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Viral evolution and epidemiology

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Abstract

This review highlights recent research on viral evolution and its use towards understanding disease pathogenesis and epidemiology. The development of techniques such as enzymatic amplification of viral genomes and automated sequencing has led to a dramatic increase in the amount of sequence information from clinical samples. These sequences (RNA or DNA, or the amino acids they encode) have been compared by complex computer algorithms to generate evolutionary trees or phylogenies of natural virus variants, which can sometimes be used to correlate viral genotype with phenotype. Understanding the rates and types of evolution that occur during the transmission of viruses has considerable impact on the design of methods for the control of virus diseases.

Introduction

The high genetic variability of viruses has been suspected for some time but only recent technical advances have allowed us to observe and describe this natural phenomenon. Because of the very large population of viruses circulating in host populations at any given time, even small changes in the genetic content of viruses could have a considerable impact on the way disease is manifested. Although viruses have one of the highest mutation rates of all organisms (presumably because they lack error-correcting mechanisms during replication), it is astounding how stable they are as a population or species, and how they continue to produce diseases which have been clinically described for decades or centuries. Viral mutation thus occurs at a high rate, on a regular basis, but we have not yet encountered many examples of rapid progression towards new phenotypes (or ‘Andromeda’ strains); changes in the pathogenesis or transmission of HIV-1, influenza A and Venezuelan equine encephalitis viruses may be caused by actual mutation events, but the clinical syndromes they cause remain relatively stable. It seems that the advent of modern day air travel and the urbanization/encroachment of humans on previously remote areas has had more impact on the public’s health because they have led to a wider distribution of viral diseases and the emergence of viruses which were previously limited to other animal hosts (e.g. HIV-1 and influenza A). Here we review reports on the molecular evolution of both RNA- and DNA-containing viruses, and how their long- and short-term evolutionary trends can be interpreted epidemiologically, for an empirical approach towards improving viral disease diagnosis and control.

Constructing phylogenies

The genetic or phylogenetic relationship among virus variants or strains can provide us with a wealth of information about how a virus is transmitted and what evolutionary selective pressures it undergoes. Understanding these relationships can help to determine which strains might be more virulent or efficiently transmitted, when and where these strains are originating, and under what ecological conditions, what virus(es) to use as the basis for a vaccine, and what host population should be targeted for vaccination or treatment. Therefore, the methods used to construct phylogenies should be selected carefully, and their interpretation should include a consideration of the assumptions and biases involved in these experimental approaches. Because we cannot possibly include representatives of all variants circulating in the natural population at a given time, nor include all of the genetic information in a complete viral genome, we must remember that these trees are approximations of reality. Most trees are thus designed to respond to a specific question and will give us limited information about a viral phenotype or biological characteristic.

Probably the most important decision in generating phylogenies is the nature of the sequence to be compared among strains [1**]. Complete gene sequences or portions of genes may be used but the best choice will come from reconsidering the question to be answered. Are we looking for short or long term evolutionary trends? Do we want to see the effect of host immune selection or tissue tropism? Are we looking for transmission patterns or bottlenecks, or both? Do we want to detect recombination events? Are we looking for a 'molecular clock', to determine branching times? Is the nucleotide substitution rate of the virus gene known? The questions we ask and the assumptions we include in the comparisons will determine the complexity of the task and the limits of interpretation. Numerous computer programs have been written which perform the sequence comparisons; they use different mathematical and processing algorithms to generate the graphical representations of genetic relationships we know as trees. In short, they differ according to the number of assumptions or the information we know about the virus sequences to be compared. As long as we limit our interpretations to the major branching patterns they generate, most programs give us superimposable or similar trees. Often, because of the availability of ever increasing amounts of sequence data (character size and number of taxa), the choice of phylogeny program may be based on the attainable computing facilities (or time constraints).

Limitations on tree interpretation

One way of determining the validity of the resulting phylogenetic tree(s) is to apply statistical measures of confidence. Most of the phylogenies reviewed here use the 'bootstrap' resampling test to measure the probability that they reflect reality (i.e. confidence); a bootstrap value of over 70% has been empirically equated to a confidence level of more than 95%. In general, this means that a branching pattern to the right of a node with a bootstrap value of less than 70% is not supported and the sequence or virus relationships are indeterminate. Therefore, those that have bootstrap values of 50% or less should probably not even be discussed; these values have often been seen in recent literature and they have led to unsupported interpretations [2*].

Other common problems with interpretations have been caused by sampling bias [3,4], statistically questionable correlations [5] or too small a sample size; a widely publicized paper [2*] used only one sample (and sequences ranging in size from 38–152 bp) to demonstrate the origin of the 1918 influenza pandemic. Sometimes assumptions about the gene nucleotide substitution rates are included in the phylogeny program which might impact on the outcome [6,7*] such as tree branching pattern or bootstrap values, but the effect of these data are hard to evaluate. Great care should also be taken when interpreting relationships in terms of phenotype or biological properties [8,9*]; this is probably the most difficult to evaluate, because we currently know little about the molecular determinants of complex biological characteristics [10].

RNA virus evolution

Viruses using RNA molecules as genomes have the highest mutation rates observed *in vivo* or *in vitro*. The fact that most natural populations or single clinical sample of these organisms consist of a range of genetic variants has led to their description as a 'quasispecies'. When we analyse RNA sequences we should therefore keep in mind that we are looking at representatives of a normal distribution of genotypes; the area under the normal curve (number and type of variants) varies from one virus family or species to another.

Retroviridae

Human immunodeficiency virus type I—The largest number of papers concerned this virus and its classification into genetic subtypes on the basis of the comparison of different genes (*env*, *gag*, *pol*, *vpr*, *vpu*) or regions within a gene (*env*). New variants from Malaysia, Indonesia, Martinique, Cameroon and Nigeria have been classified on the basis of *env* sequences [10–14], whereas the classification on the basis of *pol* sequences seems to be more stable in time [15*,16]. The comparison of classification schemes on the basis of both *env* and *gag* sequences demonstrated the existence of hybrid or recombinant HIV-1 viruses in Thailand [17*], whereas the comparison of phylogenies using complete versus partial *env* gene sequences detected probable recombination sites within this region of the genome [18*].

To investigate the association of nucleotide deletions in the *nef* gene with attenuation further, HIV-1 viruses from a large group of patients with differing clinical presentations were analysed, and no correlation was found between defective *nef* genes and disease state in nine out of 10 patients [19*]. The relationship between disease progression and the genetic diversity of HIV-1 was studied in perinatally infected children; where the evolution of a portion of the *nef* gene seemed to reflect positive selection for change in children with slow disease progression [20*]. Three epidemiologically linked individuals were sampled over 8 years to determine evolution rates and selective pressures on the V3 loop of *env* [21], and there was evidence for strong selection towards mutation at the amino acid level, thus supporting the Darwinian natural selection hypothesis.

Human T-cell lymphotropic virus type II—Analysis of the X-V/*raf* gene region of 16 samples from different Indian groups in North, Central and South America suggested that

two distinct subtypes of human T-cell lymphotropic virus (HTLV)-II viruses were brought by tribes migrating to the New World, and that the origin of the subtype infecting intravenous drug users in the USA is still unknown [22*].

Orthomyxoviridae

Studies of the genetic relationships of influenza A viruses, using nucleotide sequences of the neuraminidase gene, support the existence of two distinct lineages that are being transmitted around the world; strains currently used for the preparation of vaccines represent both lineages, and this approach should be used as a basis for future strain selections [23].

Flaviviridae

Hepatitis C—Mutation rates in the E1 and NS5B genes were estimated from a known transmission cohort, and this information was then used to calculate divergence times for the known hepatitis C virus (HCV) genotypes (types 1–6); after careful consideration of the limitations of this interpretation, the conclusion was that HCV subtypes have been evolving independently for 500–2000 years [24].

Yellow fever—Three different gene regions of 13 yellow fever virus isolates from Africa and South America were compared; four distinct lineages were observed and the distinct evolution and transmission of African viruses led to the conclusion that this group was introduced to the Americas [25*].

Filoviridae, Bunyaviridae, Arenaviridae, Togaviridae

Ebola—The analysis of Ebola isolates from recent outbreaks in Zaire and the Ivory Coast and the comparison of sequences encoding the glycoproteins demonstrated the extreme stability of this virus in nature, after almost 20 years of transmission; this suggests that this virus has coevolved with its as yet unknown reservoir host [26**].

Hantavirus—A literature review [27] containing a section on hantavirus evolution concludes that the phylogenetic relatedness (based on S segment sequences) between these viruses resembles that of their natural hosts (rodents).

Arenaviruses—Phylogenetic analysis of a portion of the nucleocapsid (N) gene of all Tacaribe complex arenaviruses revealed three-distinct lineages, one of which seems to segregate on the basis of human pathogenic potential [28*]. The comparison of another portion of the N gene was used to classify a newly described human pathogen, Sabiá virus [29].

Alphaviruses—Western equine encephalitis strains from the New World were shown to be of recombinant origin when sequences from the E1 glycoprotein gene and those from the nonstructural protein nsP4 were compared [30*]. Enzootic, equine-avirulent Venezuelan equine encephalitis viruses were shown to be the source of epidemic/epizootic strains which caused a very large outbreak in Colombia and Venezuela in 1995; the mechanisms leading to the generation of these equine-virulent viruses seem to include point mutation and selection [31].

DNA virus evolution

In general, DNA viruses show fixation of mutation (in the natural population) at rates much lower than those for RNA viruses (on average, one mutation in every 10^8 nucleotides copied versus one in every 10^4 , respectively). These differences are presumably caused by processing mechanisms of the viral replicating enzymes, with DNA polymerases having additional proofreading activities. In spite of this conservation in genome structure, researchers have found regions with higher mutation frequencies that can be used to distinguish strains.

Papovaviridae

A comprehensive study of human papillomaviruses from cervical specimens collected around the world gave reliable estimates of the genetic variability in a portion of the L1 open reading frame (major capsid protein); because these viruses use the host cell DNA polymerase for replication, it seems that the virus phylogenies may reflect host (human) population evolution and dynamics [32].

Herpesviridae

The initial characterization of a virus associated with Kaposi's sarcoma demonstrated its probable classification as a gamma-2 herpesvirus (genus *Rhadinovirus*), and this is the first member of this genus which has been shown to infect humans; comparison of amino acids from nine open reading frames to corresponding genome regions of 11 other herpesviruses allowed the classification of this new agent [33**].

Conclusion

The exponential increase of available sequence data will lead to further interpretations of phylogenies towards understanding the epidemiology of viral diseases. Researchers should, however, be aware of the limitations of comparing mutation rates across genes that undergo different selective pressures. Without the elucidation of the mechanisms leading to virus variant selection in nature, the conclusions we reach may not reflect the true complexity of evolution.

Abbreviations

HCV	hepatitis C virus
HTLV	human T lymphotropic virus

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