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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

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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.

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.

Background

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.

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. Nevertheless, recombination among viruses in the human population clearly occurs as exemplified by a recent study describing interclade recombinants of varicella zoster virus [34].

Vaccine viruses in the wild

Although experience with virus-vectored vaccines in humans is limited, perspective on the issue of recombination between virus-vectored vaccines and wild type viruses can be informed by experience with traditional live, attenuated human virus vaccines and virus-vectored veterinary vaccines. Vaccine viruses may establish a long-term reservoir in the wild, and recombination between attenuated vaccine strains and circulating wild type viruses or even between two different live attenuated vaccine strains has been documented. Specifically, evidence exists that vaccinia virus used as vaccine during the smallpox eradication campaign in Brazil has established a durable reservoir in the wild and is the cause of numerous cowpox-like infections in cattle and humans [35]. Likewise, the bovine herpesvirus vaccine may establish a latent reservoir in vaccinated animals which, through reactivation, may spread to other animals [36]. Numerous examples document probable recombination between live attenuated vaccine viruses and wild viruses. Phylogenetic analysis revealed recombination between wild, circulating strains of Newcastle disease virus

(NDV), an avian paramyxovirus, and attenuated NDV vaccine strains [37]. Attenuated viruses contained in the oral poliovirus vaccine frequently recombine with related indigenous human enterovirus strains to produce circulating vaccine-derived polioviruses (cVDPV), which can cause paralytic disease [38]. A reassortant Rift Valley Fever virus strain containing both wild type virus-derived and vaccine virus-derived genomic segments was isolated from a patient who received a needle stick injury while vaccinating sheep [39]. Analysis of two independently isolated disease strains of the pestivirus bovine viral diarrhea virus (BVDV) demonstrated that these strains arose via both homologous and non-homologous recombination between a persistent BVDV strain and a vaccine strain, resulting in evolution of strains with enhanced pathogenicity relative to the parental strains [40]. Analysis of a strain of the poxvirus myxoma, originally isolated from a wild rabbit, suggests that it resulted from a recombination between a wild myxoma strain and a vaccine strain [41]. Genome analysis of a disease strain of porcine reproductive and respiratory syndrome virus (PRRSV), an arterivirus, demonstrated that it is a recombinant between an attenuated PRRSV vaccine strain and a field strain [42]. Additionally, recombination between independently derived attenuated avian herpesvirus vaccine strains can give rise to circulating pathogenic recombinant viruses [43]. Lastly and most extraordinarily, evidence exists that the retroviral disease reticuloendotheliosis was introduced into avian populations via contamination during development of fowlpox (a poxvirus) and Marek's disease (a herpesvirus) vaccines, and now circulates in the wild as an integrated provirus in some fowlpoxvirus genomes [44]. All of these examples attest to the potential for genetic interaction between vaccine viruses and viruses in the wild. By contrast, it is noteworthy that more than 100 million doses of poxvirus-vectored recombinant rabies virus vaccine has been distributed in the wild in the United States, Eurasia and Western Europe resulting in reproducible reduction or elimination of wild rabies where applied and with no reports of recombination with wild type virus strains, attesting to the utility of recombinant vectored vaccines [45]. Furthermore, a wide variety of viral-vectored recombinant vaccines have been routinely used in veterinary practice for well over a decade with no recorded evidence of problems resulting from recombination with wild viruses [11]. Thus, while the occurrence of a recombination event is likely to be rare, the unanticipated consequences of such an event must be considered by developers and regulators alike.

Recombination between virus vaccine vectors and wild type virus strains

Although as described above, recombination has occurred between traditional live attenuated vaccine viruses and wild type viruses, to date there are no examples of recombination between virus vectored vaccine strains and wild type virus strains outside of the laboratory. However, on at least two separate occasions the possibility of recombination between viral vaccine vectors and wild type virus strains has been debated in the literature. One debate related to the development of ChimeriVax based vaccines [46–48] and one related to the development of Newcastle disease virus as a vector [49;50]. These debates defined some of the central concepts surrounding the issue of recombination between viral vaccine vectors and wild type virus strains. In the case of the ChimeriVax debate, most of the issues were ultimately addressed experimentally, thus providing a template for safety assessment of viral vaccine vectors, discussed in more detail at the end of this document.

In summary, it is wholly feasible that virus-vectored vaccines could undergo recombination with naturally occurring viruses to produce hybrid viruses that could theoretically have undesirable properties affecting transmission or virulence. Especially given the entirely novel nature of chimeric viruses, prudence dictates that this possibility be taken into account in the design of virus-vectored vaccines. While medicinal regulatory decisions are made on the basis of risk and benefit considerations, the approach that has primarily been taken by regulators towards genetically-modified organisms has been that of the precautionary principle. Both types of regulatory decision-making (risk/benefit; precautionary principle) implore prudence and caution on the part of developers and the requirement for provision of evidence to support decision-making.

A framework for consideration of recombination between virus-vectored vaccines and circulating wild type viruses

The subject of recombination between virus-vectored vaccines and circulating wild type viruses comprises two overlapping sub-topics, namely, the probability that recombination will take place and the possible outcomes of recombination. The numerous variables affecting these issues are probably impossible to quantify accurately given existing tools and knowledge. However, the major variables that could influence recombination between a virus-vectored vaccine and circulating wild type virus and the consequences of such recombination can be identified and evaluated at least qualitatively during vaccine development. These major variables are:

1. Intrinsic recombination properties of the parent virus used as a vector
2. Sequence relatedness of vector and wild virus
3. Host range, pathogenesis and transmission
4. Replication competency of vector in target host
5. Mechanism of vector attenuation
6. Additional factors potentially affecting virulence
7. Circulation of multiple recombinant vectors in the same target population

Each of these variables is considered separately in the following paragraphs.

Intrinsic recombination properties of the parental viruses

As noted above, different virus families are associated with different intrinsic frequencies of recombination, and these intrinsic properties will affect the probability that recombination will take place. Generally, DNA viruses are subject to relatively high frequencies of recombination. Although RNA viruses generally display lower recombination frequencies compared to DNA viruses, retroviruses and some positive stranded RNA viruses (picornaviruses, coronaviruses, togaviruses, noroviruses), readily recombine. Other positive stranded RNA viruses recombine only inefficiently, and while recombination among negative stranded RNA viruses can be demonstrated on an evolutionary scale, the frequencies are sufficiently low as to make recombination under laboratory conditions

difficult to detect. Segmented viruses display extremely high rates of reassortment, and concern has been expressed that live attenuated influenza vaccines might readily reassort with circulating wild type strains [51]. One must assume that vectors based on virus families with segmented genomes or families prone to high recombination rates would also be most prone to recombination with related wild type viruses should the opportunity for recombination arise. For viruses of any given family, the intrinsic rates of recombination both in cell culture and in laboratory animals can often be determined experimentally, and recombination among related viruses in the wild can sometimes be deduced based on phylogenetic analyses. The intrinsic recombination properties of any given virus should be taken into account during vaccine vector development.

Sequence relatedness of vector and wild virus

For both DNA and RNA viruses, the majority of observed recombination events occur through regions of nucleic acid sequence homology between parental genomes. Thus, any sequence alterations to a vector that reduce homology with the wild type virus should theoretically reduce the probability of recombination between vector and wild type virus. Viruses can be attenuated by changing the genome sequence to substitute less common codons [52]. Sequences can be changed in silent positions such that the original amino acid sequence of the gene is preserved, but multiple substitutions of less frequently used codons, “codon deoptimization”, results in loss of virulence. Attenuation can also be achieved by the introduction of many uncommon combinations of pairs of codons, thus changing the “codon pair bias” [53]. Vectors engineered in this fashion should theoretically have a reduced frequency of recombination with wild type homologs. Alternatively, vectors intended for human use which are based on naturally occurring non-mammalian viruses, for example the use of canarypox virus as an HIV vaccine vector [54], are sufficiently divergent in sequence homology from mammalian viruses such that the probability of recombination between the vector and a mammalian virus in the vaccinated host should be lowered. Lastly, gross alterations in gene arrangement of a vector could suppress productive recombination with a wild type virus homolog [55–57]. Notably, non-homologous recombination among viruses does occur, albeit at a relatively low frequency, and manipulations to influence homologous recombination will theoretically not affect non-homologous recombination events. Furthermore, sequence homology is irrelevant in reassortment of genome segments in segmented viruses.

Host range, pathogenesis and transmission

The probability of recombination between two viruses should be directly proportional to the probability that a cell will be co-infected by the two parental viruses under consideration. The probability of co-infection, in turn, may theoretically be influenced by virus host range, pathogenesis (i.e. whether infections are normally acute, persistent, chronic or latent) and transmission. Considering the influence of host range, use of an avipoxvirus vector in humans or non-avian vertebrates should limit the potential for recombination of the vector with wild avipoxviruses [54]. Likewise, use of the avian Newcastle disease virus (NDV) as a vector in humans would present minimal opportunity for co-infection with the wild type avian virus, thus limiting the opportunity for recombination [49]. By contrast, use of a human adenovirus as a vaccine vector carries the clear potential for genetic interaction

between the vector and circulating human adenoviruses. Considering the influence of pathogenesis, one would expect that a virus that in the wild causes a latent infection in the target population would provide a greater opportunity for recombination compared with a virus that results in an acute infection. As a specific example, herpes viruses have been proposed as vaccine vectors [58]. Because a large fraction of the human population carries several different herpes viruses in a latent state, these latent wild type genomes could theoretically provide stable, persistent populations of virus genomes in a target population which could recombine with vaccine vectors. By comparison, if the vector parent usually undergoes an acute infection such as in the flavivirus yellow fever, the opportunities for recombination would be relatively rare. Transmission mechanism could also theoretically affect the probability of recombination between a viral vaccine vector and a circulating virus. For example, different species of arthropod-borne flaviviruses exhibit differences in observable recombination frequencies in nature attributable to differences in mechanism of vectoring by ticks and mosquitoes and by differences in both host and vector ecology among different flaviviruses [30].

Replication competency of vector in target host

The probability of recombination between a virus-vectored vaccine and a wild virus should be proportional to the virus load in a vaccinated individual, and thus limited by the replication competency of the vectored vaccine. Virtually all vaccine vectors are either defective for virus replication or naturally or artificially attenuated for pathogenicity in their target populations, and attenuation sometimes equates to reduced virus replication, hence reduced virus load and reduced opportunity for recombination. “Naturally” attenuated vectors comprise those in which a virus specific for one animal species is used as a vector for vaccination of another normally non-permissive species. Examples include canarypox virus and Newcastle disease virus engineered for use in humans as described above [49;59]. Canarypox in particular undergoes an abortive infection in non-avian cells, virtually nullifying the opportunity for recombination [60]. Vectors may be artificially attenuated using classic methods of serial passage in vitro or more modern methods involving engineered gene deletion or rearrangement. Modified vaccinia Ankara provides an example of a poxvirus vector attenuated by serial passage [61], the MRKAd5 HIV-1 clade B gag/pol/nef vaccine provides an example of an adenovirus vector artificially attenuated by deletion of the essential viral E1 regulatory region [62], and vesicular stomatitis virus vectors have been attenuated by rearrangement of gene order in the viral genome [57].

Mechanism of attenuation

The specific mechanism of attenuation impacts the consequences of recombination between a vaccine vector and a wild type virus. Specifically, if attenuation is genetically linked to transgene expression, reversion to virulence seems unlikely. For example, as noted above, in an adenovirus vector the transgene encoding an immunizing antigen may be inserted into a deleted E1A region [62] so that any recombination event that restores virulence to the vector also deletes the transgene and conversely transfer of the transgene to a wild type virus also transfers attenuation. Thus, theoretically, no recombinant should be more virulent than the vaccine vector itself. By contrast, in an attenuated vaccinia vector (MVA) multiple genes are mutated or deleted to confer attenuation, and the transgene is expressed only from one site

[61]. Recombination with a wild orthopox virus (cowpox virus, vaccinia virus or monkeypox virus) could in theory either “repair” some virulence mutations or transfer the transgene into a wild type virus background, generating a recombinant with improved replication properties relative to the original vaccine vector and also leaving the transgene expression intact.

Additional factors potentially affecting virulence

Vector designs have been proposed that incorporate expression of immune modulatory molecules, for example interleukins, to stimulate or otherwise regulate the immunogenicity of the recombinant vaccine [63]. However, in some cases these genes may act as virulence factors. For example, in a model poxvirus system, expression of interleukin-4 (IL-4) from ectromelia virus (a natural pathogen of mice; mousepox virus) enhanced the pathogenicity of the virus and conferred resistance of the recombinant virus to pre-existing immunity in infected animals [63;64]. Theoretically, recombination of a vector expressing such a virulence factor with a wild type virus could result in production of a wild virus with increased virulence.

Circulation of several recombinant vectors in the same target population

Although hypothetical, the possibility of recombination between two live recombinant vectors administered in the same population deserves some consideration. Two examples are provided. Attenuated vaccinia Tiantan [65;66] used in China for smallpox eradication is being assessed as a replication-competent vector for both HIV-1 [67]and H5N1 [68] vaccines and in its replication-defective form for hepatitis C vaccine [69]. Similarly, replication-competent adenovirus serotype 4 (Ad4) has been tested in humans as a vector for H5N1 [70] HIV and anthrax. Ad4 and Ad7 cause serious lower respiratory tract disease in military recruit training camps. Non-recombinant Ad4 and Ad7 vaccines proved to be among the safest vaccines, with more than 10 million military recruits vaccinated without serious adverse experience and the military continues to vaccinate recruits today with these vaccines [71;72]. The use of two different vaccines based on the same vector in the same population creates an opportunity for recombination which could generate a novel virus with potentially undesirable properties. A practical example with existing traditional attenuated veterinary vaccines is the recombination between two independently derived attenuated vaccines for infectious laryngotracheitis virus, a herpesvirus affecting commercial poultry, which regenerated a pathogenic wild type virus, cited above under “vaccine viruses in the wild” [43].

A template for investigations of recombination between live virus-vectored vaccines and wild type virus: experience with the ChimeriVax platform

The experience with ChimeriVax may serve as a template for analysis of the role of recombination in the development of vectored vaccines.

As introduced above, ChimeriVax is a live, attenuated recombinant virus platform constructed from the live attenuated yellow fever (YF) vaccine strain 17D in which the envelope protein genes of YF 17D are replaced with the corresponding genes of another

flavivirus [73]. The ChimeriVax platform has been used to develop live vaccines for dengue viruses (DENV), West Nile virus (WNV) and Japanese encephalitis virus (JEV).

Concerns have been raised that recombination between ChimeriVax vectored vaccines and wild type flaviviruses could generate a novel virus with enhanced pathogenicity [47;48]. These concerns have been addressed through a variety of studies including a review of evidence for inter- and intra-typic recombination among flaviviruses in the wild [74], laboratory tests for recombination among flaviviruses [74;75], and deliberate construction and testing of theoretical recombinants containing a heterologous envelope vaccine antigen in a wild-type vector background [76;77]. The results demonstrated intra-typic recombination among flaviviruses that occurred on an evolutionary scale in the wild and little or no recombination in cell culture, and that substitution of heterologous envelope proteins into a virulent flavivirus backbone results in a virus with properties of attenuation matching those of the attenuated vaccine vector.

For analysis of an existing vector or development of a new vector, new or existing studies such as those done for ChimeriVax, along with a thorough description of the distribution and host range of both the vaccine and the wild strains should effectively address the fundamentals influencing recombination between virus-vectored vaccines and wild strains as recommended by WHO and outlined in this document.

Guiding principles for vector design and testing

Consistent with the recommendations of the U.S. Food and Drug Administration and the European Medicines Agency [4;5], the potential for recombination with circulating wild-type viruses should be addressed during development and testing of virus-vectored vaccines.

Rather than attempting to prepare specific guidelines for each vector, the Brighton Collaboration recommends that developers take the responsibility for assessing the potential for recombination between their virus-vectored vaccine and wild-type circulating viruses, taking into account the issues described above. Specifically:

1. Consider the evidence that members of the virus genus do or do not undergo recombination in the wild or under experimental conditions, for example in an animal model or in cell culture.
2. Given the vector design and mode of delivery, assess the magnitude of the opportunity for recombination in a clinical scenario.
3. Given the vector design, evaluate the probability that a recombination event with a wild virus could lead to a virus of increased pathogenicity. Consider the potential mechanisms whereby this could happen, and cite or conduct laboratory studies to evaluate those mechanisms.
4. Consider vector designs that could further reduce the probability of a recombination event, and enhance safety, while leaving the potency of the vector largely intact.

5. Develop an optimized system for recombination and a strategy for detection of recombinants perhaps using current, sensitive assays for detection of expected viruses (e.g. PCR or infectivity assays) and new, broad methods for detection of novel viruses (e.g. degenerate PCR and massively parallel sequencing).

A place already exists for reporting the general conclusions of these investigations in the existing Brighton Collaboration templates for description of vectors [78]. The option for including a more detailed report can be considered.

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