Adenoviral vectors: a possible road to an HIV vaccine
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CHAPTER 8

General Discussion

Introduction

Twenty-five years after the first reports on AIDS, approximately 39.5 million people are living with HIV. Still close to 5 million new infections occur every year, making the need for an effective HIV vaccine paramount. However, the development of a vaccine achieving sterilizing immunity faces major challenges, since such a vaccine most likely requires inducing both NAb and CD8+ T lymphocytes. To date, a wide variety of approaches to generate a vaccine capable of generating neutralizing antibodies have proven ineffective. In contrast, the development of vaccines capable of inducing HIV-specific cellular immune responses has proven successful. The HIV-specific CD8+ T lymphocytes are believed to be important to control HIV replication and to slow the clinical disease progression. Long-term non-progressors or HIV controllers are human individuals that control HIV replication, as characterized by undetectable levels of HIV RNA in their plasma, for long time periods in the absence of any treatment. It was observed that these individuals generally have potent CD8+ T lymphocytes responses (45, 121), which strongly indicates that the induction of CD8+ T lymphocytes by a vaccine will help to control the viral load and prevents or delays disease progression towards AIDS. This hypothesis is further strengthened by the observation that depletion of CD8+ T lymphocytes during SIV infection in rhesus monkeys resulted in rapid disease progression and lack of control of HIV replication (289).

In particular, plasmid DNA vaccines and recombinant live viral vector-based vaccines are potent inducers of HIV/SIV-specific CD8+ T lymphocytes responses. One such live viral vector, based on the human Ad5, has demonstrated great promise as a vaccine vector in both pre-clinical as well as clinical studies (50, 76, 301, 302, 313, 314). A possible limitation to the use of Ad5-based vaccine vectors, however, is the worldwide prevalence of anti-Ad5 immunity in the human population. Pre-clinical studies have demonstrated the blunting effect of anti-Ad5 immunity on the immunogenicity of the Ad5-based vaccines (18, 48, 375) and these results were confirmed by the recently completed phase I human clinical trials (50, 76). To date, several research strategies are under investigation that potentially will solve the problem of Ad5 pre-existing immunity, including plasmid DNA prime followed by a rAd5 boost, the development of novel rAd vaccines vectors based on rare human adenovirus serotypes or non-human adenoviruses and the construction of chimeric rAd5 vectors. The work presented in this thesis focused on the determination of the main immunodominant target of vector-specific NAb and on the construction and immunogenicity of novel rAd vectors derived form rare human adenovirus serotypes.

Chimeric rAd5 vector-based vaccines

Complete understanding of anti-Ad5 immunity is pivotal to be able to construct chimeric rAd5 vaccine vectors capable of evading the prevalent Ad5 pre-existing immunity. It has been shown that both NAb and CD8+ T lymphocytes take part in the anti-Ad5 immunity, although the NAb play the primary role (316). Therefore it is important to determine which capsid proteins are the main target of the NAb. One study demonstrated that in human sera mainly anti-fiber and anti-penton antibodies were induced upon adenovirus administration (137). In addition, another study utilizing human sera showed that anti-fiber and anti-penton antibodies display a synergistic neutralizing activity (97). Both these studies indicate that exchanging the fiber and/or penton gene would result in a vector that is able to escape anti-Ad5 immunity. However, one pre-clinical study in mice utilizing a chimeric rAd5 vector carrying the fiber of Ad35 proved that such a chimeric vector is still hampered by anti-Ad5 immunity (240). In addition, we have shown in chapter 2 that a chimeric rAd5 vector carrying both the fiber and penton of Ad35 also remained neutralized by anti-Ad5 immunity in mice. This could suggest that the NAb profiles differ between human and mouse sera.
Though, in chapter 2 we used both human and mouse sera to determine Ad neutralization \textit{in vitro} and observed no differences in NAb profiles. In fact, the study described in chapter 2 confirmed other prior reports (61, 331, 364), which illustrated that the hexon protein is the major determinant for anti-vector immunity. Based on this knowledge, many attempts were subsequently made to construct Ad5 chimeric vectors carrying hexon proteins derived from rare adenoviruses. This has proven to be a major challenge and many attempts have failed (378). Reason for the latter likely reflects the lack of structural compatibility between the newly introduced hexon proteins and the other Ad5 capsid proteins e.g. penton base, protein IX, protein IIIa, protein VI. Despite this challenge, several hexon chimeric vectors, based on the hexon of 1, 2, 3, 6 and 12 have been successfully constructed (367, 378). With at least two of these vectors, i.e. Ad5-Hex6 and Ad5-Hex3, successful escape of anti-Ad5 immunity was demonstrated (367, 378). However since many of these vectors proved poorly viable, an alternative strategy was recently employed in which not the entire hexon-coding domain was exchanged but only the short amino acid stretches called hypervariable regions (HVRs), which are considered to contain the major NAb determinants. Seven HVRs are present in all adenoviral hexon sequences known to date (61). These HVRs are predicted to be exposed on the surface of the viral particle (271). The importance of the HVRs as NAb epitopes was confirmed by the finding that an Ad5-chimeric vector, in which all the Ad5 hexon HVRs were replaced by Ad48 derived HVRs (Ad5HVR48), was successful in escaping anti-Ad5 immunity in both mice and non-human primates (271).

**Rare human adenovirus 35 vector-based vaccines**

The high seroprevalence of Ad5 among human populations has initiated seroprevalence surveys to identify other human adenoviruses with low seroprevalence (237, 344). In these surveys, human Ad35 has been identified as rare in human populations (174, 237, 344). As such, Ad35-based vaccine vectors have been developed for several infectious diseases (20, 27, 123, 241, 264). Pre-clinical studies in naïve mice showed that the Ad35-based vaccine vectors are capable of inducing potent antigen-specific cellular immune responses (20, 241, 264), albeit somewhat less than for Ad5-based vaccine vectors (20, 241). The somewhat lower immunogenicity of Ad35-based vaccine vectors in comparison to Ad5-derived vectors was also confirmed in Ad5-naïve non-human primates ((301), chapter 3). However, in studies using mice with anti-Ad5 immunity, the immunogenicity of Ad35-based vaccine vectors was not hampered, while the immunogenicity of Ad5-based vaccine vectors was clearly diminished (20, 241).

The reason for the higher immunogenicity of Ad5-based vectors in naïve settings is currently not well understood. A possible explanation may be the lack of the cellular receptor of the Ad35-based vaccine vectors in mice. Ad5 is known to be able to utilize the mouse-CAR for cellular transduction (34). However, Ad35 uses the CD46 receptor for cellular transduction (96), which is not expressed in inbred mice. In a recent study the immunogenicity of an Ad35-based vaccine was investigated in both wildtype and CD46-transgenic mice. An improved immunogenicity for the low (10^7 vp) dosages of the Ad35-based vaccine in CD46-transgenic mice was observed in comparison to the immunogenicity of the vector in wildtype mice (343). This study indicates that the lack of CD46-expressing in inbred mice could, at least in part, be an explanation of the lower immunogenicity of Ad35-based vaccines in naïve settings compared to the immunogenicity of Ad5-based vaccines. However, also in Ad5-naïve non-human primates, which do express CD46 comparable to humans, a somewhat lower immunogenicity of Ad35-based vaccine vectors in comparison to Ad5-derived vectors was observed ((301), chapter 3). A possible explanation for this may be that non-human primates, in contrast to humans, express CD46 also on their erythrocytes (141). The presence of CD46 on erythrocytes may consequently
result in unwanted clearance of the Ad35-based vaccines. However, this hypothesis remains to be further investigated. In contrast, we demonstrated in chapter 3 that the Ad35k5 vector proved equal immunogenic to the Ad5-based vaccines in both naïve mice and naïve non-human primates upon a prime immunization. This latter finding may indicate that the usage of CAR is preferable to the usage of CD46 in relation to the immunogenicity of the vaccine vector. A possible explanation for this is offered by a publication by Shayakhmetov et al. In this study it was demonstrated that binding to the Ad35 receptor (later identified as CD46) by an chimeric Ad5k35 vector resulted in an inefficient transport of the viral DNA to the nucleus (297). Consequently, this could result in a reduced transgene expression, which may explain the lower immunogenicity observed for Ad35-based vaccines. However, an artificial human skin model utilizing skin emigrated DC transduced in situ by either an Ad5- or Ad35-based vaccine, demonstrated that the Ad35-based vaccines is as potent as the Ad5-based vaccine to activate T lymphocytes (67). In this respect, the Ad35-based malaria and an Ad35-based tuberculosis phase I clinical trials, which have recently been initiated, will undoubtedly provide valuable insights into the value of these vectors for vaccine purposes and will also provide inside into the ability of animal models to predict to immunological potential of Ad-based vaccines.

Additional rare human adenovirus serotype vector-based vaccines

The seroprevalence surveys helped to identify, in addition to Ad35, several other human adenoviruses that also have a low seroprevalence worldwide and low NAb titers in individuals who are seropositive, including Ad11, Ad26, Ad48, Ad49 and Ad50 ((136, 344), chapter 5 and chapter 7). From these serotypes, replication-incompetent vector systems have been generated ((136), chapter 5 and chapter 7). The Ad11-based vectors were initially developed as a possible booster for an Ad35-based vaccine. In chapter 4, we demonstrated that the Ad11-based vaccine proved to induce potent antigen specific cellular immune responses upon a prime immunization. Also it was demonstrated that in mice with high levels of anti-Ad5 immunity, an Ad35/Ad11 prime-boost regimen proved superior to Ad11/Ad5 or Ad35/Ad5 vaccine modalities. However, the studies in chapter 4 also clearly indicated that Ad11/Ad5 and Ad35/Ad5 vaccine combinations induced more potent immune responses in naïve mice than Ad11/Ad35 vaccine regimens. Further investigations revealed the presence of cross-reactive NAbs and CD8+ T lymphocytes between Ad35 and Ad11. The cross-reactive NAbs played the critical and primary role in the diminished immunogenicity of the Ad35/Ad11 prime-boost regimen. Since Ad35 and Ad11 are derived from the same human adenoviral subgroup B, it was hypothesized that more distantly related adenoviruses may be required. Therefore, as described in chapter 5, an Ad49-based vector was developed, which is derived from human adenovirus subgroup D. As a stand-alone, also this vaccine vector induced potent antigen specific cellular immune responses and importantly was not hampered by the presence of anti-Ad5 immunity. In chapter 6 the Ad49-based vaccine vector was tested in a prime-boost regimen together with an Ad35-based vector in comparison to the Ad35/Ad11 prime-boost regimen. We demonstrated that the Ad35/Ad49 prime-boost regimen was indeed superior to the Ad35/Ad11 prime-boost regimen. In addition, no cross-reactive NAb could be determined between Ad35 and Ad49. These studies indicated that the most optimal adenoviral based prime-boost regimen includes two vectors that circumvent both anti-Ad5 immunity and cross-reactive anti-vector immunity.

In chapter 7, a total of seven different human adenovirus vaccine vectors, based on Ad5, Ad11, Ad26, Ad35, Ad48, Ad49 and Ad50 were tested in a direct comparison. All proved capable of inducing potent antigen specific cellular immune responses in mice as well as not to be hampered by anti-Ad5 immunity. An interesting finding in this study was the high immunogenicity of the Ad26-based vector, which proved more potent than any of the other
non-Ad5 based vectors in the generation of SIV Gag-specific T lymphocyte responses in both mice and non-human primates. The Ad26-based vector proved also successful in heterologous prime-boost regimens, including those involving another human adenovirus subgroup D member, i.e. Ad48 or Ad49. The latter contradicts with the hypothesis that members of the same adenoviral subgroup are immunologically too closely related, as was observed in chapter 4 for the Ad35-Ad11 prime-boost regimen and in chapter 7 for the Ad35-Ad50 prime-boost regimen. However, it does corroborates with the finding from another group that an Ad6-based vaccine vector was capable of overcoming anti-Ad5 immunity, despite the fact that both Ad5 and Ad6 are from subgroup C (46). This may indicate that members of the human subgroup D as well as subgroup C adenoviridae might be less immunologically cross-reactive than subgroup B members.

Besides the vaccine vectors derived from the rare human adenoviruses described in this thesis, several additional ones that are shown in table I have been developed by other groups. Overall, also these vaccine vectors proved successful in inducing potent transgene-specific immune responses (46, 180, 301).

### Table I: Additional rare human adenoviruses from which vaccine vectors are derived.

<table>
<thead>
<tr>
<th>Human adenoviral serotype</th>
<th>Subgroup</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Ad6</td>
<td>C</td>
<td>(47)</td>
</tr>
<tr>
<td>Ad24</td>
<td>D</td>
<td>(301)</td>
</tr>
<tr>
<td>Ad34</td>
<td>B</td>
<td>(301)</td>
</tr>
<tr>
<td>Ad41</td>
<td>F</td>
<td>(180)</td>
</tr>
</tbody>
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### CONCLUDING REMARKS

While the generation of broadly reactive HIV-neutralizing antibodies remains an elusive goal, CD8+ T lymphocyte-based HIV vaccines are actively being evaluated in (pre)-clinical trials. One of the most promising technologies for the induction of HIV-specific CD8+ T lymphocytes is based on replication-incompetent adenoviruses. The knowledge gathered on the basic biology of adenoviridae has proven pivotal to develop, improve and expand the number of immunologically distinct Ad-based vaccine vectors. The near future will also demonstrate the utility of the vaccine vectors based on rare human serotype adenoviruses, Ad5-chimeric vectors and non-human adenoviruses, since several of these promising vaccine carriers are or will very soon be advanced into human clinical trials. For the Ad35-based malaria and tuberculosis vaccines of Crucell, phase I clinical trials have already been initiated. The work done with the Ad26-based (as described in this thesis) and Ad5HVR48-based HIV vaccines of Crucell and the Harvard Medical School department of Prof. Barouch have received the prestigious Integrated Preclinical/ Clinical AIDS Vaccine Development Program (IPCAVD) grant of the NIH, and both these vaccine vectors will be advanced into a phase I clinical trial at the end of 2007 or beginning of 2008.

However, many basic questions still need to be answered in the development of a preventive HIV vaccine. For example, it is important to define which antigens will elicit the most effective neutralizing antibody and CTL responses. Moreover, strategies will need to be developed to minimize the ability of the virus to escape from neutralizing antibodies and CTL. And finally, immunogen sequences will have to be chosen carefully to cover the diversity of HIV-1 subtypes worldwide most effectively.