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Future viral vectors for the delivery of asymptomatic herpes epitope-based immunotherapeutic vaccines

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Recombinant virus as a vaccine for herpes diseases

The concept of using a recombinant virus to deliver viral antigens from a different virus for the purpose of therapeutic vaccination has been developed for more than 25 years [1]. In 1983, vaccinia virus recombinant for the hepatitis B surface antigen was the first viral vector to successfully induce protective immunity against HBV [1]. Since then, a number of viral vector-based vaccines, such as adenovirus, herpes simplex virus (HSV), vaccinia virus, retrovirus, Newcastle disease virus, vesicular stomatitis virus, measles virus, poliovirus and West Nile virus, have been designed and tested in both preclinical and clinical trials (reviewed in [2]). Genetically engineered HSV mutants have recently been exploited as promising vector platforms in immunotherapy (reviewed in [3]). Despite substantial development, the list of licensed viral-vector vaccines for human use remains short. The challenges include: issues concerning the large-scale production, the stability and the stringent safety requirements, especially for viral vectors that remain replicative or that persist in the host. Despite these challenges, the continued interest in developing viral-vector vaccines is due to their promising immunogenicity, especially for the most difficult antigens from notorious widespread pathogens, such as HSV-1 and/or -2.

Primary HSV infection is usually asymptomatic and results in a lifelong latent virus, which lies dormant in sensory ganglia (SG) and the CNS. However, in a minority of individuals (designated as symptomatic individuals), latent HSV often reactivates spontaneously and causes a variety of asymptomatic recurrent diseases or even fatal encephalitis. Worldwide, over 4 billion of the human population are estimated to already be infected with HSV, which could turn into symptomatic diseases in the form of skin lesions, genital ulcerations, blinding eye disease and fatal encephalitis. Unfortunately, most virus transmission occurs during asymptomatic shedding, underlying the necessity of targeting therapeutic vaccines

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toward apparently non-harmful virus. One of our goals is to develop a viral vector-based immunotherapy to prevent or alleviate ocular, oro-facial (cold sores) and genital herpetic disease.

Enhancing immunogenicity: prime–boost regimes

Viral vector-based vaccines were originally developed to induce protective CD8⁺ T-cell responses against intracellular pathogens [2]. Impressive levels of T-cell immunogenicity are now being reported for the first time in human clinical trials [2]. Key to this advance has been the development of adenoviral vector (AdV)-based vaccines. AdVs have the remarkable ability to prime immune responses against a transgene that can be boosted to high levels with a second vector that is recombinant for the same antigen, typically modified vaccinia Ankara (MVA) or a second AdV of heterologous serotype.

In the 1990s, excitement surrounded the advent of DNA vaccines. However, DNA vaccines by themselves might not be adequately immunogenic to generate T-cell responses at sufficient levels to protect against viral diseases in humans. Unlike DNA vaccines, the immune responses (mostly neutralizing antibodies to the structural proteins) generated by viral vector-based vaccines become self-limiting after one or two administrations. Thus, the prime–boost strategies have emerged to overcome the anti-vector humoral immune responses, whilst maximizing the T-cell responses to the vaccine insert.

In contrast to the use of DNA plasmid to prime T-cell responses, the recent development is the use of combinations of different recombinant viral vectors or a viral vector along with a subunit vaccine (e.g., lipopeptide epitope-based vaccines; reviewed in [4]). A consistent observation throughout these studies is the differential ability of certain vectors to prime or boost immune responses. DNA vaccines, fowlpox virus and influenza virus, are good priming vectors, whereas poxviruses (e.g., vaccinia virus Ankara [MVA] and NYVAC [5]) are consistently able to boost T-cell responses that are primed by other means. A large body of data now indicates that recombinant AdV can prime and boost strong T-cell immunity (reviewed in [2]). More recently, a combination of three unrelated vector modalities expressing the same vaccine insert has been used (reviewed in [2]). Viral vector-mediated prime–boost vaccine strategy is an attractive procedure for introducing protective ‘asymptomatic’ epitopes into the CNS and SG to develop effective immunotherapy for herpes disease that results from HSV reactivation. Both MVA and AdV can substantially boost immune responses primed by other means (e.g., the lipopeptide vaccination). One new and promising strategy for immunotherapy is the use of modified viruses that have been engineered to selectively induce local ‘asymptomatic’ CD4⁺ and CD8⁺ T cells, in order to eliminate infected cells in SG and CNS. However, for this step to occur, the newly constructed viruses must combine high safety and preferential replication of the virus in cells of CNS and SG.

HSV-vector vaccines

A defining feature of all HSVs is that they persist for the life of the infected individual. Replication-competent and replication-defective HSVs have been exploited as vaccine vectors in order to elicit immunity against HSV and other heterologous viruses, such as SIV and HIV [6]. Three types of vector have been derived from HSV: the amplicons, the nonreplicating genomic HSV vectors, and the conditionally replicating, attenuated deletion viruses. The HSV amplicons are an excellent means for delivery of large or multiple antigens [7]. The amplicon vectors obtain their structural proteins from helper viruses or other HSV constructs, being unable to replicate in the target organism on their own. Systems have been recently developed for production of helper-free HSV amplicons [8].

The genomic, disabled HSV vectors lack essential genes and must be prepared in complementing cell lines. In the genomic nonreplicating HSV vectors, the immediate early genes (coding for ICP0, ICP4, ICP22, ICP27 or ICP47) are usually deleted in different combinations [9]. The genomic disabled HSV vectors lacking all five immediate early genes are nontoxic, attenuated and express transgenes inefficiently, probably owing to a lack of ICP0-mediated counteractions of the host responses. The nonreplicating HSV vectors have been used in gene therapy of CNS disease models and of chronic pain [10,11].

The replicative or conditionally replicating HSV vectors are either deleted or mutated of nonessential genes. They grow well in monolayer cell cultures, but they are usually replication incompetent in terminally differentiated cells. The conditionally replicating HSV vectors typically harbor a deletion of the neurovirulence gene $\gamma_134.5$, with or without additional gene deletions. A simple neurovirulence gene deletion HSV mutant, designated as R1716, has proven safe in clinical trials [12], even when administered repeatedly [13]. Protective efficacy of replication-defective HSV vector-based vaccines was recently reported in a SIV-rhesus macaque model [14].

Replication-defective HSV-2 vaccine viruses have been developed, which provide neutralizing antibodies and reduce HSV-2 shedding in experimental models [15]. Vaccine candidates have also been derived from replicative HSV vectors, by insertion of a cytokine transgene promoting Th1 responses [16]. Replication-defective adenoviruses possess many features, which make them ideal vectors for this purpose of efficiently transducing terminally differentiated cells, such as neurons and glial cells, resulting in high levels of local antigen expression *in vivo*. In the absence of antiadenovirus immunity, these vectors can sustain very long-term gene expression within the CNS and SG parenchyma, thus leading to an antigen-specific local immunity. In addition, regulatable promoter systems maintain specific gene expression under tight control. Ideally, regulatable cassettes should have minimal gene expression in the 'off' state, and expression should quickly reach therapeutic levels in the 'on' state. In addition, a specific increased transgene expression per vector genome is an important goal for the optimization of viral vectors in immunotherapy. Adenoviral vectors induce dose-dependent innate and adaptive immune responses [17]. Decreasing the total dose of therapeutically effective viral vector should result in safe and long-term gene transfer. The Lowenstein and Castro group recently demonstrated that HSV-1 TK gene sequences fused to the 3'-end of *lacZ* increase transgene expression from high-capacity adenoviral vectors [17]. These are the highly desirable prospects for the development of safe, efficacious adenoviral vectors that can be used as an immunotherapeutic approach for human herpes diseases.

Future perspective

Most viruses, including HSV, have evolved mechanisms to evade host immune responses. However, the future of viral vector vaccines is to be capable of knocking or mutating immune-evasive viral genes, thus improving antigen presentation and enhancing the immunogenicity of a well-established virus. In parallel, new viruses are being discovered and characterized as alternative viral vectors in order to circumvent the problem of pre-existing vector immunity. These new alternative viral vectors are being combined with new vaccine prime-boost regimens (e.g., peptide or lipopeptide epitope vaccines) to induce stronger, broader and more durable T-cell immunity. Approaches that improve adjuvant effects of existing viral vectors and intentional skewing of immune responses, such as toward Th1 type, are important new lines of investigation.

The development of viral vector-based herpes vaccines for human use faces substantial challenges, both in the scientific and regulatory fronts. However, these technologies are

finally achieving their efficacy in both animals and humans. The translation of these new technologies will facilitate vaccine development against some of the toughest challenges, such as ocular and genital herpes. The licensure of replication-deficient viral vector vaccines is feasible, given that they lie between the two extremes of protein-in-adjuvant vaccines and live viral vaccines, both of which, after considerable effort, have been licensed before. In particular, we expect that the next 5 years will be a challenging, but exciting, time for the renaissance of prime–boost strategy in herpes vaccine development. Adenoviral vector priming and peptide/lipopeptide boosting is the next prime–boost strategy being developed in our laboratory for induction of asymptomatic protective CD8⁺ T-cell responses against ocular and genital herpes.

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