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Enteric Adenoviruses

Brief Review

By

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Introduction

The use of the electron microscope in the investigation of childhood diarrhoeas since the early 1970's has revolutionised our understanding of their aetiologies. On the basis of morphology alone, a host of viruses have been discovered, e.g. Norwalk agents, rotaviruses, astroviruses, caliciviruses, coronaviruses and small round virus (srv) particles (27). Frequently, adenovirus particles were visualised in the diarrhoeal stools. However, their aetiological significance was not known, as adenoviruses were isolated in culture from both diarrhoeal cases and matched controls with equal frequency (20, 52). Soon, it became apparent that most of the adenoviruses visualised in the diarrhoeal stools by electron microscopy, could not be cultivated in the cell culture systems normally used to cultivate adenoviruses (5, 28, 48). These viruses were referred to as fastidious or enteric adenoviruses (14, 19, 33).

Several studies which will be described later, have established enteric adenoviruses (E Ads) as definite pathogens in the causation of diarrhoea. Although some of the established adenovirus serotypes are also implicated in diarrhoea (14, 19, 33), the ensuing discussion will be limited only to E Ads.

Morphology

The appearance of E Ads under EM does not differ from that of the established adenoviruses. A limited structural study of some E Ad strains showed an icosahedron capsid with a diameter of 70—80 nm and fibres 28—33 nm long, which are typical of established adenoviruses (10). However,

more structural studies are required for complete characterisation of the virus.

Purification and Buoyant Density

E Ads can be extracted from either stool or tissue culture fluids by genetron 113, and purified by centrifugation through a sucrose cushion and then isopycnically in a caesium chloride gradient. Complete particles form a band at a density of 1.337 g/ml, whereas incomplete particles form a band at a density of 1.229 g/ml (4, 7, 39).

Growth Characteristics

KB, Hela, human amnion and human embryonic kidney are some of the cell lines used to cultivate and serially passage established adenovirus serotypes. Although some strains of E Ads produce an adenovirus-like cytopathogenic effect (CPE) on primary inoculation into these cell lines, they can not be serially passaged in them (8, 10). Most strains undergo an abortive infection in a very small proportion of cells (<1 of 10,000 cells), which can be demonstrated by specific immunofluorescent staining (39). Takiff and Straus (40) investigated the reasons for the lack of growth of E Ads in KB and Hela cells. Their data suggested an early replicative block in the growth cycle of the virus. However, there is an indication that E Ads express some early synthetic functions as they help adeno associated viruses (a group of defective parvoviruses) to replicate in these cells: these viruses normally require early helper functions from established adenoviruses for their growth.

Several E Ads have now been grown in Graham 293 cells (8, 26, 39), Chang conjunctival cells (22, 50), Hep-2 cells (10), tertiary monkey kidney cells (10) or in HT-29 cells (42). However, Graham 293 cells are most widely used for diagnostic work, probably due to their higher susceptibility to E Ad infection. It is a human embryonic kidney cell line transformed by a type 5 adenovirus (15). It is likely that early adenovirus 5 gene functions expressed in Graham 293 cells may be providing helper functions for E Ads. However, this does not appear to involve modification to the E Ad genome, as viruses grown in this cell line maintain their original host range and DNA cleavage patterns (39) (see section on genome profiles).

De Jong *et al.* (10) conducted a detailed study on the growth characteristics in different cell lines of 200 strains of E Ads obtained from different countries. They found variations within laboratories in the growth of different strains, and between laboratories in the growth of the same strains. They also found differences in cell line susceptibility to virus growth in different batches of the same cell line obtained from the same company. This may explain the difficulty of growing Ad40 strains in Graham 293

cells by some workers (9, 42), but not by others (8). These results suggest that caution should be exercised in comparing the results of various laboratories when different strains of viruses and different batches and types of cell lines are used. Other interesting observations also emerged from the study. Some viruses multiplied in the cells without producing a CPE. This suggests the need for blind passages until a CPE becomes apparent, or the need to look for evidence of growth by specific immunofluorescence. It was also observed that incorporation of trypsin in the maintainance medium and rolling of cultures with tertiary cynomolgous monkey kidney cells enhanced virus growth. Also notable was that Chang cells and 293 cells produced CPE more readily when incubated in stationary culture than in roller culture. These observations should establish guidelines for optimal culture conditions for E Ads.

Taxonomy and Classification

There are 39 serotypes of conventional or established adenoviruses, which are further subdivided into 5 subgroups A, B, C, D and E (42). By neutralisation test, DNA-DNA hybridisation test and restriction endonuclease analysis of viral DNA, E Ads are shown to be distinct from the established serotypes (10, 42). Furthermore, the same range of tests have shown that there are at least two subgroups among E Ads. Pending proper classification by the International Committee on Taxonomy of Viruses, they are provisionally assigned into two new subgroups F and G. Viruses in these two subgroups belong to two new serotypes 40 (Ad40) and 41 (Ad41), respectively (10, 42). Ad40 and Ad41 are represented by two prototype strains, Dugan and Tak, respectively. Both these strains were isolated in Holland from children with diarrhoea (10). A strain Hovi-X was subsequently isolated from a child with diarrhoea in Helsinki, and was shown to be similar to Dugan. Therefore, DE JONG *et al.* (10) proposed to refer to Dugan-Hovi-X-like viruses as Ad40, and Tak-like viruses as Ad41.

Genome Profiles

Adenoviruses are double-stranded DNA viruses. Ad40 and Ad41 exhibit genome profiles which are distinct from each other and from the established adenovirus serotypes, when their DNA is cleaved with restriction endonuclease SmaI and the fragments separated by gel electrophoresis. SmaI cuts at the sequence 5′ CCC-GGG and generates 9 fragments for Ad40 strains and 11—12 fragments for Ad41 strains without producing any co-migrating bands between the two types (42, 47).

Detailed restriction endonuclease analysis of KIDD *et al.* (25) and KIDD (24) using a combination of restriction enzymes have shown the existence of genome variants among Ad40 and Ad41 strains. When 26 Ad40 strains from

3 different continents were studied, all of them had genome profiles typical of Ad40 after analysis with endonuclease SmaI. However, when further analysed with six other enzymes, namely BamHI, EcoRI, BglI, XhoI, SstI and HindIII, at least two different restriction profiles were evident for each enzyme. Nineteen strains had the same combination of profiles for all the enzymes. In the remaining 7 strains there were five other genome types. These variants had a different distribution pattern in different continents. Of particular interest was that strains Dugan and Hovi-X, which are indistinguishable in neutralisation test (10) had different genome profiles with all the restriction enzymes except SmaI. Based on these results, KIDD *et al.* (25) proposed that Ad40 strains with genome profiles of Hovi-X virus be called Ad40 and its variants be given letter designations such as Ad40a, Ad40b etc.

Likewise, when Ad41 strains isolated from 3 different continents were studied for genome variation, all test strains had a profile typical of Ad41 with the restriction enzyme SmaI. However, the prototype strain Tak had a profile slightly different from those of the test strains. Therefore the test strains were called 41a, variants of reference strain Tak. With the enzymes BamHI, BglI, SstI, PvuII and XhoI, all the test strains had identical patterns. On the otherhand, with a second set of endonucleases, HindIII, EcoRI, BstEII and KpnI, all strains from two continents (Europe and North America) and only one of 7 strains from the other continent (Africa) displayed identical patterns. Moreover, with the latter combination of enzymes, the remaining 6 strains from Africa proved to be 4 different variants of Ad41. Therefore, as with Ad40 and its variants, KIDD (24) proposed that Ad41 strains with Tak-like genome profile be called Ad41 and its variants be called Ad41a, Ad41b etc.

It is obvious that restriction endonuclease analysis of viral DNA with a combination of enzymes gives a detailed epidemiological information about the virus. What the genome variants mean in terms of antigenicity is not known. The fact that in spite of the genome variation, serotype as determined by neutralisation test using polyclonal sera remains the same, indicates that the region of the genome coding for the neutralisation antigen is conserved. The proof for this has to come from sequencing studies. In the meantime, more studies are needed to determine the geographic and temporal occurrence of Ad40 and Ad41 and their genome variants.

Cloning and Mapping of Genomes

TAKIFF *et al.* (41) have cloned several fragments of genomes of an Ad40 and an Ad41 strain. The fragments were generated by restriction enzymes, SmaI, EcoRI and BamHI. The cloned fragments were nick translated and used in hybridisation experiments to identify homologous fragments

in virion DNAs cleaved with different enzymes. Digestion of the plasmid-E Ad DNA with enzymes other than those used in cloning or with double enzymes simultaneously, permitted localisation of the cleavage sites for other enzymes within the cloned fragments. The orientations of E Ad genomes were determined by hybridisation with the genome of a type 2 adenovirus. Physical maps constructed in this manner permitted examination of homologies among E Ads and type 2 adenovirus. The data revealed substantial homology between Ad40 and Ad41, but considerably less homology between E Ads and type 2 adenovirus.

Similarly, ALLARD *et al.* (1) cloned several BamHI fragments of the genome of an Ad41 strain. Two of these clones were useful for the detection of adenoviruses: one clone detected all adenoviruses and the other only Ad41. The Ad41 specific clone corresponded to the 5′ portion of early region 1a, the region involved in growth restriction in human embryonic kidney cell line. In addition, restriction maps were constructed for six different restriction endonucleases that included the two enzymes, BamHI and EcoRI used by TAKIFF *et al.* (41). The location of fragments less than one kilobase for BamHI map was at variance with the location of the same in the BamHI map for Ad41 published by TAKIFF *et al.* (41). This was thought to be due to the differences in the strains used in the two studies.

Antigenic Structure and Cross-Reactivity with Established Adenoviruses

No systematic study of the antigenic structure of E Ads has been undertaken. However, some information about their antigenic structure can be obtained from serological methods used to identify them, and by analogy with established adenoviruses. In established adenoviruses, the type-specific antigenic determinants exposed on the exterior of the hexon are responsible for neutralisation reactions, whereas the fibre antigens are responsible for haemagglutination (HA) reactions. In addition, these viruses possess a group-specific antigen, which is located on the interior of the virus capsid. Likewise, E Ads possess type-specific antigens as determined by neutralisation test (10) and type-specific ELISA (42, 43). In addition, they possess antigens for HA (10). Also, they share the group-specific antigen with established adenoviruses, since they react with antiserum directed against this antigen of established adenoviruses in complement fixation (CF) test, immunofluorescent (IF) test and group-specific ELISA (14, 19, 33).

Ad40 and Ad41 strains cannot be distinguished from each other when neutralisation test is carried out in Graham 293 cells or Chang conjunctival cells using antiserum against either serotype, but can be differentiated when the test is done in tertiary cynomolgous monkey kidney cells (10). The reason for this is not clear. Again, the two serotypes cannot be distinguished by haemagglutination inhibition (HI) test (10) or by solid phase immune

electron microscopy (SPIEM) (38). E Ads do not cross-react with established adenoviruses either in neutralisation test or in HI test (10). However, SVENSSON *et al.* (38) observed a one-way cross-reaction between adenovirus type 4 antiserum and Ad40 in SPIEM. Similarly with neutralisation test and HI test, GARY JR. *et al.* (14) demonstrated low levels of antibodies against adenovirus serotypes 11, 17, 32 and 33 in antisera to 3 partially purified, untyped and non-cultivable adenovirus isolates from diarrhoeal stools. However, the results of tests carried out without purified antigens and monospecific antisera may not be reliable. The presence of naturally occurring antibodies in the animals used for immunization, may also contribute to the reactions and make the interpretation of results difficult.

Structural Polypeptides

As a group, adenoviruses possess several structural proteins which can be resolved by SDS-polyacrylamide gel electrophoresis. These are polypeptides II, III and IV, which represent the hexon, vertex and fibre antigens respectively, and polypeptides V, VI and VII, which represent the internal structural proteins. In addition, there are other proteins such as VIII and IX, and several minor components of the above mentioned proteins (45). WADELL (45, 47) and WADELL *et al.* (46) found that adenoviruses can be classified into various serotypes and subgroups based on differences in the molecular weights of the structural proteins. In different serotypes of adenoviruses belonging to the same subgroup, the molecular weights for internal polypeptides V, VI and VII were the same, but were different for polypeptides II, III and IV. This classification scheme correlated well with other schemes based on neutralisation or HA tests, oncogenicity in new born hamsters, and GC content and sequence homology of viral DNA. The molecular weights of internal polypeptides of Ad40 and Ad41 were sufficiently different from those of established adenovirus subgenera A to E, thus warranting their classification into two new subgroups, F and G. The molecular weights of Ad40 polypeptides V, VI and VII were 46K, 25.5K and 17.2K, respectively, and for Ad41, 48.5K, 25.5K and 17.7K, respectively. These values were obtained by separation in 15 per cent polyacrylamide gels.

It is not known whether there is any molecular weight heterogeneity in the structural proteins of various Ad40 and Ad41 isolates. There is a need to investigate this possibility and to correlate any heterogeneity with the genomic variation.

Association with Disease and Clinical Symptoms

The prominent symptom of E Ad infections is diarrhoea. It may be associated with fever, vomiting or respiratory symptoms. The disease is

usually mild (9, 13) but fatal cases of adenovirus gastroenteritis have been reported (33, 49). The age groups mainly affected are infants and young children. Both males and females are affected. One study showed a preponderance of infection in females (43). The incubation period is approximately 7 days, and excretion of virus lasts for 10—14 days (42).

UHNOO *et al.* (43) carried out a detailed clinical study of 33 Swedish children with E Ad gastroenteritis. Fourteen children shed Ad40 virus and the remaining 19 shed Ad41 virus. The mean duration of diarrhoea in Ad40 infected children was 8.6 days as against 12.2 days in Ad41 infected children. Prolonged diarrhoea was common in association with Ad41 infection. One-third of these patients had symptoms for 14 days, and in 3 children the diarrhoea persisted for a month. The frequency of stools varied from 3 to 30 per day. The mean number of stools passed daily by Ad40 infected children was 9.7 as against 6.8 passed by Ad41 infected children. Fever was mild in all patients. Vomiting was also mild, and persisted for a mean of two days in all patients. Dehydration in both groups was mild and was isotonic in nature. Approximately 21 per cent of children in both groups had upper respiratory symptoms, which consisted of tonsillitis pharyngitis, otitis, coryza and cough. Either a similar or a lower frequency of respiratory symptoms was observed in several other studies (9, 13). The only exception was in a study conducted in hospitalised children in Baltimore, U.S.A., where the frequency was as high as 93 per cent (51).

In Uhnoo's study, a follow-up of 3 children showed that they had lactose intolerance for up to 5 to 7 months following E Ad infection. This suggests a possible role of E Ads in the aetiology of chronic diarrhoea and malnutrition in children. MAVROMICHALIS *et al.* (29) found evidence of malabsorption in 3 children with E Ad gastroenteritis using the D-xylose absorption test at the acute stage of the disease. They were not followed for a longer period.

Pathogenesis and Pathology

E Ads are excreted in large quantities of upto 10^{11} particles per gram of stool at the acute stage of the disease (14, 33). This suggests that they are actively multiplying in the gastrointestinal tract, most likely in the small intestinal mucosa. MAVROMICHALIS *et al.* (29) recovered adenovirus particles from the small intestinal fluids of 3 children with E Ad gastroenteritis. In addition, adenovirus particles were visualised within the nucleus of small intestinal mucosal cells in a fatal case of adenovirus gastroenteritis. In the same child, crystalline arrays of virus particles were observed in the small intestinal mucosa which is suggestive of their replication (49).

Respiratory symptoms in some children with E Ad gastroenteritis suggest possible viral multiplication in respiratory tract organs. This is similar to the possible respiratory tract involvement in some children with rota-

virus gastroenteritis. However, a study of 8 children conducted by PETRIC *et al.* (32) showed no evidence of viral multiplication in the respiratory tract. Obviously, a larger number of patients need to be studied to clarify the point.

In several cases of E Ad gastroenteritis, a serum antibody response to E Ad can be demonstrated by HI test, CF test, neutralisation test, immune electron microscopy or ELISA (9, 10, 43).

Epidemiology

E Ads have been detected in the stools of infants and young children with acute gastroenteritis in several studies conducted in industrialised countries (3, 5, 7, 28, 30, 33, 42, 43, 44). The incidence of infection varies between 4 and 12 per cent suggesting that they are the most frequent cause of viral diarrhoea next to rotavirus. However, one study stands out with an unusually high isolation rate of 52 per cent (51). This was conducted in hospitalised children in Baltimore, U.S.A., between October 1980 and the beginning of January 1981 (late fall). This is a period when diarrhoeal diseases are frequently associated with unknown aetiologies in this geographical area. This high isolation rate may have been due to the use of more sensitive virologic technique, differences in hospital environment and patient population or the chance occurrence of an epidemic during the study period. Ad40 and Ad41 have been isolated with approximately equal frequency in many studies (10, 42), although a study conducted in Canada showed a preponderance of Ad40 (8).

There are only three studies on the incidence of adenovirus gastroenteritis in developing countries. These included two studies from South Africa and one from Brazil. The South African studies involved only a small number of children over a short period. In the first study (36) adenoviruses that cannot be cultivated in conventional culture were found in 3 of 30 black children. In the second study (12), adenovirus particles were visualised in 14/66 cases in one hospital, and in 3/28 cases in another. Since no attempt at cultivation was made in this study, it is difficult to establish whether the viruses were E Ads. In the study from Brazil, E Ads were isolated from approximately 2 per cent of cases (26).

In some cases, concomitant infections with other organisms such as bacterial pathogens, astrovirus, calicivirus, established adenovirus serotype or rotavirus have been observed (7, 43). In such instances, it is difficult to establish the true aetiology of diarrhoea.

a) Seroepidemiology

KIDD *et al.* (23) carried out a serological survey of E Ad infection using sera from both developed countries (U.K. and New Zealand) and developing

countries (Hong Kong, Kuwait, Guatemala and Gambia). A total of 375 single sera were tested in a neutralisation test using a Glasgow strain of Ad40 (strain 26341-77). This is a variant of Ad40 and is designated Ad41a. This strain reacts equally well with antibodies against both Ad40 and Ad41 in their test system. The survey found that more than one-third of sera from the U.K., New Zealand, Hong Kong and Gambia possessed antibodies, as against none of 16 sera from Guatemala. Only 15 per cent of Kuwaiti sera were postive. One general trend noted was that the proportion of positive sera increased with increasing age. It was more than double in children aged 2 to 4 years than in children aged 2 years or less. Interestingly, 7 of 100 cord sera from Kuwait also had antibodies. Whether transplacentally acquired antibodies can confer protection during infancy remains to be seen.

In another study, high titres of antibodies in pooled sera from persons living in West Germany and the Netherlands were demonstrated (10).

Both these studies show that infection with E Ads is widespread both in developed and developing countries.

b) Seasonal Variation

In contrast to the winter peak of rotavirus infection, no striking seasonal variation has been observed with E Ad associated gastroenteritis. In a one year study conducted in Uppsala, Sweden, E Ad infections were found throught the year with two small peaks in summer and late winter (42). In a two year study in New York (35) and in a 9 year study in Washington DC (7), E Ad infections were found in every month of the year. These observations indicate that E Ad infections may be endemic in developed countries.

c) Outbreaks

The first reported outbreak of diarrhoea due to E Ads occurred in April 1974 in a long stay children's ward in the U.K. (13). It affected 6 children and a nursing staff. There was no secondary spread of the disease.

The second outbreak reported also occurred in the U.K. (49) and involved twin brothers, one of whom died. At necropsy, adenovirus particles were demonstrated in sections of the small intestinal mucosa of the diseased child and was recovered in monkey kidney cells. The stool of the twin who survived was positive for adenovirus, but the virus could not be cultured. This adenovirus was later identified as Ad41 (43).

In a third outbreak again reported from the U.K. (34), 17 children from 15 families living in an R.A.F. camp were affected. In 14 of the 15 families, the illness was confined only to children and did not spread to older siblings and parents. However, this did occur in the one remaining family. The adenovirus was later identified as Ad40 (43).

An Ad40 strain caused an outbreak of diarrhoea in an orphanage in Sopporo, Japan (9). Seven of the 11 children housed in a single room devel-

oped diarrhoea. However, all 11 seroconverted, as demonstrated by immune electron microscopy.

d) Mode of Spread

How the disease is transmitted is uncertain, although studies involving outbreaks indicate person to person transmission by contact (9, 34). Since the virus multiplies in the gastrointestinal tract, it may be transmitted via the faecal-oral route, and perhaps via the respiratory route if the respiratory tract is infected.

There is evidence of nosocomial spread of infection (30).

Despite earlier doubts about the role of E Ads in paediatric diarrhoea (20, 52) this has been established by the several studies described on the basis of the following: the observation of shedding of adenovirus particles during diarrhoea but not convalescence, exclusion of other causative organisms, and the documentation of a significant sero-response. However, perhaps undisputed proof could be obtained by studies of transmission using human volunteers and experimental animals.

Detection Methods

a) Electron Microscopy

E Ads are abundant in diarrhoeal stools. Their concentration is estimated to be 8 times that of the established adenoviruses (6). BRANDT *et al.* (6) found that if in direct EM viewing of 10 per cent stool suspensions, one or more adenovirus particles were found per minute of viewing, the adenovirus particles would most likely be enteric species. In this study, every 4 out of 5 stools that were positive for adenovirus contained E Ads. In other studies (6, 26, 33, 48), of the stools positive for adenovirus by EM, 45—79 per cent contained E Ads. Obviously, confirmation of visualised adenoviruses as enteric species has to come from other tests.

b) Immune Electron Microscopy (IEM)

The sensitivity of EM method can be increased by incorporating antiserum against any of the adenoviruses. The technique can be made specific for enteric species by the use of specific antisera (26).

c) Enzyme Immunoassay (EIA)

The presence of adenovirus particles can be detected in an indirect EIA, using antiserum against common hexon antigen. If capture antibody and detector antibody are directed against the common hexon antigen, the system will detect both established and enteric adenovirus serotypes. However, the assay can be made specific for E Ads, by using detector antibody specific for E Ads (2, 19, 42, 43).

d) Counter Immunoelectro-Osmophoresis (CIEOP)

All adenovirus serotypes can be detected by this technique using antiserum against the common hexon antigen. The test can be made specific for E Ads by incorporating specific antiserum (17).

e) Indirect Immunofluorescence Microscopy

Monolayers of human amnion cells infected with stool specimens containing adenoviruses, can be reacted with antiserum against common hexon antigen and stained with fluorescein conjugated anti-species immunoglobulin. Established adenovirus serotypes produce clusters of fluorescent cells due to the spread of virus to adjacent cells. In contrast, non-growing E Ads produce a characteristic single cell fluorescence with no spread to adjacent cells. The distribution of fluorescence is the same for both groups of viruses, being present in the nucleus with some additional granular regions in the cytoplasm. This method also compared favourably with electron microscopy in demonstrating virus in the stools (33).

f) Four Layer Radioimmunoassay

The presence of all adenovirus serotypes can be recognized by this technique using anti-hexon antiserum. Polystyrene beads coated with anti-hexon antiserum are used as the primary antibody, anti-adenovirus rabbit immunoglobulin as the secondary antibody, and ^{125}I labelled sheep anti-rabbit immunoglobulin as the indicator antibody. The test was found to be more sensitive than electron microscopy and was able to detect 1 ng of hexon protein per ml. With type-specific antisera, the test can be modified for type-specific assay of E Ads (16, 44).

g) Spot Hybridisation Assay

The DNA of the virus in the stools can be extracted by a simple procedure and immobilised on to nitro-cellulose filter paper. The presence of viral DNA can than be demonstrated by hybridisation with ^{32}P labelled adenovirus type 2 DNA. This method is comparable in sensitivity with four layer RIA (37). It can be made specific for E Ads by using probes constructed from them. This technique employing specific probes were successfully used to detect Ad40 and Ad41 in the faeces (1, 9).

h) Polyacrylamide Gel Electrophoresis (PAGE)

Adenoviruses can be recognized by a simple nucleic acid extraction and separation on PAGE. After electrophoresis of the DNA, the gel is stained with silver nitrate to visualise nucleic acid. An adenovirus nucleic acid preparation is used as a standard with each run. Adenoviruses are recognized by a characteristic high molecular weight band. The technique

is of comparable sensitivity to electron microscopy, but like electron microscopy, it does not discriminate between E Ads and established adenoviruses (31).

i) Restriction Endonuclease Analysis

Viral DNA can be extracted either from purified adenovirus from stool or directly from stool, treated with the restriction enzyme SmaI, and fragments separated by PAGE. E Ads can be recognized by their characteristic restriction patterns (7, 8).

j) Tissue Culture Methods

The stool specimens containing adenoviruses can be inoculated into any of the cell lines that are normally used to isolate established adenoviruses, as well as into any of the cell lines for E Ads, preferably Graham 293 cells. A presumptive diagnosis of E Ads can be made by the absence of growth in the cell lines used for established adenoviruses, and presence of growth in Graham 293 cells. However, caution should be exercised in the interpretation of results. E Ads can sometimes produce CPE in the cell lines used for established adenoviruses, which will disappear on serial passage of the material. Occasionally, even some of the established adenovirus serotypes will grow only in Graham 293 cells. The identity of isolates can be confirmed by the neutralisation of CPE by specific antisera or by restriction endonuclease analysis of viral DNA. It is customary to passage the material more than once in tissue culture. However, care should be taken when mixtures of an E Ad and established adenovirus serotype are present in the specimen. In such instances, if the material is passaged beyond primary inoculation in cell culture, established adenoviruses will outgrow E Ads (8). To circumvent this, it is important to identify the isolate at the primary passage (50).

Both EIA and CIEOP techniques for detection of E Ads require the use of highly specific antisera. The problem of producing high quality antisera is compounded by the presence of naturally occurring antibodies against rotaviruses and adenoviruses in the animals used for immunisation. Antibodies against stool derived adenovirus antigen are usually produced by affinity bead immunization (ABI). They can be made specific by passing them through an affinity chromatography column containing heterologous antigens of the contaminating species (18).

Electron microscopes, radioactive probes, radioactive isotopes for RIA, and gel electrophoretic equipments are beyond the reach of many routine diagnostic laboratories, as these items are expensive and require specially trained personnel. However, a type-specific ELISA would seem to be the most suitable method. This is likely to replace other methods in future, when monoclonal antibodies against E Ads become available. Even a type-

specific ELISA can be time consuming and uneconomical in screening a small number of samples. Ultimately, a rapid, sensitive and economical test such as latex agglutination test may have to be developed.

For the moment, the indirect immunofluorescence microscopy seems to be the simplest and attractive method. The characteristic single cell fluorescence exhibited by E Ads is easliy recognized. Yet, this method has not found widespread acceptance. Its use is limited in that when E Ads and established adenoviruses are present in the same specimen, E Ads may be missed (8).

Recommendations for Diagnosis of E Ad Gastroenteritis

A combination of the aforementioned methods for diagnosis of E Ads can be used if facilities are available. However, the most suitable method seems to be the type-specific ELISA although it depends upon type-specific antiserum which unfortunately is not widely available. Failing these, any routine diagnostic laboratory with tissue culture facility can adopt the following methods with their attendant limitations which have been described earlier. Adenovirus positive stools can be inoculated into various cell lines, and the presence of an E Ad can be recognized by the absence of viral growth in the cell lines used for established adenoviruses and by the presence of growth in the cell line used for E Ads. Alternatively, E Ads can be recognized by the characteristic single cell fluorescence in the indirect immunofluorescence microscopic method.

Future Directions

The impact of diarrhoeal disease is most greatly felt in developing countries. Yet most of the studies on the role of E Ads in the causation of diarrhoea have been conducted in developed countries. More extensive studies therefore need to be undertaken in developing countries. Also, there is an urgent need for the commercial production and supply of kits for type-specific EIA or other rapid tests.

Search should continue for the existence of other possible serotypes of E Ads. There is preliminary evidence to indicate this (10, 43). Studies should be undertaken regarding genome variations and their implications for possible changes in the neutralisation antigen. This could be achieved with the production of monoclonal antibodies.

Nothing is known about immunity to E Ad diarrhoea. Many children develop serum antibodies in response to infection. Whether serum antibodies have a role in protection is not known. Since gastroenteritis is a local infection of the gut, antibodies in the gut are likely to be involved in protection. This should be investigated. Also, it is not known whether E Ad

specific antibodies are present in breast milk and whether they confer protection. If immunity develops after infection, it should be determined whether it is homologous or heterologous in nature.

The role of E Ads in adult diarrhoea also needs to be defined.

A suitable animal model should be developed to study the pathogenesis and pathology of the disease. The model should be useful to study the dynamics of the disease and the immune response, and also to evaluate a vaccine when it becomes available.

Conclusion

In 1978, the World Health Organisation initiated a programme for global prevention and control of childhood diarrhoeas. As a result, the relative importance of various pathogens in the aetiology of diarrhoea in many parts of the world has been recognised. Rotavirus, which ranks as the most prevalent viral pathogen in childhood diarrhoea may be closer to control as many vaccines are in sight. This has been made possible due to a decade of intense research since its discovery. E Ad, which probably ranks as the second most important viral pathogen has not received much attention. As has been discussed, much information has to be gathered about this virus, before W.H.O's. goal of controlling childhood diarrhoea is realised.

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Note Added in Proof

Since submission of this review, the following additional information has been published. Van Loon *et al.* (Virology **140**, 197—200, 1985) studied the homology of DNAs from Ad40 and Ad41 by liquid hybridisation and found it to be 62—69 per cent. In view of this high homology and the similarity of these two viruses in other properties, e.g. restricted host cell range, cross-reactivity in immunological assays and association with diarrhoea, Van Loon and Wadell (personal communication) recommend that Ad40 and Ad41 be placed into a single subgroup F instead of two different subgroups F and G (in accordance with the criteria of subgroup classification for other adenovirus serotypes of subgroups A—E). Also, Takiff *et al.* (J. Med. Virol. **16**, 107—118, 1985) and Stalhandske *et al.* (J. Med. Virol. **16**, 213—218, 1985) developed dot-blot hybridisation assays for detecting E Ads using cloned DNA fragments from Ad41 strains as probes; Johansson *et al.* (J. Med. Virol. **17**, 19—27, 1985) developed a specific ELISA for detecting Ad41 in stool; Buitenwerf *et al.* (J. Virol. Method. **10**, 39—44, 1985) detected E Ads in faeces by direct extraction of viral DNA by a simple and rapid method and by characterisation of DNA by restriction endonuclease analysis; Brown *et al.* (J. Virol. Method. **9**, 87—98, 1984) studied nosocomial infections due to adenoviruses that included E Ads by a rapid technique using silver staining of DNA restriction fragments; Brown (J. Clin. Micro-

biol. **22**, 205—209, 1985) studied the selection of non-enteric adenovirus serotypes over E Ads in 293 cells from specimens containing mixtures of both enteric and non-enteric adenoviruses; and RODRIGUEZ *et al.* (J. Ped. **107**, 514—520, 1985) conducted a 29-month long community based study of adenovirus diarrhoea in metropolitan Washington, D.C.

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