Pathogenesis of lentivirus infections

Ashley T. Haase

Department of Microbiology, University of Minnesota, 420 Delaware St, SE, Minneapolis, Minnesota 55455, USA

Following infection of animals or humans, lentiviruses play a prolonged game of hide and seek with the host's immune system which results in a slowly developing multi-system disease. Emerging knowledge of the disease processes is of some relevance to acquired immune deficiency syndrome (AIDS), which is caused by a virus possessing many of the characteristics of a lentivirus.

LENTIVIRUSES, a subfamily of retroviruses (Table 1), derive their name from the slow time course of the infections they cause in humans and animals[1-10]. The persistence and spread of these viruses, despite host defences, and the origins and slow evolution of the diseases they cause, pose fascinating problems in pathogenesis which have assumed a new significance with the addition of the agent of acquired immune deficiency syndrome (AIDS) to the subfamily. This review summarizes the increasingly coherent picture of the pathogenesis of lentivirus infections, and discusses the relevance of these concepts to the behaviour and control of the AIDS virus.

Visna–maedi and central issues

Lentiviruses cause chronic diseases affecting the lungs, joints, nervous, haematopoietic and immune systems of humans and animals (Table 1). The prototypic lentiviruses, visna and maedi, derived their Icelandic names from the prominent symptoms (wasting and shortness of breath) of the neurological and pulmonary diseases they cause in sheep. In fact, it is the same virus[2] that is responsible for both maedi, the more prevalent pulmonary disease, and visna, a paralytic condition showing some resemblance to multiple sclerosis[11-15]. These two diseases reached epidemic proportions in Iceland in the 1940s about a decade after the virus was inadvertently introduced into Iceland by sheep imported from Germany. An Icelandic physician, Bjorn Sigurdsson, investigated the outbreaks of visna and maedi, showed that a filterable agent caused them[12], and discovered the long incubation period and protracted course of disease that distinguish slow infections[16]. Sigurdsson introduced the term slow infection[17] to capture the novel timescale of disease and the experimental design needed to demonstrate transmissibility, thus setting the stage for the discovery of slow diseases in man[18].

In natural and experimental infections[7] of sheep (Fig. 1*a*), the aetiological agent of visna-maedi replicates at the site of entry (the lung in natural infections) and subsequently spreads via the bloodstream or by other routes, such as the cerebrospinal fluid (CSF). The infected animal mounts a defensive response which has both nonspecific components, such as phagocytic cells, and specific humoral and cellular-immune elements[19,20]. These defence mechanisms are effective against extracellular virus but are generally unable to eradicate the infectious agent altogether. Virus persists in many organ systems and continues to circulate in blood and tissue fluids. In the lungs and central nervous system (CNS), tissue is destroyed in areas where inflammatory cells have collected, and eventually this burden of pathological change becomes apparent as shortness of breath or partial paralysis and weight loss. In natural infections, animals generally become symptomatic in the second year of infection and die after a protracted and progressive illness.

This brief description of the major events of infection in visna-maedi is intended to bring out three salient problems in understanding the pathogenesis of slow infections: (1) How does virus persist and spread in the face of a vigorous and sustained immune response by the host? (2) What causes destruction of tissue? (3) Why do these pathological events evolve so slowly?

Virus gene expression and persistence

The best current explanation for the persistence of lentiviruses is the immunologically silent nature of the infection. Most infected cells harbour the virus in a latent state in which viral antigens are not produced in sufficient quantities for detection and destruction of the infected cell by immune-surveillance mechanisms[21]. To show the magnitude of these damping effects, evidence for and molecular measures of the restricted virus gene expression *in vivo* have been set against a background of the growth of virus under permissive conditions in tissue culture in Figs 1 and 2 and Table 2. In tissue culture, visna virus reproduces rapidly to high titre and destroys the host cell[22,23]. In this lytic and productive cycle, genetic information is transferred from the RNA genome of the infecting virus to a DNA intermediate

Table 1 Retrovirus subfamilies

Subfamily	Disease	Natural hosts
Oncoviruses	Cancer	Man, animals, birds and reptiles
Spuma viruses	Inapparent infections	Man, animals
Lentiviruses	Slow infections	Man, animals
Visna-maedi virus	Pneumonia, meningoencephalitis	Sheep, goats
Progressive pneumonia virus (PPV)	Pneumonia	Sheep, goats
Caprine arthritis encephalitis virus (CAEV)	Arthritis, pneumonia, meningoencephalitis	Goats, sheep
Zwoegerziekte	Pneumonia, meningoencephalitis	Sheep
Equine infectious anaemia virus (EIAV)	Fever, anaemia	Horses
AIDS virus (HIV)	Immune deficiency, encephalopathy, myelopathy	Man

The retrovirus subfamilies shown are currently accepted taxonomic divisions[121]. The EIAV and AIDS virus are provisionally included in the lentivirus subfamily because they, too, cause slow infections and have other properties in common with visna-maedi[122-131]: cell fusion and other cytopathic effects in tissue culture; virion morphology; polypeptide composition; large envelope glycoproteins; shared antigenic determinants in the major structural protein (gag); similar size and structure of their genomes; nucleotide and amino-acid sequence homologies largely confined to conserved regions of *gag-pol*.

in the cell[24,25] which serves as a template for the synthesis of thousands of copies of genomic and messenger RNAs (Table 2*a*). The latter are translated into millions of copies of structural virion proteins[26] and infectious progeny (50–100 per cell) subsequently assemble at the cell surface. The infected cells degenerate, either individually or after fusion[27], within 3 days.

By contrast, replication of virus in animals is highly focal and unproductive, even in relatively homogeneous populations of cells which serve as substrates for permissive growth in tissue culture: the number of copies of viral RNA in choroid plexus of infected animals is about two orders of magnitude less than in infected choroid plexus cells in culture (Table 2). Synthesis of viral RNA, antigens and virus is confined to one in a hundred to thousands of cells (Table 2*b*)[28]. This focal and restricted growth cycle has been revealed by methods for quantitative analysis of virus replication at the single-cell level (*in situ* hybridization)[29] and, more recently, by methods that combine macroscopic screening of tissue with single-cell resolution (Fig. 2)[30]. The fundamental question of why virus growth should be so different in cells in culture from that in tissues is unanswered, but viral RNA synthesis has been defined as the major point in the virus growth cycle at which virus gene expression is blocked[28].

Visna thus has two kinds of life cycle, somewhat analogous to bacteriophage λ in *Escherichia coli*: a productive and lytic life cycle *in vitro* and a latent life cycle in sheep, figuratively referred to as lysogeny on a grand scale[3]. This is probably overstated, as visna virus is not integrated into the host cell genome, at least in tissue culture[31], but it does epitomize the notion of the clandestine state of the virus as the mechanism by which it eludes the host's defences.

Trojan horse mechanism

A similar mechanism can be invoked to explain the continued spread of virus in the bloodstream, cerebrospinal fluid and other fluids that contain immune cells and neutralizing antibody. In this mechanism, a mobile cell, predominantly if not exclusively a monocyte[32,33] in the case of visna virus, conceals the virus genome and conveys it without detection to other sites. Evidence for this Trojan horse mechanism again comes from *in situ* hybridization analyses of CSF, where infected cells are concentrated[32]. (The frequency of leukocytes carrying visna virus in the bloodsteam—about 1 in 10^6 cells—is too low to be detected directly.) The CSF data[32] provide evidence of (1) restricted levels of viral RNA accumulation in monocytes; (2) circulation of the latently infected monocytes in tissue fluids containing neutralizing antibody; and (3) transfer of infectious virus between monocytes and choroid plexus cells under conditions of close contact between the cells. These data satisfy the major predictions of the Trojan horse hypothesis.

Inapparent infections and latency

The theme of restricted virus gene expression is the dominant motif of lentivirus infections. In animals, infections are frequently not apparent, with no obvious pathological sequelae for periods that approximate the normal lifespan of the host. In the United States, for example, lentivirus infection of sheep is widespread and probably largely asymptomatic[34]. There are flocks of sheep in Germany in which antibodies to virus are present in 50 per cent of the serum samples[35] and in which clinical symptoms of maedi have never been observed. Importation of silently infected sheep like these from Germany was presumably responsible for the outbreaks of maedi and visna in Iceland[4].

The AIDS virus also establishes persistent and non-cytopathic infections in normal human lymphocytes[36]. In the persistently infected cultures, virus production may be absent or limited (but can be induced), in keeping with the definition of latency[37]. By extrapolation, similar virus-host cell interactions provide a mechanism for the AIDS virus to escape neutralization and other defences, but also a basis for the comforting observation that inapparent infection and a carrier state are common in infected patients[38].

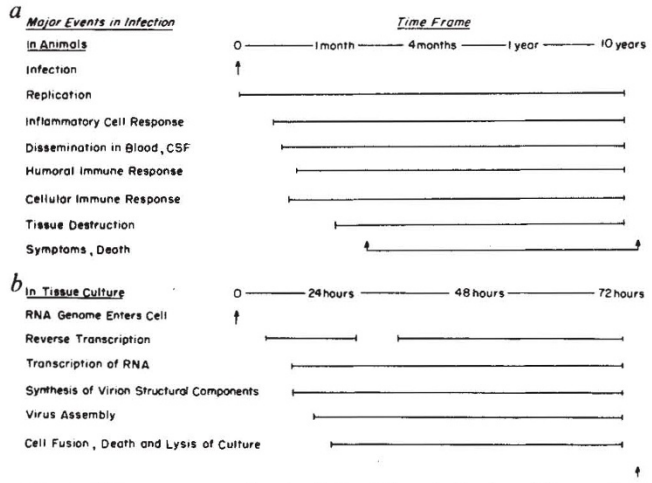

Fig. 1 Time course of animal lentivirus infection. The major events and strikingly different timescale of infection in animals (*a*) and tissue culture (*b*) can be seen.

Population spread

Animal lentivirus infections are transmitted between animals, probably by virus inside monocytes and macrophages in secretions (little if any cell-free virus is detectable). The aetiological agents of visna and maedi enter adult sheep by the respiratory route and lambs by a gastrointestinal route in colostrum[2,4]. The appearance of disease in epidemical form requires special conditions such as those in Iceland, where sheep from many parts of the island are housed together for several days each year[2]. This practice may have been as important in the rapid spread of the disease as the greater susceptibility of the Icelandic than German sheep to lentivirus infection.

AIDS is transmitted similarly. Virus is introduced into the bloodstream through sexual contact, intravenous drug administration with contaminated needles or administration of blood and blood products[36]. It is not known whether the virus is transferred inside cells, but this is obviously of great importance in predicting the efficacy of conventional vaccine strategies to interdict further spread of AIDS. As in visna, perinatal transmission is also important, although it is not known whether this occurs *in utero* or postnatally.

There is no evidence of germline transmission of animal lentiviruses from mother to offspring, and endogenous lentiviruses are exceptional[39], in contrast to the situation with

Table 2 Comparison of the growth of visna virus in animals and in tissue culture

Type of infection	Cells positive for viral RNA (%)	Average no. of copies of viral RNA per infected cell	Cells with viral antigen (%)
a, Tissue culture (choroid plexus cells 3 days after infection)	90	5,000	90
b, Sheep (choroid plexus, alveolar macrophages monocytes, glial cells; 3 days to 3 weeks after infection)	0.1–2	50–150	0.001

Data from refs 26, 28, 32, 56.

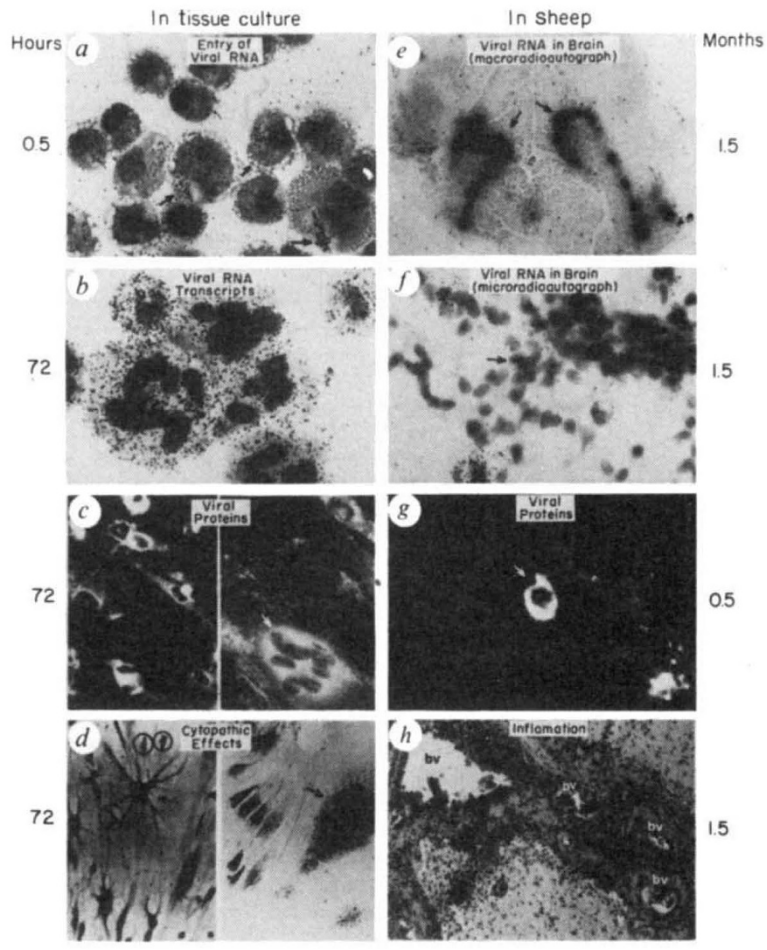

Fig. 2 Life cycles of an animal lentivirus in tissue culture and sheep. *a*, *b*, Autoradiographs of visna virus RNA detected by *in situ* hybridization. The number of copies of viral RNA in individual cells is proportional to the number of grains over the cell and the length of exposure[29]. At 0.5 h after infection (at a multiplicity of infection of 3 plaque-forming units per cell) about 30 copies of viral RNA enter the cell, often apparently inside a vacuole (arrows, *a*). By 72 h the cells contain about 5,000 copies of viral RNA[26], often in giant cells. Visna virus RNA can be localized anatomically in infected animals to regions surrounding the ventricles (arrows, *e*) using combined macroscopic-microscopic screening[30]. In this method whole brain sections are hybridized to a virus-specific probe labelled with ^{125}I. The γ emission of ^{125}I produces a latent image of viral RNA on X-ray film placed against the tissue section after hybridization. The single-cell location of viral genes is determined from a microscopic autoradiograph produced by coating the section with nuclear track emulsion. The Auger electrons emitted by ^{125}I produce latent images with good resolution at the cellular level. The average copy number in the cells in the brain is about two orders of magnitude less than that in infected tissue culture (for example, number of grains over the cell in tissue culture 72 h after infection requires an exposure of a few hours; for a probe of the same specific activity, about 3 weeks would be needed to produce the number of grains in the cells in brain)[30]. In tissue culture there are $\sim 10^6$ copies of major viral protein (gag) easily detected in most cells by immunofluorescence with monospecific antibodies (*c*; the arrow indicates a giant cell with the visna gag polypeptide in the cytoplasm)[4,127]. Only rare cells with viral antigens can be detected in the infected animal (*g*). The infection in tissue culture leads directly to cell death with lysis of the culture in 72–96 h (*d*). Arrows indicate giant cells in various states of degeneration; individual dying cells are circled. In animals, tissue damage accumulates over months to years (*h*) and is indirectly caused by inflammatory cells (the dark-staining lymphocytes, plasma cells, monocytes, macrophages that surround the blood vessles (bv) and collect in foci in the tissue of this section of brain from a paralysed sheep[1–7].

oncogenic retroviruses. The exogenous nature of lentiviruses may reflect their infrequent opportunities to interact with the host genome. Only a few cells are infected in the animal, replication is restricted[28] and integration may be rare[31]. The failure of the viral genome to form covalent linkages with the host chromosome probably has a structural basis, as the topological precursor for retrovirus integration is likely to be circular DNA[40], and in cells infected with visna virus circular molecules are rare (the predominant structure is a nicked or gapped linear duplex)[41]. That is not to say that integration of lentivirus genomes cannot occur—integrated as well as unintegrated forms of AIDS virus[42], equine infectious anaemia virus (EIAV)[43] and caprine arthritis and encephalitis virus (CAEV)[44] have been described.

Variant and common determinants

Antigenic variation is an additional or alternative mechanism for the persistence and spread of the lentiviruses. In this instance, the emergence of mutant viruses with an altered envelope glycoprotein, the antigen to which neutralizing antibody is directed[45], is the postulated mechanism by which virus temporarily escapes immunological inactivation. Gudnadottir's proposal that 'antigenic drift' might account for the survival of visna virus for such a long time in infected animals[2] was subsequently supported and fully developed by Narayan and his collaborators. They showed that antigenically distinct viruses could be isolated from sheep persistently infected with visna virus[46], and that these variants arise by point mutations in the *env* gene, which encodes the virion envelope glycoprotein[45,47]. In visna, however, variants do not replace the infecting serotype by antibody selection and, in most long-term infections, the inoculum virus strain persists and spreads without the emergence of antigenic variants[48,49]. For these reasons it seems unlikely that antigenic variation is a necessary or important means of dissemination of visna virus.

For EIAV, the case for antigenic variation as an important mechanism of virus dissemination is more persuasive. There are cycles of virus replication in which cell-free virus is isolated from serum or plasma, and each new isolate from a cycle is refractory to neutralization by antibody that neutralized previous isolates[50,51]. This immunologically dictated succession fulfils the expectations of the antigenic variation model for persistence and spread of viruses, and, as in visna virus infections, occurs primarily through point mutations in the *env* gene[52]. But this is not the sole basis for persistence and spread. By the end of the first year, cyclic replication of EIAV is superseded by an inapparent carrier state in which continued dissemination of virus within the host, or to new hosts, is again accomplished by latently infected macrophages[9]. Because this conversion to a Trojan horse mechanism of spread may be a consequence of production of antibody to all strains of EIAV, identification of these common antigenic determinants for neutralization would clearly be important in designing broadly effective vaccines.

It would be premature to predict the role of antigenic variation in AIDS infections, but isolates of the AIDS virus from different individuals differ genotypically[53], and this genomic diversity is greatest in the region of the *env* gene[54]. If these genetic changes

are mirrored phenotypically, it will pose serious problems for vaccine development and provide an additional mechanism for the AIDS virus to persist and spread in individuals and populations.

Immunopathogenesis

Pathological changes in lentivirus infections are for the most part indirectly mediated by the immune and inflammatory response of the host. In visna, the coordinate reduction of inflammation and tissue lesions[55] with immunosuppression suggests that it is the inflammatory cell response (Fig. 2) that causes tissue damage. In the brain, this process is demyelinating[11-15] because at least one of the infected cell targets is the oligodendrocyte[56], the cell in the nervous system which provides the myelin sheath (identified unambiguously by a new method that combines immunocytochemistry, cell-specific antibodies and *in situ* hybridization[57]; Fig. 3). The rare infected cell containing viral antigen probably provokes and sustains this inflammatory response[56], which then causes tissue damage by mechanisms that are not understood in any detail but could involve interleukin-mediated amplification of the response with indiscriminate damage to uninfected cells ('innocent bystanders') in the area. Lesions in the lungs and joints of infected animals in maedi virus and CAEV infections are also the result of the exuberant inflammatory response. In the lung, infiltration of lymphocytes and monocytes into the alveolar wall interferes with gas exchange; in the joints[58,59], the inflammatory cell infiltrate leads to the destruction of the cartilage perhaps by activating chondrocytes to elaborate matrix degrading factors[60], much as in rheumatoid arthritis.

Both lymphoproliferative changes (enlargement, necrosis) and immune complexes participate in the immunopathology of equine infectious anaemia[9,61]. The characteristic anaemia is the result of phagocytosis and haemolysis of erythrocytes that first become coated with a viral haemagglutinin and then with antivirus antibody and complement. Circulating immune complexes of EIAV and antibody also elicit fever and cause glomerulonephritis when they are deposited in the kidney. The, as yet unexplained, glomulerosclerosis and thrombocytopenic purpura of AIDS may similarly be immune complex diseases[62,63].

Immunopathology, however, cannot account for all the manifestations of lentivirus infections. There is an inflammatory component in the progressive encephalopathy that occurs frequently in children and adults with AIDS[64-66], but in AIDS encephalopathy and myelopathy[67], inflammation is overshadowed by vacuolation and degenerative changes that are more like those in a paralytic disease of mice caused by some types of murine leukaemia viruses[68].

Immunodeficiency and cachexia

The agent of AIDS differs most profoundly from the lentiviruses of animals in its effects on the immune system. Immunodeficiency is the hallmark of AIDS[10,69], whereas in animal lentivirus infections the immune response is relatively normal or selectively impaired. (In natural US infections, particularly with CAEV, animals ordinarily produce little if any neutralizing antibody[70].) Until recently, the reasons for this distinction seemed relatively straightforward: the AIDS virus homes to a receptor on the surface of helper T cells[71,72] and was thought to cause AIDS by destruction and depletion, or dysfunction, of this central element of the immune system. The animal lentiviruses infect monocytes in such small numbers that the impact on immune function is minor[32].

It is becoming increasingly evident that this view is oversimplistic, inasmuch as infection of T4 lymphocytes (about 1 positive cell in 20,000–100,000 by *in situ* hybridization[73]) is as infrequent in AIDS as it is in visna. To account for the large effects on the absolute number of T4 cells and immune function in AIDS, more complex mechanisms need to be invoked, including some that recall the theme of immunopathology in lentivirus

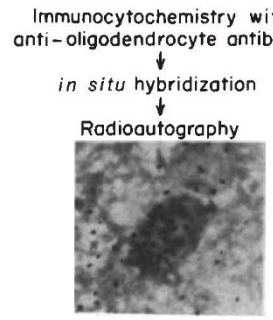

Fig. 3 Determination of virus host cell tropism by the simultaneous detection assay[57]. Brain sections from a paralysed sheep infected with visna virus were reacted with anti-oligodendrocyte antibody, and the reaction was visualized by immunocytochemical methods. After *in situ* hybridization with a visna virus-specific probe, the developed autoradiograph was examined to locate cells (oligodendrocytes) which stained with the antibody and also had an increased number of silver grains such as that shown in the figure. This method identifies the oligodendrocyte unambiguously as one of the types of cells infected by visna virus[56].

infections. For example, viral envelope glycoprotein shed from productive cells could make large numbers of uninfected cells targets for an autoimmune response directed to viral antigen bound to the T4 receptor. Alternatively, the network of interactions in the immune system should in time lead to an anti-idiotypic response to antibody to the viral glycoprotein. This anti-idiotypic antibody might also be directed against the T4 antigen and viral receptor. This hypothetical reconstruction of the immune response provides a rationale for understanding the production of anti-lymphocyte antibodies in AIDS[74], and a basis for a paradoxical and potentially hazardous approach to treatment by immunosuppression[75]. The latter could also theoretically relieve a block in the expansion, differentiation or function of T4 cells mediated by another subset of lymphocytes infected with the AIDS virus. Just as plausible, however, are direct mechanisms leading to immunodeficiency, for example, continued recruitment of uninfected T4 lymphocytes into the infectious process, destruction of precursors or the release of immunosuppressive virion components analogous to the p15E protein of feline sarcoma virus[76,77].

One of the unexplained symptoms of infection, the cachexia or wasting ('slim disease' is the name given to one form of AIDS in Uganda[78]), might be partly attributable to interactions of lentiviruses with macrophages. In addition to inanition from concurrent enteropathic infections (possibly including AIDS virus) and diarrhoea which contribute to these manifestations of the infection, lentiviruses might, as the result of infection of monocytes, cause release of cachexin, a factor that inhibits adipocyte gene expression and the production of lipogenic enzymes[79].

Form and tempo of pathology

Visna virus gene expression is markedly curtailed in most infected cells in tissues[28], but there are a few cells in which levels of viral RNA approach those in productively infected tissue culture[56]. These gradations in gene expression (Fig. 4) are tightly correlated with detection of viral antigens, the intensity of the inflammatory response, and tissue damage, as would be expected if gene expression determines the form and tempo of pathology. At one end of a spectrum of virus gene expression are a silent majority of infected cells with minimal levels of viral RNA and antigens, enabling the virus to persist and spread. Towards the other end of the spectrum is the occasional cell with higher levels of viral RNA and the antigens responsible for the continuing inflammatory cell response and inadvertent destruction of tissue at a rate commensurate with the scanty production of the inciting antigens. At the extreme end of the spectrum is the lytic

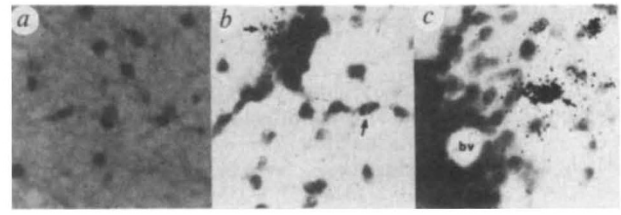

Fig. 4 Quantitative *in situ* hybridization used to demonstrate correspondence between virus gene expression and inflammatory response. Brain sections from a paralysed sheep infected with visna virus were hybridized with a virus-specific probe. In the developed and stained microautoradiographs there is a gradient in gene expression that correlates with the intensity of inflammation. *a*, A region with no inflammation and background levels of hybridization; *b*, as the number of copies of viral RNA increases, larger numbers of grains appear over cells (arrows) adjacent to small collections of dark-staining mononuclear cells; *c*, the largest numbers of inflammatory cells around blood vessels (bv) and tissue are adjacent to infected cells (arrow) with grain counts equivalent to hundreds to thousands of copies of viral RNA typical of productive infections in tissue culture. Viral antigens are occasionally demonstrable in cells with the highest concentrations of viral RNA[56].

infection in tissue culture, where destruction of cells is a rapid and direct result of virus growth under permissive conditions of replication. These cytopathic effects are more likely to be due to the abundance of virion components (probably the envelope glycoprotein) capable of fusing and killing the cell from within and outside[27] than to the high concentrations of extrachromosomal DNA which have been advanced as one explanation for these cytotoxic effects of retroviruses[80]; experimental conditions can be contrived in visna-infected tissue culture (blocking superinfection with antibody)[81] where fusion and cell death occur at DNA concentrations equivalent to those in tissues[28] at which cytopathic effects are never observed.

Regulatory mechanisms and control

The major thrust of this review on lentivirus pathogenesis is that the major issues of persistence, spread and the rate and mechanisms of cell death translate into more objective questions about the factors that control lentivirus replication. Indeed, the central fact and unresolved mystery in visna is why the replication of virus is restricted in the same relatively homogeneous and non-dividing population of cells that supports abundant replication after explantation[21] or in non-dividing cells in cultures derived from the same tissues[81]. Many factors have been investigated that might potentially account for restriction; they fall into two general categories (extracellular and intracellular), and pertain to tissue culture systems or living hosts. The latter is an important distinction, because there are many examples of regulatory mechanisms that operate in tissue culture but bear little relationship to reality. For instance, one can establish persistent infections in tissue culture with visna virus in which the cells contain high concentrations of viral antigens, whereas the concentrations are low in infected sheep (A.T.H., unpublished). Moreover, antigenic variants of visna virus appear in a logical succession based on antibody selection *in vitro*[82], but not in infected animals[48,49]; and gene dosage effects in tissue culture[81] apparently do not operate *in vivo*[83]. The discussion below therefore concentrates on regulatory mechanisms which meet the test of relevance at the organismal level and which might be exploited in the control of lentivirus infections.

Sanctuaries and vaccines

In natural and experimental visna, dissemination of virus by extracellular routes largely ceases with the appearance of neutralizing antibody[19], acting perhaps in concert with viral inhibitory factors in serum and CSF[84,85]. Neither humoral nor cellular immunity, however, plays a significant part in maintaining latency, since immunosuppression does not relieve the restriction in virus gene expression[55,86].

The experience with animal lentivirus vaccines is unfortunately too limited to give much direction to vaccine development. Veterinary practices of identifying infected sheep by serological testing and removing them from flocks have been successful in controlling lentivirus infections of animals[1,7,87]. No vaccine for visna or maedi is available because of intractable difficulties in developing an inactivated virus or envelope component vaccine that will induce neutralizing antibody. Vaccinated sheep do produce complement-fixing antibody but are not protected. Moreover, natural infections are transmitted to lambs by cells in the colostrum of mothers carrying neutralizing antibody. This mechanism of transmission and the possibility of cell-cell spread[88] would circumvent conventional strategies for disease control. Lentiviruses also take refuge from immune defences in the central nervous system. Thus, vaccines alone cannot be expected to be wholly effective in preventing lentivirus infection, but may favourably alter the outcome by reducing at the outset of infection the number of cells with the potential to contribute to pathological changes.

In AIDS, neutralizing antibody[89,90] titres are low[90], virus in semen[91] and saliva[92] is partly cell-associated[93], and the brain is infected as a potential sanctuary for virus in a significant segment of the infected population[64,94-96]. These considerations, and potential difficulties in vaccine development, underscore the importance of the practical measures and behavioural advice currently recommended to limit the spread of AIDS[36].

Intracellular mechanisms and antisense vaccine

Because viral gene expression directly or indirectly mediates cell damage, measures that limit gene expression should moderate or present the pathological consequences of infection. DNA synthesis is a logical point in the virus life cycle at which to intervene with drugs that inhibit reverse transcription or, like interferon, act intracellularly. The experience with the effects of inhibitors of viral DNA synthesis[97,98] and interferons[99,100] is limited to *in vitro* experiments which suggest that some benefit might be achieved[100]. In AIDS, inhibitors of reverse transcription have already undergone clinical trials, with demonstrable but temporary inhibition of virus replication and little alteration in clinical status[101]. Sustained treatment with newer and less toxic drugs may induce long-term remissions[102].

Transcription and translation of the lentivirus genome are also potential targets for therapy. The major controlling elements for lentivirus gene expression are located at the 5′ end (long terminal repeat, LTR) and centre of the genome (Fig. 5*a*, *b*). The lentiviral LTR, in common with other retroviruses, has promoter and enhancer domains and two additional domains, designated NRE and TAR in Fig. 5, which have negative or positive effects respectively on gene expression[103-105]. The *trans*-activating region (TAR) is responsive to a gene product encoded in a short open reading frame *S* (Fig. 5*b*)[103]. The *trans*-activating gene product (TAT) of this open reading frame acts post-transcriptionally[106] to stimulate gene expression by 500–1,000-fold and is required for replication[107,108]. Modest stimulation by *trans*-activation has also been described recently for visna virus[109], which is comparable to that of the AIDS virus when the entire LTR of visna virus is used in the assay (K. Staskus, C. Rosen, W. Haseltine and A.T.H., unpublished). If *trans*-

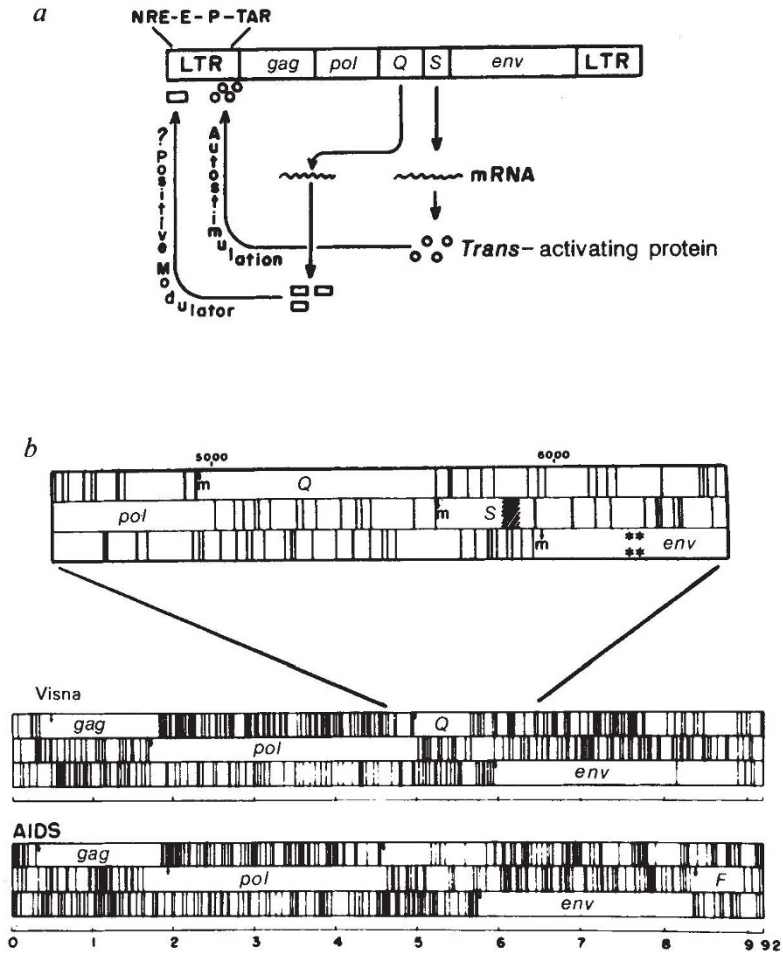

Fig. 5 Speculations on control of lentivirus gene expression. *a*, Schematic illustration of a prototypic lentivirus genome and a speculative representation of control of gene expression. Transcription of the major genes of the virus is controlled by promoter (P) and enhancer (E) regions in the LTR. A *trans*-activating protein (○) encoded by the short open reading frame *S* binds to a region (TAR) to stimulate gene expression. The gene product of open reading frame *Q* also binds to the LTR to up-regulate transcription. The overall organization of the genomes of visna virus and the AIDS virus is shown in *b* with the controlling region in the centre of the genome with *Q* and *S*. (Modified from refs 103–110.) m, Initiator Met codon; *F*, an additional open reading frame in HIV (human immunodeficiency virus); asterisks in *env* indicate the hydrophobic signal sequence.

activation in visna is also effected post-transcriptionally, it could not account for the restricted gene expression in infected sheep which is primarily a result of diminished accumulation of viral RNA[28], but it could account for the discrepancy between the relatively high concentration of virus RNA in some cells and the lack of infectivity[83].

The reduction in transcription in infected animals was most recently speculated to be due to repressor activity of a putative DNA-binding protein, the basic and hydrophilic gene product of conserved open reading frames (*Q*) in visna[110] and the AIDS virus[111–114]. But there is now evidence that the *Q* gene product is a second positive regulatory factor, because deletion of *Q* slows the growth of the AIDS virus and delays the development of cytopathic effects[115]. Highly productive and rapidly progressive lytic infections may require co-expression of both factors, as might occur, for example, during activation of macrophages (CAEV) or lymphocytes[116,117].

One possible approach to the control of lentiviruses that anticipates the importance of the regulatory proteins encoded by the *Q* and *S* genes, and exploits antisense in mRNA[118–120], is the development of defective virus vectors which would stabilize the dormant state of infection. In one such project in progress in the visna model (K. Staskus, E. Retzel and A.T.H., unpublished), the *trans*-activating gene in infectious viral DNA is replaced by its antisense counterpart. Defective virus, produced by co-transfecting cells with the viral antisense DNA and with *S* gene DNA in an inducible expression vector, will have an identical host range to wild-type virus. It will therefore serve as a vector to introduce the antisense gene into cells already harbouring standard virus. In the co-infected cells, *trans*-activation should at first drive production of additional defective virus to disseminate the antisense vector. Thereafter, the antisense vector should halt *trans*-activation and return the infected cells to a latent state. Of course, before there could be any assurance that the pathological consequences of infection may be prevented, this approach must overcome some theoretical and practical difficulties—particularly superinfection barriers in cells producing AIDS virus and the need to establish co-infection in a large pool of cells with a non-replicating virus.

Conclusions

The lentiviruses are responsible for slow infections of animals and man. Investigations of animal lentivirus infections have previously been directed to the inherently interesting issues of pathogenesis raised by this novel class of infections, parallels to such chronic diseases of man as multiple sclerosis and rheumatoid arthritis, and, to a much lesser extent, practical means of diagnosing, treating and preventing disease. With the knowledge that the causative agent of AIDS is a distant relative of the animal lentiviruses, these objectives have assumed a new and compelling urgency. Restricted gene expression is the general underlying mechanism in the persistence and spread of lentiviruses and the slow evolution of the diseases they produce. This restriction maintains the lentivirus genome in the cell in a covert state, and justifies the optimistic prediction that the commonest form of infection in AIDS, inapparent infection, will prevail for much, if not all, of the natural lifespan of the host. On a more pessimistic note, there is no guarantee that chronic infections will not ultimately progress to some form of illness; or that conventional approaches to prevention and treatment will be successful. These approaches may be frustrated by the ability of all lentiviruses to survive in intracellular and organ

(brain) sanctuaries or to elude host defences through antigenic variation. The prospects of developing vaccines to neutralizing determinants common to all strains of virus, designing vectors to maintain silent infections, and the general progress in lentivirus and AIDS research, are grounds for the hope that these problems will be resolved.

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1. Thormar, H. & Palsson, P. A. *Perspect. Virol.* **5,** 291-308 (1967).
2. Gudnadottir, M. *Prog. med. Virol.* **18,** 336-349 (1974).
3. Haase, A. T. *Curr. Topics Microbiol. Immun.* **72,** 101-156 (1975).
4. De Boer, G. F., Terpstra, C. & Houwers, D. J. *Bull. Off. int. Epiz.* **89,** 487-506 (1978).
5. Brahic, M. & Haase, A. T. *Comparative Diagnosis of Viral Diseases* Vol. 4, Ch. 15 (Academic, New York, 1981).
6. Narayan, O. & Cork, L. C. *Rev. infect. Dis.* **7,** 89-98 (1985).
7. Nathanson, N. *et al. Rev. infect. Dis.* **7,** 75-82 (1985).
8. Coggins, L. *Comparative Diagnosis of Viral Diseases* Vol. 4, Ch. 16 (Academic, New York, 1981).
9. Cheevers, W. P. & McGuire, T. C. *Rev. infect. Dis.* **7,** 83-88 (1985).
10. Wong-Staal, F. & Gallo, R. C. *Nature* **317,** 395-403 (1985).
11. Sigurdsson, B., Palsso, P. A. & Grimson, H. *J. Neuropath. exp. Neurol.* **16,** 389-403 (1957).
12. Sigurdsson, B. & Palsson, P. A. *Br. J. exp. Path.* **39,** 519-528 (1958).
13. Sigurdsson, B., Palsson, P. A. & Van Bogaert, L. *Acta neuropath.* **1,** 343-362 (1962).
14. Georgsson, G., Palsson, P. A., Panitch, H., Nathanson, N. & Petursson, G. *Acta neuropath.* **37,** 127-135 (1977).
15. Georgsson, G. *et al. Acta neuropath.* **57,** 171-178 (1982).
16. Sigurdsson, B. *Br. vet. J.* **110,** 255-270 (1954a).
17. Sigurdsson, B. *Br. vet. J.* **110,** 341-354 (1954b).
18. Gajdusek, D. C. *Science* **197,** 943-960 (1977).
19. Petursson, G., Nathanson, N., Georgsson, G., Panitch, H. & Palsso, P. A. *Lab. Invest.* **35,** 402-412 (1976).
20. Griffin, D. E., Narayan, O. & Adams, R. J. *J. infect. Dis.* **138,** 340-350 (1978).
21. Haase, A. T., Stowring, L., Narayan, O., Griffin, D. & Price, D. *Science* **195,** 175-177 (1977).
22. Sigurdsson, B., Thormar, H. & Palsson, P. *Arch. ges. Virusforsch.* **10,** 368-371 (1960).
23. Thormar, H. *Virology* **19,** 273-278 (1963).
24. Haase, A. T. & Varmus, H. E. *Nature new Biol.* **245,** 237-239 (1973).
25. Haase, A. T., Traynor, B. L., Ventura, P. E. & Alling, D. W. *Virology* **70,** 65-79 (1976).
26. Brahic, M., Filippi, P., Vigne, R. & Haase, A. T. *J. Virol.* **24,** 74-81 (1977).
27. Harter, D. H. & Chopping, P. W. *Virology* **31,** 279-288 (1967).
28. Brahic, M., Stowring, L., Ventura, P. & Haase, A. T. *Nature* **292,** 240-242 (1981).
29. Haase, A., Brahic, M., Stowring, L. & Blum, H. *Meth. Virol.* **3,** 189-226 (1984).
30. Haase, A. T. *et al. Virology* **140,** 201-206 (1985).
31. Harris, J. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 7212-7215 (1984).
32. Peluso, R., Haase, A., Stowring, L., Edwards, M. & Ventura, P. *Virology* **147,** 231-236 (1985).
33. Narayan, O. *et al. J. gen. Virol.* **59,** 245-256 (1982).
34. Cutlip, R. C., Jackson, T. A. & Laird, G. A. *Am. J. vet. Res.* **38,** 2091-2093 (1977).
35. de Boer, G. F. & Houwers, D. J. in *Aspects of Slow and Persistent Infections* (ed. Tyrrell, D. A. J.) 198-221 (Elsevier, Amsterdam, 1979).
36. Hoxie, J. A., Haggarty, B. S., Rackowski, J. L., Pillsbury, N. & Levy, J. A. *Science* **229,** 1400-1402 (1985).
37. Folks, T. *et al. Science* **231,** 600-602 (1986).
38. Curran, J. W. *et al. Science* **229,** 1352-1357 (1985).
39. Barban, V. *et al. J. Virol.* **52,** 680-682 (1984).
40. Panganiban, A. & Temin, H. *Cell* **36,** 673-679 (1984).
41. Harris, J. D. *et al. Virology* **113,** 573-583 (1981).
42. Shaw, G. M. *et al. Science* **226,** 1165-1171 (1984).
43. Rice, N. R., Simek, S., Ryder, O. A. & Coggins, L. *J. Virol.* **26,** 577-583 (1978).
44. Yaniv, A., Dahlberg, J. E., Tronick, S. R., Chiu, I.-M. & Aaronson, S. A. *Virology* **145,** 340-345 (1985).
45. Scott, J. V., Stowring, L., Haase, A. T., Narayan, O. & Vigne, R. *Cell* **18,** 321-327 (1979).
46. Narayan, O., Griffin, D. E. & Chase, J. *Science* **197,** 376-378 (1977).
47. Clements, J. E., Pederson, F. S., Narayan, O. & Haseltine, W. A. *Proc. natn. Acad. Sci. U.S.A.* **77,** 4454-4458 (1980).
48. Lutley, R. *et al. J. gen. Virol.* **64,** 1433-1440 (1983).
49. Thormar, H., Barshatzky, M. R., Arnesen, K. & Kozlowski, P. B. *J. gen. Virol.* **64,** 1427-1432 (1983).
50. Kono, Y., Kobayashi & Fukunaga, Y. *Arch. ges. Virusforsch.* **41,** 1-10 (1973).
51. Salinovich, O. *et al. J. Virol.*. **57,** 71-80 (1986).
52. Montelaro, R. C., Parekh, B., Orrego, A. & Issel, C. J. *J. biol. Chem.* **259,** 10539-10544 (1984).
53. Wong-Staal, F. *et al. Science* **229,** 759-762 (1985).
54. Hahn, B. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **82,** 4813-4817 (1985).
55. Nathanson, N., Panitch, H., Palsson, P. A., Petursson, G. & Gorgsson, G. *Lab. Invest.* **35,** 444-451 (1976).
56. Stowring, L. *et al. Virology* **141,** 311-318 (1985).
57. Brahic, M., Haase, A. T. & Cash, E. *Proc. natn. Acad. Sci. U.S.A.* **81,** 5445-5448 (1984).
58. Crawford, T. B., Adams, D. S., Cheevers, W. P. & Cork, L. C. *Science* **207,** 997-999 (1980).
59. Klevjer-Anderson, P., Adams, D. S., Anderson, L. W., Banks, K. L. & McGuire, T. C. *J. gen. Virol.* **65,** 1519-1525 (1984).
60. Jasin, H. E. & Dingle, J. T. *J. clin. Invest.* **68,** 571-581 (1981).
61. McGuire, T. C. & Henson, J. B. in *Progress in Medical Virology* (ed. Hotchin, J.) 229-247 (Karger, New York, 1974).
62. Rao, T. K. *et al. New Engl. J. Med.* **310,** 669-673 (1984).
63. Cooper, D. A. *et al. Lancet* **i,** 537-540 (1985).
64. Shaw, G. M. *et al. Science* **227,** 177-182 (1985).
65. Epstein, L. G. *et al. Ann. Neurol.* **17,** 488-496 (1985).
66. Price, R. W., Navia, B. A. & Cho, E.-S. *Neurol. Clin.* **4,** 285-301 (1986).
67. Petito, C. K. *et al. New Engl. J. Med.* **312,** 874-879 (1985).
68. Gardner, M. *Rev. infect. Dis.* **7,** 99-110 (1985).
69. Seligmann, M. *et al. New Engl. J. Med.* **311,** 1286-1291 (1984).
70. Narayan, O., Sheffer, D., Griffin, D. E., Clements, J. & Hess, J. *J. Virol.* **49,** 349-355 (1984).
71. Dalgleish, A. G. *et al. Nature* **312,** 763-767 (1984).
72. Klatzman, D. *et al. Nature* **312,** 767-768 (1984).
73. Harper,, M. E., Marselle, L. M., Gallo, R. C. & Wong-Staal, F. *Proc. natn. Acad. Sci. U.S.A.* **83,** 772-776 (1986).
74. Williams, R. C. Jr, Masur, H. & Spira, T. J. *J. clin. Immun.* **4,** 118-123 (1984).
75. Klatzmann, D. & Montagnier, L. *Nature* **319,** 10-11 (1986).
76. Pahwa, S., Pahwa, R., Saxinger, C., Gallo, R. C. & Good, R. A. *Proc. natn. Acad. Sci. U.S.A.* **82,** 8198-8202 (1985).
77. Mathes, L. E., Olsen, R. G., Hebebrand, L. C., Hoover, E. A. & Schaller, J. P. *Nature* **274,** 687-689 (1978).
78. Serwadda, D. *et al. Lancet* **ii,** 849-852 (1985).
79. Torti, F. M., Dieckmann, B., Beutler, B., Cerami, A. & Ringold, G. M. *Science* **229,** 867-869 (1985).
80. Temin, H. M., Keshet, E. & Weller, S. K. *Cold Spring Harb. Symp. quant. Biol.* **44,** 773-778 (1980).
81. Haase, A. T. *et al. Virology* **119,** 399-410 (1982).
82. Narayan, P., Clements, J. E., Griffin, D. E. & Wolinsky, J. S. *Infect. Immunity* **32,** 1045-1050 (1981).
83. Geballe, A. P., Ventura, P., Stowring, L. & Haase, A. T. *Virology* **141,** 148-154 (1985).
84. Thormar, H. & Sigurdardottir, B. *Acta path. microbiol.* **55,** 180-186 (1962).
85. Thormar, H., Wisniewski, H. M. & Lin, F. H. *Nature* **279,** 245-256 (1979).
86. Narayan, O., Griffin, D. E. & Silverstein, A. M. *J. infect. Dis.* **135,** 800-806 (1977).
87. Houwers, D. J. & Schaake, J. Jr *Vet. Microbiol.* **5,** 445-451 (1984).
88. Filippi, P., Vigne, R., Querat, G., Jouanny, C. & Sauze, N. *J. Virol.* **42,** 1057-1066 (1982).
89. Robert-Guroff, M., Brown, M. & Gallo, R. C. *Nature* **316,** 72-74 (1985).
90. Weiss, R. A. *et al. Nature* **316,** 69-72 (1985).
91. Zagury, C. *et al. Science* **226,** 449-451 (1984).
92. Groopman, J. E. *et al. Science* **226,** 447-449 (1984).
93. Levy, J. A. *et al. Ann. intern. Med.* **103,** 694-699 (1985).
94. Levy, J. A., Hollander, H., Shimabukuro, J., Mills, J. & Kaminsky, K. *Lancet* **ii,** 586-588 (1985).
95. Ho, D. D. *et al. New Engl. J. Med.* **313,** 1493-1497 (1985).
96. Resnick, L. *et al. New Engl. J. Med.* **313,** 1498-1504 (1985).
97. Haase, A. T. & Levinson, W. *Biochem. biophys. Res. Commun.* **51,** 875-880 (1973).
98. Sundquist, B. & Larner, E. *J. Virol.* **30,** 847-851 (1977).
99. Carroll, D. *et al. J. infect. Dis.* **138,** 614-617 (1978).
100. Narayan, P., Sheffer, D., Clements, J. E. & Tennekoon, G. *J. exp. Med.* **162,** 1954-1969 (1985).
101. Broder, S. *et al. Lancet* **ii,** 627-630 (1985).
102. Mitsuya, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **82,** 7096-7100 (1985).
103. Sodroski, J. G. *et al. Science* **227,** 171-173 (1985).
104. Rosen, C. A., Sodroski, J. G. & Haseltine, W. A. *Cell* **41,** 813-823 (1985).
105. Arya, S. K., Guo, C., Josephs, S. F. & Wong-Staal, F. *Science* **229,** 69-73 (1985).
106. Rosen, C. A. *et al. Nature* **319,** 555-559 (1986).
107. Dayton, A. I., Sodroski, J. G., Rosen, C. A., Goh, W. C. & Haseltine, W. A. *Cell* **44,** 941-947 (1986).
108. Fisher, A. G. *et al. Nature* **320,** 367-371 (1986).
109. Hess, J. L., Clements, J. E. & Narayan, O. *Science* **229,** 482-485 (1985).
110. Sonigo, P. *et al. Cell* **42,** 369-382 (1985).
111. Sanchez-Pescador, R. *et al. Science* **227,** 484-492 (1984).
112. Ratner, L. *et al. Nature* **313,** 277-284 (1985).
113. Muesing, M. A. *et al. Nature* **313,** 450-458 (1985).
114. Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. & Alizon, M. *Cell* **40,** 9-17 (1985).
115. Sodroski, J. *et al. Science* **231,** 1549-1553 (1986).
116. Narayan, O., Kennedy-Stoskopf, S., Sheffer, D., Griffin, D. E. & Clements, J. E. *Infect. Immunity* **41,** 67-73 (1983).
117. Zagury, D. *et al. Science* **231,** 850-853 (1986).
118. Izant, J. G. & Weintraub, H. *Science* **229,** 345-352 (1985).
119. Coleman, J., Hirashima, A., Inokuchi, Y., Green, P. J. & Inouye, M. *Nature* **315,** 601-603 (1985).
120. Pestka, S., Daugherty, B. L., Jung, V., Hotta, K. & Pestka, R. K. *Proc. natn. Acad. Sci. U.S.A.* **81,** 7525-7528 (1984).
121. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (eds) *RNA Tumor Viruses* 2nd edn (Cold Spring Harbor Laboratory, New York, 1982).
122. Haase, A. T. *Microbiol. Pathogenesis* **1,** 1-4 (1986).
123. Gonda, M. A. *et al. Science* **227,** 173-177 (1985).
124. Roberson, S. M., McGuire, T. C., Klevjer-Anderson, P., Gorham, J. R. & Cheevers, W. P. *J. Virol.* **44,** 755-758 (1982).
125. Gogolewski, R. P., Adams, D. S., McGuire, T. C., Banks, K. L. & Cheevers, W. P. *J. gen. Virol.* **66,** 1233-1240 (1985).
126. Chiu, I.-M. *et al. Nature* **317,** 366-368 (1985).
127. Stowring, L., Haase, A. T. & Charman, H. P. *J. Virol.* **29,** 523-528 (1979).
128. Weiland, F., Matheka, H. D., Coggins, L. & Hartner, D. *Archs Virol.* **55,** 335-340 (1977).
129. Stephens, R. M., Casey, J. W. & Rice, N. R. *Science* **231,** 589-594 (1986).
130. Montagnier, L. *et al. Virology* **144,** 283-289 (1985).
131. Barin, F. *et al. Science* **228,** 1091-1096 (1985).