirmed by examination with two different Ad4 antisera. On the other hand, Ad4 virions showed no heterotypic reaction with Ad40 or Ad41 antiserum.

SPIEM is a useful technique for detection of viruses which are difficult or impossible to propagate in cell cultures. The technique is also of value in detecting epitopes exposed on the surface of virions. This report has demonstrated the occurrence of determinants that are shared between the enteric adenoviruses Ad40 and Ad41. It is also of substantial interest that Ad40 exposes epitopes that are common to adenovirus type 4, which is the only member of subgroup E.

Ad4 is the human adenovirus which displays the broadest cross-reactivity with other human adenoviruses. It shares properties with subgroup B members in the composition of the T-antigen (Huebner, 1967) and the immunological reactivity of the vertex capsomer (Wadell & Norrby, 1969). Ad4 also shows cross-reactivity with Ad16 as measured in the neutralization test (Norrby & Wadell, 1969). The immunological cross-reactivity (Wadell & Norrby, 1969) and the haemagglutination property (Wadell, 1969) are, however, closely related to adenoviruses belonging to subgroup C. Furthermore, Ad4 is the only adenovirus type where the early antigens
cross-react with type-specific immune sera of members of all tested subgroups (Gerna et al., 1982). In this report we demonstrate that the enteric adenoviruses Ad40 and Ad41 can be distinguished from all tested adenoviruses by SPIEM and that Ad40 but not Ad41 exposes epitopes on the virion that are shared with Ad4.

Fig. 2. Demonstration of cross-reactivity between enteric adenoviruses and Ad4 by SPIEM. The figure shows trapping of enteric adenovirus 40 particles on grids pre-coated with Protein A and antisera to (a) Ad40, (b) Ad41 and (c) Ad4; (d) serum control. Bar marker represents 600 nm.
REFERENCES


(Received 20 June 1983)