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Event

Evolving viruses and virologists

Anne-Mieke Vandamme

Molecular phylogenetic analysis is widely used by virologists to study viral epidemiology and evolution. However, many virologists using these methods lack a true understanding of the theoretical background, which often leads to a misinterpretation of the results. Additionally, inexperienced virologists who lack background knowledge are very reluctant to begin using such methods, as they involve complicated mathematics, and there are few useful tutorials for beginners. ‘The Fifth European Workshop on Virus Evolution and Molecular Epidemiology’ (<http://www.kuleuven.ac.be/AIDSlab/VEME.htm>) set out to provide not only thorough training in the elementary aspects of molecular epidemiology, but also to give the participants a grounding in the more-sophisticated methods that exist, the type of information that can be obtained using these methods and how they can be applied to specific virology problems.

Practice makes perfect

The workshop combined theoretical and practical sessions. During the theoretical sessions, the mathematical background of sequence alignment, tree construction and

The Fifth European Workshop on Virus Evolution and Molecular Epidemiology was held in Leuven, Belgium, from 30 August to 4 September 1999.

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evaluation methods, and the use of nucleotide sequences compared with protein sequences, was thoroughly explained. The participants then applied these methods using real data during the practical sessions, and were taught how to retrieve sequences and other relevant information from international databases, to retrieve software and data, and to carry out homology searches using the Internet. They were also taught how to carry out and judge alignments, as well as simple phylogenetic analyses such as neighbour joining or maximum-likelihood trees, and the statistical evaluations required to support a particular topology. More-sophisticated analyses were also explained and utilised, including evaluation of different evolutionary models, estimation

of synonymous/non-synonymous substitution ratios, saturation at the different codon positions, molecular-clock analysis, likelihood-ratio tests, splitree analysis and quartet puzzling. Molecular epidemiology methods were used to date specific virus-transmission events, to measure the selective pressure exerted on a virus and to trace virus recombination. There were also lectures devoted to *in vitro* evolution systems and viral population dynamics.

Molecular epidemiology in action

The workshop participants contributed personally by explaining how they use molecular epidemiology in their own research. The most obvious use of molecular epidemiological methods is for mapping the epidemiology of a particular virus by phylogenetic analysis. The increased mobility of humans in the last century appears to be responsible for the worldwide distribution of virus variants. Examples of this include the spread of different HIV-1 subtypes to different European countries¹ (Maria Mezei, National Center for Epidemiology, Budapest, Hungary), the presence of different measles virus variants in Australia (Doris Chibo, Victorian Infectious

Diseases Reference Laboratory, Melbourne, Australia) and the movement of dengue virus between Cuba, Thailand and New Guinea (Carlos Sariol, Eberhard-Karls University, Tübingen, Germany). Valentina Dias-Ferrao [World Health Organization (WHO), Geneva, Switzerland] gave a particularly interesting presentation on FluNET, a specific Internet tool established by the WHO to study epidemiological data pertaining to influenza. FluNET is a relational database that is kept up to date by different national reference centers, thus allowing investigators to track the geographical and temporal spread of different influenza variants. This information is of crucial importance for the annual development of influenza vaccines.

New viral variants

During the workshop, it became clear that phylogenetic analysis is a powerful tool with which to investigate specific global epidemiological questions and to identify potentially new genotypes of different viruses. For example, Eline Op de Coul (Divn of Public Health and Environment, Amsterdam, The Netherlands) described how, in Romania, the distribution of HIV-1 subtype F among institutionalized children appears to have arisen by several separate introductions from Romanian blood donors. Also, Dieter Klein (University of Veterinary Sciences, Vienna, Austria) discussed how the increased divergence of feline influenza virus (FIV) strains in mid-European cats is caused by the increased mobility of humans with their pets, which carries different FIV clades across natural barriers such as the Alps. Additionally, new variants have been reported within several virus families; for example, new simian hepatitis B virus (HBV) variants have been found in orangutans and baboons (Ernst Verschoor, Biomedical Primate Research Centre, Rijswijk, The Netherlands; Anna Kramvis, University of the Witwatersrand, Johannesburg, South Africa); in the UK, new transmission transfection virus (TTV) genotypes have been found

(Eva Baldrich Rubio, University of Cambridge, Cambridge, UK); and, in West Africa, several new genetic lineages of Kaposi's sarcoma-associated herpesvirus (KSHV) have been reported (Vincent Lacoste, Institut Pasteur, Paris, France). Sleeping disease in salmonid fish farms is suspected to be related to a virus infection, which was reported here by Stephane Villoing (INRA, Jouy en Josas, France) to be caused by a new, atypical alphavirus.

Phylogeny and pathogenesis

Several presentations touched on the phylogenetic relationship of different virus strains, relative to their pathogenic potential. Viruses can be linked to more than one disease or to different levels of pathogenicity, but in all cases reported during this workshop, the phylogenetic relationship of different strains reflected their geographical origin better than it did the associated disease in infected individuals. This has been shown to be the case for human T-cell lymphotropic virus type 1 (HTLV-1) linked to tropical spastic paraparesis and adult T-cell leukemia (Anne-Mieke Vandamme, Rega Institute and University Hospitals, Leuven, Belgium); for KSHV linked to Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease (Vincent Lacoste); and for porcine encephalomyocarditis virus (pEMCV) linked to reproductive failure in sows and to myocardial failure in fatteners (Hans Vanderhallen, Center for Veterinary and Agrochemical Research, Brussels, Belgium). Also, the HTLV-1 genome appears to be defective in many infected patients, and, as a result of deletions of large parts of the viral genome, new 'fusion' genes can be formed that potentially encode new proteins (Vladimir Morozov, Blockhlin Cancer Research Center, Moscow, Russia). Their role in HTLV-1 pathogenesis is unknown.

Interspecies transmission

Interspecies transmission has been investigated by several participants and has been shown to occur in several virus families. Phylogenetic

clustering for these families is often not according to the host species but according to the geographical origin of the virus in the different species. HTLV-1 appears to have originated from simians several thousand years ago (Sonia Van Dooren, Rega Institute for Medical Research, Leuven, Belgium) and it has become obvious that interspecies transmission also occurred for human and non-human primate HBV-like viruses (Ernst Verschoor). Influenza pandemics are a result of the reassortment of genetic fragments from different species (Anne-Mieke Vandamme), and human hantavirus infections are the result of interspecies transmission from rodents (Tatjana Avsic-Zupanc, Institute of Microbiology and Immunology, Ljubljana, Slovenia; Alexander Plyusnin, Haartman Institute, Helsinki, Finland); humans become infected with hantavirus strains found in rodents from the same geographical area. Consequently, xenotransplantation should be carefully evaluated, taking into account potential interspecies transmissions of as-yet-unknown virus strains.

Evolution and recombination

Questions concerning viral epidemiology and evolution are also important in vaccine research. One of the main reasons FIV is being studied is to evaluate different vaccination strategies. An interesting approach was presented by Mauro Pistello (University of Pisa, Pisa, Italy), in which five FIV Tat mutants were found to replicate in fibroblasts, but not in lymphocytes or macrophages. Potentially, these mutants could be used as vaccine strains. Margaret Hosie (University of Glasgow, Glasgow, UK) has found that DNA vaccination using replication-incompetent FIV reverse transcriptase (RT) mutants induces a significant immune response, but does not provide complete protection against heterologous FIV strains.

The evolution of viruses within a single individual has been investigated by several participants. The transition from non-syncytium-inducing (NSI) to syncytium-inducing (SI) HIV-1 was discussed in terms of co-receptor usage and

virus evolution by Ronald van Rij (Central Laboratory of Blood Transfusion Services, Amsterdam, The Netherlands). It seems clear that NSI viruses are CCR5-co-receptor-restricted, whereas early SI variants have both the CCR5 and CXCR4 co-receptors, evolving into CXCR4-co-receptor-restricted variants later. The evolution of this transition is unclear, and its investigation using molecular epidemiological methods is complicated by the effect of convergent evolution in the V3 loop. Carlos Briones (Carlos III Health Institute, Madrid, Spain) reported that the evolution of drug-resistant HIV-1 is influenced by a number of factors, including drug selective pressure, the fitness of resistant variants and stochastic effects, and Magnus Lindh (University of Göteborg, Göteborg, Sweden)

reported that, for hepatitis B, a long-term study has shown that the extent of non-synonymous mutations did not correlate with disease progression.

Four talks addressed the question of virus recombination, which is particularly striking in HIV. In Nigeria, subtype A and A/G recombinants are the most prevalent subtypes. Some strains cannot be assigned to a particular subtype and could represent unknown variants or recombinants (Abigail Edubio, Robert Koch Institute, Berlin, Germany). In Greece, evidence has been found for a mosaic A/G/I recombinant strain (Dimitris Paraskevis, University of Athens, Athens, Greece).

Perspective

At the end of the workshop, the participants were encouraged to

use the methods discussed during the workshop on their own data. The workshop was evaluated by the participants as highly significant for their own research, and it is hoped that the virologists trained during this workshop will be able to make better use of molecular epidemiological methods in the context of the specific epidemiological problems they encounter during their research.

Acknowledgements

This workshop was sponsored by the Rega Institute for Medical Research, Leuven, the European Community, the European HTLV Research Network (HERN), PE Biosystems and Roche Molecular Biochemicals.

Reference

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Letters

Stress and sensitivity

All bacteria are not created equal. If life is no picnic for most, some, such as the major pathogen *Staphylococcus aureus*, adapt with the greatest of ease to man and nature's best efforts at thwarting its survival. This is dramatically illustrated by the losing battle waged against bacteria. Each new antibiotic introduced by man has been met by the emergence of resistant strains of *S. aureus*, with vancomycin remaining the weapon of last resort. Yet, clinical isolates with reduced vancomycin susceptibility have already appeared^{1,2} and a highly resistant laboratory strain has been reported³. Such tenacity forces admiration. Are these examples a dire portent of things to come? The ominous spectre of highly-vancomycin-resistant *S. aureus* is certainly worthy of concern. Some hope remains, however, as in all the cases reported to date, vancomycin resistance was not linked to the acquisition of the notorious *Enterococcus* glycopeptide-resistance genes, but was instead caused by cell wall structural modifications that appear to prevent access of the antibiotic to its target site^{2,3}. Such a

unique adaptive potential only emphasizes the importance of deciphering the *S. aureus* genome for the understanding of the mechanisms involved in pathogenesis and for developing targets for novel classes of antimicrobial agents.

Unfortunately, although the sequence of the *S. aureus* genome has been known for approximately three years, access to this virtual goldmine, as well as the genome sequences of many other pathogens, is restricted to private biotechnology and drug companies on a pay-per-view basis. As was recently reported⁴, the ensuing dismay of the public sector, which considers that withholding such vital information is a major setback for research, has prompted the federal funding of essentially redundant efforts to resequence the genome and make the data publicly available. These efforts are being led by The Institute for Genomic Research (TIGR; <http://www.tigr.org>) and the University of Oklahoma's Advanced Center for Genome Technology (<http://www.genome.ou.edu>), who are sequencing two different strains of *S. aureus*. The Sanger Centre (<http://www.sanger.ac.uk>) has also recently undertaken the sequencing of two *S. aureus* clinical isolates.

What have we learned so far? In their recent review⁵, Clements and Foster presented a timely summary of stress-resistance mechanisms in *S. aureus*, both during pathogenesis and outside the host. One of the more intriguing notions they raised was that of a quasi-altruistic phenomenon: the fact that nutrient starvation leads to the death of ~99% of the population, yet the remaining cells remain viable for several months, presumably by scavenging nutrients from their dead brethren. As Clements and Foster point out, unlike several other bacterial species, *S. aureus* has only one accessory sigma factor, σ^B , which plays an important role in general stress resistance. However, as also found in *Bacillus subtilis*, at least four different types of heat shock response mechanisms coexist.

Transduction of environmental signals in bacteria predominantly involves two large families of highly conserved proteins: two-component systems and ABC transporters. A preliminary analysis of the nearly complete genome sequence indicates that *S. aureus* could have up to 12 two-component systems, although only three have been characterized to date. Two of these, *LytS/LytR* and *AgrC/AgrA*, affect autolysin