Adenoviruses

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ABSTRACT


Adenoviruses, which in man predominantly induce diseases involving the eye and respiratory tract, have stimulated many basic studies because of their relatively simple but unique structures and their pathogenic potential.

Adenoviruses were initially discovered by chance in a quest for the virus of the common cold and in the study of an epidemic of influenza-like disease in Army recruits. Since that time, in 1953, 77 distinct species have been accepted as members of the adenovirus family: 31 from humans (two additional types have recently been reported but have not yet been clearly differentiated from other types), 25 from monkeys, 18 from other mammals, and 8 from chickens.

While considerable interest has been focused on the clinical and epidemiologic significance of adenoviruses, several other viral characteristics have aroused interest: (1) They are simple DNA-containing viruses that multiply in the cell nucleus, and therefore offer an opportunity to study quantitatively the biologic and biochemical characteristics of a relatively unique agent. (2) They induce latent infections in tonsils, adenoids, and other lymphoid tissues; and since they are readily activated, they provide excellent models for study of the mechanisms of viral latency in man. (3) Several adenoviruses represent the first examples of common viruses from man that are oncogenic, albeit this property has only been demonstrated in rodents.

Viral Characteristics

The virions are naked (non-enveloped) perfect icosahedrons, 650 to 800 Å in diameter, composed of a protein capsid formed from 252 capsomers and a nucleoprotein core containing the DNA viral genome and two to four internal proteins. Although it was originally predicted that the capsid of an icosahedral virus would be assembled from multiples of 60 identical structural subunits, the capsids of adenoviruses consist of several unique macromolecular structures which unlike most other viruses can be isolated in their native state. (1) The hexons are polygonal hollow prisms, 70 to 85 Å in diameter, with a central hole about 25 Å across; they are distributed on the faces and edges of the triangular surfaces; each is surrounded by six neighbors; and the hexon consists of three asymmetric units, each containing a single polypeptide chain of approximately 93,000 daltons. (2) The pentons which are the 12 vertex units of the icosahedron, consist of a polygonal base 70 to 85 Å in diameter and an attached fiber; each penton is surrounded by five neighbors and therefore is at the axis of fivefold symmetry; the fiber is of variable length, depending upon the adenovirus type and appears as a string-like structure with a terminal knob. The fiber is composed of three polypeptide chains of 61,000 daltons...
each. The structural units of the penton base have not yet been carefully investigated.

Chemically the virion is relatively simple, consisting only of DNA (11 to 14% by weight) and protein. The DNA is a linear, double-stranded molecule which varies in base composition according to the type. Adenovirus can be generally divided into three classes according to the DNA base compositions, DNA-DNA hybridizations, and oncogenic properties. It is striking that the highly oncogenic viruses, types 12, 18, and 31, have the smallest DNA molecules (about $20 \times 10^8$ daltons) and base compositions (i.e., a guanine + cytosine content of 48 to 49%) closest to that of their host DNA (41%). In contrast, the non-oncogenic viruses, including those which produce most of the human infections (i.e., types 1, 2, 4, 5, and 6) have the highest guanine-cytosine content (57 to 61%) and the largest DNA molecules (23 to $25 \times 10^6$ daltons). Consonant with grouping according to DNA base composition and oncogenic potential, DNA-DNA hybridizations demonstrate that viruses in each group share 70 to 100% of their nucleotide sequences—hence, the DNAs of the "strongly oncogenic" viruses hybridize with each other to a great extent, but cross-hybridize to only a small degree with "weakly oncogenic" viruses (types 3, 7, 11, 14, 19, and 21) and even less with DNA's of the non-oncogenic viruses.

The similarity of the chemical compositions of the DNA of tumorigenic adenoviruses and host cells suggests that a deletion may have occurred in the guanine-cytosine-rich region of the oncogenic virus DNA, making the molecule smaller and in some regions more like the host cell DNA. Such a similarity is consistent with the findings that at least a portion of the DNA of an oncogenic virus is integrated into the host cell genome.

**Antigenic Structure.** As noted above, each adenovirus particle contains at least three morphologic subunits; each contributes to its immunologic reactivity. The major capsid protein, the hexon, contains a family-reactive antigen that cross-reacts immunologically with a comparable antigen in all adenoviruses except those from chickens. It is particularly striking that the hexon also possesses a type-specific reactive site that is responsible for neutralizing antibodies and presumably protection against infection.

The complex intact penton, a minor antigen of the viral particles, also serves as a subgroup cross-reactive antigen and biologically acts as both a cytopathic factor and an endonuclease. The fiber, which is the organ of attachment to host cells, is a type-specific antigen but, surprisingly, cannot induce synthesis of neutralizing antibodies. The isolated penton base has a subgroup-reactive antigen identical to that of the intact penton. The purified fiber protein can combine with uninfected cells; block their DNA, RNA, and protein synthesis; stop their division; and inhibit their capacity to support multiplication of related or unrelated viruses (e.g., adenoviruses, polioviruses, and vaccinie virus). In vitro, fiber protein binds to DNA and inhibits DNA-dependent RNA polymerase and DNA polymerase activities.

**Viral Multiplication.** After attachment and entry of the virion, the capsid is shed in the cytoplasm, and the viral DNA is dissociated from the internal proteins either at the nuclear membrane or within the nucleus. The process of viral multiplication then commences about 3 hr. after infection, when transcription of the early mRNA's is detected. Transcription is soon followed by the appearance of several early proteins that are identified immunologically and enzymatically (thymidine kinase, deoxycytidylate deaminase, DNA polymerase, and aspartate transcarbamylase). Replication of viral DNA is unnecessary for the biosynthesis of these early proteins.
mRNA's and proteins. But if synthesis of early mRNA's is blocked by actinomycin D or pyrimidine analogs (6-azaauridine or 5-fluorouridine) or the production of early proteins is prohibited by cyclohexamide or amino acid analogs, viral DNA is not made.

Following the appearance of the early proteins, replication of the viral genome commences about 6 hr. after infection. Viral DNA is made de novo from the nucleotide pools of the cell, and although infection interrupts synthesis of host-cell DNA, the host DNA remains intact. Once replication of viral DNA begins, it proceeds at a relatively rapid rate for 10 to 14 hr. and culminates in an accumulation of viral DNA which approximately doubles the total content of the infected cell. Hence, the number of viral DNA equivalents synthesized is large, but only about 10% of that made is assembled into virions. The large excess of viral DNA becomes a constituent of the characteristic inclusion bodies of adenovirus-infected cells.

Shortly after initiation of DNA replication, transcription of late mRNA's begins, and is absolutely dependent upon the prior synthesis of viral DNA. Only about 30% of the mRNA's initially transcribed from the parental genome continue to be transcribed during the late biosynthetic events (these have been termed "Class II, early mRNA"). Since late mRNA's are only transcribed from progeny viral DNA molecules, synthesis of capsid proteins (i.e., late proteins) is also dependent upon prior replication of viral DNA. Although viral DNA and mRNA's are synthesized in the nucleus, and virions are assembled in the nucleus, viral proteins are synthesized on polyribosomes in the cytoplasm. After rapid synthesis on polyribosomes, the nascent polypeptide chains are immediately released and transported into the nucleus, where like species assemble into the multimeric viral capsid proteins.

Productive infection also effects profound changes on the host cell. Production of host DNA stops abruptly 8 to 10 hr. after infection, and host RNA and protein synthesis ceases 6 to 10 hr. later. With the cessation of host macromolecular biosynthesis, division of cells is halted, and chromosomal aberrations appear. Marked cytologic changes in nuclei of infected cells accompany the biochemical changes. The characteristic intranuclear inclusion bodies observed are formed from accumulation of excessive viral DNA and capsid proteins that are not properly assembled and packaged into virions. Striking crystals formed from arrays of viral particles are also present.

Investigations of conditionally-lethal temperature-sensitive mutants of adenoviruses confirm the biosynthetic events described and furnish additional tools for the study of regulation of viral replication. For example, from temperature-sensitive mutants of type 5 adenovirus there have been identified three complementation groups: (1) Group I contains mutants that are unable to synthesize viral DNA at the restrictive temperature and cannot make capsid proteins; they nevertheless cause replication of the host DNA to cease, confirming the hypothesis that the switch-off of host DNA synthesis results from an early event in viral multiplication. (2) Group II consists of mutants that replicate viral DNA, shut off synthesis of host DNA, and synthesize fibers and intact pentons but not hexon proteins, indicating that the hexon is synthesized independently. (3) Group III mutants synthesize viral DNA, stop synthesis of host DNA, and make all of the capsid proteins; but the viral components cannot be assembled at nonrestrictive temperatures, implying that additional viral gene products induce assembly. In addition, from chicken adenoviruses mutants have been obtained that cannot transport capsid proteins into the nucleus after their synthesis on cytoplasmic polyribosomes.
eating that the transport of nascent viral proteins into the nucleus is dependent upon a viral gene product.

Clinical Considerations. The diseases produced by adenoviruses predominantly involve the respiratory tract and the eye. They can be described most conveniently as inducing three syndromes. (1) Acute respiratory disease (ARD), a self-limited, influenza-like illness in which pneumonia occasionally occurs. For reasons still unknown the syndrome occurs almost exclusively in military recruits and is caused predominately by types 4 and 7. (2) Pharyngitis and pharyngoconjunctival fever, probably the civilian equivalent of ARD, but the sore throat and eye involvement are more predominant and the influenza-like features less marked. Types 3 and 7 are the most frequent causative agents of these syndromes, but other types, especially types 5 and 21, have also been responsible for epidemics and sporadic cases. (3) Conjunctivitis and epidemic keratoconjunctivitis may occur as the only manifestation of infection, or may be associated with involvement of the respiratory tract and with systemic manifestations. Keratoconjunctivitis is a particularly striking illness, occurring in epidemics, manifest by sudden onset of chemosis, edema of periorbital tissues, tender preauricular lymphadenopathy, and superficial opacities of the cornea (ulceration does not occur). Type 8 adenovirus is the most common provocateur of this illness, but type 7 has occasionally been associated with the syndrome. Types 3 and 7 are the most common causes of simple conjunctivitis, but types 2, 5, 6, 9, and 10 have also been shown to be responsible.

From several fatal cases of nonbacterial pneumonia in infants, type 3 or 7 adenovirus has been isolated, but it is difficult to assess the exact role of the virus in the initiation of the extensive pneumonia. The pulmonary lesions observed were those of nonbacterial bronchopneumonia, but in addition there were numerous epithelial cells containing nuclear central basophilic masses, closely resembling those produced characteristically by the same viral types in cell cultures.

Virus introduced by feeding or swallowed in respiratory secretions multiplies in cells of the gastrointestinal tract and is excreted in the feces, but it does not usually produce gastrointestinal lesions. Occasionally, adenoviruses, most often types 1, 2, 5, or 6, have been isolated from cases of acute infectious diarrhea and intussusception, but an etiologic relationship between virus and illness has not been clearly demonstrated.

Adenoviruses usually cause either inapparent infections or self-limited illnesses that are followed by complete recovery and persistent type-specific immunity. Most individuals are infected with one or more adenoviruses before the age of 15 years. As a corollary, 50 to 80% of tonsils and adenoids removed surgically yield an adenovirus when explants are cultured in vitro.

Laboratory Diagnosis

Adenovirus infection can be diagnosed serologically and by isolation of the offending virus from respiratory and ocular secretions and from feces. For isolation, infected material is inoculated into cultures of continuous lines of human cells (e.g., HeLa or KB) or into human embryo kidney cells. Cytopathic changes (rounding and clumping of cells) indicate the presence of virus; these characteristic cell alterations appear 2 to 14 days after inoculation, depending upon the quantity of virus in the infected secretions. The virus is identified as an adenovirus by complement-fixation titrations with hyperimmune rabbit serum. This procedure detects the cross-reactive hexon and penton antigens. The specific type of adenovirus can be ascertained most conveniently through hemagglutination-inhibition titrations. The number of titrations necessary to identify the virus type can be reduced considerably.
by determining the hemagglutination subgroup to which the newly isolated virus belongs. To establish a virus as a new serotype, a plaque neutralization titration is the method of choice.

The immunologic diagnosis of an adenovirus infection is most accurately accomplished by neutralization titrations with acute and convalescent phase sera. Hemagglutination-inhibition assay for antibodies, however, is practically as sensitive, is simpler and less expensive, and is almost as accurate if prior to use the sera are reacted with a Pseudomonas filtrate, which contains an enzyme that hydrolyzes nonspecific inhibitors. Complement-fixation titration is the most convenient assay procedure available, since all adenoviruses possess the common family cross-reactive antigen (i.e., the hexon), and therefore any type of adenovirus may be used. Unfortunately, complement-fixation assays detect fewer than 50% of the actual infections owing to the constant high levels of antibodies in many individuals.

Prevention

Practical control of adenovirus infection is dependent upon vaccination. Immunization with adequate formalinized and live virus vaccines has been experimentally successful, as would be expected from the lasting type-specific immunity produced by natural infections. The use of adenovirus vaccine primarily appears to be indicated, however, to protect military recruits, since the incidence of adenovirus disease accounts at most for 4 to 5% of viral respiratory illnesses in civilians.

Despite some clinical needs and proved effectiveness, vaccines are still generally unavailable because of the following problems: (1) adenoviruses multiply poorly in nonhuman cells, and therefore inactivated viral vaccines of adequate antigenic potency have been difficult to produce consistently (vaccines for human use require that viruses be propagated in cells of other animals to minimize the risk of transferring latent viruses pathogenic for man); (2) adventitious simian agents (e.g., the highly oncogenic SV40) are often present in primary monkey kidney cells which are generally employed for vaccine production; (3) some common adenovirus pathogens that must be included in a vaccine (e.g., types 3 and 7) are oncogenic for animals other than man. Although evidence that any tumors in man are associated with adenoviruses is lacking, many consider it unwise to immunize with a potentially oncogenic virus, even if the danger is remote, to prevent a self-limiting disease.

Viral capsid proteins, present in abundance as soluble antigens in infected cells, can stimulate production of neutralizing antibodies. Immunization with such viral antigens, instead of the presently utilized mixture of viral particles, soluble antigens, viral DNA, and host cell substances, is now under active investigation.

Vaccines for military use should contain at least types 3, 4, and 7 viruses, although other adenoviruses may become prevalent if only infection with these types is prevented. Under some circumstances (particularly in closed populations such as chronic disease hospitals or homes for orphans) a vaccine containing types 1 to 7 adenoviruses may be useful for infants and young children.

References

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