HIV as the cause of AIDS

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The two known types of HIV are members of a family of primate lentiviruses. HIV, like other retroviruses, contains a virus capsid, which consists of the major capsid protein, the nucleocapsid protein, the diploid single-stranded RNA genome, and the viral enzymes protease, reverse transcriptase, and integrase. HIV isolates show extensive genetic variability, resulting from the relatively low fidelity of reverse transcriptase in conjunction with the extremely high turnover of virions in vivo. These features of HIVs may have strong implications for vaccine development. Simian immunodeficiency viruses from naturally infected animals differ from HIV in one fundamental respect: they do not cause disease in their natural hosts. Study of these viruses may therefore lead to information about the interaction between lentiviruses and host immune response that could be exploited to combat AIDS.

Structure and molecular features of HIV

Like other retroviruses, HIV virions (figure 1) contain a virus capsid, which consists of (a) the major capsid protein, p24; (b) the nucleocapsid protein, p7/p9; (c) the diploid single-stranded RNA genome; and (d) the three viral enzymes, protease, reverse transcriptase, and integrase. Reverse transcriptase is the hallmark of a retrovirus and this enzyme is capable of transcribing its genomic RNA into double-stranded DNA. This DNA copy of the retroviral genome is called a “provirus”. After integration into the host genome, the provirus serves as a template for cellular DNA-dependent RNA polymerases to generate new viral RNA genomes as well as shorter subgenomic messenger RNAs. The unspliced and singly-spliced viral RNAs are translated into the protein components of the viral core and the envelope proteins and the multispliced viral RNAs into the small accessory/regulatory proteins.

The viral capsid is surrounded by a matrix protein (p17; figure 1), which is located underneath the virion envelope. The matrix protein is involved in the early stages of the viral replication cycle and plays a part in the formation and transport of the preintegration DNA complex into the nucleus of the host cell (figure 2). The virion envelope consists of a lipid bilayer membrane, derived from the host cell, and a virally encoded tetrameric envelope protein complex, of which each subunit consists of two non-covalently-linked membrane proteins, gp120 (the “outer” envelope protein, or SU) and gp41 (the “transmembrane” protein, or TM) (figure 1). The envelope protein complex facilitates viral entry by binding to CD4, the main cellular receptor for all primate lentiviruses, via the outer envelope protein, gp120. The transmembrane protein, gp41, is involved in the fusion of viral envelope with cellular membrane.

These viral structural proteins are encoded by different genes (figure 3). The matrix protein, p17, the major capsid protein, p24, and the nucleocapsid protein, p7/p9, are encoded by the first open reading frame (gag in figure 3) and the protease, reverse transcriptase, and integrase by the second open reading frame (pol in figure 3). The viral protease is required for generation of individual proteins by proteolytic processing from the gag precursors and gag/pol precursor proteins.

The envelope precursor protein, gp160, is encoded by the env gene and processed by a cellular protease into the two envelope proteins, gp120 and gp41.

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RNA/template for the translation of nascent RNA transcripts. The long terminal repeats (LTR) (figure 3) by binding to transcription from the viral promoter, the U3 region of retroviruses, all complex primate lentiviruses contain a series of accessory proteins. All primate lentiviruses contain the tat, rev, vif, and nef genes; HIV-1 and SIVcpz contain vpu; HIV-2, SIVsm, SIVmac, and SIVagm contain vpx; and all primate lentiviruses with the exception of SIVagm contain vpr.

The function of the tat protein is to upregulate transcription from the viral promoter, the U3 region of the long terminal repeats (LTR) (figure 3) by binding to nascent RNA transcripts. The rev protein promotes the export from the nucleus of unspliced or singly spliced viral RNAs which act, respectively, as genomic RNA/template for the translation of gag/pol proteins and template for envelope proteins. In the absence of rev, no structural proteins are made. The nef protein seems to be required for efficient replication in vivo. Tat, rev, and nef are not incorporated into virion particles but are the first viral components produced from multiply spliced viral mRNA. The vif and vpu proteins respectively influence the infectivity of cell-free virions and the release of virions from infected cells. Vpr seems to be involved in the replication of HIV in non-dividing cells.

**Genetic diversity of HIV**

The pronounced variability of HIV isolates was noted soon after the diversity and sequence analysis of the first isolates from different patients. There is sequence variation even within patients, and this observation led to the term “quasispecies” to reflect the presence of infected individuals of a “swarm” of viruses rather than a single isolate. During the course of infection the frequency distribution of individual virus variants changes. Reflecting this evolution of virus variants in vivo, the replication potential of virus isolates and their ability to infect cells increases as the clinical disease progresses. The relatively low fidelity of reverse transcriptase, combined with the extremely high turnover of virus in vivo (recent estimates put the rate of virus production in the order of 10^10 virions/day), provides the basis for the continuous emergence of new virus variants. Consequently, HIV-1 acquires resistance to all antiviral drugs rather rapidly and escape mutants to neutralising antibodies and cytotoxic T cells may emerge in vivo. Whilst drug-resistant virus mutants create obvious problems for antiviral chemotherapy, the importance of immune escape mutants for the pathogenesis of HIV-1 is not entirely clear.

Individual HIV-1 genes differ in their variability. The gag and pol genes are much more conserved than env. Tat and rev likewise vary, but to a lesser extent than the nef and env genes. Within the env gene, constant and variable domains have been defined. Some of the more variable regions—eg, the V1/V2 and V3 domains—are the target for neutralising antibodies in patient sera and are exposed at the surface of monomeric envelope protein subunits. There is some evidence that sequence variability in these regions may be due to immune selection, and that this can result in a change in tropism.

At a functional level, HIV isolates can differ in their tropism for cells of different lineages. While the CD4 molecule is required for efficient entry of all HIV-1 variants, some variants are able to infect macrophages and others show a preferential tropism for T cells. Microphage-tropic virus variants may be more effective at infecting across a mucosal barrier, and subsequent in-vivo evolution may lead to the emergence of more T-cell tropic variants. In human beings, a minority virus species among the variants present in the “infecting partner” seems to be transmitted sexually. Thus, virus variants exhibiting a certain tropism may be required for transmission, leading to the de-novo evolution of virus variants with an increased replication/fusion potential in each infected individual. This model would explain why HIV does not increase its virulence with each passage from one individual to the next.

The variability of HIV has been exploited to establish transmission chains (as in the case of the Florida dentist), to follow HIV evolution in vivo, and to document the entry of HIV into defined populations (eg, intravenous drug users) and its subsequent spread.

Extensive genetic analysis has revealed that HIV-1 isolates fall into two distinct groups, now designated M
and O. Most HIV-1 isolates belong to the M group. Within the M group there are at least ten subtypes (A to J), which are separated from each other by equivalent genetic distances and differ in their geographic distribution. Subtype B is predominant in western Europe and the USA. Several subtypes exist in Africa. In Thailand, subtype B is thought to have been introduced mainly through intravenous drug use, and subtype E mainly through sexual transmission. We still do not know whether such a separate introduction of B and E viruses is due to subtle differences in the biological properties of individual HIV-1 subtypes.

HIV-1 group O strains have so far been found in individuals originating from Africa. They are sufficiently divergent from group M strains (aminoacid homology in env of only 55%) to be missed by some serological assays. It is widely held that the existence of different HIV-1 groups and subtypes in different countries would necessitate the development of individual vaccines, by analogy with influenza A and B. This assumption is supported by the apparent specificity of neutralising antibodies in patient sera for individual subtypes. However, recent reports suggest efficient cross-neutralisation of HIV-1 subtypes by at least some patient sera, so whether subtype-specific vaccines will be required remains controversial.

Evolution of primate lentiviruses
The discovery of HIV-2 in West Africa and its similarities with simian immunodeficiency viruses (SIVs), first identified in captive macaques in North America, raised the possibility of a link between human and non-human primate lentiviruses. Research into this question began when a closely related virus was identified in wild-living West African monkeys, the sooty mangabeys. Over the past several years, SIVs have been found in diverse species of wild-caught monkeys in Africa. They all have the same complex genomic organisation as HIVs, and an exceptional level of viral diversity. However, despite a similar genome organisation, individual primate lentivirus species differ with respect to the absence or presence of some accessory genes (see above).

The existence of five distinct groups of primate lentiviruses was further confirmed by sequence analyses, from which our current understanding of the origin and molecular evolution of HIVs and SIVs derives. First, the phylogenetic trees, constructed from sequence data (figure 4), suggested that primate lentiviruses probably evolved from an ancient common ancestor. According to estimated dates of genetic diversification, this phenomenon may have occurred at least 600–1200 years ago. The hypothesis that primate lentiviruses have been around for a very long time accords with the species specificity found for SIVagm. The findings of a fifth lineage of primate lentiviruses (SIVsyk) and the apparent evolutionary link between SIVagm and SIVsm led to the hypothesis of a radiation of primate lentiviruses from the Cercopithecus monkey species. The evolutionary trees also provided evidence for a phylogenetic relation between human and monkey viruses (figure 4): HIV-1 was found to cluster with SIVcpz and HIV-2 with SIVsm. Over the past few years, the assumption of a recent simian origin of HIVs has become more and more convincing, with an abundance of epidemiological and virological
information that suggests zoonotic transmission of primate lentiviruses from naturally infected monkeys to man.42,43

However, our understanding is far from complete, in particular for HIVs, with their branching structures in the SIVcpz and SIVsm groups. The proposal that chimpanzee lentiviruses may have been introduced into human beings 30–50 years ago is supported by the branching and the divergence between HIV-1 subtypes of the M group and the SIV from a chimpanzee (figure 4). Yet, the recent discovery of a second lineage of HIV-1 (HIV-1 group O) which does not cluster with SIVcpz (figure 4) exemplifies the complexity of the evolutionary picture of primate lentiviruses.

In the light of these findings, independent introductions of primate lentiviruses into the human population may have occurred and the origin of HIV-1 group O remains to be clarified. A similar problem of branching order became apparent for HIV-2 (figure 4) with the identification of various subtypes.40 The findings of a human virus as the earliest diverging branch and the clustering of SIVSm within only one lineage of HIV-2 (figure 4) again raise the question of its origin.41,42,43 In view of these uncertainties, more SIV and HIV sequences (figure 4) again raise the question of its origin.42,43 In the light of these findings, independent introductions of primate lentiviruses into the human population may have occurred and the origin of HIV-1 group O remains to be clarified. A similar problem of branching order became apparent for HIV-2 (figure 4) with the identification of various subtypes.40 The findings of a human virus as the earliest diverging branch and the clustering of SIVSm within only one lineage of HIV-2 (figure 4) again raise the question of its origin.41,42,43 In view of these uncertainties, more SIV and HIV sequences (figure 4) again raise the question of its origin.42,43

Survey of British clinicians’ views on management of patients in persistent vegetative state

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Summary

Background T he best care and management of patients in persistent vegetative state (PVS) has been the subject of sustained moral and legal debate for a number of years. However, the views of clinicians in the UK involved in caring for patients in PVS are largely unknown.

Methods A postal questionnaire was sent to 1882 consultant members of the British Association of Orthopaedic Surgeons, the Association of British Neurosurgeons, and the British Society of Rehabilitation Medicine. Their views were sought on various aspects of the management and care of PVS, in particular the appropriateness of a decision not to treat and a decision to withdraw artificial nutrition and hydration (ANH).

Findings 1027 doctors responded (55%) of whom 558 (54%) had experience of managing patients in PVS. Over 90% of responding doctors considered that it could be appropriate not to treat acute infections and other life-threatening conditions. 65% of doctors considered that withdrawal of ANH could be appropriate. About two-thirds of doctors who thought treatment-limiting decisions could be appropriate thought that such decisions could be considered with the first 12 months of the patient being in PVS. Despite recent case law, less than half the doctors responding to the survey thought that an advance directive made by the patient should have a decisive influence in determining treatment-limiting decisions. Most doctors would like decisions about withdrawing ANH to be made in conjunction with family members and in accordance with agreed guidelines but without the need to go to court.

Interpretation T here is a broad consensus among doctors that treatment-limiting decisions are sometimes appropriate for patients in PVS, irrespective of whether they have experience of the condition or of the specialty to which they belong. However, two thirds of doctors said that such decisions can be considered at a time earlier than that recommended by the British Medical Association. It is not clear why some doctors thought a decision not to treat could be appropriate while a decision to withdraw ANH would not be.

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Introduction T he best management and care of patients in a persistent vegetative state (PVS)8 remains controversial despite several consensus statements about definitions and terminology.4,9 There are different approaches to management,5–7 and clinical decision-making is further