Medical Importance and Biology of Human Adenoviruses

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Respiratory viruses

- **Influenza virus** (orthomyxoviruses): enveloped; segmented, negative sense, single-strand RNA
- **Parainfluenza** (paramyxovirus): enveloped; nonsegmented, negative sense, single-strand RNA
- **Respiratory syncytial virus** (paramyxovirus): enveloped; nonsegmented, negative sense, single-strand RNA
- **Rhinoviruses** (picornaviruses): non-enveloped; positive sense, single-strand RNA
- **Coronaviruses**: enveloped; positive sense, single-strand RNA - SARS
- **Reoviruses**: non-enveloped; segmented, negative sense, double-strand RNA
- **Adenoviruses**: non-enveloped, linear double-strand DNA

Case Study

A 7 year old boy attending summer camp complains of sore throat, headache, cough, red eyes and tiredness. His temperature is 40°C. Within hours, other campers and counselors visit the infirmary with similar symptoms, which last for 5 to 7 days. All patients have gone swimming in the camp pool. More than 50% of the people in the camp complain of similar symptoms.

Questions:
1. Toward what adenovirus syndrome do the symptoms point?
2. What is the most likely source of the infection?
3. What properties of the virus facilitate its transmission?
4. What precautions could the camp owners take?
5. What tests could be used to diagnose the infection?
Adenovirus Disease

**Symptoms** - high fever, sore throat, malaise, conjunctivitis
- Acute febrile pharyngitis (group C viruses)
- Pharyngoconjunctival fever (types 3, 7, 14; sometimes 4)
- Acute respiratory disease (ARD) (types 4 and 7; sometimes type 3)
- Pneumonia (as a complication of ARD)
- Gastrointestinal disease (types 11 and 12): gastroenteritis
- Acute infant diarrhea (types 40 and 41)
- Acute hemorrhagic cystitis (types 11 and 21)
- "Shipyard eye" (type 8)
- Ad35 isolated from AIDS patients

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Routes of Transmission of Adenovirus

Fig. 53-4: Taken from Murray, et al., Medical Microbiology (5th Edition), 2005

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Course of an Adenovirus Infection

In immunocompetent persons, adenovirus infection is usually self-limiting

Fig. 53-5: Taken from Murray, et al., Medical Microbiology (5th Edition), 2005
Characteristics of Adenovirus Disease

- Adenoviruses can be found in many species of animal:
  - Cows, sheep, chickens, snakes, horses, frogs, rats, mice
- Humans are the only known reservoir for human adenoviruses
  - Adenoviruses from other animals don't successfully infect humans
- Transmission is from person-to-person:
  - Respiratory, fecal-oral, direct contact, hand-to-hand(?)
  - 5-10% of respiratory disease in very young children due to Ad infection
  - 15% of gastroenteritis in hospitalized patients (Ads 40, 41, 42)
  - The virus is found worldwide and its incidence is not seasonal.
- Predominant sites of infection:
  - respiratory tract, intestinal tract, urinary tract, conjunctiva of the eye
- Virus can be recovered from healthy persons – latent infections
  - Virus persists in lymphoid tissue – tonsils, adenoids, Peyer's patches
- No vaccine is currently in use for the general population
  - Routine vaccination of military recruits was discontinued in 1996

Epidemiology of Adenovirus Disease

Disease
- The adenovirus capsid is resistant to inactivation by gastrointestinal tract, by drying, and by some chemical agents (chloroform)
- Disease symptoms resemble other viruses
  - Initial infection: high levels of cytokines IL-6, IL-8, TNFα
  - Some bacterial infections have the same cytokine profiles.
  - T-cells (Th1) are important for recovery from infection.
- Virus can be shed by asymptomatic individuals.

Who is at risk?
- Children younger than 14 years of age
- Immuno-compromised patients (HIV, transplants)
- People in crowded areas (military recruits)

Mononuclear cell accumulation at sites of MAV-1 adenovirus infection in mouse lung

Ad virus production in respiratory epithelium and in vascular endothelial cells (arrow)

Hematoxylin and eosin-stained sections of mouse lungs
Susceptibility to Adenovirus Disease
Prevalence of antibodies to adenovirus serotypes 4 and 7 among unimmunized US Army trainees: Results of a retrospective nationwide seroprevalence survey

The 1996 production halt of adenovirus types 4 and 7 vaccines prompted concerns about the resurgence of large respiratory disease outbreaks among US military basic trainees. Results from a random sample of 303 trainees were analyzed for relationships between susceptibility and 4 showed that 66% and 73% of trainees were susceptible to serotypes 4 and 7, respectively.

Nearly 90% were susceptible to at least one Ad serotype.

Adenovirus disease in immuno-suppressed patients

Table 2. Common manifestations of adenovirus disease in immuno-suppressed patients

Adenovirus disease in immuno-suppressed patients

Adenovirus: an increasingly important pathogen in pediatric bone marrow transplant patients

Tony Weller, R G SHanley, and Delaia Engholm

Adenovirus is increasingly being recognized as a significant pathogen in children following bone marrow transplantation. The virus is endemic in the pediatric population, and frequently causes severe disease in immuno-compromised patients, especially following bone marrow transplantation. There is high rate of disseminated adenovirus infection resulting in high fatality rates appearing to be important for recovery. Although intensive care affects a variety of organs with gastrointestinal and urinary tract disease being the most common. When nosocomial infection occurs, mortality rates were as high as 60%. The success to treatment is heavily dependent on the immunocompromised patient's current state. Unfortunately, there are currently no specific antiviral therapy options for adenovirus infection to allow early initiation of treatment. It is hoped that this approach, together with effective antiviral treatment therapy, will reduce the likelihood of this occurring in a high-risk population.

Adenovirus disease in immuno-suppressed patients

Table 2. Common manifestations of adenovirus disease in immuno-suppressed patients

Adenovirus: an increasingly important pathogen in pediatric bone marrow transplant patients

- Close contact is essential for spread.
- Aerosol droplets
- Fecal-oral spread
- Asymptomatic family members may shed virus in feces for months after infection.
- Nosocomial infections can also be important sources of virus.
- There are few proven treatment options.
- Immunosuppressed patients – disease is usually self-limiting.
- In immuno-compromised patients, ribavirin as a first-line treatment and cidofovir (Vistide) is a second-line agent. Even with these treatments, outcomes are generally poor.
Adenovirus Found in Contaminated Sea Water

“IRVINE, CA. February 7, 2001 — Using a technique developed to track pathogens in sewage, a California Sea Grant funded researcher has shown that potentially harmful human viruses are contaminating coastal waters in Southern California at major river mouths. Their presence does indicate that human waste is making its way into urban waterways. The risk of contamination from human waste appears to be significant, according to a survey of 12 river mouths in Los Angeles, Orange and San Diego counties.

There are more than 100 viruses found in human waste that can survive for as long as 130 days in seawater. None of these are routinely tested by California health officials.”

### Classification of human adenovirus serotypes

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Serotypes</th>
<th>Oncogenicity in rodents</th>
<th>Hemagglutination (complement fixation)</th>
<th>Sites of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12, 18, 31</td>
<td>100% tumors in 4 months</td>
<td>IV gastrointestinal tract, respiratory tract</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3, 7, 11, 14, 16, 21, 34, 35</td>
<td>10-50% tumors in 4-18 months</td>
<td>I lung, urinary tract, respiratory tract</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1, 2, 5, 6</td>
<td>None</td>
<td>III upper respiratory tract</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 38-39, 46</td>
<td>None</td>
<td>II gastrointestinal tract, respiratory tract, eye (“shipyard eye”)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>None</td>
<td>III respiratory tract, eye</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>40, 41</td>
<td>None</td>
<td>III gastrointestinal tract</td>
<td></td>
</tr>
</tbody>
</table>

### Detection of Adenovirus in Clinical Specimens

**Diagnosis:** recover samples from stool, urine; throat, conjunctival swab

- **Complement fixation:** the ability of some adenovirus serotypes to agglutinate red blood cells from rhesus monkeys and/or rats
- **Polymerase chain reaction (PCR)** using DNA primers specific for individual adenovirus types or groups or generic primers that amplify hexon gene DNA – from PBMCs or urine.
- **Antibody ELISA** (enzyme-linked immunosorbent) assays specific for individual adenovirus serotypes. Also fluorescent antibodies can be used.
- **Isolate the virus** from the sample and determine its restriction endonuclease cleavage pattern
Persistence of Adenovirus Particles in a Laboratory or Office Setting

Studies by several groups have described the following survival times for viable adenovirus found in routine laboratory settings:

- Plastic surfaces: 35 days
- Cloth surfaces: 10 days
- Metal surfaces: 45 days
- Paper surfaces: 8 days

Azar MJ, Dhaliwal DK, Bower KS, Kowalski RP, Gordon YJ
AMER. J OPHTHALM 121:711-712 JUN 1996

“Possible consequences of shaking hands with your patients with epidemic keratoconjunctivitis.”

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Spread of Adenovirus Epidemic Keratoconjunctivitis in the Ophthalmic Office


In Fall 1981, an outbreak of acute infectious conjunctivitis with keratitis (EKC) occurred in patients who had visited a private ophthalmology clinic just prior to onset of illness. Among an estimated 2,200 patient visits to the office from August 10 to October 15, 1981 for problems unrelated to infectious conjunctivitis, 39 (1.8%) persons subsequently developed EKC. This outbreak illustrates the potential for transmission of adenovirus infection during the provision of eye care.

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The Survival of Adenovirus in Multiple Bottles of Topical Fluorescein

Raji P, Kowalski, MS, Eric G, Tomaszewski, MS, Barbara Wallihan, MD, and Y. Joeski Gordon, MD

PURPOSE: To determine if common ocular adenovirus serotypes survive in vitro in multiple bottles of topical fluorescein (Bausch & Lomb Boston, Elmwood, California).

METHODS: Clinical isolates of adenovirus types 8 and 19 were inoculated separately into 10 bottles each of fluorescein preserved at room temperature (25°C). All bottles were titrated for adenovirus on A549 cell monolayers at 7, 14, 21, 28, and 49 days.

RESULTS: Adenovirus was recovered from fluorescein for up to 21 days for adenovirus type 19 and 28 days for adenovirus type 8.

CONCLUSIONS: A multiple bottle of fluorescein contaminated with adenovirus can be a potential source of adenovirus transmission in an ophthalmic office setting. (Am J Ophthalmol 1998;126:823–826. © 1998 by Elsevier Science Inc. All rights reserved.)
Ad-mediacted Epidemic Keratoconjunctivitis

Epidemic Keratoconjunctivitis (EKC) often presents as a bilateral, inferior, palpebral, follicular conjunctivitis, with epithelial and stromal keratitis. Subepithelial corneal infiltrates are much more common in EKC and are typically concentrated in the central cornea. EKC is regularly caused by adenovirus types 8, 19 and 22. a.k.a. “shipyard eye”


35 kbp dsDNA Virus
Icosahedral Capsid
51 Serotypes
Tropism: Lung, Liver, G.I. tract, Ocular

Stewart et al., 1991

Structure of the Adenovirus Capsid

Characteristics
- Disease Costs from infection
- Cancer Model (E1A, E1B)
- Model for Cell Processes
- Gene Therapy Vector

Stewart et al., 1991

Adenovirus Capsid Antigenic Proteins

These three proteins are found on the cell surface. Changes in each of them help to define the serotype of the virus.
Adenovirus Enters Cells using the Coxsackie-Adenovirus Receptor (CAR)

- Adenovirus is like many viruses because it uses a cell-surface receptor that is also used by the coxsackie viruses to enter cells.
- This cell surface receptor is widely distributed throughout the body: lung, trachea, liver, heart, cornea, intestine, some neural tissues.
- Coxsackie viruses (B1 through B6) are associated with febrile illness (like many Ads), meningitis, and some cardiomyopathies.

Virus

Adenovirus Entry Pathway

- Binding
- Integrins
- Internalization
- Early Endosome
- Viral Escape
- Nucleus
- Endosomal Lysis
- Deliver virus DNA to the nucleus

Adenovirus Genetic Program

- E1A, E1B
- MLP
- L1, L2, L3, L4, L5
- E3
- E2B
- E2A
- E4
- m.u.
- 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100
Adenovirus E1 genes can transform cells

The Early Region 1 (E1) genes can transform cells by disrupting the normal control of cell division. They accomplish this function by binding to tumor suppressors like Rb (retinoblastoma) and p53.

Multiple tandem repeats of Ad left end inserted into host cell DNA
Express E1A and E1B proteins to promote cell transformation.

Trans-complementing 293 Cells

- Human embryonic kidney cells transformed by Ad5 E1
- Allow replication of E1 deleted adenovirus by providing E1A and E1B proteins in trans

293 Cells expressing E1 proteins

Therapeutic gene

Transfect 293 transformed cells with defective Ad genome carrying therapeutic gene

Ad virus particles containing recombinant Ad genome for gene therapy
These virus can infect cells but cannot replicate further
Recombinant adenovirus: A potential vector for gene therapy

- extensive genetic and biochemical characterization
- relatively easy to manipulate the genome by recombinant DNA technique
- potential to obtain high titer virus stock
- ability to transduce terminally differentiated cells in vivo
- can incorporate genes up to 7.5 kb

But also several potential liabilities, including

- pre-existing antibodies
- inflammation associated with administration of virus
- inefficient transduction to the primary target cells

Testing Adenovirus Gene Therapy for Cystic Fibrosis

Adenovirus-Mediated Gene Transfer Transiently Corrects the Chloride Transport Defect in Nasal Epithelia of Patients with Cystic Fibrosis

Summary To evaluate the potential of short bursts of efficacy in transgene expression, we delivered Adtransiently corrected the chloride transport defect in nasal epithelial cells from patients with cystic fibrosis (CF) by using adenovirus (Ad) vectors encoding the CFTR gene. The Ad vectors were delivered into nasal epithelial cells using a dry powder inhalation device. After treatment, there was a decrease in the number of nasal epithelial cells, and the normal response to a degrading agent was restored. We found no evidence of viral replication or transgene expression in affected patients, even at the highest doses used. These data suggest a new approach to achieving clinically relevant improvements in CF lung function by gene therapy.

Onyx-015 treatment passes a milestone

Defective viral cell passes only in cancerous cells used in trials

Phase I Clinical Trials:
- **Ovarian cancer**: direct injection intraperitoneally. The trial found that the therapy is well-tolerated for this patient group at the administered doses.
- **Bronchioalveolar cell lung carcinoma**: a direct administration of the drug to the lower airways. Symptomatic improvement and improved lung function was reported in some patients.
- **Recurrent glioblastomas**: intratumoral injection of adenoviral p53 is safe at the administered doses and the p53 protein is expressed.

Phase II Clinical Trials:
- **Lung Cancer**: The trial evaluated the therapeutic effect of three intratumoral injections of adenoviral p53 in conjunction with a five-week course of radiation therapy in patients with inoperable lung cancer who were too ill to receive chemotherapy. Researchers have discovered that tumors injected with adenoviral p53 therapy, combined with radiation exposure to the tumors, could improve tumor control locally.
Distinguishing Characteristics of Viruses

- Obligate intracellular parasites
- Extreme genetic simplicity
- Contain DNA or RNA
- Replication requires disassembly and reassembly
- Replication by "one-step growth"

Case Study

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