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10 Adenovirus Vaccines

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HISTORY OF DISEASE

In the 1950s, multiple researchers[1,2] identified new viruses as the cause of acute respiratory disease (ARD), pharyngitis, conjunctivitis, and pneumonitis. Soon afterward, these viruses were recognized to be a related group of viruses and given their present name: adenoviruses.[3]

WHY THE DISEASE IS IMPORTANT

The adenoviruses are often the cause of respiratory illness among U.S. military trainees[4,5] and children.[6–13] In the 1960s, adenoviruses were recognized to infect as many as 80% of trainees, with 20% requiring hospitalization.[5] Median attack rates for trainees ranged from 6 to 16.7/100 per month at the most affected northern and Midwestern U.S. military bases, and from 2.3 to 2.6/100 per month for posts in the South and West.[14] Up to 40% of the trainees in a unit were lost to illness within a 2-week period, and many who were hospitalized had to restart training.[15] Adenoviruses were also recognized to cause as much as 15% of instances of gastroenteritis in infants and children, and up to 10% of instances of pneumonia among hospitalized children.[16–18] Adenovirus morbidity can be severe and lead to death, especially in young children, transplantation recipients, and other immunocompromised patients.

The search for vaccines against adenoviruses was driven by adenovirus morbidity among military trainees, their disruption to military training, and their medical care costs.[15] In 1971, after a series of misstarts, military recruits began routinely receiving live oral enteric-coated vaccines for adenovirus types 4 and 7 (Ad4 and Ad7), which were safe and effective.[19] With few exceptions, adenovirus morbidity was markedly controlled. However, in 1996, the sole manufacturer of these vaccines ceased production. As vaccine stores were depleted, U.S. military trainees again experienced large outbreaks of adenovirus disease, with some deaths reminding public health officials of the impact of adenoviruses.[20–22] In one comprehensive 2006 study, Russell and colleagues documented 98% Ad4 attack rates among 180 susceptible military recruits during 4 weeks of training.[23] The importance of these pathogens has been further emphasized through the recent emergence of multiple novel Ad3, Ad7, and Ad14 strains and the subsequent epidemics and deaths they caused.[24–29]

BACKGROUND
Clinical Description

Human adenoviruses have been classically grouped into seven species (A through G) containing as many as 68 unique types (Ad1 through Ad68) and multiple more genotypes often determined by restriction enzyme digests (Ad7a, Ad7d2, etc). However, the nomenclature and classification system is undergoing change. In 2013, the International Committee of Taxonomy of Viruses added a genus prefix (eg, human adenovirus A has become human mastadenovirus A).[30] A number of investigators have additionally argued that it is time to move away from neutralization and hemagglutination assays in determining serotype and to chiefly use a genetic classification approach.[31] Such virologists have used large DNA sequence comparisons to describe as many as 68 novel adenovirus types,[32,33] although this approach has met with some debate.[34–37]

No matter which adenovirus nomenclature or classification system is agreed upon, it is clear that adenoviruses affect most organ systems. Individual adenovirus types often have different tissue tropisms that lead to specific signs and symptoms (Table 10.1).[39,40]

Endemic Respiratory Adenovirus in Children

Although most children become infected with many of the common adenoviruses early in life, only approximately 50% of these infections result in disease.[39,41,42] Isolation studies have indicated that Ad1, Ad2, Ad5, Ad3, and Ad6 are most prevalent types detected,[39,43] and often manifest by pharyngitis, bronchitis, bronchiolitis, croup, or pneumonia.[43,44] The incidence of adenovirus disease is higher in late winter, spring, and early summer, and both sexes are equally affected.[41,45]

Epidemic Respiratory Adenovirus in Children

Occasionally, epidemics occur in daycare facilities, neonatal intensive care units, chronic care facilities, and orphanages, especially with Ad3 and Ad7.[46–49] In addition to causing outbreaks in closed populations, Ad7 can cause large epidemics affecting children in open communities.[50,51]

Acute Respiratory Disease of Military Recruits

In young adults who live in closed communities such as boarding schools and military recruit camps, adenoviruses may cause epidemics of illnesses similar to influenza, including tracheobronchitis and pneumonia severe enough to require hospitalization.[2,4,52,53] Adenovirus pneumonia may be fatal, especially when it is associated with Ad7 strains.[16,54–56] The incubation period of the disease is 4 to 5 days.[53] Before the use of vaccines in the U.S. military, Ad4 and Ad7 accounted for 90% of all respiratory illnesses among recruits who were hospitalized, whereas Ad3, Ad14, and Ad21 were less frequently observed.[4] At some northern basic training sites, rates of 6 to 8 per 100 trainees per week translated into 600 to 800 ARD hospital admissions per week.[15] Recently, the newly

TABLE 10.1 Clinical Syndromes Associated With Human Adenovirus Infections

Clinical Syndrome	Common Types	Population at Risk
Endemic respiratory	1, 2, 3, 5, 6	Infants, children
Epidemic respiratory	7, 14, 55	Children
Acute respiratory disease	3, 4, 7, 14, 21	Military recruits
Pharyngoconjunctival fever	1, 3, 4, 7, 14	School-age children, young adults
Keratoconjunctivitis	8, 11, 19, 37, 53, 54	All age groups
Hemorrhagic cystitis	11, 34, 35	Immunocompromised patients, children
Gastroenteritis	31, 40, 41, 52	Children, immunocompromised patients
Other syndromes	2, 4, 7, 12,19, 32, 37	Children, adults
Immune deficiency	34, 35, 43–49, 50, 51	Transplantation recipients, persons with AIDS, and immunocompromised patients

There is agreement regarding the classification of adenoviruses 1 to 51, which have been classically typed with neutralization and hemagglutination assays. However, the classification of adenoviruses 52 to 68 remains controversial as these have been chiefly identified through genetic sequencing. In particular, adenovirus 55 is a recombination of 11 and 14.[126,148]

recognized Ad55 (Ad11 and Ad14 recombinant) has been documented to have caused significant ARD morbidity among military trainees in China.[57]

Typical ARD is a febrile disease with symptoms of sore throat, fever, cough, coryza, rhinorrhea, headache, and chest pain.[2,39,58] With extension into the lungs, physical examinations can reveal rales and rhonchi with little evidence of consolidation, and chest radiography often shows patchy interstitial infiltrates, principally in the lower lung fields.[2,53,59] Symptoms last 3 to 10 days.[39] The infection is often self-limited, but occasionally deaths occur.[2,60] Routes of transmission are thought to include direct contact or aerosolized virus inhaled into the lung. The virus has been isolated from the oropharynx more than 2 weeks after exposure.[2,61] In a 2006 report, Russell and colleagues demonstrated viable adenovirus was ubiquitous on surfaces in the military trainee barracks.[23]

Pharyngoconjunctival Fever

This syndrome is characterized by pharyngitis, conjunctivitis, and spiking fever.[62] First described in the 1920s as associated with swimming, the cause has been subsequently linked to insufficient chlorination.[63–65] One or both eyes are affected, and diarrhea, coryza, tonsillar exudates, and lymphadenopathy may be observed. The most frequently identified types have been Ad3 and Ad7, but other types, such as Ad1, Ad4, and Ad14, are also associated with pharyngoconjunctival fever.[62] The disease is associated with summer camps, swimming pools, and lakes; occurs in children and young adults; and often spreads to other family members.[62,66,67] The incubation period is 6 to 9 days, and the virus may be isolated from pool water.[62,65] There is little bacterial superinfection and no permanent damage to the eye.[59]

Epidemic Keratoconjunctivitis

Epidemics of conjunctivitis in adults caused by adenoviruses were first described as "shipyard eye."[68,69] The disease was observed at industrial settings where shipbuilding took place and was probably transmitted because of inadequate infection control practices when workers sought care for chemical irritation and minor trauma from paint and rust chips.[68] The disease has an incubation time of 8 to 10 days and is characterized by conjunctivitis, edema of the eye, pain, photophobia, and lacrimation. Superficial erosions and subepithelial infiltrates of the cornea may occur.[39] Preauricular lymph gland swelling and involvement of cervical and submaxillary lymph glands may be observed.[39] Epidemic keratoconjunctivitis (EKC) is associated with Ad8, Ad19, Ad37, and, rarely, other types.[70–74] In addition, Ad19 and Ad37 have been isolated from the genital tract of young adults with EKC, and the possibility of sexual transmission has been considered.[75,76] Numerous epidemics of EKC have been associated with ophthalmology practices, where spread from contaminated ophthalmic solutions, fingers, and instruments has been implicated.[70,72,74,77,78]

Hemorrhagic Cystitis

Hemorrhagic cystitis syndrome in children is caused by adenovirus infections in 23% to 51% of cases in the United States and Japan.[79] Although the specific route of spread is unknown, Ad11 and Ad21 were the most frequently isolated adenoviruses.[79] Boys were two to three times more often affected than girls. Clinical findings included gross hematuria of 3 days' duration. Dysuria, microscopic hematuria, and urinary frequency lasted a few days longer.[79,80] No viremia or structural abnormalities were found. Adenoviral antigen in exfoliated bladder epithelial cells can be demonstrated by immunofluorescence.[79] Cases of acute hemorrhagic cystitis after renal and bone marrow transplantation are increasingly being reported.[81–83] Ad34 and Ad35, as well as species B adenoviruses, were first isolated from renal transplantation recipients, but neither was associated with symptoms of hemorrhagic cystitis.[84]

Gastroenteritis

Adenoviruses are also recognized to cause watery diarrhea with fever. Ad40 and Ad41 are the most prevalent strains, but Ad50, Ad51, and Ad52 have been detected among the immunocompromised. AdV52 has been associated with one outbreak cluster of acute gastroenteritis.[124] These strains often require special cells lines for culture and they may not demonstrate cytopathic effect.[85] Electron micrography or special assays are often necessary for their study.[85] Numerous outbreaks have been described, and adenoviruses may account for up to 12% of all infant diarrhea.[86–88]

Rare Acute Manifestations

A number of case or outbreak reports document other, more unusual manifestations of human adenovirus infection. These occur most frequently among children. Although the studies

are inconsistent, adenovirus is implicated as a possible fetal pathogen.[89,90] Adenovirus infections among children are associated with sudden infant death,[91] encephalitis, meningoencephalitis,[92,93] cerebral edema,[94] acute flaccid paralysis,[95] pertussis-like syndromes,[96,97] mononucleosis-like syndromes,[98] and neonatal disseminated infections.[99] Among adults, adenovirus infections have been associated with a toxic shock–like syndrome,[100] encephalitis, genital lesions, orchitis, urethritis, and cervicitis.[101] Nosocomial transmission to susceptible healthcare workers and patients has been reported. This is likely related to the long periods of viral shedding among adenovirus-infected immunocompromised hosts, the possible aerosolization of the virus, and fomite transmission in the hospital setting.[40,95,102]

Complications

Adenovirus Infections in Immunocompromised Patients

Adenoviruses have been implicated as opportunistic agents in patients with immune deficiency states, such as patients with AIDS, patients receiving cancer chemotherapy, bone marrow transplant recipients, and patients undergoing solid organ transplantation.[11,12,82,103–106] The patients are prone to pneumonia and disseminated adenoviral infection. They have also developed parotitis[107] and urinary tract disease.[108] A number of the more recently described types, such as Ad43 through Ad51, have been recovered from people with AIDS.[108,109]

A review of 201 bone marrow recipients over a 4-year period indicated that adenovirus infections occurred in 20.9% of patients, with a higher incidence in children than in adults (31.3% vs 13.6%).[110] Ad35 was the most common type identified.[110] In 1992, Hierholzer reported that adenovirus infections in immunocompromised patients with pneumonia were associated with case fatality rates as high as 60%, compared with only 15% among immunocompetent hosts with pneumonia.[9] Radiographs of adenovirus-infected patients often demonstrate patchy interstitial infiltrates, usually in the lower lung fields.[111] Fig. 10.1 shows the chest radiograph of a 20-year-old recipient of an autologous bone marrow transplant for diffuse large cell lymphoma (B-cell type) with systemic Ad11
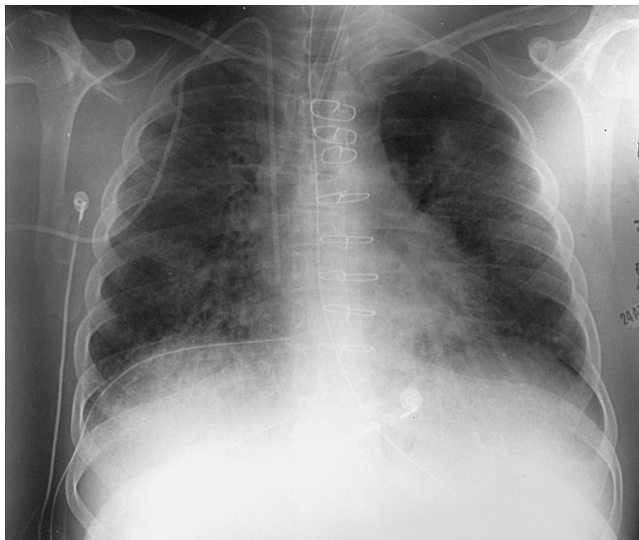

Figure 10.1. Pneumonia caused by adenovirus type 11 in a 20-year-old recipient of an autologous bone marrow transplant. *(Courtesy Stuart Ray, MD, Johns Hopkins University, Baltimore, MD.)*

infection, which was obtained on day 45 posttransplantation. He died on day 80. Adenovirus was recovered from conjunctival, urine, and bronchoalveolar lavage cultures. A case fatality rate of 50% occurred in immunocompromised people with hepatitis and associated adenovirus infections, compared with 10% in similarly infected immunocompetent patients with hepatitis.[9] Positive tests of serum by polymerase chain reaction (PCR) for adenovirus has been reported to predict severe disseminated adenovirus infection in immunocompromised patients.[112] Nephritis and kidney failure caused by adenovirus infections have been reported in adults and children receiving bone marrow transplants.[113,114] A study of 532 hematopoietic stem cell transplant recipients between 1986 and 1997 found a 12% incidence of adenovirus infections, with children being more likely than adults to have a positive culture (23% vs 9%).[115]

Chronic Diseases Caused by Adenoviruses

Thanks often to molecular techniques, human adenoviruses have been associated with a number of chronic disease conditions, including chronic airway obstruction,[101] pulmonary dysplasia,[116] myocarditis, and cardiomyopathy.[117] Although the results of studies are mixed, an intriguing series of human and animal studies associate a number of human adenovirus types with obesity.[118–121]

Virology

Human adenoviruses are nonenveloped, double-stranded DNA viruses belonging to the genus Mastadenovirus, family Adenoviridae.[122] They are classified by species (A–G) through hemagglutination properties and by serotype (1–51) with horse or rabbit antisera (Table 10.2).[40,59,123] Different strains within serotypes are further distinguished by whole-genome restriction-enzyme digest patterns. However, as typing serum samples are difficult to acquire and sequencing costs are decreasing, virologists are considering full genome sequencing as a more modern and accurate method to detect unique strains and have described as many as 68 unique adenovirus types.[124–128]

Adenoviruses are considered large viruses, with an estimated diameter of approximately 920 Å.[129] The viruses have an icosahedral capsid shell that is made up of 240 hexon bases, 12 penton bases, and 12 fibers that are associated with the penton bases (Fig. 10.2). Four minor proteins (IIIa, VI, VIII,

TABLE 10.2 Human Adenovirus Species and Types

Adenovirus Species	Types
A	12, 18, 31, 61
B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55, 66
C	1, 2, 5, 6, 57
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56, 58–60, 63–67
E	4
F	40, 41
G	52

Some information for the recently described adenovirus types is not yet available.

Modified from Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. *2014;27(3):441–462.*

II (hexon, GON)
II (hexon, GOS)
III (penton base)
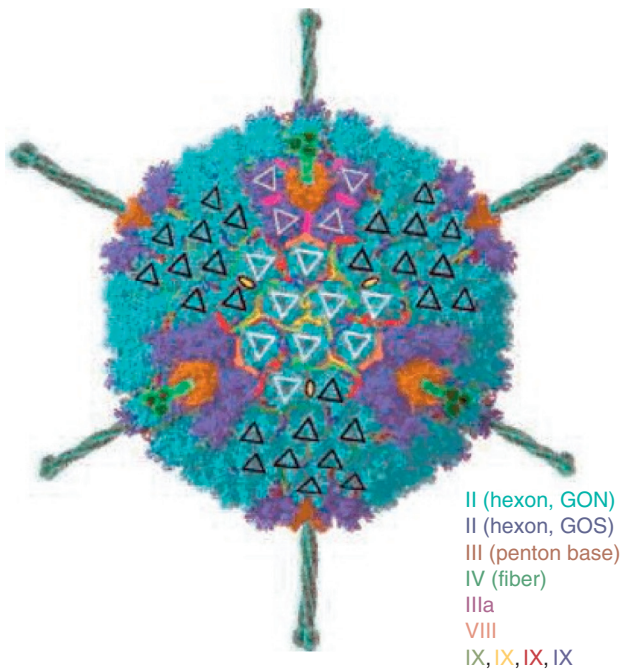IV (fiber)
IIIa
VIII
IX, IX, IX, IX

Figure 10.2. Image of adenovirus virion determined by cryoelectron microscopy showing newly understood interactions of the major components (hexon, penton, and fiber proteins) and minor proteins (IIIa, VIII, and IX). The proteins interact in groups of nine (GON) and groups of six (GOS) capsomers. *(Courtesy Hong Zhu, University of California, Los Angeles. Modified from Harrison SC. Looking inside adenovirus.* Science. *2010;329:1026–1027.)*

and IX) add to the complexity of the capsid. The pentons and hexons are each derived from different viral polypeptides.

Adenovirus hexons are both type-specific and species-specific antigens, primarily inducing species-specific complement-fixing antibodies, whereas the pentons are especially active in hemagglutination.[130] The fibers also evoke type-specific antibodies, vary in length among human strains, and are sometimes absent in particular animal strains.[131–134] The genome core of the virus is composed of five more proteins (V, VII, u, Iva2, and terminal protein) and a single molecule of linear, double-stranded DNA of 26×106 to 45×106 molecular weight. The G + C base compositions of the human virus genomes range from 47% to 60%.[110,122,135,136]

Adenoviruses are unusually stable to physical and chemical agents, as well as adverse pH, and, thus, survive for long periods outside the host, making them available for transmission to others. They can be destroyed by heat at 56°C for 30 minutes, UV irradiation, 0.25% sodium dodecyl sulfate, chlorine at 0.5 µg/mL, and formalin, but are resistant to ether and chloroform.

Adenoviruses replicate in the cell nucleus and tend to be very host-specific. However, there is mounting evidence that humans and animals have exchanged adenoviruses. In 2009, a simian adenovirus epidemic occurred among nonhuman primates at a U.S. research center and subsequently infected the primate caretakers with some evidence of human-to-human transmission.[137,138] A 2011 report documented the recent detection of 45 distinct adenovirus in nonhuman primates most of which mapped phylogenetically very closely to human adenovirus species.[139] In 2012, human infections with a novel avian adenovirus were associated with avian chlamydiosis outbreak in Hong Kong.[140] Finally, it is also important to recognize that Ad4 and Ad52 are thought to have

originated from nonhuman primates.[124,141,142] Hence, some virologists have recently reviewed the probability that adenoviruses may be zoonotic.[143]

Some adenovirus types have been determined to be oncogenic in animals and to transform cell lines, but oncogenicity has not been observed in humans. Hybridization or genetic recombination of multiple strains of adenovirus may occur. Sometimes, these recombinant strains or other emergent novel adenovirus strains lead to epidemics. A number of recent examples have been reported in the medical literature.[26,27,29,144–148]

Pathogenesis as It Relates to Prevention

Depending on the route of inoculation, the type of virus, and the immune state of the host, adenoviruses can cause diseases or asymptomatic infections. Respiratory infection is presumed to result from inhalation of aerosolized virus, whereas ocular infection, gastroenteritis, and nosocomial infections may arise from fomites, water, or fecal–oral contact. Reactivation of latent adenovirus is also believed to occur.[53] Some 50% to 80% of tonsils removed surgically may have adenoviruses isolated from the tissue, suggesting that these viruses may remain in a latent state for years.[149,150] Virus has been also isolated from lymphocytes, kidney, blood, cerebrospinal fluid, and most body organs.[9,11,12,54,55,93,151–153] In the lungs, extensive pathology has been found with microscopic necrosis of the tracheal and bronchial epithelium.[39] Acidophilic intranuclear inclusions are seen in bronchial epithelial cells in addition to the basophilic masses of cells surrounded by clear halos, which may indicate aggregations of viral material.[54] A mononuclear infiltrate, rosette formation, and focal necrosis of mucous glands are characteristically seen.

Three types of interaction of adenovirus with infected cells can occur. A lytic infection may take place during which the virus completes an entire replicative cycle.[59] Between 10^5 to 10^6 progeny viruses per cell are produced, of which only 1% to 5% are actually infectious.[59] The second type of interaction is chronic or latent infection, in which small amounts of virus are produced, and an inapparent infection results. Viral shedding from the gastrointestinal tract may occur for years.[39] In fact reactivation of such latent infection likely explains much adenovirus disease in the severely compromised. Correspondingly, monitoring viral loads in the stool of transplant patients has predicted disseminated disease and been useful in guiding antiviral therapy.[154]

In addition to aerosolization, intestinal shedding of respiratory virus is an important factor to consider in the prevention of nosocomial spread in hospitals and chronic care homes.[39,145,155] Persistent infection has been reported in epithelial cells from monkeys.[156] Lymphoid cells are thought to be the reservoir for these persistent infections.[157,158] The third type of interaction is oncogenic transformation, whereby the viral DNA is integrated into the host genome and replicated with the cellular host DNA, but only the early steps in the viral cycle occur and no infectious virions are produced.[159]

The genes from adenoviruses are expressed in the cell nucleus in two phases: "early" (E), which precedes viral DNA replication, and "late."[157] Early genes encode proteins that function to thwart immunosurveillance, especially those from the E3 transcription unit.[157] The late genes primarily encode viral structural proteins. The functions of the E1 proteins include the induction of DNA synthesis in quiescent cells, immortalization of primary cells in cooperation with activated *ras* or with the E1B proteins, transactivation of delayed early genes, induction or repression of several cellular genes, and induction of apoptosis. These proteins modulate the sensitivity of adenovirus-infected cells to tumor necrosis factor (TNF),

a key inflammatory cytokine with antiviral properties.[157] None of the E3 genes are required for adenovirus replication in cultured cells, but several of the E3-coded proteins (10.4 K, 14.5 K, and 14.7 K) inhibit TNF cytolysis.[157,160,161] Because a major function of TNF may be to prevent viral replication, the inhibition of TNF by these viral proteins may be a significant mechanism of pathogenesis.

Another significant E3-coded protein is Gp 19 K. This glycoprotein is located in the endoplasmic reticulum and forms a complex with class I antigens of the major histocompatibility complex (MHC), preventing cells from being killed by cytotoxic T lymphocytes.[157] A cotton rat animal model was used by Ginsberg and colleagues and Ginsberg and Prince to study the pathogenesis of Ad2 and Ad5, which cause pneumonia similar to that seen in humans.[160,162] Two phases of infection were seen; the initial phase, characterized by the infiltration of monocytes and neutrophils, and a later phase associated with the infiltration of lymphocytes. The pathology seemed to reflect the response by host immune defenses to viral infection. The Gp 19 K markedly reduced the transport of the class I MHC to the surface of the infected cells and impeded the attack of cytotoxic T cells.[157,160,161] It is now known that only the early genes are required to induce the complete pathogenesis of adenovirus infection in cotton rats.[161] Although several cytokines, such as TNF-α, interleukin-1, and interleukin-6, were elaborated during the first 2 to 3 days of the infection in the cotton rat model, only TNF-α had a major role in pathogenesis.[161] Steroids almost completely eliminated the pneumonic inflammatory response to infection.[161]

Pathology caused by latent infection with adenoviruses has been linked to chronic obstructive pulmonary disease (COPD).[158,162] Some have suggested that childhood viral diseases represent an independent risk factor for COPD.[163] The adenoviral E1A proteins can stimulate the transcription of many heterologous viral and cellular genes. These proteins have the ability to interact with the DNA binding domains of several cellular transcription factors and activate a wide variety of genes.[164,165] The adenoviral genome has been found to be present in the lungs of more patients with COPD than in control subjects.[166] E1A proteins are expressed in epithelial cells of human lung tissue, and by increasing the expression of several genes important in controlling the inflammatory process, these may contribute to the pathogenesis of COPD. The events described may amplify the airway inflammation associated with cigarette smoking.[161]

The isolation and cloning of a 46-kDa protein adenovirus receptor, which mediates attachment and infection of group B viruses, may facilitate the development of new strategies to limit diseases caused by adenoviruses.[167] The more common receptor is referred to as CAR. This protein has been identified as the receptor for the coxsackie B virus and adenoviruses. Recently, another receptor, desmoglein 2, was identified for Ad3, Ad7, Ad11, and Ad14 strains.[168]

Laboratory Diagnosis

Adenoviral infections generally cannot be diagnosed on clinical grounds alone because the clinical signs and symptoms of these infections are variable and often resemble those caused by other microorganisms.[101] Traditionally, laboratory support and qualified personnel are necessary to accurately diagnose adenoviruses infections. Considerations include specimen type and timing, collection and storage procedures, types of laboratory tests performed, including serological assays, and availability of newer types of diagnostic assays.

Specimen Collection. The optimal specimen for diagnosis of adenovirus infection depends on the clinical presentation and the suspected type.[101] Adenoviruses can be detected in a variety of specimens, including nasal and throat secretions, conjunctival scrapings and swabs, stool, blood, cerebrospinal fluid, and biopsied tissue specimens. Specimens should be collected early in the illness and shipped promptly at 4°C or on dry ice if prompt testing is not possible. Swab and fresh tissue specimens must be transported in appropriate viral transport media[40,58,84,101] whereas fluid samples like urine, stool, and cerebrospinal fluid should not be diluted in transport media. For culture, nonsterile specimens should be treated with antibiotics prior to inoculation. Stool specimens are brought to 10% to 20% suspensions with buffered saline and clarified by low-speed centrifugation. Blood for culture or molecular testing should be collected with an anticoagulant to prevent clotting. Serum separated from clotted blood samples can be used for serological diagnosis (see below).

Cell Culture. Through biological amplification, cell culture offers a sensitive method for adenovirus detection by monitoring for cytopathic effect (CPE). Because adenoviruses are host-specific, isolation of human adenoviruses is most easily accomplished in human cells.[169] Most human adenoviruses have been successfully isolated in cell culture. Several diploid and continuous cell lines, including A549, HeLa, HEp-2, KB, MRC-5 and commercial mixed cell lines with A549 cells, reportedly give good overall recovery and produce typical CPE.[170,171] However, the fastidious enteric adenoviruses Ad40 and Ad41 are an exception. For efficient recovery, most Ad40 and Ad41 strains require the Graham-293 Ad5-transformed secondary HEK (human embryo kidney) cell line for primary isolation.

CPE may develop slowly in monolayers of inoculated cells necessitating several subpassages before becoming visible. Infected cells become rounded, enlarged, and refractile and aggregate into irregular "grape-like" clusters.[40] A 4-week incubation with blind passage is recommended. A rapid procedure for culture identification of adenovirus is the shell vial technique. Here, cell monolayers prepared in glass vials or multiwell plastic culture plates are inoculated with the clinical specimen, centrifuged, and stained after 2 to 3 days incubation with commercial monoclonal antibodies.[172] Cell culture is declining as a routine diagnostic method for detection and identification of human adenoviruses as a result of cost and delays. Antigen and molecular detection techniques have supplanted culture in many laboratories (see below).[169] However, while direct detection methods offer important diagnostic benefits, culture is the only means available for obtaining sufficient virus for immunotyping, assessing virus susceptibility to antivirals, and identifying infectious virus in clinical and environmental specimens.

Direct Visualization of Virus Particles

Electron and immunoelectron microscopy[173] were once commonly used to detect and identify the fastidious adenoviruses in stool specimens from young children, thereby establishing their association with acute gastroenteritis.[40,102,174–177] These methods are still used to localize virus in cells from biopsy and autopsy specimens to confirm disease association and to study disease pathology.

Antigen Assays. Detection of adenovirus antigenic proteins can be accomplished by reacting with virus-specific polyclonal or monoclonal antibodies labeled with various reporter systems. Examples include enzyme immunoassay, immunofluorescence assay, time-resolved fluoroimmunoassay, and latex agglutination.[178–181] These assays can be used to detect adenovirus proteins directly in clinical specimens or to

identify virus isolates. Immunoassays are simple to perform, less costly and provide results more quickly than culture or molecular tests, but are generally less sensitive.[181,182] The immunofluorescence assay is often used in the clinical diagnostic laboratory for rapid identification of adenovirus in cells from unfrozen specimens. Point-of-care antigen immunoassays[181,183] for rapid adenovirus detection and immunohistological staining for adenovirus antigens in biopsy and autopsy specimens are still in common use[182,184]

Molecular Assays. Molecular methods are rapidly replacing classical culture and antigen detection for diagnosis of adenovirus infection. The PCR assay in particular has become a popular diagnostic alternative for these viruses, offering the potential for rapid and sensitive detection and being easily tailored for species-specific and type-specific identification as described below. PCR assays[185] using adenovirus group-specific primers individually[185,186] or combined in assays for multiple human pathogens[187] have proven comparable or better than cell culture or immunodiagnostic methods for detection of adenoviruses in clinical samples. Several commercial multiplex assays for respiratory pathogens including adenovirus have been cleared by the U.S. Food and Drug Administration (FDA) for clinical diagnostic use.[188] Use of quantitative real-time PCR assays have the added benefit of allowing monitoring of adenovirus levels that can be used to predict patient prognosis, manage chemotherapy and monitor efficacy of antiviral therapy.[189]

Adenovirus Typing Assays. Once detected, further identification of adenoviruses to species and type can be accomplished by a variety of immunologic and molecular methods to support outbreak investigations and patient clinical management. For example, adenovirus infections in bone marrow or lung transplant recipients can be rapidly fatal. It is in the patients' interest that healthcare providers determine the species and type of any infecting adenovirus as some are more sensitive to specific antiviral therapy.[190] It is also important to learn if the adenovirus represents a community-acquired strain, a healthcare-acquired strain, a donor-associated strain, or the reactivation of a latent virus. Efforts to further identify adenoviruses have led to the discovery of cross-species infections with simian adenoviruses acquired from nonhuman primate reservoirs.[138,191]

Serum neutralization (SN) using defined hyperimmune animal antisera is the gold standard method used for classifying human adenovirus types.[40] SN targets type-specific neutralizing epitopes on the adenovirus hexon and fiber proteins and to a lesser extent on the penton protein. Hemagglutination inhibition (HI) has also been used of immunotyping adenoviruses. HI targets type-specific epitopes on the adenovirus fiber protein, and when used in combination with SN, can identify hexon/fiber recombinant viruses. Because immunotyping methods require reference antisera that are difficult to obtain and standardize and often difficult to interpret, molecular methods have generally replaced immunotyping for rapid routine adenovirus typing.

Numerous molecular methods for species and type-specific identification of adenoviruses have been described, including conventional[192–194] and real-time PCR[195,196] assays using species-specific and type-specific primers and probes, PCR amplification coupled with restriction endonuclease analysis,[197,198] microarrays,[199] and mass spectrometry[200] and, increasingly, PCR-coupled sequence-based typing strategies that target hypervariable regions of the adenovirus hexon or fiber genes or both.[194,201–208] Several commercial assays have been marketed that use PCR to identify human adenovirus to species.[209–211] As molecular typing strategies often focus only on specific hypervariable regions of the virus genome, potentially important virus strains resulting from recombinations or mutations can be missed. McCarthy and colleagues proposed a molecular typing algorithm that combined examinations of the hexon and fiber genes.[212]

Restriction enzyme analysis (REA) of whole-virus genomes can be used to identify genetic variants of a serotype, aiding epidemiologic studies and outbreak investigations.[213,214] Extensive studies of Ad3, Ad4, and Ad7 using REA have been described[20,215–218] and a number of novel viruses have thus been identified.[26] However, REA is work intensive, dependent on virus isolation to recover genomic DNA and requires expertise to perform and interpret, and is therefore restricted to a few experienced laboratories.

Classical and next generation sequencing technologies are increasingly being used to obtain full genomic sequences of human adenoviruses. Genome sequences are available for all human adenovirus prototype types and sequences of newly identified genomic variants are now routinely placed in public domain providing reference data for adenovirus identification.[126] A new whole genome-based characterization system for adenoviruses is under consideration to replace immunotyping for designating new adenovirus types.[31,36]

Serological Assays. Acute adenovirus infection can also be diagnosed by serological demonstration of a fourfold or greater rise in antibody titer between paired acute-phase and convalescent-phase sera. The first acute specimen should be collected as early as possible after onset of illness and the convalescent specimen should be collected 2 to 4 weeks later. Serologic tests for adenoviruses have included complement fixation (CF), HI, SN, indirect immunofluorescence, and enzyme immunoassay (EIA).[169] The classical CF test measures adenovirus-group specific antibodies, and though once widely used, lacks sensitivity. The EIA also measures adenovirus group-specific antibodies, but is more sensitive than the CF test and is readily automated and therefore more widely used today.[40,101,219] HI and SN assays also are more sensitive than CF, but because they measure type-specific antibodies, they are not suitable for routine diagnosis and most reagents are not commercially available.[40] Although valuable for outbreak investigations and essential for seroprevalence and vaccine efficacy studies, serology is rarely used today for clinical diagnosis because of the inconvenience of serum collection and the 2- to 4-week delay needed to obtain convalescent serum.

Treatment and Prevention With Antimicrobials

Currently, there are no FDA-approved treatment or prophylaxis therapies against adenovirus infections. There are numerous off-label therapeutic case or case series reports, but their results are mixed. There are few, if any, controlled, prospective therapeutic studies.[220] In the 1960s, γ-globulin was studied as prophylaxis against adenovirus infections without good effect.[221] However, Maisch and colleagues found a positive effect of intravenous immunoglobulin therapy in patients with adenovirus-associated inflammatory cardiomyopathy.[222] Some studies suggest that interferon-β may have a beneficial effect on EKC and viral cardiomyopathies.[223,224] More recently, adoptive transfer of adenovirus-specific T-cells has been explored with some success but this is now reserved for immunocompromised patients who do not respond to antiviral therapy.[225]

Off-label antiviral use reports of the antivirals ribavirin, cidofovir, ganciclovir, acyclovir, and vidarabine[220,226] have also been disappointing. Only ribavirin and cidofovir have been used often enough to be worthy of discussion. Nebulized ribavirin was used with some success for two children with pneumonia caused by adenovirus.[227] Intravenous ribavirin has

been reportedly used in immunocompromised patients and in patients with hemorrhagic cystitis and disseminated disease.[219,228-231] Intravenous ribavirin was given to 12 adult blood and marrow transplant recipients without appreciable benefit.[232] The mixed reports may be explained by a recent finding that only human adenoviruses of species C are susceptible to ribavirin.[190] In contrast, all species of human adenovirus appear to be susceptible to cidofovir.[190] Perhaps this finding explains the better results with this antiviral drug. In a retrospective study of 45 bone marrow transplant recipients treated with cidofovir for adenovirus infections, 31 (69%) had successful outcomes; however, in 18 (40%) of the 45 patients, cidofovir-associated toxicity developed.[233]

A new experimental oral antiviral therapy against DNA viruses, brincidofovir (formerly CMX001), shows great promise against adenoviruses.[225,234,235] An analog of cidofovir, it does not have cidofovir's renal toxicity profile. Currently, brincidofovir is being evaluated against DNA viruses including adenoviruses. It has been awarded "Fast Track Designation" by the FDA.

EPIDEMIOLOGY
Incidence and Prevalence Data

Because only approximately 50% of childhood adenovirus infections result in disease, prevalence as detected by antibody studies is high.[42,236,237] Those in species C (Ad1, Ad2, Ad5, and Ad6) are usually endemic and acquired in early childhood, often before 2 years of age.[39] By school age, most children have been exposed to several types of adenoviruses. Infections caused by Ad4, Ad7, Ad14, and Ad21 may occur at a later age. Many of the other types occur sporadically or in epidemics. Many adenovirus infections are subclinical or asymptomatic, especially those in species A and D. Conversely, Ad4, Ad7, and Ad21 usually cause symptomatic respiratory disease.[39]

Most types of adenoviruses are thought to be endemic worldwide.[45] In 1983, Schmitz and colleagues[45] summarized 10 years of adenovirus reports sent to the World Health Organization. They noted an increasing prevalence of Ad7, Ad8, and Ad19 and a declining prevalence of Ad3 and Ad4.[45] Age predilections were highly significant for infants for species A (Ad12, Ad18, and Ad31); for infants and small children for species C (Ad1, Ad2, Ad5, and Ad6); for school children for Ad3; for school children and adults for Ad7; and for adults for Ad4, Ad8, and other types of species B, D, and E.[45] A predilection for males was observed for all types in species B and C and in Ad4 and Ad19.[45]

A summary of 2237 clinical adenovirus submissions to 22 U.S. laboratories during a 25-month period (2004–2006) revealed that Ad3 (prevalence: 34.6%), Ad2 (prevalence: 24.3%), Ad1 (prevalence: 17.7%), and Ad5 (prevalence: 5.3%) explained most illnesses among children. The authors noted an increasing prevalence of Ad21 over time.[177]

Even though most types of adenovirus can be found throughout the world, sometimes marked differences are observed in the geographical distribution of specific genotypes. Of particular note are the distributions of Ad3, Ad4, and Ad7 genotypes, with anecdotal suggestions that some genotypes may be more virulent and have competitive advantage.[238] As yet, no clear markers of increased adenovirus virulence have been identified.

In 2009, Lebeck and colleagues[26] reported on 516 clinical Ad3 isolates from 15 U.S. laboratories with restriction enzyme digests. The most prevalent types were Ad3a2 (36.9%), Ad3a50 (27.1%), Ad3a51, (18.0%), and Ad3a17 (4.6%). Ad3a50 and Ad3a51 were newly described strains that became more prevalent in 2006. Multivariable modeling revealed that children

younger than 2 years; persons with chronic disease; and persons infected with Ad3a2, Ad3a50, or multiple or rare strains were at increased risk of severe Ad3 clinical disease. Similarly, beginning in 1986, Chile, Uruguay, and Argentina experienced the emergence of a novel Ad7h strain that supplanted the endemic Ad7c strains.[239,240] Among pediatric patients, the Ad7h strain was associated with longer hospitalizations, higher patient temperatures, greater oxygen requirements, and 55% secondary attack rates among siblings, and it explained 94% of adenovirus mortality.[241,242] Similarly, first detected in 1992 in Israel, Ad7d2 spread to the United States in 1996 and since then has been associated with three of the five documented U.S. civilian outbreaks of Ad7[24] and at least one military outbreak.[20] These Ad7d2 outbreaks led to at least 19 deaths and the suggestion that this new genotype was a more virulent strain. In a 2005 report from Iowa, Ad7d2 had displaced other Ad7 strains.[243]

Most recently, a novel genotype of a seldom-detected Ad14 strain (classified as Ad14p1) emerged in the United States and has spread to other countries causing considerable morbidity and death.[28,146,147,244-249] It differs from the Ad14p prototype strain by markedly greater epithelial cell binding, cell cytotoxicity, plaque morphology, and minor mutations in the fiber knob and E1A genes.[250,251,251a]

High-Risk Groups

As noted, virtually all children have adenovirus infections, and, occasionally, these infections are severe and lead to death. Additionally, adenovirus infections are quite common among the immunocompromised and often associated with significant morbidity. Adenovirus epidemics often occur at intensive care units or long-term care facilities. For instance, in 1999 a novel Ad7b variant caused 84% of the 50 residents (age range: 1–46 years) of a New York long-term care facility to become ill, 26 were hospitalized, and 7 died.[252] In a 2007 national U.S. adenovirus study, the authors found risk factors for severe clinical adenovirus disease included age younger than 7 years, chronic disease, recent transplantation, and infection with Ad5 or Ad21.[177]

Epidemics among military populations, especially when adenovirus vaccines were not available, have been particularly well documented.[4] Before the use of vaccines in the U.S. military, Ad4 and Ad7 accounted for 60% of all ARD among hospitalized recruits. Ad3, Ad14, and Ad21 also occurred, but less commonly.[14] Up to 80% of recruits became infected with adenovirus, while seasoned military personnel experienced lower rates.[4] Such outbreaks have also been reported among military recruits in China, Finland, The Netherlands, The United Kingdom, Turkey, Singapore, and South Korea.[253-261]

Modes of Transmission and Reservoirs of Infection

The disease characteristics of the adenoviruses vary by species and type (see Table 10.1).[39] The reasons for these differences are not well understood. Adenoviruses are transmitted by direct contact, aerosolized virus, the fecal–oral route, and via water. Although recent data suggest that human Ad4 and Ad52 may have simian origins,[124,141] and a novel adenoviruses may have crossed species between humans and animals,[137,262] humans are thought to be by far the primary reservoir for human adenovirus.[263]

Significance as a Health Burden

The most common environment for adenovirus transmission is the home. However, high rates of adenovirus transmission

occur wherever large groups of susceptible people gather. Transmission rates are higher in children's institutions and daycare centers, and in lower socioeconomic groups.[46,47] Enteric adenoviruses may be an important pathogen in the daycare setting.[264] Epidemics associated with Ad3 often occur in association with swimming activities.[66] Ad8 has been associated with transmission in physicians' offices.[70]

Nosocomial outbreaks of adenovirus keratoconjunctivitis have been reported from the accident and emergency department of a major eye hospital in the United Kingdom.[265–267] Nosocomial conjunctivitis, pharyngitis, and pneumonia caused by adenoviruses have been noted in hospital intensive care units.[17,268,269]

With increasing numbers of immunocompromised patients in hospitals, adenovirus infections have become of more frequent cause of severe disease.[9] In addition, reactivation of a latent infection could possibly initiate a nosocomial outbreak. Persons with deficient cell-mediated immunity are at greatest risk for adverse outcomes.[39] Bone marrow transplant recipients are especially susceptible to adenovirus infections.[82,110] The death rate among immunodeficient patients with pneumonia may be as high as 60%.[9]

Chronic diarrhea in patients with AIDS is often a diagnostic problem. A prospective study using extensive diagnostic techniques, such as duodenal, jejunal, and rectal biopsies, demonstrated that 6.5% of the patients had adenovirus infections.[270]

Although the civilian experience has not established a requirement for a vaccine, the epidemic nature and extensive morbidity experienced by military recruits during the 1960s demonstrated an overwhelming need for adenovirus vaccines for military use. One well-studied and typical epidemic at Fort Dix, NJ, exemplified this requirement.[15] A platoon of 48 men was followed up prospectively for their 8 weeks of basic training. Of 92 episodes of respiratory illness, 24 required hospitalization. The documented hospitalization rate for ARD due to adenovirus Ad4 was 5/100 soldiers per week.[15] At large basic training posts, this rate translated to approximately 500 to 800 ARD admissions per week, which had a devastating impact on military hospitals.[15,19] Excess medical costs and the fact that soldiers had to be "recycled" owing to lost training time resulted in significant economic loss to the military. Serious disruptions to training schedules led to administrative attempts to control epidemics, such as sleeping head-to-foot and keeping military units separated (cohorting).[19] The impact of adenovirus infections in the military led to the development of adenovirus vaccines.

PASSIVE IMMUNIZATION

While years ago immunoglobulin therapy was not found to be particularly useful in preventing adenovirus infections among military trainees,[220] modern passive immunization strategies may prove helpful. In particular, recently isolating Ad-specific T cells from stem cell donors have been effective in treating hematopoietic stem cell transplantation recipients who develop severe adenovirus that is nonresponsive to antivirals.[37]

ACTIVE IMMUNIZATION

The first adenovirus vaccine prototype was an injectable bivalent Ad4 and Ad7 vaccine grown in monkey kidney cells and formalin-inactivated.[19] After safety tests, a small trial in 1957 reduced admissions for adenoviruses by 98%, and a large trial of 8238 soldiers was 90% effective in reducing hospitalization.[271,272] A trivalent vaccine, which also included Ad3, was also tested.[273] Large-scale production of vaccine lots led to variation in antigenicity, resulting in a lowering of protection

rates, with only a 52% reduction in hospital illnesses caused by adenoviruses.[274] Even this low rate of protection was reported in 1965 to have saved the Army approximately $5 million a year.[274] Adenovirus seed lots were found to be contaminated with simian virus 40 (SV40), a virus oncogenic in animals, and in 1963, the license for the vaccine was rescinded.[15] Although this caused considerable concern at the time, an epidemiologic study of 1959–1961 era U.S. Army male veterans showed no evidence of an increased risk of various cancers that plausibly could be the result of an SV40 exposure.[275]

Use of a live vaccine for ARD caused by Ad7 was investigated, and oral administration was reported to induce high antibody levels in 1960.[276] Couch and colleagues[277] and Chanock and colleagues[278] demonstrated that some adenoviruses infect the gastrointestinal tract but do not produce symptoms in adults. This finding led to the administration of the virus as a vaccine in an enteric-coated capsule, which produced an asymptomatic, intestinal infection and neutralizing antibodies.[277,278] Many safety studies were performed in human volunteers, including the administration of Ad4 and Ad7 vaccines separately and simultaneously.[277] Neutralizing antibodies were elicited, and asymptomatic infections were evidenced by the recovery of virus from rectal specimens. Initial vaccine virus production using HEK cells was later modified to use human diploid fibroblast strains WI-26 and WI-38 because the primary cells were not suitable for large-scale production and there was concern of their contamination with other human pathogens.[278] Because some adenoviruses are oncogenic in animals, much attention was given to safety studies in hamsters and the transformation of cell lines by adenoviruses.[279,280]

Additional field trials for the Ad4 vaccine were conducted at Parris Island, SC, and Great Lakes, IL, indicating that the vaccine was highly protective, safe, and antigenic and showed no evidence of transmission among military trainees. Vaccine use led to reductions in ARD rates by 50%.[281,282] Intrinsic vaccine efficacy was as high as 82%.[282]

Use of the monotypic Ad4 vaccine was followed by the appearance of ARD caused by Ad7 at Fort Dix, NJ.[15] The Army Adenovirus Surveillance Program was initiated to identify the agents of ARD and to assess fluctuations in disease patterns.[14]

Before an Ad7 vaccine could be developed and licensed, numerous studies addressed the oncogenicity potential of Ad7 to humans.[283–286] A trial of the Ad7 vaccine demonstrated safety, infectivity, antigenicity, and lack of communicability similar to that observed for the Ad4 vaccine.[287] Additional trials indicated that when the Ad4 and Ad7 vaccines were given simultaneously, no decrease in immunogenicity occurred, and with mass immunization, there was 95% suppression of Ad7-associated disease.[287–289] Numerous outbreaks of Ad21 in military populations in the United States and Europe led to the initiation of studies using an Ad21 prototype vaccine in safety and immunogenicity trials.[290–294] Trials of Ad1, Ad2, and Ad5 vaccines were also conducted with the goal of protecting children.[295] However, Ad1, Ad2, Ad5, and Ad21 vaccine production was never pursued.

Beginning in 1971, live, enteric-coated, oral vaccines for Ad4 and Ad7 were routinely administered, and adenovirus morbidity was greatly reduced.[19] Ad4 and Ad7 vaccines were licensed by the FDA in 1980 and produced by Wyeth Laboratories. In 1994, the Department of Defense was notified that the sole producer of Ad4 and Ad7 vaccines, Wyeth Laboratories Inc, Marietta, PA, would stop production permanently. Beginning in 1984, Wyeth repeatedly notified the Department of Defense of the need to negotiate a contract that would consider the renovation of the existing vaccine production facility.[296] The contract was not renegotiated.[296] Bulk vaccine production ceased in 1995, and the last vaccine was placed in

tablet form in 1996. Through requests for extensions of expiration dates and use of vaccines only during September through March, rather than year-round, the U.S. Department of Defense attempted to obtain maximum benefit from the remaining supply. By 1999, all vaccine supplies were depleted.

After the loss of the manufacture of the Ad4 and Ad7 vaccines, there were many documented outbreaks of ARD due to adenoviruses in U.S. military training centers. The first recorded epidemic took place during April-May 1995, at Fort Jackson, SC, during a lapse of vaccine administration that occurred because of a logistical error that temporarily interrupted vaccine production.[21] Ad4 was identified as the etiologic agent in unvaccinated soldiers who experienced hospitalization rates of 11.6% during basic training.[21] Numerous additional outbreaks occurred at the other U.S. military camps,[20,22,297] some causing large numbers of hospitalizations[22,297,298] and some spreading to more advanced training camps.[299] Results of a nationwide seroprevalence survey among unimmunized U.S. Army trainees confirmed the lack of protective neutralization antibodies to Ad4 and Ad7, with nearly 90% being susceptible to at least one type.[299,300] Evaluation of the epidemic, clinical, and immunological risk factors for adenovirus infection was performed for a subset of patients, demonstrating that anti-Ad4 immunity was low in new recruits, risk of illness was higher in smokers, and 81% of patients from whom paired serum samples were collected demonstrated a fourfold or higher increase in anti-Ad4 titer after infection.[301]

Gray and colleagues provided a concise overview of population-based surveillance for respiratory disease at four U.S. military training centers as the last of the stores of vaccines were depleted.[302] Between October 1996 and June 1998, 53.1% of 1,814 throat cultures from symptomatic trainees yielded adenoviruses.[302] Ad4, Ad7, Ad3, and Ad21 accounted for 57%,

25%, 9%, and 7% of the isolates,[302] respectively (although the prevalence of Ad7 detections has markedly declined in recent years).[303] Russell and colleagues[304] examined an estimated 73,748 clinical cases of adenovirus infection that occurred during the period of 1999 to 2004 among U.S. military trainees at eight training sites. They observed that adenovirus explained from 52.3% to 76.4% of detected febrile respiratory illnesses at these sites.[304] The aggregate number of adenovirus infections among U.S. military trainees has fluctuated with the availability of vaccines and season (Fig. 10.3). In some winter months, more than 2200 preventable clinical infections were documented. Realizing that the detected adenovirus illnesses greatly underestimated true clinical disease, Russell and colleagues[304] posited that the true count of clinical adenovirus disease occurring in these camps was an estimated 45,000 cases per year, 90% of which would be prevented were the adenovirus vaccines available. This realization of preventable morbidity was not lost to public health officials inside and outside the U.S. Department of Defense, with many calling for reacquisition and use of the vaccines.[305–308]

On September 25, 2001, 7 years after Wyeth announced permanent cessation of production of the adenovirus vaccines, two widely publicized adenovirus-associated deaths in non-immunized recruits,[309] and strongly worded letters from the Institute of Medicine and the Armed Forces Epidemiological Board to expedite contract efforts, induced the Department of Defense to contract for production of adenovirus vaccines.[296,306,310,311] The decision to resume production was based on a continuing low prevalence of antibodies to Ad4 and Ad7 in people coming into the military,[300] the occurrence of documented outbreaks when the vaccines were not given,[20–22,302] and almost 30 years of experience of not having ARD outbreaks when potent adenovirus vaccines were being
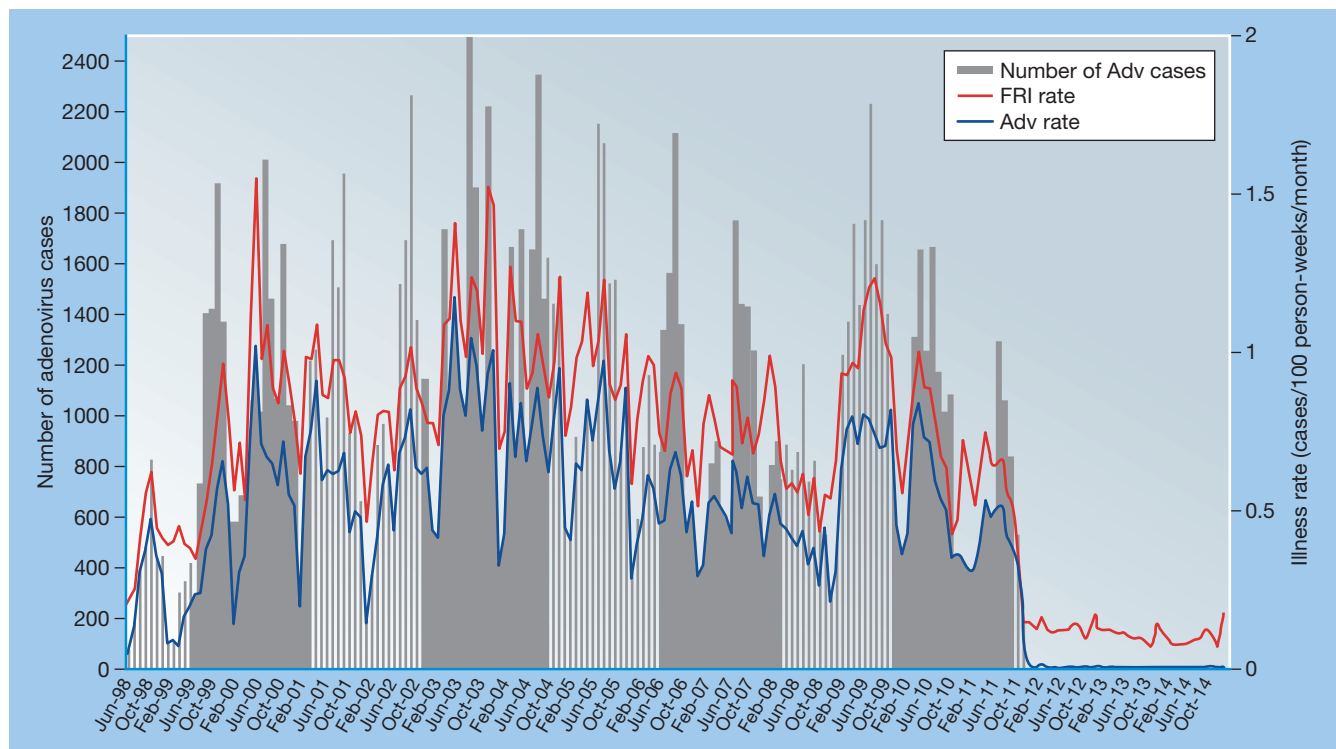

Figure 10.3. Febrile respiratory illness (FRI) and adenovirus (Adv) rates per 100 person-weeks per month with estimated monthly number of adenovirus-associated clinical encounters among trainees at eight U.S. military training sites, June 1998 through December 2014. *(Courtesy Anthony Hawksworth, Naval Health Research Center, San Diego, CA.)*

administered.[19] A genomic analysis of the Wyeth Ad4 and Ad7 vaccine strains show no evidence of attenuation.[312] In 2001 the contract was awarded and seed viruses strains from Wyeth were transferred to Barr Laboratories Inc. (now part of Teva Pharmaceuticals Industries, Inc.).

In the fall of 2004, the Teva Ad4 and Ad7 vaccines were studied in a Phase I double-blind, placebo-controlled trial involving 58 Army medic trainees who had just completed U.S. Army basic training.[313] Among the subjects who had been previously exposed to adenovirus during basic training and before enrollment, 79% and 78% were seropositive to Ad4 or Ad7, respectively. For the trial, 58 initially seronegative subjects were selected. Both vaccines were given to 30, and 28 received placebo tablets. For 32 subjects, final follow-up was permitted at day 180. The participants were closely followed up with cultures, symptom diaries, and clinical examinations during a 56-day period for evidence of vaccine reactions and adenoviral disease. The most commonly reported adverse events in the vaccine recipients were nasal congestion (33%) and dry cough (33%), sore throat (27%), headache (20%), abdominal pain (17%), arthralgia (13%), nausea (13%), and diarrhea (13%). Symptom reporting did not differ between vaccine and placebo groups. Vaccine virus was shed in the stools of 73% and 71% of volunteers seronegative to Ad4 and Ad7, respectively, for as long as 21 days after vaccination. By day 28 after vaccination, 73% of vaccine recipients seroconverted to Ad4, while 63% seroconverted to Ad7. A randomized, double-blind, placebo-controlled Phase II/III trial (NCT00382408) of these vaccines was conducted in 2006–2007 at two military training facilities. Ad4 and Ad7 vaccine or placebo was administered to 4040 U.S. military trainees in a 3 : 1 ratio. As described above the vaccine was found to have excellent efficacy (99.3% for Ad4) and safety profiles similar to the previously manufactured Wyeth vaccine.

In March 2011, after an arduous 10-year vaccine development process, eight likely preventable adenovirus-associated deaths,[314] thousands of hospitalizations, and more than $100 million in funding,[315,316] Teva was awarded FDA licensure for their Ad4 and Ad7 vaccines. The vaccines are indicated for military personnel ages 17 to 50 years of age and contraindicated for persons who are pregnant, who cannot swallow the vaccine tablets without chewing, or who have a severe reaction to the vaccines.

Constituents Including Antibiotics, Preservative, Adjuvants, Etc.

Wyeth vaccine tablets contained live viruses, materials added for the growth and maintenance of viruses and cells, and other pharmaceutical materials. Human-diploid fibroblast cells (strain WI-38) were used for virus preparation, and growth was maintained in Minimal Essential Medium, Eagle solution, antibiotics (neomycin sulfate, gentamicin sulfate, and amphotericin B), fetal bovine serum, and sodium bicarbonate. After harvesting, the viral growth was freed of particulate material by filtration and dried by lyophilization. During drying, additives were used to preserve viability. Before processing into tablets, the virus preparation was diluted with lactose powder.[317]

Using the same Wyeth master seed viruses and a manufacturing process similar to the process Wyeth used, Teva is now producing the Ad4 and Ad7 vaccines. Teva's tablets consist of three layers: a central core containing at least 4.5 $\log_{10}$ tissue culture infective dose (TCID$_{50}$) of lyophilized formulated adenovirus mixed with anhydrous lactose, microcrystalline cellulose, polacrilin potassium, and magnesium stearate, an
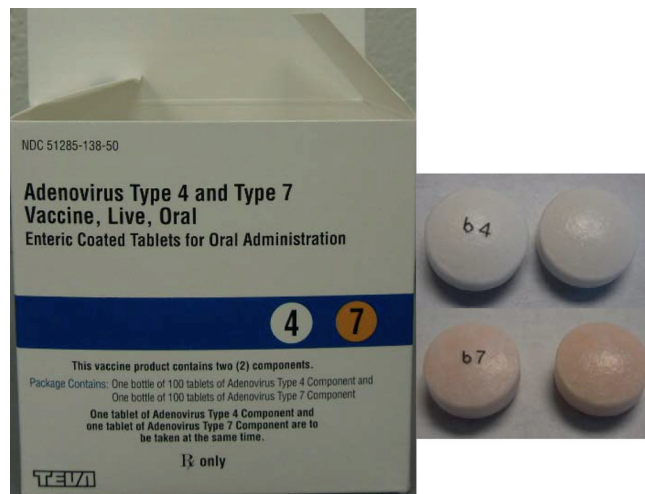

Figure 10.4. Teva Pharmaceuticals Industries Ltd. enteric-coated adenovirus type 4 and type 7 vaccine packaging and tablets. The adenovirus type 7 (b7) tablet is yellow. The enteric coat is designed to resist stomach acid. (*Courtesy John Shaw*, *Teva Pharmaceuticals*, *Inc.*)

outer layer of inactive excipients, and a protective enteric coating consisting of cellulose acetate phthalate, alcohol, acetone, and castor oil (Fig. 10.4).[318] The Ad7 vaccine tablet also contains FD&C Yellow #6 aluminum lake dye to give it a distinguishing yellow color.

Preparations Available, Including Combinations

The Teva adenovirus vaccines contain live nonattenuated Ad4 or Ad7 in the form of enteric-coated tablets. They are produced similarly to how the Wyeth vaccines (described above) were produced. The final vaccine is composed of two tablets (one tablet of Ad4 and one tablet of Ad7) designed to pass intact through the stomach and release the live virus in the intestine. Each enteric-coated tablet contains an inner core tablet containing anhydrous lactose, microcrystalline cellulose, polacrilin potassium, magnesium stearate, and live adenovirus, either Type 4 or Type 7, at a potency of no fewer than 32,000 tissue-culture infective doses (4.5 $\log_{10}$ TCID$_{50}$) per tablet. The outer tablet layer contains microcrystalline cellulose, magnesium stearate, and anhydrous lactose, with an enteric coating consisting of cellulose acetate phthalate, alcohol, acetone, and castor oil. The Ad7 tablet also contains FD&C Yellow #6 aluminum lake dye.

The Ad4 vaccine (white tablets) and the Ad7 vaccine (yellow tablets) are packaged in separate bottles and the bottles are combined in a single package.[318] The tablets can be administered simultaneously but have to be swallowed without chewing. Vomiting and diarrhea may interfere with vaccine effectiveness. These vaccines are indicated for military populations shown to be at risk of ARD from the specific adenovirus types represented in the tablets.

The adenovirus vaccines are routinely given to military recruits and U.S. Coast Guard cadets along with numerous other vaccines in the first 8 days after entering training. Type-specific neutralizing antibodies to the adenovirus vaccines are produced in the vaccinated trainees and were associated with protection against ARD. Interference with immune responses when the adenovirus vaccines were given with other vaccines has not been identified.[319]

DOSAGE AND ROUTE: INTRAMUSCULAR, SUBCUTANEOUS, AND ORAL

The initial oral Ad4 vaccine was developed using dried virus contained in enteric-coated capsules and found to be effective in preventing infections.[278] Original trials for Ad7 vaccine were conducted using 0.05 mL of a $1:10$ dilution of the Ad7 pool (10^6 TCID$_{50}$) within a hard gelatin capsule and given orally.[277] Time of disintegration within the intestine was assayed roentgenographically using a barium sulfate–containing capsule and varied between 1 and 5 hours.[277] Two small dose ranging studies using vaccine capsules suggested that for both types, nearly 100% seroconversion was achieved by approximately 10^4 TCID$_{50}$ per capsule.[289,313] A 1969 dose-ranging study used three Ad7 doses: $10^{6.8}$ TCID$_{50}$, $10^{4.8}$ TCID$_{50}$, and less than 10 TCID$_{50}$ per capsule.[277,289] There was 100% antibody response with the highest dose, 95% response with the intermediate dose, and 56% response with the lowest dose. Another study evaluated the response when both vaccines were given individually at doses of $10^{5.4}$ TCID$_{50}$ for Ad7 and 10^4 TCID$_{50}$ for Ad4, and simultaneously for both vaccines at the same doses.[287] There was no decrease in the immunogenicity of the Ad4 vaccine when it was given with the Ad7 vaccine.[289] In the mid 1960s, Wyeth Laboratories took over production of the adenovirus vaccines, which were converted to enteric-coated tablets.

Vaccine Stability

The currently licensed Teva vaccines are stored between 2°C and 8°C with a shelf life of 30 months.

IMMUNOGENICITY OF VACCINE
Humoral Responses

Oral adenovirus vaccine recipients routinely shed virus fecally, which can potentially continue for up to 6 weeks.[292,320] Wyeth vaccine recipients developed neutralizing humoral antibody (immunoglobulin [Ig] G, IgM, and IgA).[292] In soldiers who were free of preexisting antibody, an average of 80% to 95% developed a neutralizing antibody level of greater than $1:8$, while fewer than 50% demonstrated complement-fixing antibody.[289,321] Neutralizing antibody responses were detected 2 to 3 weeks after vaccination. In general, antibody titers were less than titers achieved after natural infection.[8,282] Local secretory IgA antibody was not induced by the oral vaccine, and reinfection of the respiratory tract was possible, but usually mild or asymptomatic.[322] Since viremia and viruria could occur in patients with febrile disease, invasiveness beyond mucosal surfaces could be important in the pathogenesis of the disease and infection.[323] The serum-neutralizing antibody produced as a result of vaccination may have prevented the typical febrile disease associated with natural infection.[321] Local IgA antibody could be produced experimentally by the intranasal inoculation of an Ad4 vaccine in liquid.[322] A Phase I/II study of the Teva oral Ad4/7 vaccine showed 73% and 63% of immunonaïve subjects seroconverted to Ad4 and Ad7 at 28 days postvaccination.[313] A Phase III study of the same vaccines documented seroconversion at 94.5% (Ad4) and 95% (Ad7).[324]

Cellular Responses

The cellular response to Wyeth Ad4 and Ad7 vaccines has not been well studied. However, the cellular immune responses to some adenovirus-vectored vaccines have been excellent.[325]

Correlates of Protection

A SN assay value of $1:4$ against a specific virus is considered as protective.[315] A fourfold rise in titer is considered as evidence of infection.

Special Groups (e.g., Immunosuppressed)

Although some early Ad1, Ad2, Ad4, and Ad5 vaccine trials were conducted among civilians,[320,326,327] the Teva vaccines have not been used in groups other than healthy military trainees.

EFFICACY AND EFFECTIVENESS OF VACCINE

Military recruits who received Ad4 vaccine exhibited increased resistance to respiratory disease caused by this virus.[278,281,328] A number of major well-controlled studies of more than 42,000 soldiers were performed from 1963 to 1966.[329] Use of the vaccine reduced ARD by 50% and adenovirus infection in recruits by more than 90%.

Field trials began in 1969 for the Ad7 vaccine. The trials indicated protection against disease in susceptible persons.[288,289,330] In addition, it was found that the two vaccines (for Ad4 and Ad7) could be administered simultaneously without interference or loss of efficacy.[288,289,331] As a result of using the vaccines at one large Air Force base during a 9-year period, ARD caused by adenovirus disappeared.[332] The adenovirus surveillance program demonstrated that the combination of the two adenovirus vaccines was highly effective in controlling epidemic ARD.[14] From 1971 to 1999, the live enteric-coated Ad4 and Ad7 vaccines were administered to new military recruits and successfully controlled ARD caused by adenoviruses.

The Phase III double-blind, placebo-controlled efficacy trial of the Teva Ad4 and Ad7 vaccines conducted in 2006 found the vaccines to be 99.3% effective in reducing Ad4-caused ARD.[324] Two years after their reintroduction, the Teva vaccines were credited with a 100-fold decline in military trainee adenovirus cases and with the prevention of 1100 to 2700 hospitalizations each year.[303]

Duration of Immunity and Protection, Including Description of Reinfection, If Any

The duration of immunity and persistence of circulating antibody following immunization has not been well studied. The adenovirus vaccines were developed to protect new members of the military (recruits) against Ad4 and Ad7 respiratory disease during the first months of military service. The adenovirus vaccines were very effective in accomplishing that objective, and immunity beyond 56 days has not been studied.

Safety
Common Adverse Events

Four study groups were followed up for inpatient and outpatient episodes of illness during vaccine safety and immunogenicity trials at Lackland Air Force Base, TX, in 1976. In addition to the placebo group, one group received three vaccines simultaneously (Ad4, Ad7, and Ad21), another group received two vaccines simultaneously (Ad4 and Ad7), and the last group received one vaccine only (Ad21).[294] No appreciable differences were noted in the inpatient or outpatient experiences of the different groups.[294]

Studies of the various live adenovirus vaccines have documented fecal and pharyngeal shedding of vaccine virus and

transmission to nonvaccinated study participants.[295,320,321,324,333] Although recent trials of the Teva vaccine demonstrated sparse transmission among military personnel,[324] the possibility of transmission of vaccine virus to other susceptible personnel should be considered for up to 4 weeks after receipt of modern vaccines.

Rare Adverse Events

In 1973, a Navy trainee was hospitalized with fever, malaise, and dyspnea for 11 days after receiving the Ad4 and Ad7 vaccines.[334] The patient died on the 10th hospital day with the diagnosis of Ad7 pneumonia, based on the isolation of the virus by cell culture. However, it was not possible at the time to determine whether his infection was caused by a wild Ad7 before the development of vaccine-induced immunity or to an Ad7 vaccine strain.[334]

Immunocompromised Recipients

As adenoviruses may cause severe disease among immunocompromised persons, when HIV was first discovered, concern grew among U.S. military public health officials that adenovirus vaccine might cause severe disease among HIV-infected military trainees. Subsequently, a study was conducted among HIV-infected and uninfected military trainees. Although significantly fewer HIV-infected subjects responded to the Ad4 vaccine than did soldiers without HIV infection, no clinically apparent adverse reactions were detected.[319] Response to the Ad7 vaccine was difficult to define because many vaccine recipients in both groups had high neutralizing antibody titers at the time of vaccination.[319] More severely immunocompromised persons, who might inadvertently be infected with adenovirus vaccine virus through fecal shedding from "normal" vaccine recipients, have not been studied.

Pregnancy

The live, oral adenovirus vaccines were never subjected to animal reproduction studies because of the lack of a suitable animal model. Therefore, it is not known if these vaccines had the potential to cause fetal damage if given to a pregnant woman or to affect reproductive capacity. Female military trainees are screened with pregnancy tests before they received the adenovirus vaccines. During the recent Teva vaccine trials four of the subjects were found to be pregnant after they had received the Ad4 and Ad7 vaccines.[318] All four subjects delivered healthy babies at 36 to 40 weeks' gestation. Unusual morbidity among pregnant vaccine recipients has not been reported.

Spread to Contacts

Because the virus may be shed fecally for up to 6 weeks, the virus may be spread to family members or very close contacts.[320,326] Hence, vaccine recipients are instructed to maintain good hygiene and to avoid contact with children younger than 7 years of age, the immunocompromised, and pregnant women for 28 days following vaccination.[318] The spread of vaccine virus has not been well studied. In the recent Phase II trial of the Teva vaccines, participants were followed shedding on days 0, 7, 14, 21, 28, and 56. Periodic fecal shedding was noted 7 to 21 days following vaccination.[313]

INDICATIONS FOR VACCINE—WHO AND WHY

Adenovirus vaccines have been indicated for the prevention and control of specific adenovirus-associated ARD in populations with a high risk of exposure, a high level of susceptibility,

and a high risk of subsequent infection and disease. Adenovirus vaccines were recommended for use in military populations at risk of developing ARD from adenoviruses. Use of these vaccines was not recommended for other populations. Current and future studies of virus transmission and disease occurrence in pediatric populations, chronic care, and other civilian institutional settings; high-level healthcare organizations; and colleges may identify other potential indications for adenovirus vaccines.

In 1971, the U.S. military began routinely administering both vaccines to males reporting to recruit training centers, but only during the winter months.[15] The incoming recruits were given the vaccines within hours of arrival at a training center to obtain protection as early as possible in the training program. Initially, women were not given the vaccines because ARD outbreaks caused by adenoviruses had never been documented among military women, and there was concern about the possibility of administering the vaccines to women who were pregnant.[19]

The program of administering the vaccines only during the high-risk winter months was directed at control, rather than eradication of ARD caused by adenoviruses.[15] With this schedule, late spring and early fall outbreaks occurred. These outbreaks prompted the U.S. Army and the U.S. Navy to adopt policies of year-round administration of both adenovirus vaccines in 1983.[19] Taking a different course, the U.S. Air Force stopped the administration of adenovirus vaccines at its only recruit training center in Texas in the mid-1980s and adopted a program of surveillance with use of the vaccines only as indicated.[19]

When the military started immunizations to protect against ARD caused by adenoviruses, training programs were segregated by gender. These separate training programs have since been combined, leading to concern that the risk for ARD caused by adenoviruses would be the same for men and women. The U.S. military regulation for immunizations required that, based on risk, Ad4 and Ad7 vaccines be administered simultaneously and only once to Army, Navy, and Marine Corps recruits.[335] In the Air Force and Coast Guard, the vaccines were administered when directed by the appropriate authority.[335] The same regulation described precautions to be taken to avoid unintentional administration of the vaccines during pregnancy and counseling instructions regarding the possibility of pregnancy within 3 months following immunization.[335]

In response to the cessation of adenovirus vaccine production in 1996, the Army, Navy, and Marine Corps modified their policies of year-round vaccine administration to conserve the remaining vaccine for use in higher-risk months only. The modified policies directed that the vaccines be given to arriving military recruits only during the period of September 1 through March 31 until all vaccine stocks were exhausted in 1999.

CONTRAINDICATIONS AND PRECAUTIONS

Teva uses no antibiotics in the virus propagation steps. The vaccine is contraindicated for pregnant women, immunocompromised patients, persons outside the target age range of 17 to 50 years, persons who cannot swallow the tablets whole, or persons who are allergic to the vaccines.

PUBLIC HEALTH CONSIDERATIONS
Epidemiologic Effects of Vaccination, Including Herd and Contact Immunity

In the prevaccine era, ARD from adenoviruses caused significant morbidity at military training centers. These outbreaks

were extremely costly in terms of medical care requirements and time lost from training.[19] The routine administration of the Ad4 and Ad7 vaccines, which began in 1971, resulted in an extremely efficacious, cost-effective, and safe immunization program.[19,336–338] Initially, the vaccines were administered only during the high-risk winter months. The occurrence of spring and fall outbreaks prompted a modification of the program to administration of the vaccines year-round.[19] From 1971 to 1999, except for a period in the 1970s when ineffective vaccines thought to be contaminated with solvent were used,[15] there has never been a reported outbreak of ARD caused by Ad4 or Ad7 in U.S. military units that received the vaccines. Outbreaks of ARD caused by Ad14 and Ad21 have occurred, but these have been sporadic.[19,294,339]

A problem in the administrative system used to procure vaccines for the U.S. military resulted in an adenovirus vaccine production delay that began in the spring of 1994 and lasted until late February 1995.[19] There was only one outbreak of ARD caused by adenoviruses reported in conjunction with that production delay.[21] When Wyeth Laboratories announced in 1994 that it would no longer produce the adenovirus vaccines, many in the U.S. Department of Defense and the different military services asked if the vaccines were still needed. Some thought that improved military barracks with modern heating, ventilation, and air-conditioning systems may have significantly reduced the risk of transmission of the adenoviruses and subsequent disease in susceptible people. The assessment of current risk was hampered by the lack of modern seroprevalence data for Ad4 and Ad7 among new military entrants and the lack of studies of adenovirus carriage, transmission, infection, and disease because the vaccines had been so successful. The U.S. Department of Defense also found it had lost the capability to perform serologic studies for adenovirus infections. Unfortunately, with the loss of the vaccines, adenovirus-associated ARD increased in all uniformed services, including the U.S. Air Force.

Disease Control Strategies

Ad4 and Ad7 vaccines have been the primary means for controlling adenovirus-induced ARD among U.S. military trainees. Secondary control measures have included reducing crowding, aligning trainees' beds head-to-foot, making frequent hand washing a requirement, increasing air exchanges, and keeping contact between different cohorts of recruits to a minimum (cohorting).[340–342] Although interventions like frequent hand washing[340] caused a 45% reduction in upper respiratory tract infections among trainees, the numerous attempted interventions fell far short of the effectiveness of administering adenovirus vaccines in reducing ARD.

Cost-to-Benefit Information

The excess morbidity caused by ARD and associated costs are of significant concern to the U.S. military. A number of cost-to-benefit analyses have been performed each showing significant benefit in using the vaccines.[336–338] Using an estimated cost for receipt of both vaccines at $150 and using the vaccines year-round in approximately 200,000 trainees per year, Radin and colleagues,[303] estimated the U.S. Department of Defense would prevent approximately 6000 to 13,000 clinical adenovirus cases and 1100 to 2700 hospitalizations per year, saving the U.S. government approximately $20 million per year in training and medical costs.

FUTURE VACCINES
Subunit Vaccine

In 1963, soluble viral subunit antigens were found to be highly immunogenic on parenteral administration in animals.[321,343] Crystalline hexon and fiber antigens from Ad5 have been shown to induce neutralizing antibody and protection in human volunteers who were challenged.[344] There has been no additional development of these antigens as potential vaccines. However, adenovirus vaccines consisting of soluble viral subunit antigens would be free of DNA and could alleviate fear of the oncogenic potential of adenoviruses.[321]

Recombinant Vaccines

Advances in molecular biology have permitted the in vivo and in vitro gene transfer into mammalian cells using various adenovirus constructs. Genetically engineered adenoviruses have been altered to carry genes from a wide array of other pathogens including influenza A virus,[345] Ebola virus,[346] and Middle East respiratory syndrome–coronavirus,[347] and to introduce genes as a therapy against various chronic diseases. Adenovirus vaccine construct research is quite dynamic. A detailed description is beyond the scope of this textbook. However, excellent reviews have been published discussing the advantages and drawbacks of recombinant adenovirus vaccine strategies.[348–355]

References for this chapter are available at ExpertConsult.com.

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