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## NEWS AND COMMENTARY

Microarray analysis

# The evolving story of oral polio vaccines

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RNA virus evolution proceeds under a very high mutation rate of $10^{-4}$–$10^{-5}$ substitutions per base per replication (Drake and Holland, 1999). These changes result from the error-prone viral RNA-dependent RNA polymerases and the absence of endonuclease proof reading to correct the mistakes. Recombination between the consequent divergent genomes occurs, and may be particularly important to prevent accumulation of adverse mutations (the so-called Muller's ratchet) (Gordo *et al*, 2002). Over time, the population structure may change as fitter variants with different properties emerge within and dominate the biological profile of the viral mix. These processes mean that that the RNA viruses are comprised of exceptionally dynamic quasispecies.

Oral poliovirus vaccine (OPV) is a live vaccine. Unlike most comparable vaccines, it is actually transmitted from person to person. It is therefore particularly important that it is likewise subject to a high mutation rate, and that some of the mutations may result in reversion to neurovirulence.

A recent paper by Cherkasova *et al* (2003) describes the development of microarray-based systems, namely the microarrays for resequencing and sequence heterogeneity (MARSH) and microarray analysis of viral recombination (MARV) assays, for analysis of genetic alterations in circulating vaccine-derived strains of poliovirus. These methodologies are equally applicable to the monitoring of genetic changes occurring during cell culture passage of seed virus to produce vaccine lots. Current measures to protect against the accumulation of potentially neurovirulent mutations during this process include batch testing for neurovirulence in monkeys; an expensive and unpopular process.

An advantage of both MARSH and MARV is that they allow simultaneous rapid analysis of a population of viral variants, enabling the identification of clusters or 'hotspots' of mutations. The methods can also provide a picture of genome evolution. In this respect, they differ from historical methods for studying sequence heterogeneity such as restriction fragment length polymorphism (RFLP) analysis, which is relatively insensitive, limited to too few mutations, and may be difficult to automate. More recent methods for the detection of known single-nucleotide polymorphisms (SNPs), by Taqman PCR and other protocols, are more flexible in that they can be used to genotype at most loci and can be automated. However, the analysis is limited to predetermined targets. The current method adopted by the WHO for quality assuring OPV vaccine, mutation analysis by PCR and restriction analysis (MAPREC) (Chumakov *et al*, 1991; World Health Organisation, 1999), allows better quantification of SNPs, which is important for detecting reversion of OPV to neurovirulence. However, MAPREC is difficult to automate and is subject to inaccuracies due to PCR artefacts (Cherkasova *et al*, 2003).

Data on all components of a viral population are vital, as minor variants may alter the phenotype of the mix. This has been clearly demonstrated in retroviral infections. For example, there is a neurovirulent variant of simian immunodeficiency virus (SIVsmm-PBj clone 14) derived from non-neurovirulent SIVsmm-9. If clone 14 makes up only 10% of the viral population, it will confer a neurovirulent phenotype on the remaining 90% of the (SIVsmm-9) population (Tao and Fultz, 1995).

Glimpsed on a genome- and population-wide basis, the processes by which mutations accumulate may also inform our understanding of how virulent variants emerge. Accumulation of synonymous mutations over time, given steady rates of replication, should be linear. However, there is evidence in poliovirus and other RNA viruses of accelerated accumulation of mutations during some periods, and of different rates of synonymous mutations in different parts of the genome. This information has enabled cogent analysis of how neurovirulent variants of vaccine strains emerge (Cherkasova *et al*, 2002). Using the background information about the accumulation of synonymous mutations to estimate rates of evolution of the VP1 region, Cherkasova *et al* have suggested two hypotheses. Firstly, recombination may contribute to the loss of attenuating features, though the relationship between recombination and the emergence of neurovirulent variants is not clear. Secondly, long-term persistence and evolution of vaccine variants may lead to the emergence of virulent strains, even in a well-vaccinated population. This has particular implications for WHO vaccination policies in areas from which wild-type poliovirus has been eradicated.

So, how useful are the MARSH and MARV systems? A major drawback of the MARSH assay is its use of PCR product as its template, which precludes analysis of individual genomes within the quasispecies. In this respect, it is no different from direct sequencing. The microchip is designed to function as a screening tool with which to identify the approximate location of base substitutions. Precise information as to which base is altered, however, requires additional sequencing of the product. Data on allele frequency at each locus are not generated. The key questions are, therefore: what is the sensitivity of the MARSH system (ie, what is the minimum proportion of a minority base can be detected at a mixed locus); and what is its specificity (ie, how often are peaks found not to be associated with a mutation)? No formal evaluation of this is provided and until such data are available the utility of this method for screening vaccines and vaccine-related isolates cannot properly be assessed. The MARV assay allows detection of recombinants. Although the simplicity of the method has its attractions, the use of a conventional resequencing chip with appropriate software would obviate the need for this second assay.

The global use of live attenuated vaccines, especially those that are able to immunise by secondary spread, offers the opportunity to achieve widespread coverage. However, as the authors state, the evolution of virulent vaccine strains and their ability to cause outbreaks somewhat blurs the definitions of 'wild type' and 'attenuated', and raises questions about the achievability of the WHO campaign to

eradicate polio. The methods described here may provide a cheap and reproducible alternative to sequencing for screening large numbers of isolates. More precise information, including the proportion and identity of nucleotides present at individual loci and the haplotype of individual viruses, will help us to understand how the accumulation of mutations leads to the emergence of virulent variants. Future developments must combine precise methods for estimating allele frequency with the high throughput offered by the techniques described here.

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