Adenoviruses

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General Concepts

Clinical Manifestations

Adenoviruses cause acute respiratory disease (usually), pneumonia (occasionally), acute follicular conjunctivitis, epidemic keratoconjunctivitis, cystitis, and gastroenteritis (occasionally). In infants, pharyngitis and pharyngeal-conjunctival fever are common.

Structure

The icosahedral capsid (70 to 100 nm) is made up of 252 capsomeres: 240 hexons forming the faces and 12 pentons at the vertices. Each penton bears a slender fiber. The double-stranded linear DNA is associated with two major core proteins and carries a 55-kDa protein covalently attached to its 5' end.

Classification and Antigenic Types

More than 100 antigenic types of adenoviruses have been identified that infect mammals (mastadenoviruses) and birds (aviadenoviruses); 47 human adenovirus types are classified, 5 more candidate types are presently studied.

Multiplication

Infection may be productive, abortive, or latent. In productive infections, the viral genome is transcribed in the nucleus, mRNA is translated in the cytoplasm, and virions self-assemble in the nucleus. In latent infections and in transformed and tumor cells, viral DNA is integrated into the host genome. Virus-host DNA recombinants are also found in productive infections.

Pathogenesis

Infection is usually transmitted in droplets of respiratory or ocular secretions. Persistent infection occurs in the tonsils. Some adenovirus types are oncogenic in newborn rodents and can transform cells. A few transformed human cell lines exist. Human oncogenesis has not been found but may nevertheless occur (e.g., by a "hit-and-run" mechanism).

Host Defenses
Most adolescents and adults have circulating neutralizing antibodies; immunity is widespread. Cytotoxic T lymphocytes destroy adenovirus-infected cells.

**Epidemiology**

Infection is common in children. Epidemics do not occur in the general population, but outbreaks of acute respiratory disease occur in military recruits. Serious complications are very rare.

**Diagnosis**

Adenovirus infection is suggested clinically by fever, upper respiratory tract infections, and conjunctivitis; the diagnosis is confirmed by a rise in antibody titers and by virus isolation.

**Control**

There is no treatment. Whole-virus vaccines are not used because of the potential risk of oncogenesis. Other vaccines, including recombinant vaccines, are under development, but adenoviruses do not represent a serious health hazard.

**Vector in Gene Therapy**

Adenoviral genomes have been developed into vectors in experimental therapy since adenoviruses readily infect human and other mammalian cells. Vector genomes carry deletions in the E1 and E3 regions; the gaps in the genome are used to take up foreign genes, e.g., the gene for the cystic fibrosis transmembrane conductance regulator (CFTR). Deletions in E1 minimize the potential of these vector genomes to elicit an infection cycle in human cells. The first clinical applications in patients suffering from the genetic disease cystic fibrosis have been reported but problems with adenovirus toxicity remain.

**INTRODUCTION**

The adenoviruses are common pathogens of humans and animals. Moreover, several strains have been the subject of intensive research and are used as tools in mammalian molecular biology. More than 100 serologically distinct types of adenovirus have been identified, including 49 types that infect humans. The family Adenoviridae is divided into two Genera, the mammalian adenoviruses (mastadenoviruses) and the avian adenoviruses (aviadenoviruses). The adenoviruses are named after the human adenoids, from which they were first isolated.

Several adenoviruses can cause respiratory and conjunctival diseases. In addition, a few types of human adenoviruses induce undifferentiated sarcomas in newborn hamsters.
and other rodents and can transform certain rodent and human cell cultures. There is currently no evidence that adenoviruses are oncogenic in humans, but the possibility remains of interest.

**Clinical Manifestations**

The main target for human adenoviruses is the respiratory tract. Various adenoviruses can also cause acute follicular conjunctivitis, epidemic keratoconjunctivitis, and, less frequently, cystitis and gastroenteritis (Fig. 67-1). In infants, the most common clinical manifestations of adenovirus infections are acute febrile pharyngitis and pharyngeal-conjunctival fever. In military recruits, acute respiratory disease is the predominant form of adenovirus disease, with adenovirus pneumonia as a not infrequent complication. Except for outbreaks in military groups and occasionally among children, adenovirus infections do not occur epidemically. The virus is probably transmitted via droplets of respiratory or ocular secretions.

**FIGURE 67-1 Pathogenesis of adenovirus diseases.**

**Structure**

The adenovirus particle consists of an icosahedral protein shell surrounding a protein core that contains the linear, double-stranded DNA genome (Fig. 67-2). The shell, which is 70 to 100 nm in diameter, is made up of 252 structural capsomeres. The 12 vertices of the icosahedron are occupied by units called pentons, each of which has a slender projection called a fiber. The 240 capsomeres that make up the 20 faces and the edges of the icosahedron are called hexons because they form hexagonal arrays. The shell also contains some additional, minor polypeptide elements. The core particle is
made up of two major proteins (polypeptide V and polypeptide VII) and a minor arginine-rich protein (µ). A 55 kDa protein is covalently attached to the 5' ends of the DNA.

**FIGURE 67-2 Structural model of the adenovirus virion.** The Roman numerals refer to the standard designations of the viral structural proteins according to their decreasing molecular masses. FP stands for fracture plane in freeze etching. (From Brown DT, Westphal M, Burlingham BT et al.: Structure and composition of the adenovirus type 2 core. J. Virol. 16:366, 1975, with permission.)

Figure 67-3 shows the genetic map of a prototype adenovirus, adenovirus type 2 (Ad2). The genome is divided into early functions (E1A, E1B, E2A, E2B, E3, and E4 regions), which are expressed first during viral replication, and late functions (L1 to L5 regions), which are usually expressed after the early functions and after the beginning of viral DNA replication. The late genes encode the viral structural proteins. In the case of Ad2, DNA replication begins 6 to 8 hours after infection of cultured human cells. The VA segment of the genome codes for small RNAs (VAI and VAI RNAs) about 160 nucleotides long, which are not translated but regulate the translation of viral mRNAs. The VA RNAs are transcribed by eukaryotic RNA polymerase III. The genome also codes for a tripartite RNA leader sequence that is spliced onto all the late viral mRNAs. In 1977, RNA splicing was discovered in adenovirus-infected cells. Both strands of the double-stranded DNA code for specific viral functions (Fig. 67-3).
The coding capacities of individual genome segments are indicated by the sizes of polypeptides (K represents 1,000 Dalton) or by the designations of the virion structural proteins (Roman numerals; see Fig. 2). The double-stranded DNA molecule with its 3' and 5' ends and a scale in map units are in the center of the graph. (From Akusjärvi G, Pettersson U, Roberts RJ: Structure and function of the adenovirus-2 genome. In Doerfler W (ed): Adenovirus DNA. Martinus Nijhoff, Boston, 1986, with permission.)

The termini of the DNA molecule carry inverted repeat sequences so that denatured single strands can form circular DNA molecules.

Classification and Antigenic Types

At present, 47 types of human adenoviruses have been identified (Table 67-1) five additional candidate types are under investigation. The genomes of the different adenoviruses are genetically distinct and vary somewhat in size.

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>Serotypes</th>
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<tbody>
<tr>
<td>A</td>
<td>12, 18, 31</td>
</tr>
<tr>
<td>B</td>
<td>3, 7, 11, 14, 16, 21, 34, 35</td>
</tr>
<tr>
<td>C</td>
<td>1, 2, 5, 6</td>
</tr>
<tr>
<td>D</td>
<td>8, 9, 10, 13, 15, 17, 19, 20, 22-31, 32, 33, 35-39, 42-47</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>40, 41</td>
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Multiplication

Host cells differ in permissivity for adenovirus types (Table 67-2). In permissive cells, the virus multiplies productively and kills the host cell. Other cells are semipermissive, allowing replication at low efficiency, whereas in still others replication is blocked and the infection is abortive. As discussed below, in some abortive infections all or part of the genome may be integrated into the host DNA, resulting in a latent infection, which may lead to oncogenic transformation.

<table>
<thead>
<tr>
<th>Type of Interaction</th>
<th>Functional Definition</th>
<th>Biologic System</th>
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<tbody>
<tr>
<td>Productive infection</td>
<td>Complete replication of infectious virions</td>
<td>Cultured human cells</td>
</tr>
<tr>
<td>Abortive infection</td>
<td>Synthesis of viral gene products without production of infectious virions</td>
<td>Cultured hamster or monkey cells</td>
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<tr>
<td>Semipermissive infection</td>
<td>Complete replication with low yields of infectious virions</td>
<td>Cultured rat cells</td>
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<tr>
<td>Malignant transformation</td>
<td>Associated with integration of viral DNA and differential viral and cellular gene expression</td>
<td>Cultured rodent cells</td>
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<tr>
<td>Tumor induction</td>
<td>Associated with integration of viral DNA and differential viral and cellular gene expression</td>
<td>Newborn hamsters (mice)</td>
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<tr>
<td>Viral latency</td>
<td>Persistence of viral genome</td>
<td>Human tonsils</td>
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Productive Infection

The virion enters host cells either by attaching to the cytoplasmic membrane and then being engulfed into the cytoplasm in a membrane-bound vesicle (viropexis) or by directly penetrating the cytoplasmic membrane. The viral DNA is gradually uncoated and enters the nucleus of the cell, most probably as a nucleoprotein complex that still contains viral core proteins (Fig. 67-4).
FIGURE 67-4 Early events in the interaction of the adenovirion with a host cell.
The figure shows a model based on biochemical studies that depicts the major steps in viral penetration and uncoating. (From Lonberg-Holm K, Philipson L: Early events of virus-cell interaction in an adenovirus system. J Virol 4:323, 1969, with permission.)

The viral DNA is transcribed and replicates in the nucleus of the host cell. The viral mRNA undergoes processing in the nucleus and/or during transport through the nuclear membrane into the cytoplasm, where it is translated by polysomes into viral proteins. These proteins return to the nucleus, where new virions self-assemble. The mass of newly synthesized virus particles can assume crystal-like arrangements. The bulk of the virions may not be easily released from the nucleus and the cell. There is evidence that extracellular adenovirus type 12 virions have a considerably higher specific infectivity than intracellular virions. During active viral release, the newly synthesized virions may receive properties conferring high infectivity toward the host cells.

The initiation of adenovirus DNA replication is atypical in that the \( \beta \)-hydroxyl group of a serine residue in the precursor to the terminal protein (pTP), an 80- to 87-kDa polypeptide, acts as a primer in DNA replication. Viral DNA replication can proceed bidirectionally and by single-strand displacement from either end of the DNA duplex. The adenovirus-encoded DNA polymerase, pTP, the adenovirus E2A protein, and several host proteins catalyze viral DNA replication.

Most of the adenovirus genes (Fig. 67-3) are transcribed by the host DNA-dependent RNA polymerase II in a complex transcriptional program. This program is regulated by the nucleotide sequences and the structure of the viral promoters and by a host of cell-encoded transcription factors that recognize specific upstream and downstream nucleotide sequence motifs in the promoters. Genes in the E1A region of the adenovirus
genome are the first to be transcribed. One protein product of this gene region is a transactivator that is essential for the activation of all other viral genes. This immediate-early viral function can also activate or inactivate certain cellular genes.

The jointly controlled E2A and E2B regions code for proteins that are essential for viral DNA replication. Among the E3-encoded functions, one is a 25,000 (19,000)-molecular-weight glycoprotein responsible for the interaction with cell membrane-associated proteins (major histocompatibility complex). The E3 region-encoded functions may be unnecessary for viral replication in cell culture, but essential for the interaction with the intact defense system of an organism and for the modulation of host functions. The late viral L1 region can also be transcribed early in the infection cycle, probably to a limited extent. Genes encoded in the L1 region of Ad5 DNA are essential for virion assembly.

All the late viral functions are under the control of the major late promoter (MLP) components, which are located at about 17, 20 and 27 map units on the viral genome. The gene encoding the fiber structural protein can also be controlled by the x, y, and z leaders (Fig. 67-3)

The regulation of promoter activity in all biologic systems is dominated by the interaction of promoter sequence motifs with specific factors. These (protein) factors in turn bind to a host of further proteins, cofactors, that determine the structure of transcription complexes. Viral promoters are conditioned to the factors present in specific host cells. Enhancers and silencers are quantitative modulators of promoter function. Both act independently of position and orientation and can exert their influence over relatively long distances. Enhancers strengthen promoter activity, whereas silencers have a negative effect, abrogating or diminishing promoter function. Enhancer and silencer elements are species specific.

The VAI and VAI RNAs (Fig. 67-3) are transcribed by RNA polymerase III. VAI RNA is an important translational activator of host cell and viral messenger RNAs (mRNAs) late after infection. It prevents activation of a protein kinase that is responsible for the phosphorylation and ensuing inhibition of the eIF-2 translation factor. This kinase can be induced by interferon. VAI RNA, thus, can be viewed as a viral defense mechanism against interferon.

Abortive Infection

Virus interaction with a host cell can be blocked at many different steps, thus leading to an incomplete or abortive cycle. Depending on the permissivity of the host cell, different types of adenovirus-host cell interactions can be distinguished (Table 67-2). Many cultured human epithelioid cell lines are productively infected by human adenoviruses. Rat cells are semipermissive (e.g., for Ad5), and permit viral replication only at low efficiency. The outcome of an adenovirus infection depends on the animal species, cell type, and virus type involved. For example, hamster cells are abortively infected with human Ad12. The viral DNA is transported to the nucleus, where part of it is integrated into the host genome. Both in productively and in abortively-infected cells, the viral DNA gravitates towards and becomes transiently associated with the host cell chromosomes as demonstrated by fluorescent in situ hybridization. Most of the early viral genes are transcribed, but the late genes remain silent in the host cells. Ad12
DNA replication in hamster cells cannot be detected with the most sensitive techniques. The major late promoter of Ad12 DNA is inactive in both uninfected and Ad12-infected hamster cells, whereas it functions in infected human cells. Ad2 cannot replicate in monkey cells; in this case, the translation of some of the late viral mRNAs is amiss. The adenovirus genome persists, perhaps for a very long time, in cells of the human tonsils. It is not known how adenovirus replication in this human organ is restricted.

Pathogenesis

Adenovirus disease results from localized virus multiplication at the portals of entry (Fig. 67-1). The pathogenesis of localized infections is presented in Chapter 45.

Integration of Adenovirus DNA into the Host Genome

Latency and persistence of, as well as oncogenicity by, DNA viruses are frequently associated with integration of all or part of the viral genome into the host cell DNA. Integration of adenovirus DNA has been demonstrated in abortively infected cells, in adenovirus-transformed cells, and in Ad12-induced tumor cells. In productively infected human cells, recombination between adenovirus DNA and host cell DNA has also been observed. However, it is not known whether this recombination can lead to stable integration, because in the productive infection cycle the host cells are eventually killed. There is evidence that early in productively infected human cells Ad12 DNA becomes preferentially integrated into human chromosome 1.

Soon after infection, the viral genome may be inserted into selective sites of the cellular genome. The initial steps of viral malignant transformation could involve insertional mutagenesis at a certain number of selective cellular sites. From the viewpoint of the geneticist, this model of viral oncogenesis is still one of the more attractive possibilities. Moreover, after being inserted initially at a limited number of sites and eliciting decisive mutagenic events (e.g., deletions), the viral DNA could perhaps be transposed to other loci in the host genome or could be lost.

Recently, an interesting alternate mechanism of insertional mutagenesis in adenovirus-transformed or Ad12-induced tumor cells was discovered. Insertion of Ad12, plasmid or bacteriophage lambda DNA into established mammalian genomes can lead to extensive changes in patterns of cellular DNA methylation far away from and on chromosomes different from those of the site of viral DNA integration. Since patterns of DNA methylation are related to expression patterns and genome organization, alterations in patterns of DNA methylation might affect many cellular functions whose altered expression may play a role in insertional mutagenesis and viral oncogenesis.

Analyses of several different integration sites in transformed cell lines suggest that transcriptionally active regions of the host cellular genome, which have a characteristic chromatin structure, are most apt to recombine with foreign (viral) DNA. Adenovirus DNA frequently recombines with cellular DNA via its termini, and terminal viral nucleotides are often deleted from the integrated viral DNA molecule. In general, considerable variability is observed in the structure of the site of integration. No specific cellular DNA sequence has been found at the site of viral DNA insertion in established cell lines. Cellular DNA can be deleted at the insertion site, or the cellular site can be preserved to the last nucleotide. Ad12 DNA is frequently integrated nearly intact in the
DNA of nonpermissive hamster cells. However, Ad2 DNA is usually integrated in fragments in hamster cells permissive for Ad2.

The adenovirus system has also served as a model for studying the function of sequence-specific promoter methylations in mammalian cells. Upon integration of the adenovirus genome into the host cell genome, a highly specific pattern of methylation is de novo imposed on the integrated viral genome during many cell generations. This de novo methylation is not primarily dependent on nucleotide sequence. Site of integration, structure of integrate and genetics of the host cell are contributing factors. There is evidence from analyses in many different biologic systems that sequence-specific promoter methylations can cause long-term gene inactivation.

Ad12-transformed hamster cells or Ad12-induced hamster tumor cells maintained in culture can eventually lose the integrated copies of viral DNA. This loss suggests that adenoviruses might cause transformation by a "hit and run" mechanism.

**Malignant Transformation and Oncogenesis**

Cells from a number of rodent species and humans can be transformed in culture by adenoviruses. The frequency of malignant transformation is extremely low, and this has prohibited quantitative studies in this system. Transformed human cell lines have also been described. Some adenoviruses, such as Ad2 and Ad5, are not oncogenic in animals at all. Tumorigenic potential has been attributed to the capacity of some adenoviruses (e.g., Ad12) to turn off the expression of genes of the major histocompatibility complex and thus to allow the transformed cells to overcome host defenses and grow into solid tumors. Most of the adenovirus-induced tumors, tumor cell lines, and transformed cell lines carry one or several copies of the viral genome integrated into the chromosomes. The tumor or transformed state is also associated with the differential expression of the integrated viral genes. The early viral genes are often the predominant genes expressed. It is thought that the E1 region of the viral genome is particularly important in eliciting the transformed state. However, the continued presence of the viral genome, or of parts of it, may not be essential for the maintenance of the transformed state.

The so-called oncogenes represent a set of cellular genes that are involved in many different ways in growth control. Oncogenes in adenovirus-induced tumor or transformed cells have received surprisingly little attention. The few studies on this topic have reported occasional changes of oncogene activity, particularly for the myc gene. Moreover, E1 proteins can bind tightly to the product of the retinoblastoma (RB) or the p53 gene, which are considered to be anti-oncogenes. It has been suggested that the fixation of the anti-oncogene products by E1 proteins might contribute to the transformation of cells. The interplay of several viral and cellular factors may eventually alter the cellular growth control and weaken or overcome the host defenses in such a way that an adenovirus-transformed rodent cell can grow into a solid tumor.

Since many human tumors do not contain even traces of adenovirus genes or gene products, the possibility that adenoviruses cause human tumors is low. New, more sensitive techniques are now available. Moreover, the "hit and run" hypothesis has not been ruled out. Since even experimentally induced tumors can lose the viral genome and retain oncogenicity, this possible mechanism of transformation of human cells is still being studied.
Persistence of Adenoviruses in Human Tonsils

Adenoviruses were first isolated from human adenoids, and the persistence of these viruses or their DNA in the human adenoids has been studied. It is not known whether adenoviruses or their genomes can persist in other human organ systems. When the adenoids are removed during acute adenovirus infection, intact viral genomes are present. In contrast, when adenoid tissue obtained during a symptom-free interval or from a chronically-infected carrier is analyzed, only a small number of cells seem to harbor the viral genome, which may not be intact. In some cases, in situ hybridization is needed to show that individual cells in the adenoids contain the viral DNA and/or adenovirus-specific RNA. These cells do not produce infectious virus. It is not known to what extent adenovirus virions continue to replicate in the adenoids throughout adult life.

Host Defenses

In adolescents and adults a high prevalence of circulating neutralizing antibodies contributes to widespread immunity against adenovirus infections. Cytotoxic T lymphocytes also recognize and destroy adenovirus-infected cells. Interferon is induced by adenoviruses in vitro but fails to inhibit many adenovirus types, perhaps due to the function of VA RNA. Nevertheless, in a few preliminary studies interferon has been reported to be effective in the treatment of adenovirus conjunctivitis.

Epidemiology

Adenovirus infections are widely distributed in human populations. The highest susceptibility is found among children from 6 months to 2 years of age and extends to the group of 5 to 9 year old children. Types 2, 1, 3, 5, 7, and 6 (in that order) are most frequently isolated from adenovirus-infected children, with types 1 and 2 constituting some 60 percent of all isolates. Nevertheless, adenovirus infections are responsible for only 2 to 5 percent of acute respiratory infections in children.

Adenovirus also infects military recruits in the United States, where this infection has been studied well, and most likely in other countries as well. Adenovirus types 4, 7, and 3 cause acute respiratory diseases, including pneumonia, in this population.

Adenoviruses have been isolated from severely immunocompromised patients, such as those with acquired immune deficiency syndrome (AIDS). Many of these isolates, including the adenovirus types 42 to 47, are found in the urine of AIDS patients.

Diagnosis

Infection with an adenovirus may be suspected on the basis of a characteristic clinical presentation e.g., respiratory disease, conjunctivitis. The diagnosis can be confirmed by demonstrating a rise in antibody titer between acute-phase and convalescent-phase sera or by virus detection or isolation.

Control
Since adenoviruses are excellent antigens, vaccination could be very effective. However, viral vaccines usually have not been used because adenoviruses are involved in tumorigenesis in animals and in cell culture. Moreover, adenovirus infections only rarely cause serious complications. Nevertheless, efforts are under way to produce vaccines by recombinant DNA technology. Purified hexon or fiber preparations induce high levels of neutralizing antibodies, and vaccines based on these proteins have been tested successfully.

**Vector in Human Somatic Gene Therapy**

Adenoviruses have been used as vector systems in approaches towards human somatic gene therapy. The early region E3 of the viral genome is not essential for viral replication in cell culture and can be removed to yield space in the genome for the insertion of foreign genes constructed for therapeutic purposes. Moreover, the E1 region of the adenoviral genome can be excised to incapacitate viral replication in human tissues thus offering further space for foreign gene insertions. Manipulated, E1-deficient adenovirions can be propagated in the human cell line 293 which contains in an integrated form and constitutively expresses the E1 region of Ad5. Results adduced to date indicate that manipulated adenoviral genomes, e.g. with the test gene for β-galactosidase under eukaryotic promoter control inserted, persist and continue to express this test gene in different organs of rodents for periods up to months. It is not known whether these viral genomes can integrate into the host genome under these conditions.

In the genetic disease cystic fibrosis, mutations in the human gene for the cystic fibrosis transmembrane conductance regulator (CFTR) cause severe symptoms in the respiratory and gastrointestinal tracts, mainly because of a drastically increased viscosity of secretions. This disease can lead to death early in the lives of afflicted individuals. Recombinant adenoviruses carrying the c-DNA for the CFTR gene have been shown to facilitate synthesis of the CFTR gene product in infected human cells. Recent clinical trials in human cystic fibrosis patients have demonstrated that CFTR gene-recombinant adenovirus infection can lead to improved pulmonary function in these patients. Rise in antibody titer against adenoviruses and bronchial irritations - presumably due to adenovirus toxicity - have also been reported. Adenoviruses may not be the final answer to the solution of vector problems in human somatic gene therapy, but they may help open the path towards the construction of more suitable vector systems.

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