Gene therapy research at the frontiers of viral immunology

Health & Medicine

Although originally conceived in the mid 1970s as an alternative to transfection (Goff and Berg, 1976), the use of viral vectors as a tool for clinical gene therapy did not emerge until the 1980s. As Li and Ertl (2011) recapitulate in their thought provoking perspective, viruses meet all the requirements needed for gene therapy. Evolving since life's beginning, viruses have established the ability to efficiently infect and transfer their genomes in a wide variety of mammalian cell types. Likewise, and generally considered beneficial for survival, mammals have equally evolved highly complex mechanisms to protect themselves against invading pathogens such as viral gene transfer vectors. However, it is this protective immune response that often presents major obstacles for successful long-term therapy. Fortunately, the gene transfer community has been extensively studying the mechanisms of immune responses against viral vectors and has started to develop strategies and protocols to block or circumvent such responses. In this *Research Topic* , the editors present a collection of mini-Reviews, in-depth Reviews, Perspective, and Primary research articles that highlight both the well established and emerging strategies currently being used in blocking the immune response to gene transfer with viral vectors.

Viral vectors such as adenovirus and adenovirus-associated virus (AAV) are superior tools for gene therapy due to their high efficiency of transduction into a variety of cells *in vivo* . Administration of viral vectors often provokes the initiation of innate and antigen-specific adaptive immune responses against the virus and/or the therapeutic transgene products. Activation of these pathways elicits a flurry of anti-viral and pro-inflammatory signals that can recruit effector lymphocytes, inhibit viral transduction, and encourage

elimination of transduced cells over a period of time. Additionally, the *de novo* expression of a wild-type protein may trigger an adaptive immune response in the form of neutralizing antibodies. Likewise, pre-existing immunity (immunological memory) to the gene transfer vector resulting from prior exposure to the virus often prevents efficient gene transfer. All of these scenarios pose serious hurdles for successful gene therapy.

The first review article of this special topic provides a comprehensive overview of the innate immune responses to AAV. The authors highlight and discuss recent discoveries regarding strategies to abrogate potentially detrimental signaling pathways (Rogers et al., 2011). Historically, the innate immune response to single-stranded AAVs has been considered weak and transient when compared to the potent and prolonged response elicited by Ad vectors, thus prompting many investigators to focus more on the adaptive immune response to AAV. In more recent years our understanding of the early innate mechanisms of immune responses to viral vectors has greatly improved. The authors present an up-to-date analysis of the mechanisms surrounding the innate immune response to single-stranded and self-complimentary AAVs, including the role of the viral capsid, the effects on target tissue, and the therapeutic potential of blocking innate responses.

An equally impressive review detailing the current understanding relative to Ad vector mediated induction of the innate and adaptive immune responses is presented by Aldhamen et al. (2011) . Ad vectors possess several advantages, the most important of which is that they can be easily, and

routinely produced to high titers, however they also rapidly activate innate immune responses as well as potent cellular and humoral adaptive immune responses. This review examines the impact these responses have on the safety and efficacy of Ad vector-based therapies (Aldhamen et al., 2011). They also highlight strategies proposed to either mitigate or harness Ad-induced innate and adaptive immune responses for the improved and broadened development of advanced, Ad-based therapies.

Adenovirus-associated virus vectors have shown considerable promise as a gene delivery tool for clinical gene therapy applications. Unfortunately, the presence of pre-formed neutralizing antibodies directed against the AAV capsid in a large proportion of the human population due to widespread prior exposure to the wild-type virus has prevented AAV from reaching its full potential as a gene therapy vector. Bartel et al. (2011) provide an intriguing review that examines the use of capsid engineering as a means to evade pre-existing immunity. The authors discuss the consequences of humoral anti-AAV immune responses and potential strategies to prevent them. Additionally, Arnett et al. (2011) discusses the influence that cross reactivity of pre-existing antibodies has on gene therapy, including neonatal administration of viral vectors as a means to circumvent such antibody responses.

As a target tissue for gene therapy, muscle is appealing because it is abundant, easily accessible, and procedures involving gene transfer to muscle are relatively safe and non-invasive (Hoffman et al., 2007). The mini-review by Wang et al. focuses on recent reports of immunity to AAV

capsid proteins and transgene products in the context of gene delivery to muscles for treating both muscular dystrophies and other non-muscle diseases. They provide strategies of immune modulation and tolerance induction in order to prevent unwanted immune responses to the vector and/or the therapeutic gene product (Wang et al., 2011). Additionally, the research article presented by the Boyer group, evaluates a treatment protocol designed to inhibit the deleterious immune activation during muscle gene transfer. Their strategy is based on the administration of CTLA-4/Ig in order to block the co-stimulatory signals required early during immune priming combined with gene transfer of PD-1 ligands to inhibit T cell functions at the tissue sites (Adriouch et al., 2011).

It has become evident that the manipulation of various co-stimulatory pathways to regulate host immune responses is of therapeutic interest. Addressing this, Huang and Yang, offer a broad review of relevant T cell co-stimulatory pathways in the activation of both T and B-cells, and provide strategies for targeting these co-stimulatory pathways in gene therapy applications. Ultimately, they suggest that studies should focus on targeting multiple pathways including both the positive and negative co-stimulatory pathways (Huang and Yang, 2011). Using a multiple pathway approach in an animal model of hemophilia, the Herzog lab presents new findings that suggest that transient immune modulation using a cocktail of rapamycin, IL-10, and specific peptides could prevent or possibly reverse gene therapy-induced inhibitor formation (Nayak et al., 2011).

The success of *in vivo* gene therapy not only depends on the ability to control the immune response toward the input vector, but also to the therapeutic transgene. Using both vector-based and pharmacological approaches, various groups have explored various approaches to control the immune-mediated clearance of transgene-expressing cells after viral delivery. One approach using micro-RNA transgene regulation to generate a tolerogenic response is reviewed by Goudy et al. They further speculate on possible mechanisms used by the liver to induce the transgene-specific regulatory T cells (Goudy et al., 2011). Another method to modulate tolerance induction is via gene transfer to B-cells and such an approach is presented by Su et al. Here, the authors demonstrate that host IL-10 is critical for the tolerogenicity of B-cell based peptide–IgG gene therapy (Su et al., 2011).

Overall, this Research Topic: " Approaches to Blocking the Immune Response to Gene Transfer with Viral Vectors" provides a well-developed overview of the current therapeutic potentials of viral gene therapy. This collection of articles not only provides a dynamic review and analyses of the complex immune responses present in current gene therapy applications, but they also provide insight for the future direction of the field.

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