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Viral vectors as vaccine carriers

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This chapter reviews the performance of viral vectors based on adenoviruses or adeno-associated virus as vaccine carriers for infectious diseases. Replication-defective adenovirus vectors based on multiple human or non-human serotypes have consistently induced potent transgene product-specific B and T cell responses and are increasingly being explored in human clinical trials. The immunogenicity of most vectors based on adeno-associated virus vectors has been poor with the exception of a recently described hybrid vector from rhesus macaques that due to its ability to induce potent responses in mice warrant further investigation.

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Introduction

The development of vaccines based on viral vectors is a subspecialty of gene therapy with albeit a distinct goal. Gene therapy for correction of genetic diseases aims to permanently replace a missing or faulty gene and this can only be achieved if the immune system tolerates both the delivery vehicle and the transgene product. The objective of vaccines on the other hand is to transiently express high levels of an antigen in a form that elicits robust adaptive immunity to this antigen with support from inflammatory responses induced by the delivery vehicle. In spite of the opposing goals of permanent gene replacement therapy and vaccines these two fields commonly use similar tools — recombinant viral vectors that express either a therapeutic transgene product or an immunogenic antigen from a pathogen. Multiple viruses have been used as vaccine carriers ranging from very complex large DNA viruses such as poxviruses [1–3] down to simple RNA viruses such as parainfluenza viruses [4] and just about everything in between [5,6,7,8•,9,10]. In this chapter we will focus on viruses that are used for both gene replacement therapy and as vaccines, specifically vectors based on recombinant replication-defective adenoviruses (Ad, [11,12•,13,14,15•,16]) and gutted adeno-associated viruses (AAV, [17–21]) Other viral vectors that are being explored as vaccine carriers in clinic trials such as poxvirus vectors or vectors based on herpes viruses [22] are beyond the scope of this review.

Immune responses to pathogens

Pathogens elicit innate immune responses through pathogen-associated molecular patterns (PAMP) that are recognized by pathogen recognition receptors (PRR), such as Toll-like receptors (TLR), C-type lectin receptors and NOD- or RIG-I-like receptors [23–25]. Activation of PRRs induces inflammatory cytokine and chemokine responses and leads to maturation of dendritic cells. They then take up antigen, upregulate expression of major histocompatibility (MHC) antigens and costimulatory molecules, and migrate to lymph nodes where they induce primary adaptive immune responses [26•,27]. The highly antigen-specific adaptive immune system is broadly subdivided into B and T cells; T cells in turn are composed of $CD8^+$ and several subsets of $CD4^+$ T cells.

B cells recognize soluble antigen and their epitopes are commonly conformation-dependent. Antibody molecules serve as B cell receptors. Once they are engaged B cells can rapidly differentiate into short-lived plasma cells, which transiently produce IgM antibodies [28,29]. Alternatively, B cells enter germinal centers where they proliferate and acquire additional antigen from follicular dendritic cells. They internalize the antigen and degrade it into peptides, which are presented to follicular T helper cells [30•] that provide signals for survival and class switching. B cells then upon additional rounds of proliferation and hypermutation of Ig genes differentiate into long-lived antibody-producing plasma cells or memory B cells [29,31]. Once induced memory B cells persist [32,33] and can rapidly become antibody-producing cells upon encounter of their cognate antigens.

$CD8^+$ and $CD4^+$ T cells recognize peptides associated with MHC class I or II antigens, respectively [34,35]. While $CD8^+$ T cells once activated can directly eliminate virus-infected cells, $CD4^+$ T cells' primary task is to positively or negatively regulate immune responses [36,37].

$CD8^+$ T cells upon recognizing their MHC class I-associated antigen on cells that provide in addition costimulatory signals proliferate with help from cytokines released by activated $CD4^+$ T cells. $CD8^+$ T cells then differentiate into effector cells, which produce anti-viral

cytokines as well as the lytic enzymes perforin and granzyme B that can directly kill antigen-presenting target cells. Once infected cells have been eliminated most effector $CD8^+$ T cells undergo apoptosis [38,39]. A small fraction persists as effector memory cells that patrol the periphery and immediately assume effector functions should they encounter their cognate antigen [39]. Other $CD8^+$ T cells form central memory cells, which reside in lymphatic tissue where they are maintained for the lifespan of an individual through occasional cytokine-driven homeostatic proliferation [40,41].

Pathogens that persist have unique effects on the immune system. High levels of persistence exemplified by hepatitis viruses in humans can cause so-called T cell exhaustion [42,43] characterized by increased expression of co-inhibitors such as PD-1, gradual loss of T cell functions followed by T cell death. Low level persistence, such as upon infection with herpes viruses, Ad viruses or AAV, does not cause T cell exhaustion by rather maintains high levels of effector or effector memory T cells [44–46].

Subunit vaccines

Viral vectors are subunit vaccines. They can present one or in case of large DNA viruses a few foreign antigens to the immune system. The design of an efficacious subunit vaccine thus necessitates detailed knowledge of the viral antigens and how they contribute to the induction of immune responses best suited to prevent an infection. Protection against most pathogens is achieved by neutralizing antibodies. Complex pathogens may require $CD8^+$ T cells to allow for clearance of infected cells [47,48]. Neutralizing antibodies and $CD8^+$ T cells can be induced by the same antigen, but commonly $CD8^+$ T cells respond best to internal proteins [49,50], which are typically more conserved, while neutralizing antibodies selectively target antigens expressed on the pathogen's surface, which can show high variability between different isolates [51,52].

Most viral vectors including those based on Ads and AAVs readily induce transgene product-specific $CD8^+$ and $CD4^+$ T cells. Direct transduction and activation of dendritic cells by a viral vector vaccine is not essential as dendritic cells can present antigen that they acquire from other cells [35,53•,54]. Viral vectors generally do not induce potent regulatory T cell responses although this depends to some degree on the type of the vector and the route of immunization [55]. Viral vectors that express correctly folded proteins induce transgene product-specific B cell responses.

Pre-existing immune responses to the vaccine carrier

Many of the viruses that are being developed as vaccine carriers circulate in humans, which consequently carry antibodies including neutralizing antibodies as well as T cells to viral vaccine vectors. In case of Ad and AAV vectors prevalence rates of neutralizing antibodies which dampen the efficacy of gene transfer and thereby the antigenic load that drives transgene product-specific immune responses varies depending on the serotype and the study population including its age and place of residency [56•,57,58,59,60•]. For examples about 35% of adults living in the USA have neutralizing antibodies to Ad of human serotype 5 (HAdV-5), while prevalence rates increase to over 90% in individuals living in Core d'Ivoire. Antibodies to the so-called rare HAdV-26 serotype are below 20% in the USA and Europe but above 90% in South Africa. Prevalence rates of antibodies to AAV2, 5 and 8 range between 20 and 30% in US cohorts while pre-existing neutralizing antibodies to AAV1 are very variable ranging globally from 10 to 80% of the study cohorts (Figure 1).

Although neutralizing antibodies do not completely block induction of an immune response by a viral vector vaccine they tend to reduce responses and may even augment unwanted side effects such as reactogenicity [61]. An unexpected effect of pre-existing neutralizing antibody to the vaccine carrier was observed in the STEP trial where an HIV-1 antigen expressing HAdV-5 vector transiently increased HIV-1 acquisition rates in sero-positive individuals [62•].

Pre-existing immunity can be circumvented by vectors based on viruses that circulate in non-human primates [63–66]. These viruses are closely related to their human counterparts but most humans lack neutralizing antibodies to simian viruses. For examples for Ads derived from chimpanzees, sero-prevalence rates are below 10% in most human populations with slightly increased rates in Sub Saharan Africa [65]. Individuals that carry neutralizing antibodies to chimpanzee Ads in general have only low to moderate titers. In the same token humans only rarely have neutralizing antibodies to simian AAVs [66].

T cells induced by natural infections to Ads or AAVs cross-react between multiple serotypes including those from non-human primates [67]. Although pre-existing T cell responses may pose problems for permanent gene replacement therapy by clearing the transduced cells that produce the therapeutic transgene product [68], they do not appear to have a major impact on vaccine-induced immune responses [69].

Vaccine carriers based on Ad vectors

Ads are small double-stranded species-specific DNA viruses. They are broadly divided into families A-F and each family contains multiple serotypes. Most Ads enter cells upon binding to the Coxsackie Adenovirus receptor (CAR, [70]). Integrins such as $\alpha_v\beta3$ and 5 further promote viral uptake [71]. Ads from some families such as family B