Biology and Pathogenesis of Lentiviruses

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Lentiviruses are a family of retroviruses linked by similarities in genetic composition, molecular mechanisms of replication and in biological interactions with their hosts. They are best known as agents of slow disease syndromes that begin insidiously after prolonged periods of subclinical infection and progress slowly leading to the degeneration of multiple organ systems, cachexia and death. The viruses are species-specific in host range and several have been recognized as pathogens of domestic animals, non-human primates and humans. The prototypes of the family are the agents causing maedi–visna in sheep and infectious anaemia in horses. These diseases have been known for several decades and studies on the biology of the viruses have provided a fund of information that predicted most of the properties of their human counterpart which was identified only 6 years ago as the aetiological agent of AIDS.

Lentiviruses persist indefinitely in their hosts and replicate continuously at variable rates during the course of the lifelong infection. Persistent replication of the viruses depends on their ability to circumvent host defences. In this respect the agents have evolved a repertoire of strategies that surpass those of any other known pathogen. Studies on immunization have shown that the viruses are poor immunogens for the induction of protective antibodies but this varies among the virus families. The 'Achilles heel' of these viruses lies in their absolute dependence on blood and tissue fluids for host-to-host transmission.

The viruses are tropic for macrophages in vivo and replication is regulated by non-structural viral genes and by factors produced by the activated host cells. Clinical disease is related to virus replication in macrophages and two types of disease are produced: primary disease, caused directly by the lentivirus and secondary disease, caused by opportunistic agents.

Primary disease is the major pathological manifestation of infection in domestic animals and is associated with the activation of virus replication in selective populations of macrophages which are tissue-specific. Host and viral factors determine which populations will be involved in the support of virus replication. Primary disease also occurs in humans and is exemplified by the unique lesions in the brain, lungs and lymph nodes of patients with AIDS and AIDS-related complex. Development of primary lesions is associated with the enhanced production of cytokines that are produced partly by the macrophage and partly by the lymphocytes, responding to antigens presented by the immunologically activated, infected macrophages.

Secondary disease (AIDS) is caused by opportunistic agents which proliferate as a result of the loss of function of activated helper T lymphocytes. In humans, macaques and cats, these cells are highly susceptible to lysis by the respective viruses and/or by virus-infected macrophages under cell culture conditions. Loss of the lymphocytes in vivo leads to profound immunosuppression. Helper T lymphocytes of ungulate animals are not as sensitive to the specific lentivirus infecting the animal and this correlates with the lack of secondary diseases in these animals.

INTRODUCTION AND HISTORICAL PERSPECTIVE

The biology of lentiviruses can be best grasped by comparing them to viruses that cause acute transient disease. The classical viral infection consists of three phases of interaction between parasite and host: dissemination of the agent to specific target cells following infection of the
host, productive virus replication in the target cells and thirdly, the period of virus elimination. The decline in virus replication in the third stage is associated with the mobilization of non-specific and immunologically specific host defence mechanisms. Disease results either from direct pathophysiological effects of virus replication in tissue cells or from immunopathological consequences associated with virus elimination. In most cases, the sequence of these events is complete within days to weeks, as seen in influenza, poliomyelitis, measles and mumps. Furthermore, the immune responses associated with recovery from these infections can be induced by vaccines that protect against disease. Successful vaccine prophylaxis has been accomplished by various procedures including the use of live viruses with stably attenuated virulence (e.g. measles virus), inactivated viruses (e.g. poliovirus) or viral subunits (e.g. surface antigen of hepatitis B virus).

The infection and diseases caused by the human immunodeficiency viruses (HIV) are the antithesis of this general concept of pathogenesis of viral disease. The incubation period of months to years that precedes the onset of clinical AIDS, the chronic progressive nature of the disease leading to cachexia and death, the diversity of organ systems affected and the failure of people to recover from the infection emphasize that there is a marked difference between the mechanisms of pathogenesis of HIV and those of viruses that cause acute disease.

This concept is new with respect to human infections but had been established more than 3 decades ago in the field of infectious diseases of domestic animals. The idea that viruses, like certain intracellular bacteria, can cause a slowly progressive disease with prolonged periods of subclinical infection was put forward originally by Bjorn Sigurdsson during his studies on two slow diseases in sheep in Iceland (Sigurdsson, 1954). The first was scrapie (rida in Icelandic), a slowly progressive degenerative disease of the brain. This is the prototype of a group of human diseases that includes Creutzfeldt-Jakob disease, Gerstmann–Sträussler disease and kuru, and which are caused by unconventional infectious agents thought to be devoid of nucleic acid (Prusiner, 1982). The second was maedi–visna (maedi, laboured breathing; visna, paralysis and wasting, in Icelandic) a pneumo-encephalitic disease complex, the prototype lentiviral disease, caused by a conventional enveloped virus. Experimental studies in sheep showed that both scrapie and maedi–visna agents replicated persistently but at a remarkably slow rate in infected hosts. Clinical disease developed with gradual onset after an incubation period of months to years and then followed a chronic protracted course. Disease was manifested by cachexia and chronic neurological disease. The aetiological agent of scrapie is still uncharacterized in terms of its genetic content but the causal agent of maedi–visna was shown to be a replication-competent, non-oncogenic retrovirus that is unique in structure, morphogenesis, genetic composition and molecular mechanisms of replication (reviewed in Narayan & Clements, 1988). Experimental inoculation of sheep with maedi–visna virus reproduced the persistent infection and slowly progressive disease with incubation periods that extended for months to years. Some animals remained persistently infected throughout life, never becoming ill. Primarily because of this slow pathogenic process, the aetiological agent of maedi–visna was named lentivirus (lentus, slow, in Latin).

Lentiviruses of sheep are relatively common pathogens in most parts of the world but explosive epizootics of the type seen in Iceland are rare (Dawson, 1980). The usual picture is that infection is widespread but attrition from disease is relatively low. Few animals become ill and these are usually adults that had been infected 2 to 3 years previously. Many virus strains have been obtained from diseased sheep in different countries; these are closely related genetically and antigenically but have varying degrees of genetic heterogeneity. Some of these genetic properties are associated with distinct characteristics in cell culture (Querat et al., 1984). The arthritis–encephalitis virus of goats (caprine arthritis–encephalitis virus, CAEV) is one such virus which has genetic and biological properties that differ from those of maedi–visna virus (Narayan et al., 1980, 1984; Roberson et al., 1982; Pyper et al., 1984). CAEV has a low virulence for tissue cultures of sheep origin, is tropic for synovial tissues and has a unique interaction with the immune system resulting in the failure to induce neutralizing antibodies. Some strains of virus that cause pneumonia and arthritis in sheep in the United States have the general properties of CAEV. In contrast, visna virus causes lytic infection in cell cultures of goats and
Lentiviruses are host species-specific, exogenous, non-oncogenic retroviruses. The viral genome contains several small genes that regulate expression of structural genes during virus replication. The env gene encodes a single highly glycosylated glycoprotein that forms the virus envelope. This structure contains determinants that bind virions to receptors on cells and also cause cell fusion. It also contains multiple determinants that induce neutralizing antibodies. Mutations in certain regions of this gene result in rearrangement of these determinants and result in development of neutralization-escape mutants.

Lentiviruses are tropic for cells of the macrophage lineage in vivo. Virus gene expression is curtailed to a minimum degree in precursor cells and is increased when the cells become differentiated and/or immunologically activated.

The viruses replicate continuously in vivo and escape host defences by a variety of mechanisms including sequestration of neutralization epitopes by carbohydrate molecules, resistance to inactivation by proteolytic enzymes, antigenic variation, infection in macrophages and enhanced infection in macrophages by non-neutralizing antibodies.

Lentivirus disease is characterized by a variable and often prolonged incubation period and a chronic progressive course. Disease is primary or secondary. Primary disease correlates with amplified virus replication in selective populations of macrophages in different tissues; secondary disease is associated with loss of helper T cell function and inability to cope with opportunistic pathogens.

The virus that causes equine infectious anaemia (EIA) has recently been shown to have the biophysical properties of lentiviruses although the pathogenicity of the agent has been known for several decades (Vallee & Carre, 1904). EIA is the most important infectious disease of horses and occurs throughout the world. Difficulty in the cultivation of field strains of this virus has limited the biological characterization of these agents but transmission studies with tissue homogenates have suggested differences in virulence among virus strains (Konno & Yamamoto, 1970). The EIA virus (EIAV) is a retrovirus (Crawford et al., 1978; Cheevers & McGuire, 1985) and recent studies have shown it to be similar to the lentiviruses of sheep and goats with respect to molecular organization and in many aspects of its biological interaction with the horse (Gonda et al., 1986). These properties (Table 1) have been used as taxonomic criteria for classifying EIAV and certain other retroviruses as lentiviruses. These latter agents include newly identified immunodeficiency viruses of cats (Pederson et al., 1987), cattle (Van Der Maaten et al., 1972; Gonda et al., 1987) and primates (Barré-Sinoussi et al., 1983; Clavel et al., 1986; Desrosiers & Letvin, 1987). The primate lentiviruses comprise HIV types 1 and 2 (HIV-1, HIV-2), the simian immunodeficiency viruses (SIV) of Asian macaques (SIVmac) (Daniel et al., 1985; Benveniste et al., 1986) and those of African monkeys [the African green monkey (SIVagm) (Ohta et al., 1988), the sooty mangabey monkey (SIVsm) (Fultz et al., 1986; Murphey-Corb et al., 1986) and the mandril (SIVmd) (Tsujimoto et al., 1988)].

The lentiviruses of humans and macaques cause severe immunodeficiency in their natural hosts, a property not shared by the viruses that infect ungulates. Aside from this, however, many of the newly defined molecular and biological properties of HIV have been mirrored by similar previous reports on viruses that cause maedi–visna in sheep, arthritis–encephalitis in goats (Narayan & Cork, 1985; Narayan & Clements, 1988) and infectious anaemia in horses (Cheevers & McGuire, 1985). In this review, we highlight the general characteristics of the lentiviruses and illustrate points of congruence between the animal and the human agents. The mechanisms of disease production are poorly understood but, given that lentivirus–host interactions follow common pathways (Narayan et al., 1988), lessons learnt from the earlier studies on animal lentiviruses may be applicable to a better understanding of current studies on the human pathogens.

**GENETIC STRUCTURE AND REPLICATION**

The lentiviral genome, larger than that of oncogenic retroviruses, is a positive-stranded polyadenylated RNA of 9000 to 10000 bp (Clements & Narayan, 1981; Harris et al., 1981;
Molineaux & Clements, 1983; Yaniv et al., 1985). Lentiviruses contain three structural genes organized, 5' to 3', gag, pol and env, typical of all retroviruses (Fig. 1). In addition, the lentiviruses have unique small open reading frames (ORFs) located between pol and env and at the 3' terminus (Pyper et al., 1986; Davis et al., 1987; Sonigo et al., 1985). In HIV, visna virus and EIAV, these ORFs code for regulatory proteins (Rather et al., 1985; Sanchez-Pescador et al., 1985; Wain-Hobson et al., 1985; Rushlow et al., 1986; Derse et al., 1987). At both the 5' and 3' ends of the RNA (R region) are sequences that contain the cap site, the polyadenylation signal and the termination signal for viral RNA transcription (Hess et al., 1986). Sequences at the 3' end of the viral RNA in the U3 region contain the enhancer–promoter elements for initiation of RNA transcription. The nucleotide sequence immediately downstream of the U5 region is complementary to mammalian lysine tRNA and serves as a primer binding site for the synthesis of minus strand viral DNA (Sonigo et al., 1985; Hess et al., 1986). Purine-rich sequences located immediately upstream of these U3 sequences serve as the initiation site for the synthesis of plus strand DNA.

In general, retroviruses have a strong requirement for dividing cells (Weiss et al., 1982); these cells presumably provide optimal conditions for the synthesis of viral DNA and integration of the proviral DNA. In contrast, lentiviruses replicate efficiently in non-dividing, end-stage cells both in the animal and in cell cultures (Thormar, 1963). Rather than depend on cell division, the lentiviruses require activation and/or differentiation of the host cell for productive replication (Narayan & Cork, 1985). These physiological changes are associated with DNA synthesis that may not necessarily result in cell division. Since these viruses utilize cellular enzymes to complete the synthesis of viral DNA and since integration in the host cell genome is similar to that of the oncogenic retroviruses, the lentiviruses must either provide additional virus-encoded replication functions or must activate the non-dividing cells to synthesize proteins and other factors that are required for DNA replication and integration. The effectors of these additional functions are probably encoded by the lentiviral RNAs in the small ORFs unique to these viruses.

The second major difference in replication between lentiviruses and oncogenic retroviruses is the cellular location of synthesis of viral DNA. Synthesis of proviral DNA of the oncogenic retroviruses take place in the cytoplasm of the infected cell (Weiss et al., 1982). In contrast, visna virus replicates its proviral DNA almost exclusively in the nucleus (Haase et al., 1982). This may be a common property of the lentiviruses and is probably linked to their ability to replicate in non-dividing cells. Since the nucleus is the location of cellular DNA synthesis, the enzymes and
cofactors necessary for this would be expected to be located there even when the cell is not dividing. Thus, by replicating in the nucleus, the lentivirus may circumvent the need for the dividing cell or even exert some function to stimulate expression of cellular enzymes required for the completion of synthesis and integration of the viral DNA.

The DNA replication schemes of oncogenic retroviruses and lentiviruses are similar to each other but they differ with respect to the ratio of linear to circular DNA synthesized during replication. During replication of oncogenic retroviruses, both types of DNA can be detected but the circular form predominates. It has recently been shown that the linear DNA is the precursor to integrated DNA (Fujiwara & Mizuuchi, 1988). In contrast only a small proportion of lentiviral DNA becomes circularized and only a small number of copies become integrated in each infected cell (Yaniv et al., 1985; Chiu et al., 1985). However, many more copies (100 to 200) per cell are found as free linear dsDNA molecules in the nucleus of the infected cell (Clements et al., 1979; Harris et al., 1981). In other retroviral systems, the integrated viral DNA is the most efficient template for viral RNA transcription (Panganiban & Temin, 1983). In the case of lentiviruses, it is not currently known whether transcription of the linear non-integrated DNA also contributes to the large amount of viral RNA found in the infected cell (Somasundaran & Robinson, 1988).

TRANSCRIPTION AND TRANS-ACTIVATION OF GENE EXPRESSION

In a similar way to oncogenic retroviruses, the lentiviruses utilize the eukaryotic cell machinery to transcribe viral DNA into genomic RNA and mRNA. This is accomplished by cellular RNA polymerase II. The U3 region of the virus genomes contain elements commonly found in polymerase II promoters and transcription of lentivirus mRNA begins in the 5' long terminal repeat (LTR). The region of the DNA to be transcribed by polymerase II contains the nucleotide sequence TATA which is located upstream (5') with the initiation site of transcription and is the recognition signal (promoter) for RNA transcription. Polymerase II presumably binds to the viral DNA near the TATA box in the U3 region and RNA transcription is initiated at the cap site located at the U3–R junction. Transcription proceeds through the R–U5 region of the viral genome to the poly(A) site in the R region of the 3' LTR. The RNA termination signal is found beyond the poly(A) site.

Lentiviruses have a more complex pattern of transcription than other retroviruses; lentivirus-infected cells contain at least five species of mRNA (Rabson et al., 1985; Muesing et al., 1987; Davis et al., 1987). The full-length genomic RNA serves as the mRNA for the gag- and pol-encoded polyproteins. A single splicing event removes the gag and pol genes leaving a portion of the genome that serves as mRNA for the envelope protein and the product of at least one of the small ORFs between pol and env. Smaller mRNAs produced by multiple splicing events contain the coding regions for both positive (tat and rev) and negative (3' ORF or nef) regulatory proteins. The positively acting genes have been identified in HIV, visna virus, EIAV and SIV (Hess et al., 1985; Sodroski et al., 1985; 1986b; Arya et al., 1987; Dorn & Derse, 1988) whereas the negative regulatory genes are postulated by analogy to HIV (Guy et al., 1987; Ahmad & Venkatesan, 1988).

The positive acting regulatory genes [tat and rev (previously art/trs) (Hess et al., 1985; Sodroski et al., 1985; Cullen, 1986; Feinberg et al., 1986; Guy et al., 1987; Arya et al., 1987; Knight et al., 1987; Dorn & Derse, 1988)] are transcribed in lentivirus-infected cells and are required for viral replication. These small regulatory proteins have basic amino acid compositions that suggest they may interact with nucleic acid. The tat proteins regulate expression of the viral genome via the viral LTR by both transcriptional and post-transcriptional mechanisms (Sodroski et al., 1985; Cullen, 1986). The trans-activation of viral gene expression may be responsible for rapid mobilization of the transcriptional machinery of the cell that is essential for transcription of viral RNA. This results in rapid accumulation of viral RNA in the infected cell. In cell culture systems, the trans-activation of virus gene expression is associated with the highly productive and cytolysis replication that is typical of lentiviruses.
The rev gene is required for viral replication in HIV and is translated from a second ORF in the mRNA which codes for tat (Feinberg et al., 1986; Knight et al., 1987). The rev protein of HIV suppresses splicing, resulting in the accumulation of genomic RNA, and is also required for the translation of viral structural genes (gag, pol and env). The nef gene of HIV acts negatively to regulate genes which are under the control of the viral LTR (Guy et al., 1987). It is not clear whether all lentiviruses have this gene. Thus, the small mRNAs are important for the complex gene expression exhibited by these viruses and may also play a role in the restricted expression observed in vivo.

Lentivirus infections in vivo are characterized by both latent and productive virus replication in monocyte–macrophage cells and T4 lymphocytes (Popovic et al., 1984; Gendelman et al., 1985, 1986). Cellular factors play important roles in the activation of viral gene expression and in the shift from latency to productive replication. For example, a small number of monocytes from visna virus-infected animals have viral RNA detectable by in situ hybridization, but no viral proteins (Gendelman et al., 1985, 1986). Mature macrophages in target organs of the infected animal have much higher levels of viral RNA and many of these cells also have viral proteins (Gendelman et al., 1986). An analogous situation is found in HIV/SIV-infected T lymphocytes from infected hosts. These cells are latently infected and must be activated or stimulated to produce virus (Popovic et al., 1984). Studies on gene expression of HIV in T cells have shown that a cellular factor in activated T lymphocytes is important for the transcriptional activity of the HIV LTR (Nabel & Baltimore, 1987). Similarly, the LTR of visna virus becomes activated by a factor produced by macrophages that are differentiated/activated by treatment with the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (Clements et al., 1988b). Thus, the activated state of the lentivirus’ host cell, be it lymphocyte or macrophage, provides factors that are important in the shift of the viral life cycle from latency to a productive state.

VIRAL ANTIGENS

The viral core is composed of three proteins, p16, p27 and p14 cleaved from a polypeptide encoded by the gag gene. This gene is highly conserved among lentiviruses of each host species, but to a lesser degree among viruses from different host species. (Roberson et al., 1982; Pyper et al., 1984; Chiu et al., 1985; Gonda et al., 1985, 1986). The p27 is the major core protein of the virion and elicits a strong antibody response during infection. The antibodies are group-specific and recognize a wide spectrum of viruses from a particular host species. The antibodies do not protect against infection. Rather, their presence in serum is used as an index for infection. The p14 is a highly basic protein that contains a repeated motif of cysteines (Cys-X2-Cys-X9-Cys), characteristic of retrovirus nucleic acid-binding proteins.

The pol gene encodes the RNA-dependent DNA polymerase (reverse transcriptase, RT) and the endonuclease/integrase of the virus (Sonigo et al., 1985; Vigne et al., 1982). A viral protease has been identified in the nucleotide sequence at the 5' end of the pol ORF.

The env gene encodes a single large polypeptide varying from 115K to 160K among the various lentiviruses (Sonigo et al., 1985; Vigne et al., 1982). This protein becomes highly glycosylated during synthesis and forms the envelope of the virus. It is processed into its final form during assembly of the virions. The env gene of all lentiviruses contains the amino acid sequence Arg-X-Lys-Arg which is present in all retroviruses (Sonigo et al., 1985). This sequence represents the site of cleavage of the envelope glycoprotein into a hydrophilic outer membrane protein and hydrophobic transmembrane portion.

The envelope glycoproteins of lentiviruses contain many biologically important determinants. These include the determinants of virus–cell receptor interaction and virus-induced cell fusion (Harter & Choppin, 1967; Lifson et al., 1986a, b; Sodroski et al., 1986a; Crane et al., 1988) and also the epitope(s) that induce neutralizing antibodies (Scott et al., 1979). The envelope is highly glycosylated; carbohydrate moieties in the protein protect the viruses from proteases, reduce the affinity of neutralizing antibodies for the virus and in some cases sequester neutralizing epitopes from the immune system (Huso et al., 1988). Some infected animals produce antibodies to these epitopes in a sequential order during a course of several months; early post-infectious sera from
these animals thus contain a narrow spectrum of antibodies whereas late sera contain a broad spectrum. Other animals produce antibodies to all of the epitopes soon after infection (Narayan et al., 1987). The implication of these reactions in selection of antigenically variant viruses are discussed in the later section entitled PATHOGENESIS.

The nucleotide sequences comprising the gag and pol genes are the most highly conserved of the lentivirus genes (Gonda et al., 1985) whereas the env gene is highly heterogeneous. The latter is caused in part by point mutations that occur either at random or at specific sites with subsequent accumulations during the replication of the viral nucleic acid (Clements et al., 1988a). These mutations arise as a result of the high intrinsic mutation rates (approximately 1 in 10^4) of retroviruses since the RT lacks editing functions. Mutations are associated frequently with the development of neutralization-escape variants. Under the selective pressure of antibodies described above, variants of visna virus, EIAV, and more recently HIV, have been obtained easily (Narayan et al., 1981; Kono et al., 1973; Kono, 1988; Reitz et al., 1988).

MORPHOLOGY AND MORPHOGENESIS

The viruses are enveloped particles 80 to 100 nm in diameter with dense cylindrical cores (Thormar, 1976; Dubois-Dalcq et al., 1976; Gonda et al., 1985). Each virus replicates optimally in cell cultures (macrophages, T4 lymphocytes and/or fibro-epithelial cells derived from its natural host). Inoculation of virus into host macrophage cultures is followed by endocytosis of the particles. The cells do not fuse 'from without'. Progeny virus is produced within cytoplasmic vacuoles by budding off the vacuolar membrane and accumulating within the vacuole (Narayan et al., 1982; Gartner et al., 1986). The cells develop a minimal c.p.e. during the first week or two after inoculation. Eventually they develop extensive multinucleated giant cell formation, exceeding that seen during normal ageing of macrophage cultures.

The viral life cycle is different in cultures of lymphocytes and fibroblasts. Inoculation of these cells at a high multiplicity (> 10) results in fusion of the cells 'from without' and degeneration within 24 h without the production of virus particles. Inoculation at a lower m.o.i. results in productive replication. The virus enters the cell by fusion with the plasma membrane and the life cycle is completed in approximately 20 h. Maturation of the particles occurs by budding from the plasma membrane (Dubois-Dalcq et al., 1976; Popovic et al., 1984) (in contrast to the intracellular maturation of virus particles in macrophages). Multinucleated giant cell formation occurs at the peak of virus production. The lymphoid/fibroblastic cell culture systems are much more permissive for virus replication than for macrophage cultures.

PERSISTENT INFECTION

Infection by lentiviruses is followed by the indefinite persistence of the agents in cells of the macrophage lineage. (Klatzmann et al., 1984; Ho et al., 1987; Narayan & Zink, 1988). Integration of proviral DNA into stem cells may be a mechanism for maintaining the virus in these cells. The infection is not affected by neutralizing antibodies produced by the host (Narayan et al., 1977a; Crawford et al., 1978; Fauci, 1988). Not only do the neutralizing antibodies fail to cure infection but antibodies induced by inactivated virions or viral subunits may or may not protect against infection. This depends on the particular lentivirus species (Narayan et al., 1984). In fact, goats immunized with inactivated virus developed more severe lesions after challenge (McGuire et al., 1986) (see under PATHOGENESIS.) Similar failure to obtain protective immunity has been obtained subsequently in challenge studies of macaques immunized with inactivated SIV_{mac} (Letvin et al., 1987) and chimpanzees immunized with HIV-1 (Nara et al., 1987). A continuous presence of the viral genome in monocyte–macrophage cells and possibly lymphocytes is the basis for the infectious carrier status of the host. These cells are important for virus dissemination because they are present in inflammatory exudates and in secretions such as milk and semen (Ho et al., 1984; Narayan & Clements, 1988; Narayan et al., 1988).
INCUBATION PERIOD OF THE DISEASE

The phenomenon of an incubation period of the disease that may extend for several years is one of the main features of lentivirus infections. It is important to note however that the incubation period is variable and, although unpredictable, it may extend from weeks at one extreme to the remaining life span of the host at the other. In the latter case, the infected host may remain clinically normal but is a continuous source of infectivity. Virus can be obtained at any time by cultivation (and activation) of monocytes from peripheral blood or by explantation of biopsied tissues (Narayan et al., 1977a, b).

Infectious anaemia in horses (Konno & Yamamoto, 1970), encephalitis in kid goats (Cork et al., 1974) and AIDS in children (Connor et al., 1987; Epstein et al., 1984; Joshi et al., 1985) are notable exceptions to the generalization of long incubation periods in lentivirus diseases. Although EIA is best known as a chronic relapsing disease, experimental inoculation may result in fulminant disease and death of the horse within 1 month. In goat herds endemically infected with CAEV, kids born from infected mothers frequently develop encephalitic disease within 1 month of birth. These animals progress to paralytic disease by 6 weeks of age after the initial development of ataxia at 2 to 3 weeks of age. In humans the majority of children born with HIV develop AIDS within 2 years of age. Pneumonia (lymphoid interstitial pneumonia, similar to mild maedi in sheep) and overt neurological disease are major forms of disease expression. However, earlier signs such as failure to thrive and failure to achieve infantile developmental landmarks indicate short incubation periods of disease. Early disease expression in both children and goats may be associated with intrauterine infection, in addition to the exposure to virus perinatally and in milk.

The end of the incubation period (i.e. the onset of disease) is unpredictable in all host species. In humans, loss of antigenaemia frequently precedes the onset of AIDS. The only known example of disease that can be induced artificially from a subclinical state is EIA; carrier animals injected with steroids or subjected to stressful conditions have been reported to develop an acute onset of the disease (Cheevers & McGuire, 1985). Steroids may interfere with host mechanisms that maintain virus replication at a minimal level and thus potentiate enhanced production of virus.

CLINICAL DISEASE

Disease in lentivirus-infected hosts may be primary, caused by the lentivirus, or secondary, caused by opportunistic pathogens that proliferate unchecked as a result of the loss of helper T lymphocyte function. In nature, the diseases of ungulate animals (horses, cattle, sheep and goats) are of the primary category. These animals become cachectic with severe pathological involvement of different organ systems. However, diseases caused by other organisms do not occur to any noticeable degree (Konno & Yamamoto, 1970; Cheevers & McGuire, 1985; Narayan & Cork, 1985). In contrast, humans (Ho et al., 1987; McArthur, 1988), macaques (Chalifoux et al., 1984) and domestic cats (Pederson et al., 1987) develop both primary and secondary disease. Primary disease is seen as lymphadenopathy, pneumonia and neurological impairment and the secondary disease is characterized by lesions pathognomonic for the particular opportunistic organisms (protozoan, mycotic, bacterial or viral agents) as seen in AIDS.

Specific diseases

Equine infectious anaemia

This disease may express itself as an acute syndrome that ends fatally within 1 month of inoculation, or a chronic relapsing disease that ends in cachexia and chronic anaemia or to the occurrence of infected carriers of the virus that never develop the disease (Konno & Yamamoto, 1970). Generally, inoculated horses develop clinical disease and this is characterized by anorexia, fever and anaemia within 1 month. Horses that survive this attack recover 3 to 5 days
later and remain clinically normal for weeks to months. A relapse of the disease with the same transient duration of illness usually occurs within the same year. These cyclical recrudescences of disease may occur three or four times, after which cycling stops and chronic wasting disease sets in. Alternatively, the animal may remain clinically normal. In these latter animals, stressful events or administration of corticosteroids may trigger the onset of a new cycle of disease. Acute disease is characterized by haemolytic anaemia and haemorrhages and by necrosis of parenchymal cells in the liver and lymphocytes in the spleen and lymphatic tissues. Animals with chronic disease have chronic anaemia but only minimal parenchymal necrosis in visceral organs. These animals develop lymphadenopathy (hyperplasia in the lymph nodes, spleen, etc). Immune complex disease, manifest as glomerulonephritis and autoimmune haemolytic anaemia, becomes pronounced during the late disease. Interestingly, the horse also develops encephalitis characterized by gliosis and cuffing of cerebral blood vessels with mononuclear cells (O. Narayan, unpublished observations).

The disease complex (maedi-visna) in sheep

For reviews, see Dawson (1980) and Narayan & Cork (1985). The main disease expression in lentivirus-infected sheep is dyspnoea and severe loss of flesh. The disease is disseminated worldwide except in Australia and New Zealand from which it has been kept out by the restricted importation of sheep. This may be coincidental because this disease complex rarely occurs among free-range animals. The disease had occurred in epidemic form in Iceland following the introduction of latently infected sheep from Europe during the 1930s. The Icelandic epidemic described by Sigurdsson reached its peak in the 1950s and the disease was eliminated from the Island by the slaughter of affected animals and prohibition of further importation of sheep. The neurological disease, visna, occurred mainly among the pristine Icelandic sheep during the maedi epidemic of the 1950s and usually occurred as a complication of maedi. Visna occurs rarely among non-Icelandic sheep. Arthritis and mastitis also occur in sheep but vary in incidence.

In all cases clinical disease is due to active-chronic inflammation in the particular organ or tissue. Lesions are characterized by the proliferation and infiltration of mononuclear cells including lymphocytes and macrophages. Normal tissue architecture is disrupted and degenerates in the face of invasion by inflammatory cell populations. These histological changes may be observed in the lung, brain and spinal cord, lymph nodes and spleen, joints and areas of the glandular tissue of the mammary glands. In addition, nodular accumulation of lymphoid cells are seen frequently in all affected tissues including those of the brain and lung.

The disease complex (arthritis-encephalitis-mastitis) in goats

For review, see Narayan & Cork (1985). The main expression of disease in goats is synovitis and this occurs mainly in adult dairy animals. The gradual onset of synovitis and its slow progression to crippling arthritis is similar, in its slow progression, to maedi in sheep. A sporadic slowly progressive neurological disease among adult animals has also been observed in certain goat populations in Sweden and Germany which is analogous to visna in sheep. Mastitis is also highly prevalent in dairy goats. Newborn goats in endemically infected herds develop the rapidly progressive paralytic disease described above. Similar to sheep, clinical disease is associated with severe active-chronic inflammation in the affected tissues.

The disease complex in cattle

See Van Der Maaten et al. (1972). The original isolate of bovine immunodeficiency virus (BIV) was obtained from a cachectic cow that had lymphadenopathy and mild encephalitis. A similar virus was obtained from other animals that had persistent lymphocytosis. The virus was propagated in bovine tissue cultures and shown to have the structural, genomic and biological properties typical of lentiviruses. Calves were inoculated with tissue culture-propagated virus which persisted throughout a 6 month observation period but did not cause a further deterioration of health.
AIDS in cats

See Pederson et al. (1987). The recently isolated feline immunodeficiency virus (FIV) is distinct from the oncogenic retrovirus feline leukaemia virus (FeLV). Infection with FIV has been associated with lymphadenopathy characterized by hyperplasia in the lymph nodes, enteropathy and wasting. Infection with opportunistic agents occurs frequently and these diseases complicate the pathological effects of the lentivirus. Preliminary studies in cats inoculated with virus (cultivated in primary cultures of feline T cells) have shown that cats become persistently infected and develop lymphadenopathy. None has developed AIDS as yet during the 1½ years of observation.

The disease complex in humans

For references, see Fauci et al. (1985), Fauci (1988), McArthur (1988), Johnson et al. (1988) and Price et al. (1988). The earliest symptom of HIV-induced disease is an influenza-like syndrome that develops about 2 weeks after infection and which lasts for 2 to 3 weeks. Symptoms include fever, sore throat, myalgia, arthralgia and macula-papular rashes. Recovery from these symptoms is followed weeks to months later by lymphadenopathy, characterized by follicular hyperplasia in the lymph nodes and spleen. Progressive loss of helper T lymphocytes and their function frequently accompanies lymphadenopathy. Weeks or months later, these signs progress gradually to the onset of AIDS. This is seen as loss in weight, diarrhoea and infection with opportunistic pathogens. These diseases are exacerbated by the loss of function of helper T lymphocytes. Oral thrush, enteropathy, generalized disease caused by cytomegalovirus, pneumonia associated with Pneumocystis carini, toxoplasmosis, etc., are all parts of the syndrome; neurological disease is a frequent manifestation of the opportunistic infections. However, primary lentivirus-induced neurological disease also occurs frequently. The lesions are characterized by gliosis, diffuse but mild deterioration of myelin, and in some cases by multinucleated giant cells that resemble macrophages containing lentivirus particles. In children, as discussed above, the onset of disease is relatively acute; lymphoid interstitial pneumonia and encephalopathy are the major and most severe forms of the disease. Lesions in the CNS may vary from microcephaly and atrophy of the brain to loss of neurons with microglial nodule formation and multinucleated giant cells containing viral particles. Opportunistic infections in the brain of children are rare. The disease complex in adults and children is caused by both HIV-1 and HIV-2.

The disease in macaques

SIVmae is closely related genetically to HIV-2 and replicates efficiently in human T cells (Daniel et al., 1985; Chakrabarti et al., 1987). Whereas neither HIV-1 nor HIV-2 is pathogenic for macaques, SIVmae is highly virulent and causes disease in macaques similar to HIV-induced disease in humans. The first clinical indication of infection in macaques after experimental inoculation with SIVmae occurs weeks to months later and is seen as focal cutaneous rashes in the inguinal areas (Ringler et al., 1986) and onset of lymphadenopathy (Chalifoux et al., 1987). Clinical deterioration sets in with diseases caused by opportunistic organisms that include protozoa (e.g. toxoplasma), bacteria (e.g. cryptosporidia and salmonella) and viruses (e.g. adeno-, papova- and cytomegaloviruses) (King, 1986). Wasting disease sets in and is accompanied by involution of the lymphatic system. Enteropathy occurs frequently and loss of body fluids becomes life-threatening. The macaques do not develop neurological signs of disease but frequently develop encephalopathy with neural lesions similar to those seen in brains of patients with AIDS dementia (Letvin et al., 1985). However, lesions in the white matter of the brain and spinal cord have been recognized only in humans. Multinucleated cells with lentivirus particles are also seen in other affected tissues such as the lung and gastrointestinal tract.

Infection in African monkeys

There is a high prevalence of persistent infection in sooty mangabeys, African green monkeys and mandrils by the lentiviruses SIVmoe, SIVagm and SIVmnd, respectively. However, there are no reports that these viruses cause disease in these animals (Ohta et al., 1988; Fultz et al., 1986).
NATURAL HISTORY OF THE LENTIVIRUSES

Spread of lentiviruses in nature involves a complex bio-ecological system in which host, parasite and a range of social and environmental problems interact. The viruses are disseminated exclusively by exchange of body fluids. Any factors that facilitate such exchange on a large scale have potentiated epidemics and epizootics. Such outbreaks of disease are usually associated with some aspect of human socio-economic intervention.

Equine infectious anaemia virus

For review, see Cheevers & McGuire (1985). Infection in horses, mules, donkeys and other equidae is followed by a highly productive phase of replication of the virus resulting in plasma viraemia. This high concentration of infectious virus in plasma is unique among the lentiviruses. Virus is spread by blood-sucking flies that act as mechanical vectors in the transfer of virus with blood-contaminated mouth parts. It is the only lentivirus known to be spread by an insect vector. This occurs at a very high rate when agricultural, sporting and military (in previous times) events necessitated dense crowding of these animals during the summer months. The disease is distributed world-wide.

Visna-maedi, progressive pneumonia or zwoegerziekte

For reviews, see Dawson (1980) and Narayan & Cork (1985). Maedi-visna developed in Icelandic flocks following the introduction of European Karakul rams found in retrospect to be latentely infected with a lentivirus. These rams had been obtained from flocks that had no previous history of the type of disease that broke out among the Icelandic animals. The severe outbreak of maedi-visna was thought to be related causally to three factors: firstly, for centuries the Icelandic sheep had been bred in isolation from other breeds and had become more susceptible to lentivirus disease than European sheep; secondly, nose-to-nose contact is common among sheep during winter housing conditions and this potentiated the spread of virus in nasal exudates; thirdly, coinciding with the development of maedi, the Icelandic sheep had developed another respiratory disease, pulmonary adenomatosis. This disease is associated with the production of profuse quantities of pulmonary exudates. Dispersal of these fluids under close housing conditions enhanced further spread of the lentivirus. The maedi-visna complex was eliminated from Iceland by the slaughter of all sheep in affected flocks on individual farms.

The maedi form of the disease occurs in most other countries. Disease prevalence is low although sheep of certain breeds such as Border Leicester sheep in the U.S.A. and Texel sheep in Holland seem more susceptible. The virus is transmitted primarily in milk and perhaps by husbandry practices including overcrowding and by the loan of breeding rams to different farms. Virus is present in macrophages in the mammary gland and these cells are present in both colostrum and milk. Other infections that cause mastitis result in the extrusion of increased numbers of macrophages into the milk and thereby increase the load of lentivirus infectivity of the milk. Such enhanced infection has been observed in individual animals in which one half of the udder was normal and had a low virus load whereas the other was mastitic and had a heavy virus load (Kennedy-Stoskopf et al., 1985). In Holland, the authorities have succeeded to a great extent in controlling the disease by eliminating all seropositive animals and their lambs (Houwers, 1985).

Caprine arthritis-encephalitis virus

Arthritis-encephalitis of goats occurs primarily in industrialized countries. Approximately 80% of dairy goats in the U.S.A., Canada and western Europe are infected with this virus in contrast to 0 to 10% in many other countries in Africa and South America (Crawford & Adams, 1981; Adams et al., 1984). This disparity has been ascribed to husbandry practices in the dairy-goat industry in which milk from lactating animals is pooled and a portion of the pool is fed to kid goats. This practice greatly accelerated the spread of virus within goat herds by the wide dispersal of infected macrophages from a rare, possibly infected doe in the herd.
Bovine immunodeficiency virus

This virus has only recently been identified and epidemiological data are scant. However in the U.S.A., small-scale surveys of cattle herds in Iowa and Maryland suggest that the virus may be spread within herds by hypodermic needles used repeatedly for mass immunizations or parenteral injections (M. A. Gonda, personal communication).

Feline immunodeficiency virus

See Pederson et al., (1987). Limited serological surveys suggest that FIV causes infection mainly in free-roaming cats and that the incidence is highest in males. Biting is speculated to be the major mechanism for spread because of the propensity for fighting among tom cats. Infectious virus is present in saliva. In addition, gingivitis is relatively common among these cats and inflammatory cells in the lesions may contain virus.

The human immunodeficiency viruses, HIV-1 and HIV-2

A vast number of studies and reports have been devoted to the natural history of HIV. The brief remarks on the subject in this review are intended only to provide a perspective on the biology of the virus within the framework of lentiviruses in general. Two major types of viruses, HIV-1 (Barré-Sinoussi et al., 1983) and HIV-2 (Clavel et al., 1986) occur in nature. The two viruses share sequence homology in their pol and gag genes but differ greatly in env and in the number and organization of their regulatory genes (Clavel et al., 1986). This is similar to the differences between maedi–visna viruses of sheep and CAEV (Pyper et al., 1984). There is extensive genetic variability among strains of both HIV-1 and HIV-2 (McClure & Weiss, 1987). HIV-1 is distributed throughout the world whereas HIV-2 seems to be confined to West Africa. The viruses are pathogenic only for humans. Inoculation of subhuman primates and other species of animals with both viruses has shown that the disease agents infected and replicated only in chimpanzees. However, 4 years after infection, these animals have not yet developed clinical disease.

As is typical of mechanisms for the dissemination of lentiviruses, spread of HIV requires exchange of blood, blood products and/or body secretions (Lifson & Curran, 1987). The extent of virus spread correlates directly with the nature of the mechanism of exchange and can be compartmentalized into specific groups of individuals, depending on risk factors. The major means of spread is by sexual contact, primarily among homosexuals in western countries and among homosexual and heterosexual groups in Africa, Haiti and Brazil. Prostitution is a major factor in heterosexual transmission. Intercurrent mucosal infections in HIV-infected individuals may result in infiltration of lentivirus-infected monocytes into areas of inflamed epithelium. This may enhance the transmission of HIV among people with infections in the peri-anal area or in the genitalia. In addition to sexual transmission, repeated use of single hypodermic needles among many individuals (by intravenous drug users in the west and by unqualified community therapists in underdeveloped countries) is a major mechanism of spread. These mechanisms are reminiscent of the spread of maedi–visna virus among sheep and BIV among cattle.

The role of milk and colostrum as vehicles of transmission of HIV have not yet been evaluated thoroughly. HIV has been obtained from human milk and transmission by this route has been inferred in individual cases (Thiry et al., 1985). It is noteworthy that pooled human milk is used to feed undernourished infants in underdeveloped countries. Whether this practice holds a potential for transmission of HIV as it is for the spread of CAEV to kid goats is yet to be determined.

Simian immunodeficiency virus

Lentiviruses have been isolated from captive Asian macaques (SIVmac) in the U.S.A. from African sooty mangabey monkeys (SIVsm) bred in captivity in the U.S.A., from African green monkeys (SIVsmp) in Africa, the U.S.A. and Japan and from African mandrils (SIVmnd) in Kenya (Desrosiers, 1988). All of the viruses replicate productively in cultures of human T lymphocytes.
**SIV/mac**

The origin of this virus is obscure. It is closely related, genetically and antigenically, to HIV-2. Limited serological surveys have shown no evidence that the virus occurs in nature in the Asian habitat of these animals (Lowenstein et al., 1986; Ohta et al., 1986). The first description of SIV/mac was by investigators at the New England Primate Center in Massachusetts, who identified the lentivirus during studies on experimentally transmitted lymphomas of rhesus monkeys (Macaca mulatta) (Hunt et al., 1983). Serological studies on other animals in the Center showed that only three of more than 800 rhesus macaques had antibodies to the virus (Daniel et al., 1988). The original virus was apparently obtained from a macaque purchased in the early 1970s from another centre that had no history of AIDS-like syndromes in their colony. This is reminiscent of the lentivirus-infected Karakul rams that introduced maedi-visna virus into Icelandic sheep. A similar virus was obtained from serum of a stump-tail macaque (M. arctoides) (M. B. Gardner, personal communication), that died during an outbreak of AIDS among these macaques during the early 1970s at the Primate Center at Davis, California. Several animals had died mysteriously from lymphoma, progressive multifocal leukoencephalopathy, tuberculosis, etc., diseases that are seen typically in human patients with AIDS. The association between these outbreaks and the infection with SIV was obtained retrospectively because the virus was cultivated only in the 1980s from the serum sample that had been stored frozen since 1972. A third occurrence of SIV/mac has been obtained from a pig-tail macaque (M. nemestrina) dying from a lymphoma at the Primate Center in Seattle, Washington (Benveniste et al., 1986). Preliminary studies on genetic homology and antigenic relatedness among these viruses suggest that they are closely related (Desrosiers, 1988).

**SIV/sm**

Sooty mangabeys (Cercocebus atys) are monkeys of West African origin and are present in several zoos in the U.S.A. A large colony of these animals has been maintained at the Yerkes Primate Center in Atlanta, Georgia and more than 80% of adults are seropositive. Animals seroconvert after 1 year of age. The virus is thought to be disseminated by the oral–faecal route and this route of infection has been confirmed experimentally (H. McClure, personal communication). Naturally infected mangabey monkeys do not become ill and are virus carriers for life. However, inoculation of the virus obtained from mangabey monkeys into macaques results in acute disease and the onset of AIDS (Murphey-Corb et al., 1986). The relationship between this virus and SIV/mac has not been established as yet. The question whether mangabey monkeys are infected in nature has not been determined.

**SIV/aqm**

Several strains of lentiviruses, distinct biologically and genetically from HIV-1, HIV-2 and SIV/mac, have been obtained from African green monkeys (Cercopithecus aethiops) in the U.S.A., Japan and from the wild in Africa. The viruses are non-pathogenic for African green monkeys but probably not for rhesus macaques.

**SIV/md**

A new lentivirus, distinct from any of the other SIV agents from primates has been obtained from mandril monkeys (Papio sphinx) caught in the wild in Kenya. Biological characterization of this virus is in progress. No sign of illness was seen in these naturally infected animals.

The natural occurrence of lentiviruses among monkeys in Africa provides intriguing speculations that viruses from these animals may have been the source of the virus in humans. Further studies on the natural history of these agents from non-human primates will add to the understanding of the possible origin of the human pathogens.

**PATHOGENESIS**

The lentiviruses follow many common pathways that lead to disease. This includes the mechanisms the viruses use to elude host defences, the direct effect of infection on cells of the...
immune system and the pathological effects of replication of opportunistic organisms that proliferate as a consequence.

Mechanisms of continuous replication

The nature of lentivirus disease with its long incubation period, its gradual onset and its chronic progressive course requires the continuous replication of the virus. In order to accomplish this, the agent must have mechanisms for escaping from host defences. The lentiviruses have evolved a number of strategies that provide formidable survival advantages [for review see Narayan & Clements (1988), also Narayan et al. (1988); Jolly et al. (1989)].

Contribution of proviral DNA

Lentiviruses utilize the DNA intermediate phase of replication common to all retroviruses. Integration of CAEV proviral DNA has been documented in infected cell cultures (Chiu et al., 1985). However, whether integration occurs in macrophages or lymphocytes in vivo is not known. Latent viral genomes would not be detectable by the defence systems of the host.

Infection in macrophages

Cells of the macrophage lineage are major hosts for replication of lentiviruses in vivo. The first report of this was shown by the demonstration by immunofluorescence of EIAV antigens in Kupffer macrophages in the liver of horses with acute EIA (McGuire et al., 1971). The role of macrophages in lentivirus infection was explored further in visna and CAE viral systems and has been reviewed recently (Narayan & Zink, 1988; Zink et al., 1987). Not only are cells of the macrophage lineage the hosts for the virus but virus gene expression is a function of maturation differentiation of the cells. The virus is latent in precursor cells and its life cycle is completed only in the mature cell (Narayan et al., 1983; Gendelman et al., 1986). In vivo, this occurs only in macrophage populations in specific tissues (Kennedy et al., 1988). Infected macrophages in these locations present lentivirus antigens among their class II major histocompatibility complex (MHC) antigens to T lymphocytes (Kennedy et al., 1985) and probably cause infection in helper T lymphocytes during this interaction. Tropism of the lentivirus for macrophages and antigen-presenting cells has been observed also in tissues of humans and macaques infected with HIV (Ho et al., 1986, 1987; Koenig et al., 1986; Salahuddin et al., 1986) and SIVmac (Letvin & King, 1984) respectively. Viral gene expression in specific populations of cells of these lineages correlates with histological lesions in the respective organs such as the lung (Salahuddin et al., 1986), brain (Koenig et al., 1986) and skin (Ringler et al., 1986). Since macrophages constitute the main non-specific cellular defence system of the host, lentivirus replication undoubtedly subverts this arm of the defence system and results in failure of the host to eliminate the virus.

Infection in helper T lymphocytes

Most of the lentiviruses infect helper T lymphocytes and this most probably compromises the ability of these cells to perform specific immunological functions (Fauci, 1988). Although lentivirus infection in helper T lymphocytes of humans, subhuman primates and cats has been well established, the phenomenon has only recently been observed in lentivirus-infected sheep (S. Kennedy-Stoskopf & O. Narayan, unpublished). However, unlike the primate and feline viruses which cause lytic infection in cultured helper T cells, the viruses infecting sheep appear to cause non-cytopathic infection. Loss of this part of the defence system allows continuous replication of the virus.

Low induction of neutralizing antibodies

In general, lentiviruses are poor inducers of neutralizing antibodies (Narayan et al., 1987; Weiss et al., 1985; Robert-Guroff et al., 1985). The efficiency at which they induce these antibodies varies among the viruses. Some, such as visna virus, EIAV and HIV, induce neutralizing antibodies whereas others such as CAEV are intrinsically poor inducers. Studies on the poor neutralizing antigenicity of CAEV suggests that it may be due to the glycosylation pattern of the viral envelope glycoprotein. Sialic acids on the surface of the virus decreased the
avidity of binding between virus and antibodies (Huso et al., 1988) and in some cases completely obscured the neutralization determinant(s). Treatment of the virus with neuraminidase improved the kinetics of both tests (P. E. Jolly et al., unpublished).

Non-neutralizing antibodies enhance infection in macrophages

Goats persistently infected with CAEV have antibodies to all lentivirus polypeptides. Nevertheless, the antibodies do not neutralize viral infectivity in fibroblast cultures. However when tested in macrophage cultures the antibody-treated virus was internalized more rapidly than untreated virus particles (Jolly et al., 1989). The virus replication cycle was thus accelerated by the rapid completion of the early phases of the infection. Fc receptors (FcR) on the surface of the macrophages probably had a role in phagocytosis of antibody-coated virus because the process did not occur when only F(ab')2 portions of Ig were used in the experiment. Therefore the net result of the development of non-neutralizing antibodies is that infectious immune complexes are produced and these are taken up by macrophage populations that remove such complexes by FcR-mediated phagocytosis. Antibody-mediated enhancement of infection in macrophages with HIV-1 has also been observed (Takeda et al., 1988).

Low affinity of neutralizing antibodies

Neutralizing antibodies induced by the more immunogenic lentiviruses generally have a low affinity for the viruses. The biological effect of this phenomenon has been investigated in the visna virus system. Visna virus induces neutralizing antibodies during the first month or two after infection. However, to achieve neutralization, antibodies required a prolonged incubation of at least 30 min with the virus. Furthermore, the antibodies required more than 20 min incubation to bind virus particles, whereas the virus particles bound to cells within 2 min (Kennedy-Stoskopf & Narayan, 1986). The role of sialic acids in delaying (or decreasing) the firmness of binding between the antibodies and neutralization determinants was described above. It is thus theoretically possible that the highly sialylated virus particles could spread efficiently from cell to cell before they can be neutralized.

Antigenic variation

EIAV, visna virus and HIV undergo antigenic variation during infection in individual hosts (Clements et al., 1988a; Hahn et al., 1985, 1986). This phenomenon, by definition, occurs only in infections in which the viruses induce neutralizing antibodies. As described in the section entitled VIRAL ANTIGENS, the lentiviruses mutate at a high rate and this provides a basis for genetic heterogeneity among the viral agents in the host population as well as in the individual host. Studies on EIAV have shown that the virus mutates at random and mutants are selected by specific antibodies. Emergence of new, non-neutralizable viruses coincides with cyclical episodes of disease in horses (Kono et al., 1973). Antigenic variation also occurs during the persistent infection of sheep with visna virus (Narayan et al., 1977a, b) and probably also in humans infected with HIV (Hahn et al., 1985). However there is no evidence for cyclical episodes of disease in either of the latter two hosts. Fully virulent antigenic variants have been recovered from sheep experimentally infected with plaque-purified virus. Variants of both visna virus and HIV can be derived easily in cell cultures by the incorporation of immune serum from infected hosts into the maintenance medium (Narayan et al., 1981; Reitz et al., 1988); this phenomenon is unique to the lentiviruses. The variants are stable in cell culture and do not undergo further variation unless subjected to selective pressures with neutralizing antibodies.

Studies on visna virus showed that it has multiple neutralization epitopes and variation is caused by the genetically determined rearrangement of these epitopes in the viral envelope (Narayan et al., 1986). Sheep vary in their antibody responses to these epitopes. Some infected (or hyperimmunized) sheep develop antibodies to all of the epitopes. Sera from these animals neutralize all visna viruses (at the typically slow rate of neutralization described above). Antigenically variant viruses do not develop in such infected or immune animals and their sera do not select antigenic variants in cell culture. Other infected sheep produce neutralizing antibodies against a limited number of the epitopes; such antibodies neutralize only the
infecting virus but not other strains of the agent. Antigenic variants develop in these animals. Furthermore, sera from these animals with limited neutralizing capabilities select for rapid development of variant viruses in cell culture. Late sera from such animals collected 2 years or more after infection have a broader spectrum of neutralizing antibodies and do not select for new antigenic variants. Thus, depending on the rate of development of broad spectrum neutralizing antibodies, these variants may or may not develop in individual animals. Emerging antigenic variants of EIAV cause the cyclical episodes of a haemolytic crises in horses. However, animals develop only three or four such crises and these occur during the first year of the infection (Crawford et al., 1978). The broadening antibody spectrum induced by the variant virus may be responsible for the limited number of such crises.

The biological importance of antigenic variation in these viruses is not understood. The phenomenon does not seem to be essential for either the persistence of the agent or the induction of disease. Antigenic variation may be more important for survival of the virus in nature, given its narrow host range and its inefficient mechanism for spread to new hosts. It is possible that mutations and other selective pressures may lead to viruses with differences in tropism for cells, tissues and hosts and also possibly with differences in virulence. This may provide a mechanism for derivation of viruses with variable tissue tropisms in individual hosts such as neurotropic and lymphotropic viruses in humans (Koyanagi et al., 1987) and arthro-, neuro- and pneumotropic viruses in sheep (Narayan & Cork, 1985). Mutation and selection may also provide the basis for a change in virulence for different host species as indicated among viruses from sheep and goats and between humans and macaques. It is possible that SIVmac may be the progeny of an HIV-2 strain of virus which was introduced into macaques and mutated into its uniquely pathogenic phenotype. On the other hand HIV-2 may be a mutant of the lentivirus of free-ranging mangabey monkeys in West Africa (P. Marx, personal communication).

MECHANISMS OF HISTOLOGICAL LESIONS FORMATION

Whereas the loss of helper T lymphocytes may be responsible for secondary disease, the infection in macrophages may be the basis for primary disease, causing a variety of different lesions.

Lytic infection in EIA

The highly productive virus replication in macrophage populations in the liver and spleen is associated with acute necrosis in these cells (Konno & Yamamoto, 1970) and may be the first step in the induction of acute disease.

Immune complex disease in EIA

Antibodies bind to cell-free virus in plasma and the immune complexes are either deposited in specific areas such as the glomeruli of the kidneys and cause immune complex disease or are taken up by specific macrophage populations that clear such complexes. Association of immune complexes with circulating red blood cells leads also to a haemolysis of the cells, resulting in chronic anaemia. Infectious HIV and SIVmac also circulate in the blood but it is not known whether this leads to the type of lesion seen in horses. It is of interest, however, that disappearance of antigenanaemia in both humans and monkeys heralds the onset of disease.

Maturation differentiation of macrophages

Studies on the pathogenesis of visna suggested that replication of the virus in monocyte-macrophage cells is dependent on the maturation/differentiation of the cells. Latent infection in pro-monocytes in the bone marrow becomes productive when the monocytes mature into tissue macrophages. This concept may be applicable to the other lentivirus-host systems with activation confined to certain populations of macrophages. These vary from host to host. The highly permissive macrophages in the liver and spleen of horses infected with EIAV, the permissive brain macrophages of humans and macaques infected with HIV and SICmac, respectively, the permissive alveolar macrophages of humans and sheep infected with HIV and
maedi viruses, respectively, and permissive synovial and colostral macrophages of sheep and goats infected with the ruminant lentiviruses are examples of this phenomenon.

Effects of breeds on the permissiveness of tissue macrophages

Icelandic sheep are much more susceptible to CNS disease than are British sheep (Dawson, 1980; Kennedy et al., 1988). Sheep inoculated intracerebrally with the same stock of Icelandic visna virus showed that viral genes were transcribed much more extensively in the brain cells of Icelandic sheep than in British sheep and that encephalitic changes were much more severe in Icelandic sheep. Thus genetic factors in the host may regulate the extent of viral gene expression in specific cell types and thereby influence the organ specificity of the lesions.

Possible role of cytokines and autoimmunity

Lymphadenopathy and follicular hyperplasia in lymph nodes and spleen are prominent lesions following natural and experimentally infection with EIAV, maedi–visna, CAEV, SIV, FIV and BIV. Lymphadenopathy is also a clinical manifestation of HIV infection in humans. The lesions are associated frequently with polyclonal B cell activation. The mechanisms of these processes are not understood, but in all cases infected macrophages can be found in the tissues. The lesions may be caused by cytokines produced in excess by infected macrophage or dysregulation of cytokine production resulting in the pathological proliferation of subsets of lymphoid cells (such as cytotoxic T lymphocytes) and suppression of others (such as helper T lymphocytes).

The active-chronic inflammatory lesions caused by maedi–visna and CAE viruses are the most severe seen in any lentivirus disease (Narayan & Cork, 1985). The inflammatory cells include monocytes–macrophages, cytotoxic T lymphocytes, plasma cells and follicular accumulations of lymphoid cells. Similar lesions are found in kid goats developing acute encephalitis and in children with AIDS. In sheep, the macrophage is the only cell type that harbours the viral genome in these lesions (Kennedy-Stoskopf et al., 1987). Many of these cells are Ia-positive, indicating immunological activation. Previous studies had shown that interaction of T lymphocytes with infected macrophages resulted in the production of interferon (Narayan et al., 1985). Fluid containing this cytokine caused enhanced expression of Ia antigens in macrophages and caused reduction in replication of the virus in these cells. It is not known whether the other lentiviruses induce a similar type of interferon. It is of interest that SIVmae infection in macaque macrophages results in the expression of Ia by the cells without the participation of interferon (Kannagi et al., 1987). The lentivirus infections may thus directly or indirectly lead to unnaturally high levels of expression of MHC class II genes. This sets the stage not only for abnormal proliferation of lymphoid cells, but these activated antigen-presenting cells may inadvertently begin to present host antigens to lymphocytes. This could result in autoimmune lesions.

IMMUNIZATION AGAINST LENTIVIRUS INFECTIONS

Controlled studies on immunization against lentivirus infection are still in their infancy. In the established animal models in horses and ruminants, control of disease by immunization has never been pursued enthusiastically because of the expense and the relative lack of efficacy of vaccines in contrast to the greater effectiveness of eradication and prevention programmes. The available results can be summarized as follows. Sheep hyperimmunized with disrupted visna virus and virus-infected cells developed broad spectrum neutralizing antibodies and two such animals resisted infection after intracerebral challenge with the homologous infectious virus (Narayan et al., 1981; O. Narayan, unpublished results). Similarly immunized animals had not been challenged with antigenic variant viruses or heterologous viruses of any kind. Horses that recover from episodic disease induced by the mildly pathogenic Wyoming strain of EIAV remain persistently infected at a subclinical level. Injection of such animals with extremely virulent antigenically distinct field isolate viruses failed to cause disease. (R. Montelaro, personal communication). Goats immunized with visna virus became immune to challenge with
the homologous virus but remained susceptible to infection with CAEV. CAEV failed to induce protective immunity in goats after multiple injections of purified inactivated virus, live virus, detergent-disrupted virus, virus-infected cells or detergent-treated virus-infected cells. (Narayan et al., 1984). Finally, macaques immunized with inactivated SIVmac251 developed neutralizing antibodies but in the initial test groups, the animals succumbed to infection and disease after challenge with a high dose of virus given intravenously (Letvin et al., 1987; R. C. Desrosiers, personal communication).

These preliminary studies thus suggest that although animals do not recover from infection with lentiviruses, hyperimmunization with appropriate antigens of these agents may lead to protective immunity against infection with homologous viruses of some members of this family. The phenomenon of infection and immunity seen in horses occurs also in protozoan infections in many animals, but its mechanism in either type of infection is not understood. Given the disappointing results on immunization obtained so far with the lentiviruses, it would be of interest to determine whether infection in primates with avirulent viruses would protect the animals against challenge with virulent virus.

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