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Unique safety issues associated with virus-vectored vaccines: Potential for and theoretical consequences of recombination with wild type virus strains

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ABSTRACT

In 2003 and 2013, the World Health Organization convened informal consultations on characterization and quality aspects of vaccines based on live virus vectors. In the resulting reports, one of several issues raised for future study was the potential for recombination of virus-vectored vaccines with wild type pathogenic virus strains. This paper presents an assessment of this issue formulated by the Brighton Collaboration.

To provide an appropriate context for understanding the potential for recombination of virus-vectored vaccines, we review briefly the current status of virus-vectored vaccines, mechanisms of recombination between viruses, experience with recombination involving live attenuated vaccines in the field, and concerns raised previously in the literature regarding recombination of virus-vectored vaccines with wild type virus strains. We then present a discussion of the major variables that could influence recombination between a virus-vectored vaccine and circulating wild type virus and the consequences of such recombination, including intrinsic recombination properties of the parent virus used as a vector; sequence relatedness of vector and wild virus; virus host range, pathogenesis and transmission; replication competency of vector in target host; mechanism of vector attenuation; additional factors potentially affecting virulence; and circulation of multiple recombinant vectors in the same target population. Finally, we present some guiding principles for vector design and testing intended to anticipate and mitigate the potential for and consequences of recombination of virus-vectored vaccines with wild type pathogenic virus strains.

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1. Preface

The Brighton Collaboration is a global, non-profit, scientifically independent, largely volunteer research network created for the purpose of providing reliable, high quality international information and guidelines relevant to vaccine safety. One of many working groups within the Brighton Collaboration is the Viral Vector Vaccines Safety Working Group (V3SWG) that was formed to explore safety issues relevant to virus-vectored vaccines [1].

In 2003, the World Health Organization (WHO) convened an informal consultation on characterization and quality aspects of vaccines based on live virus vectors [2]. One section of the 2003 report reviewed "regulatory issues for live viral-vectored vaccines", including input from regulators representing the European Union, the USA (specifically the Center for Biologics Evaluation and Research (CBER), a unit within the Food and Drug administration (FDA)), China, and Health Canada. Among the issues raised by the Center for Biologics Evaluation and Research, U.S. Food and Drug Administration (CBER/FDA) was:

> Recombination of a live virus-vectored vaccine with a circulating or reactivated latent virus could theoretically generate a more pathogenic strain. This would be less of an issue for vectors that share little homology with circulating/latent viruses. The risk of recombination should be studied if possible in a non-clinical model system, but should also be considered in clinical study designs.

Recombination was not explored further in the 2003 consultation, but was listed in among the "Recommendations to WHO and priorities for future work" as one of several "issues of critical importance to be investigated further", specifically, "Potential of recombination with wild type pathogenic strains: Vector – circulation virus could create a more pathogenic strain; this issue should be addressed in vitro or in animal studies".

In 2013, WHO convened an additional informal consultation which reinforced concerns regarding recombination within the vaccine recipient [3]. Specifically, the report from this consultation states that "guidelines for characterization of the viral vector based vaccines have been harmonized and require the following" [among others]:

> Demonstration of stability of insert/transgene by PCR, expression, passage *in vitro* and/or *in vivo*, as well as stability of the attenuated phenotype, i.e., investigate the potential for reversion, recombination or replication in the vaccine recipient.

The U.S. Food and Drug Administration and the European Medicines Agency have published general guidance for use of recombinant virus-vectored vaccines [4,5]. The following report specifically explores the issue of potential recombination between virus-vectored vaccines and wild type pathogenic strains of virus. Our intent is not to conduct an exhaustive review of literature, but rather to provide some salient examples to guide consideration of issues relevant to the topic.

2. Background

2.1. Virus-vectored vaccines

Virus-vectored vaccines are laboratory-generated, chimeric viruses that are based upon replicating ("live") or non-replicating virus vectors into which have been spliced genes expressing antigenic proteins for a target pathogen. A live virus-vectored vaccine is biologically active and produces virus progeny in the vaccinated host but may be attenuated for pathogenicity either because of mutations in the vector, because of the chimeric nature of the vaccine itself, because the vector is used in a heterologous host, or due to a combination of these factors. A non-replicating virus-vectored vaccine is so severely attenuated that is cannot undergo a complete replication cycle in infected cells. Administration of the chimeric virus-vectored vaccine results in expression of antigen(s) of the target pathogen and induction of an adaptive and possibly protective immune response. At the time of this writing, only two virus-vectored vaccines have been approved for human use, specifically Imojev® [6] and Dengvaxia® [7–9]. Imojev® is marketed in Australia and Thailand for immunization against Japanese encephalitis virus infection. Dengvaxia® is approved in Mexico, the Philippines and Brazil for immunization against dengue fever. Both vaccines are based on the live yellow fever vaccine virus vector generically known as ChimeriVax [10]. In Imojev® and Dengvaxia®, the genes for the yellow fever virus virion structural proteins M and E have been replaced with the homologous genes from Japanese encephalitis virus or dengue virus respectively. Because Japanese encephalitis virus, dengue virus and yellow fever virus are all flaviviruses, these particular chimeras represent relatively subtle exchanges of antigens among closely related viruses. Numerous other virus-vectored vaccines using a wide range of vectors and targeting a variety of different pathogens are at various stages of research and development. Although currently the number of virus-vectored vaccines available for human use is small, a variety of viral-vectored vaccines are available commercially for use in veterinary practice [11], demonstrating the promise and likely future use of viral-vectored vaccines in humans.

2.2. Virus recombination

Recombination describes a process by which nucleic acid sequences from two different parental viruses are exchanged so that the progeny contain sequences derived from both parents. Both RNA and DNA viruses may undergo recombination when two related genomic variants of a virus co-infect a cell. In viral systems there are three different mechanisms of recombination, dictated by the structures of the viral genomes. For DNA viruses, recombination occurs by the physical breakage and rejoining of parental DNA molecules through regions of sequence homology, in a fashion similar or identical to the same process in bacteria or higher organisms. For RNA viruses containing segmented genomes, gene exchange occurs primarily through reassortment of individual parental genome segments into progeny viruses, however *intra*genic recombination has also been reported for the segmented orthomyxoviruses, reoviruses and bunyaviruses [12–16]. Recombination has been observed in several single-stranded RNA (ssRNA) virus families representing both positive and negative sense genomes both in the laboratory and in the wild; picornaviruses, coronaviruses, togaviruses and retroviruses, all with positive sense ssRNA genomes, display relatively efficient recombination [17–31]. The frequency of recombination among negative sense RNA viruses (excluding reassortment of segmented genomes) seems to be relatively low [31]. Recombination in RNA viruses, including retroviruses, is thought to occur during replication via "copy choice", namely switching RNA templates during replication with the result that the newly synthesized genome contains sequences from two different parental molecules [32,33].

While recombination clearly requires coinfection of a cell with two different viruses, the circumstances leading to such a coinfection in vivo are not clearly understood. Coinfection could theoretically result from infection with a heterogeneous population of viruses, by simultaneous or overlapping serial infections with different viruses, or by infection of an individual harboring a persistent, latent or reactivated infection with a different virus.

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