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Diversity of adenoviruses in humans and in non-human primates

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Publication date
2012

Link to publication

Citation for published version (APA):
Roy, S. (2012). *Diversity of adenoviruses in humans and in non-human primates*. [Thesis, fully internal, Universiteit van Amsterdam].

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Chapter 1

General Introduction

The isolation of adenoviruses from humans, macaques, and apes

In 1953, Rowe et al. (1) observed the spontaneous degeneration of cultured explants from tissues that had been surgically removed by adenoidectomy and correctly attributed this to a new viral agent which he termed the Adenoid Degeneration (A.D.) agent. At about the same time, Hilleman and Werner (2) found that an outbreak of a contagious upper respiratory tract disease at an United States army base was not due to influenza as they had initially surmised. They were able to isolate the actual causative agent by using patient exudates to infect primary cultures of tracheal epithelial cells and called it the Respiratory Illness (RI) agent. Different serotypes of this virus were recognized, and it was soon realized that most humans are usually exposed to these viruses at an early age (3). The name "adenovirus" was adopted for these viruses (4). Thus, two forms of adenoviral infection were recognized at a very early stage following the identification of adenoviruses: the first, a syndrome that ranged from a mild febrile catarrh to a primary atypical pneumonia requiring hospitalization, and the second, an apparently quiescent infection in asymptomatic individuals where the virus was latent in lymphoid tissue and could be recovered by culture of explanted lymphoid tissues *ex vivo*.

The discovery of adenovirus infections in humans was quickly followed by the serendipitous isolation of macaque adenoviruses (5, 6). In the 1950s, large numbers of primary monkey kidney cultures were being cultivated for the propagation of poliovirus. These cultures would sometimes undergo spontaneous lysis preceded by a change in cellular morphology characterized by rounding, clumping and reduction in size, the "grape-cluster" morphology typical of the adenoviral cytopathogenic effect. At about the same time, additional macaque adenoviruses were also isolated from feces (7, 8).

Discovery of ape adenoviruses also occurred soon after human adenoviruses began to be characterized. A chimpanzee adenovirus isolated from the feces of a captive chimpanzee (Bertha) was among the earliest adenoviruses isolated (9). The next isolation of chimpanzee adenoviruses stemmed from an attempt to identify the causative agent of hepatitis in a primate facility by culturing stool filtrates (10). Twenty-two isolates belonging to at least 7 serotypes were characterized. Unfortunately, these strains are now no longer available. Later, Gajdusek and colleagues isolated four adenovirus strains that were present as adventitious agents in the mesenteric lymph nodes obtained from chimpanzees at autopsy (11).

Pathogenicity of adenoviruses

Human adenoviruses were initially classified into four species based on differences in their ability to agglutinate rat and rhesus erythrocytes (12). The number of species (initially called subgroups) was extended to six (HAdV-A to HAdV-F) as more differences became experimentally demonstrated, such as GC content and tumorigenicity in rodents (13). HAdV-B, C, and E have come to be recognized as common human upper respiratory tract pathogens although most exposure is probably sub-clinical. HAdV-F can

cause intestinal infections and is responsible for infectious diarrheas in regions where drinking water may be fecally contaminated. Also, intestinal infections of HIV-infected patients has often been found to be related to HAdV-D (14).

Evidence for persistence of adenoviruses

In two epidemiological studies it was found in that in children who had presented with clinical adenoviral disease, adenoviral shedding in stools was common and in some instances was detectable over months of follow-up (15, 16). Another study provided evidence that children who excreted adenoviruses for prolonged periods were in fact persistently infected by the same virus, i.e., persistent excretion was not due to recurrent infections (17). Early studies on adenoviruses that could be isolated from explanted adenoids and tonsils showed that these adenoviruses could set up a lytic infection of fibroblasts but infection of lymphocytes did not result in a productive infection (18, 19). In several instances the productive replication of latent adenoviruses present in lymphocytes could be stimulated by a mitogen such as phytohemagglutinin (20-22). Persistent infection of cultured lymphoid cells from humans and monkeys has also been demonstrated (23). Interestingly, the viral infection of lymphoid cells was seen to persist even in the presence of neutralizing antibodies, suggesting direct cell to cell spread as a possible method of avoiding neutralization by antibodies (24). There was one report suggesting the presence of adenoviral transcripts in normal human and gorilla tissues (25), but this result has not been independently verified.

The most convincing evidence that adenoviruses can cause silent infections of humans comes from situations where these latent infections become exposed, e.g., in situations where immune surveillance mechanisms may have been compromised, such as in HIV-infected individuals. Also, pharmacologic secondary immunodeficiencies due to immunosuppression for transplantation or as a result of anti-neoplastic drugs can often give rise to life-threatening adenoviral infections (14, 26).

Adenoviral gene-transfer vectors

Historically, adenoviruses evoked intense interest following the discovery that when injected into non-susceptible hosts such as rodents, some adenoviral species such as HAdV-12 were able to reproducibly induce tumors (27). The ability to transform cells in culture was found to be present in all adenoviruses and could be attributed to the early gene products transcribed from the left end of the genome – the E1 proteins consisting of the E1a protein (and its splice variants) and the E1b 19K and the E1b 55K proteins. Deletion of this region of the adenovirus was found to render the virus replication-incompetent, and also provided a location where foreign gene expression cassettes could be inserted. Crucially, it was found that cell lines that were stably immortalized by the DNA from the left end of the adenoviral genome could efficiently trans-complement adenoviral mutants deleted in the E1 region. One such cell line that was

derived by immortalization of human embryonic kidney cells – HEK 293 (28), – has frequently been used to manufacture adenoviral vectors for use in animal and human studies.

In an adenovirally infected cell, the E1b 55K protein forms a complex with the 34K gene product from the adenoviral early region 4 (E4 orf6). This complex translocates to the nucleus and plays an important role in viral mRNA transport. Thus, the ability of E1-transformed cell lines, such as HEK 293, to support the replication of E1-deleted adenoviruses requires that the E1b 55K gene product that is synthesized by the complementing cell can form a functional complex with the E4 gene product expressed from the viral DNA. Cell lines transformed by the E1 genes from HAdV-C can thus be used to rescue E1-deleted vectors derived from HAdV-C, and as was subsequently discovered, HAdV-E as well (29) but not HAdV-B. Rescue of HAdV-B E1-deleted vectors has required the co-expression of the cognate E4 orf6 protein (30, 31).

Pre-existing immunity to adenoviruses

The efficacy of adenoviruses as gene transfer vectors is reduced considerably when the injected subjects have circulating antibodies capable of neutralizing the infectivity of the virus. Antibodies capable of neutralizing adenoviruses can arise following a natural infection and also after the first administration of a vector. The antibodies are directed against all three major capsid proteins, hexon, fiber, and penton. Analyses performed by utilizing purified capsid proteins have shown that the fiber and hexon provide serotype-specific neutralizing determinants (32). An attempt to circumvent pre-existing immunity (experimentally induced in rats) by engineering the vector to harbor a fiber derived from a different serotype was unsuccessful (33) indicating that anti-hexon antibodies likely provided important neutralization determinants. This hypothesis was tested and shown to be true in C57BL/6 mice (34) where it was shown that a HAdV-5 vector in which the hexon had been replaced with that from HAdV-12 was able to efficiently transduce mice that had been previously administered a HAdV-5 vector (Figure 1). Alignment of hexon sequences from different serotypes shows that sequence variation between hexon sequences is greatest in the region of the solvent exposed loops that are now defined to constitute the hypervariable regions (35, 36) and are thus the likely target for antibodies. Therefore it was proposed that similarly to hexon switching, serotype-specific anti-hexon neutralizing antibodies could also be circumvented by exchanging only the exposed hypervariable loops between serotypes in the construction of vectors with new antigenic specificities (34, 37).

Because neutralizing antibodies greatly reduce vector efficacy, adenovirus serotypes that are endemic in populations are likely to have limited use as vectors, including HAdV-5 or other HAdV-C vectors. This fact led to the search for adenovirus serotypes such as HAdV-35 against which antibodies are of low prevalence in human populations (38). Another possible solution is to utilize vectors created from animal adenoviruses such as ovine (39), canine (40) or bovine (41) adenoviruses. However, one of the drawbacks of this approach is the necessity to create an E1-complementing cell line for each of these adenoviruses.

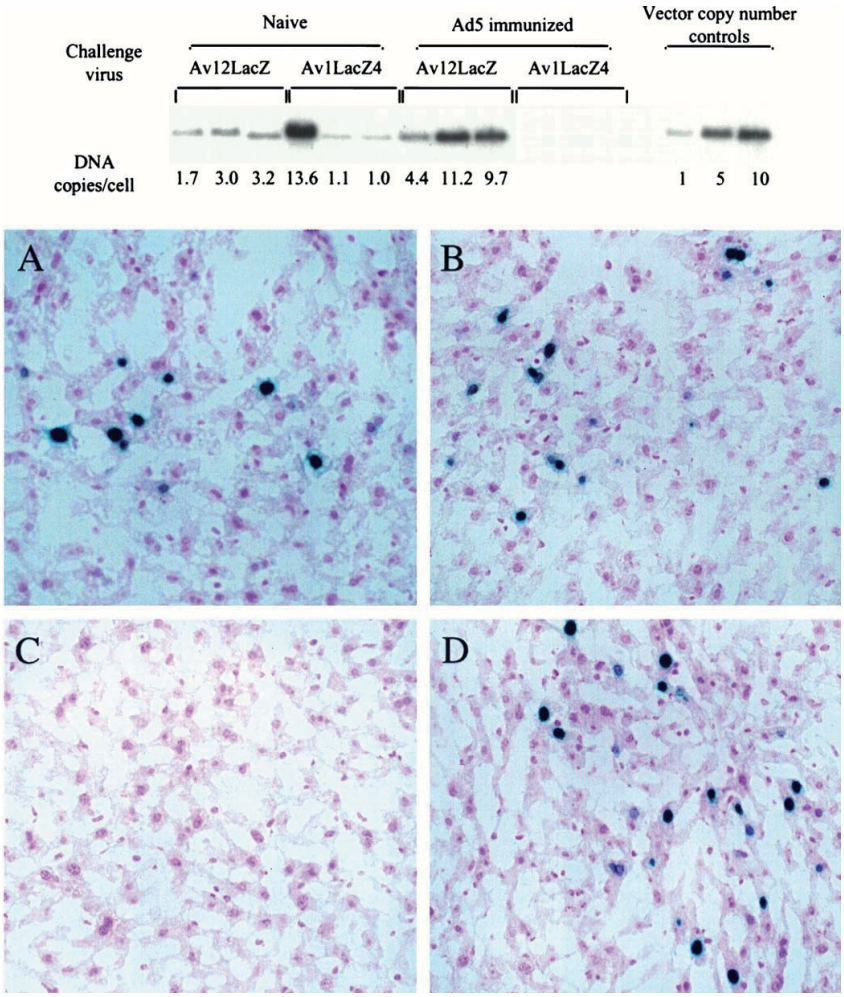

Figure 1 Southern blotting (upper) and X-gal staining (lower) of livers of mice that were administered adenovirus vectors expressing β-galactosidase. Either naïve (panels A and B) or HAdV-5-immunized (panels C and D) mice were injected with either a HAdV-5 vector (Av1LacZ; panels A and C) or a HAdV-5 based vector where the hexon sequence was from HAdV-12 (Av12LacZ; panels B and D). [from Soumitra Roy et al. J. Virol. (1998) 72, 6875].

However E1-deleted ape adenovirus vectors such as those isolated from chimpanzees were found to be sufficiently close to human adenoviruses to allow for their growth in HEK 293 cells (29). An additional advantage of manufacturing ape adenovirus vectors in cell lines expressing HAdV-5 E1 is that there is insufficient homology between the integrated chromosomal HAdV-5 sequences with the adenoviral DNA to allow for the emergence of E1-containing replication-competent vectors by homologous recombination.

Scope of this thesis

As discussed above, children with adenovirus infections have in some cases been found to shed adenoviruses in their stools even after recovery from illness. However, this appears to be the exception rather than the rule, because adenovirus culture from the stools of asymptomatic humans is positive in only 2 to 3% of cases.(42, 43). This is in contrast to lymphoid organs such as adenoids and tonsils that frequently harbor lymphocytes from which adenoviruses can be recovered. Since the gut is a very large lymphoid organ, it was of interest to determine whether lymphocytes present in the normal human gut harbor adenoviruses. This study is described in **chapter 2**. In contrast to humans, it had been reported that chimpanzees did excrete adenoviruses in their stools that could be easily recovered by culture (10). In **chapter 3** the prevalence of adenoviral shedding in normal apes (i.e., not known to be ill at the time, unlike the afore-mentioned study) was investigated. Chimpanzees, bonobos and orangutans were tested. In addition, four chimpanzee adenoviruses that had been isolated in the 1950s (SAdV-21) and 1960s (SAdV-22, -23, -24, and -25) were also completely sequenced and their characterization is described in **chapter 4**. In **chapter 5**, the study of the shedding of adenoviruses was extended to rhesus macaques. This was of interest because macaques are frequently used for vaccine challenge studies including those with adenoviral vaccines and it is plausible that the characteristics of the resident adenoviruses in macaques may influence the immune responses to administered vectors. As discussed earlier, ape (including chimpanzee) adenoviruses may have advantages over human adenoviruses for gene transfer to humans because the prevalence of neutralizing antibodies to these viruses in human populations is likely to be low. Such vectors were constructed and tested in mice as described in **chapter 6**. While the E1-deletion of species E chimpanzee adenovirus vectors could be complemented by the HAdV-5 E1 gene products in HEK 293 cells, the species B adenoviruses (including SAdV-21) could not, as in the case for human species B adenoviruses. To overcome this, a chimeric adenovirus approach was devised as described in **chapter 7**. The feasibility of the construction of adenoviral vectors from the widely divergent ape adenoviruses belonging to species B, C, and E was explored in **chapter 8**. It was also found that a macaque adenovirus, that had been isolated from primary monkey kidney cells during polio vaccine manufacture, was naturally truncated in E1a and E1b 19k genes and could grow in human cell lines. It was thus a good candidate for a non-human primate derived adenovirus vector as described in **chapter 9**. In **chapter 10**, the question of the specific targets of the antibodies against adenoviral capsid components that are responsible for diminishing vector efficacy is addressed by creating chimeric vectors where hexon and fiber genes could be exchanged between two distinct chimpanzee adenovirus serotypes. The utility of chimpanzee adenoviral vectors as vaccine vectors in challenge models are explored in **chapters 11 and 12**. In **chapter 11**, a chimeric species B chimpanzee adenovirus vector expressing the Ebola (Zaire) glycoprotein was tested in a mouse challenge model. In **chapter 12**, the T-cell response against nucleoprotein from an H1N1 influenza strain that was elicited by a chimpanzee adenovirus vector was measured and the possibility of cross-protection against two non-homologous H5N1 influenza strains was tested in a mouse challenge model.

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