Dear Colleagues and Friends,

We face great challenges in the field of HIV vaccine discovery and development. Twenty years of studies have answered many scientific questions, yet many more questions remain. Our opportunity now is to review the central objectives of the HVTN and set the scientific agenda that will move forward the field of HIV vaccine science. This is particularly important in light of the recent results of STEP and Phambili trials. As with any scientific endeavor that has met an apparent failure, we will revise our hypotheses and address new questions. And, from these new questions will come insights we were not expecting. We will work with each of you and our partners to continue to design excellent studies that challenge us all and provide guidance to our field.

I accepted the position of Director of the HVTN with a sense of respect for the challenges, a sense of honor to be asked to serve in leadership of the organization, and a sense of excitement over what the upcoming years will bring in our field.

We all face the challenges that make the design of HIV efficacy trials particularly provocative. First, trials must assess multiple types of vaccine effects that demonstrate the ability to eradicate infection, delay disease progression and/or to reduce infectiousness. Second, since immunologic and virologic correlates of protection remain unidentified, efficacy trials need to incorporate secondary objectives aimed at identifying such correlates. Third, the global nature of the epidemic and the genetic diversity of HIV mean that trials must accommodate heterogeneity in study populations and virus strains, each of which may affect efficacy. These challenges will require altering our scientific direction, and at times being willing to move in multiple directions as new data arise.

Despite these challenging prospects, the HVTN has built a robust global infrastructure with strong scientific expertise, sound community preparedness, robust ethical review, and the operational disciplines to support it. As we look to the future, HVTN will undoubtedly continue its tradition of scientific leadership working closely with colleagues around the world to ask and answer questions that are central to finding a safe and effective preventive vaccine for HIV.

As many of you are aware, I’ve directed the HIV/AIDS Network Coordination (HANC) office since 2004, and I’ve had the opportunity to interact with many of you on specific aspects of network coordination. As the co-chair on the Phambili study, I’ve been able to work with some of you and become very familiar with the dedication and expertise in our organization. This transition means that I will have the pleasure to work more closely with all of you all on a daily basis. I am particularly proud of the progress that the HVTN has made over the recent years and I am keen to continue this tradition.

The HVTN demonstrates the critical role clinical trials play in the continuum of HIV vaccine research, and is a model of the iterative process that is required when obstacles are encountered and results are not as we would like them to be. We will continue to pursue science of the greatest integrity, conducted under the highest ethical standards possible. I look forward to working with you all in ensuring that, while vigorously seeking new scientific directions, we adhere to these highest principles.

Sincerely,

Jim Kublin

*Left: Jim Kublin, new HVTN Director*
Adenoviruses and Adenovirus Vectors: 
A Primer on their Biology

Introduction
This article provides a comprehensive educational update on the epidemiology, genetic content, phylogeny and evolution of the family of Adenoviridae, whose members infect hosts throughout the vertebrates. This area has, for the most part, taken a back seat to studies examining the interaction of selected human adenovirus proteins with cellular processes and more recently to the use of adenoviruses as vectors.

Definition, phylogeny and classification
Adenoviruses are non-enveloped viruses that replicate in the nucleus of the invaded cell. Their linear, double-stranded DNA molecules rank them as medium-sized among the DNA viruses. They have an outer protein shell surrounding an inner nucleoprotein core (Figure 1, right). The facets of the virus capsid are composed primarily of trimers of the hexon protein as well as a number of other minor components. The capsid vertices consist of the penton base which acts to anchor the fiber protein; these are responsible for primary attachment of virions to the cell surface.

Phylogenetic relationships among a large number of adenoviruses infecting vertebrates from fish to humans are shown in figure 2 (lower right). The major clades of adenovirus correspond to the four accepted genera, plus a fifth that is likely to be added. Two genera (Mastadenovirus and Aviadenovirus) originate from mammals or birds, respectively, and the other two genera (Atadenovirus and Siadenovirus) have a broader range of hosts including ruminant, avian, reptilian and marsupial. The only confirmed fish adenovirus falls into the fifth clade. Within each genus, viruses are grouped into species which are named from the host and supplemented with a letter of the alphabet.

Human Adenoviruses
Human adenoviruses (HAdV) represent a large family comprised of 51 different serotypes, which are divided into six species (A to F) based on morphological and DNA sequence properties (see table 1, next page).

The hexon protein contains seven hypervariable regions and eight conserved regions. Serotype-specific epitopes are encoded by the hypervariable regions (HVRs) of the hexon. The hexon protein appears to be the most important part of the adenovirus proteome for classification and recognition of individual serotypes. The remaining parts of the hexon protein elucidated to date show little variability among different adenovirus serotypes, thus indicating that the hexon gene might be the most highly conserved component of the adenoviral genome.

Figure 1 (above): The structure of adenovirus. 1 = penton capsomeres 2 = hexon capsomeres, and 3 = viral genome (linear dsDNA)

Figure 2 (above right): Distance tree summarizing the phylogeny of adenovirus hexon genes. Members of the various genera are indicated in different colors, and viruses that belong to the same species are grouped by light-green ovals. Abbreviations of virus names are indicated at the ends of the branches, with species names listed to the right (recognized species in italics): B, bovine; C, canine; D, duck; E, equine; F, fowl; Fr, frog; H, human; M, murine; O, ovine; P, porcine; Po, possum; Sn, snake; T, turkey; and TS, tree shrew.

(Adenovirus continued on page 3.)
However, while complete hexon gene information has been available for a few serotypes, very little sequence information has been available on serotypes of species D, the largest group among human adenoviruses. Phylogenetic analysis of human adenoviruses is shown in figure 3.

Ad5 and Ad35, current vectors for candidate vaccines, are distantly related to each other but exhibit a common ancestor 3 to 5 generations upstream (Figure 3, page 4). The availability of the complete hexon sequences in all human adenoviruses will offer new possibilities in adenovirus classification, diagnosis, and characterization of surface epitopes, which may contribute to more successful, anti-adenoviral therapy or adenovirus-based vaccine designs.

Clinical manifestations and Epidemiology

Adenoviruses were first isolated in 1953 from adenoids surgically removed from children as part of a study to evaluate different tissues for growth of polio viruses by Rowe, et al. They were soon established as the aetiological cause of acute respiratory disease. Adenoviruses have been isolated from every species of mammals, birds and amphibians studied. The recognized diseases of adenoviruses predominantly involve the respiratory tract, the GI tract and the eye. Adenoviruses are associated with a wide variety of clinical syndromes; the majority concerns the respiratory tract (Ad4 and Ad7). The association of particular types with specific disease syndromes is striking. Clinical syndromes also include pharyngitis, pharyngoconjunctival fever, follicular conjunctivitis, epidemic keratoconjunctivitis (Ad8, Ad19, and Ad37), pneumonia, gastroenteritis (Ad40 and Ad41), acute hemorrhagic cystitis, meningitis and a severe disease in AIDS-affected or other immunocompromised persons.

Although epidemiologic characteristics of the adenoviruses vary by type, each is transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission. Virus may be introduced through respiratory droplets or ingestion. After recovery from illness, adenoviruses may maintain latent persistent infections in the tonsils, the adenoids, and other lymphoid tissues and they can be readily activated. Most persons are infected with one or more types of adenovirus before the age of 15 (50 to 80% of surgically removed tonsils yield an adenovirus when cultured in vitro). Ad1, Ad2 and Ad5, members of subgenus C, persist in tonsils for several years. Shedding of infectious virus in the stools for at least two years has been documented. Adenovirus strains can also be secreted in the urine.

What about Adenovirus 5?

Viral and patient epidemiological data on clinical adenovirus infection detected through a nationwide network of 22 US military and civilian medical facilities between 2004 and 2006 were analyzed. Among 1059 patients infected with adenoviral infection from a unique clinical event, 59 (5.3%) were positive for adenovirus type 5. The examination of medical records for patients infected with adenovirus type 5 showed that most patients (94%) were less than 7 years of age and 57% were male and the study highlighted an association of adenovirus type 5 with severe disease as shown in table 2 (next page).

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**Table 1**: Medical record information based on a unique clinical event associated with adenovirus positive specimens. (Gray et al. CID 2007;)

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Serotypes</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>12, 18, 31</td>
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<tr>
<td>B</td>
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</tr>
<tr>
<td>C</td>
<td>1, 2, 5, 6</td>
</tr>
<tr>
<td>D</td>
<td>8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49</td>
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<tr>
<td>E</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>40, 41</td>
</tr>
</tbody>
</table>

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(Adenovirus continued from page 2.)

(Adenovirus continued on page 4.)
Patients infected with adenovirus type 5 were more likely to be hospitalized, to have an extended ICU stay or die than those who were infected with other serotypes of adenovirus. Based on this study the clinical to subclinical for infection with adenovirus type 5 was approximately 2 to 1.

Another interesting finding was that there was a relatively high number of patients detected as having infections with multiple adenovirus isolates over time, with multiple adenovirus types being identified during a single clinical event. Recent reports regarding repeat adenovirus detections in immunocompromised patients and multiple strains causing concomitant infections in US military recruit seem to reinforce that observation, illustrating the complexities of evaluating adenovirus infection.

Although primary infections are respiratory, species C viruses (including adenovirus type 5) display prolonged fecal excretion months and even years after virus is no longer detected in nasopharyngeal washings. This highlights the potential of persistent adenovirus antigens in the gut, documenting chronic shedding. The probable source of persistent virus is mucosa-associated lymphoid tissue, although the molecular details of persistence or latency of virus are not completely elucidated. A study using a sensitive nested PCR assay to look for adenovirus DNA in PBMCs found none in PBMCs from 33 healthy donors and only one positive sample among 40 pediatric specimens. Another study of over 200 donors reported only 1.7% of healthy human adults with adenovirus DNA in their PBMCs. In contrast, a study to determine the amount of species C adenovirus DNA present in tonsils and adenoid lymphocytes, using quantitative PCR in combination with modern cell separation techniques, found a significant amount of adenovirus DNA in those tissues. However, coculture with permissive cells documented that infectious viruses were rarely present. These findings suggest that human mucosal T lymphocytes may harbor species C adenoviruses in a quiescent, perhaps latent form. PBMCs and mucosal-derived lymphocytes have very different circulation and homing patterns.

Virus shedding in the stool also support the mucosal association of the virus. The virus may primarily infect mucosal lymphocytes; alternatively the virus could induce the expression of homing receptors that target infected lymphocytes.

Although early epidemiological studies concluded that persistent adenovirus infections were benign, recent evidence using PCR to identify pathogens at sites of disease suggest a role for the species C viruses in a variety of chronic diseases in immunocompetent persons. For instance, approximately 80% of children with asthma have adenovirus DNA in their nasopharynx compared with 5% of age-matched controls. The persistence of adenovirus in the mucosal compartment may have implications for vaccines using adenoviral vectors.

In sum, adenoviral infections affect infants and young children much more frequently than they affect adults. In the US, childcare centers and schools sometimes experience multiple cases of respiratory infections and diarrhea that are caused by adenovirus. Although these infections can occur at any time of the year, respiratory tract disease caused by adenovirus is more common in late winter, spring, and early summer. However, conjunctivitis and pharyngoconjunctival fever caused by adenovirus tend to affect older children mostly in the summer.

A majority of the population will have experienced at least one adenoviral infection by age 10. Although adenoviral infection in children can occur at any age, most take place in the first years of life. Seroprevalence studies for Adenovirus type 5 indicate that most persons in developing countries (65-80%) have been exposed to adenovirus type 5 as compared to approximately 30% of persons in the US and Europe. Since there are many different types of adenovirus, repeated adenoviral infections can occur.

In the past, US military recruits were vaccinated against two serotypes of adenovirus, with a corresponding decrease in illnesses caused by those serotypes.
Since that vaccine is no longer manufactured, and there are currently no vaccines available to protect against adenovirus, good hygiene, including handwashing, is still the best way to avoid picking up adenovirus from an infected person.

Viral genome and use of adenovirus vectors

Initial infection from virion particles to the cell surface occurs through binding of the fiber knob to the coxsackievirus B adenoviral receptor (CAR). After cell infection, there is disassembly of the capsid that allows for import of the viral genome and start of the viral transcriptional program.

The E1A and E1B genes, referred to collectively as the E1 region, is essential for preparing the cell for viral replication. The genes of the E1 region are necessary for activation of viral promoters and expression of both early and late genes. The E2 region encodes proteins necessary for replication of the viral genome. Products of the viral E3 region function to subvert the host immune response and allow persistence of infected cells. The E4 transcription unit encodes a number of proteins that have been known to play a role in cell cycle control and transformation (Figure 5, below).

Adenoviral vectors incorporating a variety of expressed proteins have been used for gene therapy studies in many different human diseases including cancer, cystic fibrosis, and cardiovascular disease. These vectors have been delivered via several routes of administration including aerosol, intradermal, intramyocardial, intravenous, intrapleural and intratumoral. More recently, recombinant adenovirus vectors have been or are currently being evaluated in large scale clinical trials for HIV and other pathogens.

To deliver genes to the intended target and provide expression for an appropriate length of time, several strategies have been developed to alter the tropism of adenovirus vectors. The construction and biology of these vectors are presented in a schematic showing the genome structure of commonly used vectors (Figure 6, below left). There are three categories of vectors based on the adenovirus genome structure.

The first generation of vectors includes E1 region-deleted adenovirus vectors. Removal of the E1 coding sequence results in viruses that are severely impaired in their ability to replicate. Removal of the E1 region alone allows for a relatively large space (5.1 kb) for insertion of genes. Many of the first generation vectors also contain a partial deletion in the E3 region, mainly to minimize the likelihood of regeneration of the wild-type virus. Furthermore, E3 genes are entirely responsible for virus growth in vitro and their removal allows for more space for gene insertion (8.2 kb). Even though these vectors have proven to be highly promising as vehicle for gene delivery, there are problems associated with their production, as it is possible that recombination between the E1 region sequence and the recombinant virus give rise to viruses with functional E1 genes that are replication competent. The major disadvantage associated with the use of first generation vectors is their stimulation of a cellular immune response with only a transient transgene expression.

The second generation vectors have been constructed primarily by the removal of E2 and E4 coding sequences. These vectors have been developed to prevent the immune response generated by low level replication of E1 deleted viruses, thus inhibiting viral gene expression. It is theorized that removal of all or part of the E4 transcription unit would impair viral replication and gene expression such that an immune response would not be triggered, thus increasing the length and level of transgene expression. These vectors also provide the benefit of a larger capacity for transgene insertion.

The helper-dependent vectors represent the approach that holds perhaps the most promise for long-term gene expression. In this strategy, all the viral structural genes are deleted from the viral chromosome, leaving just the two inverted terminal repeats (ITRs) and the packaging signal. Such a chromosome can accommodate a large amount of transgene sequences (37 kb).

As discussed previously with the wild-type viruses, adenovirus vectors may persist in vaccine recipients. The mechanism of Adenovirus vector persistence remains to be elucidated. While the vectors used in gene therapy and as vaccines are replication incompetent and appear to not replicate or persist over time, there is controversy in the field about this persistence. Recently, some studies in mice and primates have documented that adenoviral vectors (E1 –deleted) may persist for extended time periods in vivo and maintain activated CD8+ T cells at least 2 years. These vectors persist at the site of inoculation, in the liver and lymphatic tissues.

Figure 5 (above): Map of the adenovirus genome and transcription units. The central, solid line represents the viral genome. Positions of the left and right inverted terminal repeats (ITRs), the packaging sequence (ψ), the early transcription units (E1A, E1B, E2, E3, and E4), and the major late transcription unit (major late promoter [MLP]), L1-L5 are shown. Arrows indicate the direction of transcription.

Figure 6 (above): Genome structure of first-generation, second-generation and helper dependent vectors. Regions that have been deleted are indicated by open boxes.

(Adenovirus continued on page 6)
Within the spleen, adenovirus vectors persist preferentially in T cells activated in response to adenovirus transduced cells. These vectors persisting at low levels continue to produce antigens and maintain a cohort of activated effector memory/effector T cells, thus allowing for the development of central memory CD8 T cells. Levels of effector CD4+ T cells to adenovirus vectors in the gut remains unknown as well as the balance between their activation and suppression. Even though several studies have documented the persistence of adenovirus 5 in the gut, none have described the mechanisms (cell trafficking, TLRs, ligands, chemokines, etc.) underlying that persistence. A better understanding of these mechanisms may help us explain the recent observation of the enhancement of infection among vaccine recipients of the Merck study vaccine.

How are these vaccines produced?

The main cell line used for E1 deleted-adenovirus vectors is the 293 cell line derived from human embryonic kidney cells that contains the E1 region of Adenovirus. Another popular cell line is the Human Embryonic Retinal cell, the PER.C6® (PGK promoter, E1 containing and Retinal, clone number 6). PER.C6® cell line is ideally suited for the development and large-scale manufacturing of a multitude of biopharmaceuticals. These include vaccines, therapeutic proteins including antibodies and gene therapy products (Figure 6, below left). PER.C6® cell line is derived from a single, human retinal-derived cell, which was purposely immortalized using recombinant DNA technology. As a result, PER.C6® cells can replicate indefinitely, allowing them to be cultured in single cell suspension under serum-free conditions in quantities appropriate for large-scale manufacturing. PER.C6® cells support growth production of many viral vaccines (Figure 7, below left).

Many viruses have been demonstrated to efficiently replicate on PER.C6® cells. Because of the high titers reached, PER.C6® cells provide excellent bulk material for processing and final formulation of inactivated whole virus, subunit and live recombinant vaccines. The MRKAd5 vaccine has been developed using this cell line.

Immune response to adenovirus vector vaccines

Adenovirus is a significant pathogen in immunocompromised patients and is widely utilized as a gene delivery vector. After systemic administration of adenovirus, a large proportion of vector is sequestered in the gut. Subsequent uptake by macrophage results in rapid release of large quantities of inflammatory cytokines. In addition to the adaptive immune response expressed by the low-level expression of viral genes, a substantial innate immune response is triggered on virus administration. Activation of the innate immune system is stimulated by virus particles and therefore not dependent on transcription from viral DNA. This response can cause inflammation in both target and surrounding tissues, resulting in a considerable loss of transduced cells. Furthermore, high titers of antibodies against capsid proteins, either pre-existing because of previous exposure to natural virus or generated as a result of vector administration, may inhibit subsequent dosing with same vector. Several strategies have been used to improve adenovirus vector based vaccines and circumvent pre-existing immunity to Adenovirus vectors. These strategies include the use of novel recombinant adenovirus vectors (rare serotypes) such as Ad11, Ad35 and Ad50 from subgroup B and Ad26, Ad48 and Ad49 from subgroup D. Other strategies include the development of novel chimeric adenovirus vectors and adenovirus vector combinations (Ad5+Ad6). While T-cell based vaccines focus primarily on the delivery of virus specific CD8+ T cells, there is increasing evidence of the importance of CD4+ T cells in the control of viral diseases. Murine models are somewhat limited because adenovirus infections are at best semi-permissive and consequently, cellular immune responses are dominated by CD8+ cytotoxic T cells. Most studies of human immunity in healthy volunteers have reported a predominant CD4 response, but the relative importance of CD8+ and CD4+ T cells in Adenovirus disease remains unclear. Perhaps the greatest obstacle to systemic delivery and long term gene expression is the innate immune response, to which any vector is susceptible. Although a lot of ground has been covered in the knowledge of Adenovirus biology, we need to continue to study this intriguing virus and how it interfaces with the host in order to make it a more useful tool.

By Gaston Djomand

(References on page 7)
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