Antibody to Human Adenovirus Early Antigens During Acute Adenovirus Infections

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The antibody (Ab) response to human adenovirus (AV) early antigens (EA) in acute AV infections was studied by the immunoperoxidase antibody technique for determining virus-specific immunoglobulin G (IPA-IgG). AV-EA-Ab appeared about 5 days after the onset of clinical symptoms, reached a peak 15 to 30 days later, and declined in titer after a few months. The staining pattern in the IPA-IgG reaction was usually nuclear; however, in most primary infections sera obtained 2 to 3 weeks after the onset of infection also showed cytoplasmic staining. According to the recent deoxyribonucleic acid homology classification of human AV in five groups (A, B, C, D, and E), the EA-Ab response in primary human infections was found to be group specific for groups A to D, with consistent cross-reactions with group E. In AV type 4 (group E) infections, EA-Ab appeared to be directed against all groups, although at different titers. Comparable results were obtained using AV type-specific animal antisera. Thus, it was concluded that group E shares EA with all the other groups. Furthermore, in each individual with remote AV infections, the current infection elicited an anamnestic EA-Ab response to all AV groups responsible for previous infections. In diagnostic virology these findings can be applied to the rapid diagnosis of a current (or recent) AV infection on a single serum sample and to the rapid group identification of clinical isolates by using type-specific animal antisera containing EA-Ab (one for each group) or sera from patients with primary AV infections.

Antibody (Ab) to early antigens (EA) of some human deoxyribonucleic acid (DNA) viruses, such as cytomegalovirus (3, 21) and varicella-zoster virus (4), has been shown to be a marker of a current (primary or reactivated) infection. The Ab response to EA (referred to as T antigens) of human adenoviruses (AV) has mostly been studied in tumor and acutely infected cells by complement fixation (CF) and immunofluorescent-antibody (IFA) tests with sera of tumor-bearing hamsters (7, 11, 14, 15). Homotypic EA-Ab response was demonstrated in human acute AV infections; however, heterotypic reactions were not investigated (13).

In the present report, the EA-Ab response to human AV of different groups has been demonstrated in patients with acute AV infection, using the immunoperoxidase technique for determining virus-specific immunoglobulin G (IPA-IgG). The results show that EA induced by AV type 4 (group E) cross-reacted with EA of all other groups, whereas EA of AV groups A to D did not show intergroup cross-reactions other than with type 4. Intragroup cross-reactivity among serotypes was consistent (i.e., AV types of the same group could not be distinguished by EA reactivity). Furthermore, a new AV infection elicited, as a rule, an anamnestic EA-Ab response to all AV groups responsible for previous infections in the same individual. These observations confirm the recent classification of human AV in five groups (A to E), based upon DNA homologies (6) and electrophoresis of structural polypeptides (23), and also offer practical applications in diagnostic virology.

MATERIALS AND METHODS

Cell cultures. HEp-2 cell cultures obtained from the American Type Culture Collection (ATCC, Rockville, Md.) were used in passages 380 to 390 for virus propagation, preparation of complement-fixing (CF) and hemagglutinating (HA) antigens and cell-free virus stocks. Human embryonic lung fibroblast (HELF) cells at passage 20 to 25 were used for neutralization and IPA-IgG tests and for preparation of cell substrates for AV EA-Ab determination. Eagle minimum essential medium (EMEM) with 10% fetal calf serum (FCS) was used as the growth medium, and EMEM with 2% FCS was used as the maintenance medium. All cell cultures employed were repeatedly tested by cultural and cytochemical methods and were found to be mycoplasma free.

Virus strains. AV type 1 to 31 seed viruses and rabbit antisera were originally obtained from ATCC. Viruses were subcultured three to five times on HEp-2 cells before preparing cell-free stocks and CF and HA antigens. Stock virus of most AV serotypes was prepared by infecting HEp-2 monolayers at a multi-
plicity of infection of 1 to 10 50% tissue culture infectious doses per cell. After 3 h of adsorption at 37°C, the maintenance medium was added, and incubation continued until the monolayers were completely detached. Infected cells were then harvested and sonicated; cell-free and cell-associated viruses were pooled and stored in aliquots at −80°C.

Preparation of cultures containing AV-EA and AV-LA. Confluent HELF monolayers were prepared in tissue culture microliter plates (Falcon Plastics, Oxnard, Calif.). Each well was inoculated with 30 to 1,000 infectious units (as determined by the immunoperoxidase-stained cell assay [IPSCA]; see below) of a single AV serotype in 0.050 ml. After 3 h of incubation at 37°C in a 5% CO₂-air–humidified atmosphere, the cells were washed three times with Hanks balanced salt solution (HBSS). Cultures for EA were then refed with maintenance medium containing 40 μg of cytosine arabinoside (Ara-C) per ml and incubated for 24 h. After fixation with absolute ethanol, microliter plates were either stained immediately or stored at −80°C. The absence of mature AV particles in infected cultures treated with Ara-C was confirmed by the lack of staining using a reference serum positive for Ab to AV-late antigens (LA), but not to EA. Cultures for LA were prepared similarly; however, after virus adsorption, they were refed with Ara-C-free medium and incubated for 48 to 96 h, depending on the AV serotype (see below).

Determination of AV-EA and AV-LA IgG Ab by IPSCA. The application of test serum dilutions and positive sera to wells precoated with IgG (gamma chain-specific) IgG fraction (Cappel Laboratories, Cochraneville, Pa.) onto HELF microcultures containing AV-EA was performed as previously reported for human cytomegalovirus (3) and varicella-zoster virus (4) EA-Ab determination. An identical staining procedure was followed for determining LA-Ab in human sera. When reference rabbit antiserum were tested, a peroxidase-conjugated IgG fraction of sheep anti-rabbit IgG (Cappel Laboratories) was used in the second step of the IPSCA-IgG test. Confluent HELF-2 cell cultures gave nonspecific staining with low serum dilutions. HELF cultures showed virtually complete absence of background staining; thus, they were more advantageous and reliable.

HI test. HA antigen was prepared in HELF-2 cells as reported by Hierholzer (8). For removal of nonspecific inhibitors, test sera were routinely pretreated with 2 mg of phospholipase C (PLC) per ml (10); after 60 min of incubation at 37°C, PLC was inactivated by heating at 56°C for 60 min. The hemagglutination inhibition (HI) test was performed by using spectrophotometrically standardized 0.4% suspensions of grivet, rhesus, or rat erythrocytes (9).

IPSCA. For IPSCA, replicate HELF cultures in microplates were inoculated with 0.050 ml of log₁₀ dilutions of cell-free virus per well. After 3 h of adsorption at 37°C in a 5% CO₂-air–humidified atmosphere, the cultures were washed three times with HBSS and refed with EMMEM containing 2% FCS. At different times postinfection (p.i.), they were fixed with absolute ethanol and then stained immediately or stored at −80°C. Fixed monolayers were incubated for 60 min at 37°C with appropriately diluted AV rabbit antiserum (previously absorbed with fixed human uninfected cells). Uninfected HELF cultures treated in the same manner were included as negative controls. Microplates were then washed three times with phosphate-buffered saline and reacted with the peroxidase-conjugated IgG fraction of sheep anti-rabbit IgG (Cappel Laboratories) for 60 min at 37°C. Three additional phosphate-buffered saline washings removed excess conjugate; the immunocytochemical reaction was then revealed by the diaminobenzidine–H₂O₂ method (5). Immunoperoxidase-stained cells were easily counted at ×100 with an inverted microscope.

Neutralization test. Serial twofold dilutions of heat-inactivated sera in EMMEM containing 2% FCS were mixed with equal volumes of virus suspension containing 50 to 100 infectious units per 0.050 ml (as determined by IPSCA). Each serum was tested in duplicate; virus controls contained equal volumes of diluent and virus suspension. The mixtures were incubated at 37°C for 60 min and were then transferred (0.050 ml/well) onto HELF microcultures for IPSCA. After 3 h of adsorption at 37°C in a 5% CO₂-air atmosphere, cell monolayers were washed with HBSS and maintained in EMMEM supplemented with 2% FCS. The immunocytochemical staining reaction was performed 48 to 96 h p.i., depending on the AV serotype tested; the highest serum dilution neutralizing 50% or more of the virus was considered the antibody endpoint. In about 95% of cases, duplicate antibody determinations gave identical results; in the other cases, the variation was within a twofold dilution. HELF were preferred over HEp-2 cell cultures because of easier detection of AV-infected cells and absence of nonspecific background staining.

CF test. The CF test was performed by the Laboratory Branch, Centers for Disease Control, CF procedure (22). AV (type 4) and control antigens were prepared in HEp-2 cell cultures (16), whereas reference antiserum were obtained commercially (Microbiological Associates, Walkersville, Md.).

Isolation and identification of AV isolates. AV strains were isolated in HEp-2 or HELF cells or both. Isolates were identified as members of the AV family by CF and IPA-IgG for LA, using goat and rabbit antiserum, respectively. Clinical isolates were subgrouped by HA with rat, rhesus, and human erythrocytes, as described by Hierholzer (8). Type-specific identification was achieved by HI or neutralization or both. The neutralization test was performed on HELF cells, after titration of AV isolates by IPSCA at 48 to 96 h p.i. Equal volumes of serial twofold dilutions of type-specific rabbit antiserum (selected on the basis of subgrouping by HA) and virus suspension, containing 50 to 100 infectious units of AV isolate per 0.050 ml, were incubated at 37°C for 60 min; subsequent steps were identical to those of the neutralization test for Ab determination.

Clinical cases. Patients were selected for this study on the basis of AV isolation. Most AV strains were isolated from oropharynx or stools or both during acute respiratory or gastrointestinal infections. Acute- or convalescent-phase sera or both were tested for Ab to AV family-specific antigens by both CF and IPA-IgG for LA-Ab and for antibody to type-specific anti-
gens by HI and the neutralization test. The same sera were also tested for EA-Ab to all five AV groups.

RESULTS

Quantitation and staining patterns of AV types 1 to 31 by IPSCA. HELF microplate cultures were inoculated with log10 dilutions of each stock virus. Ten replicates of each virus dilution (of each AV serotype) were fixed at the following times: 12, 24, 36, 48, 72, and 96 h p.i. Cell monolayers were then stained by IPA-IgG using antisera diluted to contain Ab to AV-LA, but not to AV-EA. Morphological IPA-IgG staining patterns did not differ appreciably among different serotypes tested (Fig. 1). A linear relationship between virus dilutions and the number of infected cells was found at 48 h p.i. for AV types 1 to 8, 11, 13 to 17, 19 to 21, and 24; at 72 h p.i. for AV types 9, 10, and 26 to 28; at 96 h p.i. for AV types 12, 18, 22, 23, 25, and 29 to 31. A representative dose-response curve is shown in Fig. 2.

Development of EA in AV-infected HELF cells. In preliminary experiments replicate HELF microcultures were inoculated with 300 to 1,000 infectious units of AV type 1, 2, or 5 and then fixed at 12, 24, 36, and 48 h p.i. In the IPA-IgG reaction, rabbit or human AV type 1 or 2 immune sera were used, each at two different dilutions. The lower dilution (1:50 to 1:100) reacted by IPA-IgG to both LA and EA, whereas the higher one (1:500 to 1:3,000) contained Ab reactive to LA only. Thus, the number of EA-positive cells was easily calculated by subtracting the number of cells stained by the LA-reactive serum dilution from the number stained by the dilution reactive to both LA and EA. In this way, it was shown that at 24 h p.i. more than 95% AV-infected cells were EA positive, while at 48 h p.i. less than 5% of the AV-infected cells contained EA (Fig. 3). Similar experiments were performed on microcultures refed, after virus adsorption, with medium containing Ara-C. In Ara-C-treated cultures only EA were detected at 24 and 36 h p.i., whereas at 48 h p.i. LA were observed in a small proportion (≤5%) of AV-infected cells. Therefore, it was decided to prepare EA cell substrates by fixing Ara-C-treated, AV-infected cultures at 24 h p.i. The same procedure was later used for optimal preparation of EA cultures infected with all other AV serotypes. Positive IPA-IgG reaction for AV-EA was revealed by the appearance of nuclear staining in the absence of nuclear inclusions. The morphological staining patterns of EA induced by AV serotypes of different groups did not differ appreciably (Fig. 4).

Degree of specificity of AV-EA-Ab response. The specificity of EA-Ab response to AV of different groups was investigated by using type-specific rabbit antisera and convalescent-phase sera from infants with primary AV infections. The EA-Ab response by IPA-IgG in typespecific antisera identified five AV groups identical to those distinguished by Green et al. in their DNA homology studies (6). This classification, which will be referred to from now on, consists of group A (AV types 12, 18, and 31), group B (AV types 7, 11, 14, 16, and 21), group C (AV types 1, 2, 5, and 6), group D (AV types

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Fig. 1. Staining patterns of AV type 4 late antigens by IPA-IgG. HELF were inoculated at a multiplicity of 0.01 infectious units (as determined by the IPSCA) per cell and stained 48 h thereafter. Staining patterns were similar for all AV serotypes.

Fig. 2. Dose-response curve for AV type 1 by the IPSCA at 48 h p.i. Data are arithmetic means (± standard deviation) of three experiments (10 replicates for each virus dilution in each experiment). All AV serotypes tested gave linear dose-response relationships at 48, 72, or 96 h p.i.
8 to 10, 13, 15, 17, 19, 20, and 22 to 30) and group E (AV type 4). Our findings are summarized in Table 1, in which are reported only EA-Ab titers to five AV groups for a few type-specific antisera (representative of each group). Antisera to serotypes of groups A to D reacted at a titer of >1:16 with EA induced by the homologous group and with EA of AV type 4 (group E). Type 4 antiserum reacted, at different titers, with EA induced by AV serotypes of all five groups. Thus, a two-way cross-reaction appears to exist between groups A to D and group E, as far as EA are concerned.

In a similar study, convalescent-phase human sera were used from patients with primary AV infections caused by serotypes of different groups. Results, reported in the second part of Table 1, appear closely comparable to those obtained with animal antisera and confirm the EA cross-reactions between AV type 4 (group E) and serotypes of the other groups (A to D). For each antiserum no difference in EA-Ab titer was observed among serotypes of the same AV group.

**EA-Ab response in humans after primary AV infection.** Diagnosis of primary infection was established by virus isolation from patients (more than 6 months old) showing a presence or appearance of CF or IPA LA-Ab or both, and HI or neutralizing Ab only to the AV serotype isolated or both; pretreatment of sera with PLC for removal of nonspecific inhibitors rendered the HI test more reliable. Primary AV infections were also confirmed by the presence of EA-Ab responses similar to those observed in reference animal antisera. In convalescent sera, HI or neutralizing Ab or both were consistently associated with an EA-Ab response to the AV group of the type isolated (Table 2). In addition, individuals with AV infection by serotypes of groups A (AV type 12), B (AV type 7), C (AV types 1 and 2), and D (AV type 8) also responded to EA induced by AV type 4 (group E), whereas a patient with primary type 4 infection showed serological response, at different titers, to EA of all AV groups. In primary AV infections, the EA-Ab response did not appear before 4 days after the onset of clinical symptoms (cases R-7, 1878, 1032, and 1141b), but was already evident after 7 days (case 1544). In most EA-Ab-positive sera from patients with primary infection, cytoplasmic staining was associated, at a lower titer, with the usual EA nuclear staining pattern. However, in each of these patients, cytoplasmic staining was only homotypic, appearing 2 to 3 weeks and lasting 2 to 3 months after the onset of symptoms.

**EA-Ab response in humans with previous AV infections.** In patients with a current acute infection, diagnosis of a past AV infection was established by detection in the acute-phase serum of CF or IPA LA-Ab or both and of HI or neutralizing Ab to one or more AV serotypes or both. Examples of EA-Ab response in patients with actual acute and remote (single or multiple) AV infections are presented in Table 3. The EA-Ab response to the AV group of the actually infecting serotype substantially followed the patterns observed in primary infections, but was consistently associated with a simultaneous response to all the groups responsible for previous infections in the same patient (anamnestic response). The development of EA-Ab response was also comparable to that observed in primary infections: the peak titer was reached in 1 to 2 months, and then it declined gradually after the third month (Fig. 5). A new rise in EA-Ab titer was observed when a subsequent AV infection occurred. The EA-Ab titers in convalescent-phase sera from 23 other patients with a current acute and with remote AV infections are reported in Table 4. In all cases, the anamnestic response to the groups causing previous infections was associated with the EA-Ab response elicited by the actual infection. In a single patient (case 1113), neutralizing Ab to serotypes of an AV group (group A) was detected in the absence of an EA-Ab response to the homologous group. In five cases, EA-Ab to groups A (case R-8), B (cases 1402 and R-5), and D (cases R-18b and 1567) were not associated with detectable HI or neutralizing Ab to any serotype
of the homologous AV group or both; thus, the AV serotypes of these groups, responsible for previous infections, remained unknown. It is noteworthy that, in a patient suffering from systemic lupus erythematosus (case 1132) with acute AV type 7 infection, EA-Ab to groups A to E persisted at a high titer (>1:1,024) over a period of 4 months.

**EA-Ab response in infants less than 6 months old.** In infants less than 6 months old,
diagnosis of primary infection was established by AV isolation and detection of a fourfold or greater rise in family-specific and/or type-specific Ab titer between acute and convalescent-phase serum; diagnosis of remote infection was based upon persistence of HI and/or neutralizing Ab in acute and convalescent-phase sera drawn at a 3- to 4-week interval. In these patients the EA-Ab response to different AV groups often appeared impaired (Table 5). Two patients (R-3 and R-19) responded normally, although at low titers. In one case (R-12) the EA-Ab response to the group responsible for actual infection was not detected; in two cases (R-17 and R-2), the anamnestic response was lacking to one, but not to another, AV group; in another patient (R-19) both events occurred. In a single patient (R-19) the anamnestic EA-Ab response occurred
TABLE 3. Ab response to EA of 5 AV groups in patients with a current acute AV infection and a single or multiple remote AV infections

<table>
<thead>
<tr>
<th>Case no., age</th>
<th>Clinical symptom, AV type isolated (group)</th>
<th>Group responsible for remote infections*</th>
<th>Day after onset</th>
<th>Ab titer to EA of each AV group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1495, 14 mo</td>
<td>Pneumonia, 2 (C)</td>
<td>A 12</td>
<td>2</td>
<td>A &lt;16 B &lt;16 C &lt;16 D &lt;16 E &lt;16</td>
</tr>
<tr>
<td>958, 32 yr</td>
<td>URTI 3 (B)</td>
<td>A 12, B 7, C 1, D 5, E 8, 10, 24</td>
<td>3</td>
<td>A &lt;16 B &lt;16 C 16 D 16 E 16</td>
</tr>
<tr>
<td>R-15, 3 yr</td>
<td>URTI 3 (B)</td>
<td>A 18, B 31</td>
<td>5</td>
<td>A &lt;16 B &lt;16 C &lt;16 D &lt;16 E &lt;16</td>
</tr>
<tr>
<td>1031, 5 yr</td>
<td>URTI and diarrhea, 7 (B)</td>
<td>A 31, B 2, 6</td>
<td>2</td>
<td>A &lt;16 B &lt;16 C &lt;16 D &lt;16 E &lt;16</td>
</tr>
<tr>
<td>1141, 11 mo</td>
<td>URTI 7 (B)</td>
<td>A 12, B 2, 5, C 8, 22, D 23</td>
<td>1</td>
<td>A &lt;16 B &lt;16 C &lt;16 D &lt;16 E &lt;16</td>
</tr>
</tbody>
</table>

* Diagnosis of past AV infection was established by the detection in the acute-phase serum (still free of Ab to the AV serotype responsible for the actual infection) of neutralizing (≥1:20) and/or HI (≥1:10, in PLC-treated sera) Ab to one or more AV serotypes.

** AV type responsible for remote infections.

† URTI, Upper respiratory tract infection.

be distinguished) and with intergroup cross-reactivity between groups A to D and group E (primary response). However, primary infection by AV type 4 (group E) produces a broad, family-specific EA-Ab response to all five AV groups. In secondary infections, the EA-Ab response is directed to the AV groups of the serotypes responsible for current and previous infections in the same patients (anamnestic response). Thus, the presence of AV EA-Ab can be either the expression of a primary or an anamnestic response, but it is always a marker of a recent infection. These results agree with those previously reported for EA-Ab response to cytomegalovirus and varicella-zoster virus. In addition, the pattern of EA-Ab response in patients with nonprimary AV infections is similar to that observed in reactivated herpesvirus infections (3, 4). Secondary AV infection and reactivation of a latent herpesvirus infection appear to be comparable events, both eliciting an anamnestic EA-Ab response.

In this study, infections by low-numbered AV serotypes were largely predominant, as reported in extended epidemiological studies (1). However, a fair number of infections by AV type 8 and high-numbered AV serotypes were also found in infants and young children. In addition, in two infants (one, case R-4, from a lower socioeconomic setting; the other, case 1141, with multiple congenital malformations and several admissions to different hospitals) infections by seven AV serotypes of four different groups were detected. It is well known that heterotypic neutralizing (12, 18) and HI (17, 18) Ab response may occur, mainly among serotypes of the same

Fig. 5. Ab response to EA of AV groups (□) A, (△) B, (○) C, (▲) D, and (●) E, in a 3-year-old child with acute AV type 2 respiratory infection and previous AV infections by types 5 (group C), 4 (group E), 3 (group B), 8 (group D), and 12 (group A).

in the absence of detectable HI and neutralizing Ab to any serotype of the homologous group in the acute-phase serum.

DISCUSSION

Our findings show that acute AV infections elicit two main patterns of EA-Ab response. In primary infections, the EA-Ab response appears specific for groups A to D, with virtually complete intragroup cross-reactivity (i.e., EA of different serotypes within the same group cannot
TABLE 4. AV infections in humans with a single infection or multiple remote infections: Ab titers to EA of different AV groups in convalescent-phase sera

<table>
<thead>
<tr>
<th>Case no., age</th>
<th>Actual AV infection</th>
<th>AV group responsible for remote infections</th>
<th>Ab titer to EA of AV group:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Group</td>
<td>A</td>
</tr>
<tr>
<td>1488, 12 mo</td>
<td>1</td>
<td>C</td>
<td>12*</td>
</tr>
<tr>
<td>1878b, 8 mo</td>
<td>1</td>
<td>C</td>
<td>?</td>
</tr>
<tr>
<td>1349, 3 yr</td>
<td>2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>1402, 16 mo</td>
<td>2</td>
<td>C</td>
<td>?</td>
</tr>
<tr>
<td>1359, 10 mo</td>
<td>2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>1833, 16 mo</td>
<td>2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>R-5, 21 mo</td>
<td>2</td>
<td>C</td>
<td>?</td>
</tr>
<tr>
<td>1072, 2 yr</td>
<td>5</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>1299, 10 mo</td>
<td>5</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>R-16, 3 yr</td>
<td>5</td>
<td>C</td>
<td>31</td>
</tr>
<tr>
<td>R-18b, 4 yr</td>
<td>5</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>981, 5 yr</td>
<td>7</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>1113, 45 yr</td>
<td>7</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>1132, 12 yr</td>
<td>7</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>1567, 4 yr</td>
<td>7</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>R-4, 7 mo</td>
<td>7</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>R-9, 6 yr</td>
<td>15</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>R-13, 16 yr</td>
<td>8</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>1632, 28 yr</td>
<td>8</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>1594, 74 yr</td>
<td>19</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>R-10, 3 yr</td>
<td>12</td>
<td>A</td>
<td>7</td>
</tr>
</tbody>
</table>

*AV type responsible for remote infections.

TABLE 5. Ab response to AV-EA in infants less than 6 months old

<table>
<thead>
<tr>
<th>Case no., age</th>
<th>Actual AV infection</th>
<th>AV group responsible for remote infections</th>
<th>Ab titer to EA of AV group:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Group</td>
<td>A</td>
</tr>
<tr>
<td>R-3, 3 mo</td>
<td>1</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>R-2, 2 mo</td>
<td>2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>R-12, 3 mo</td>
<td>2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>R-17, 5 mo</td>
<td>2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>R-19, 4 mo</td>
<td>2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>1870, 2 mo</td>
<td>12</td>
<td>A</td>
<td>4</td>
</tr>
</tbody>
</table>

*AV type responsible for remote infections.
from tumor-bearing hamsters (11, 14, 15); inter-
group cross-reactions have also been described
(11, 15, 19). However, these results are incom-
plete and cannot be compared, since they have
been obtained by using different procedures and
reagents. Our results show a consistent two-way
cross-reaction between group E and groups A to
D in both human sera and animal antisera. The
antigenic relationship between EA induced by
AV type 4 and those of all the other groups
suggests that, in terms of evolution, AV type 4
could be the common ancestor of the prototypes
of all the AV groups, which has been proposed
by Green et al. (6) on the basis of the similar
genome organization of different AV groups.

In most patients with acute AV infections, HI
or neutralizing Ab or both are associated with
EA-Ab in convalescent-phase sera. However, in
infants less than 6 months old, as well as in older
patients, the anamnestic EA-Ab response occa-
sionally may not be associated with detectable
HI or neutralizing Ab to any serotype of the
responding AV group or both. This finding
might be due to the well-known impairment of
the AV Ab response frequently found in infants
and young children (18); it could also depend on
remote infections by AV types 32 to 35 (not
tested in the present study) or by as yet uniden-
tified serotypes, such as enteric adenoviruses (2).

Although the EA-Ab titers observed in pri-
mary and secondary infections are not appreci-
able different, it is noteworthy that their level
appears related to the AV group responsible for
actual or remote infections. Thus, titers of ≥1:64
are often detected in response to group B, C, and
E infections, whereas lower titers are usually
found after group A or D infections. The only
difference between primary and secondary EA-
Ab response detected by IPA-IgG is the presence
of a homotypic cytoplasmic staining, associated
with nuclear staining, in convalescent-phase sera
from patients with primary AV infections. This
finding has been previously observed by using
IFA, with sera from tumor-bearing hamsters
(14) or antisera against a DNA-binding protein
extracted from AV type 2-infected cells (20). Thus,
some AV-EA apparently react only with
EA-Ab produced during the primary response.

In conclusion, our observations define the
specificity of the EA-Ab response to different
human AV groups in naturally occurring AV
infections and we document for the first time
the appearance of an anamnestic EA-Ab re-
sponse in every secondary AV infection. In ad-
dition, these findings can be applied in diagnostic
virology for the rapid diagnosis of a current (or
recent) AV infection on a single serum sample
and for rapid group identification of clinical iso-
lates by IPA-IgG, with type-specific animal an-
tisera containing EA-Ab (one for each group) or
sera from patients with primary AV infections.

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