EDITORIAL REVIEW

Bovine immunodeficiency virus

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Introduction

Retroviruses are a large family of RNA viruses that have been implicated in a broad spectrum of pathologic disorders in both man and animals (reviewed in [1–3]). In the 2 decades since the finding that animal retroviruses encode a unique enzyme, reverse transcriptase (RT) [4,5], the discovery of a multitude of retroviruses that were pathogenic in animals laid the foundation for investigations in man. These studies led to the isolation of the first human oncogenic retroviruses, human T-cell leukemia virus types I and II (HTLV-I and HTLV-II, respectively) and the human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2, respectively). HTLV-I and HIV-1 infections result in major clinical disease and are considered to be the primary causes of adult T-cell leukemia and the acquired immune deficiency syndrome (AIDS), respectively (reviewed in [6–10]).

The retrovirus family (*Retroviridae*) has been divided into three subfamilies: the *Oncovirinae*, *Lentivirinae*, and *Spumavirinae*. Humans are now known to be infected by at least one representative virus species from each subfamily. Bovines parallel humans in this respect (Table 1). One of these bovine retroviruses, the bovine immunodeficiency virus (BIV), like HIV-1 and HIV-2, has recently been shown to be a genetically distinct member of the growing lentivirus subfamily of retroviruses [10–14]. Chronologically, BIV was the third distinct lentivirus discovered after equine infectious anemia virus (EIAV) and the ovine visna, maedi, and progressive pneumonia (OPPV) virus group [15,16]. Our current knowledge of the discovery, molecular biology, host range, pathogenesis, and emerging potential of this bovine lentivirus in AIDS-related research will be reviewed here.

Table 1. Subfamilies of bovine retroviruses.

Lentivirinae
Bovine immunodeficiency virus (BIV)
Spumavirinae
Bovine syncytial virus (BSV)
Oncovirinae
Bovine leukemia virus (BLV)
Bovine Type D virus

Historical perspective

The intensive search in the late 1960s for an infectious viral agent that might be the cause of bovine leukemia/lymphosarcoma [17,18] led to the isolation and identification of three morphologically and biologically distinct classes of bovine retroviruses [11,19,20]. A striking feature of the newly described bovine retroviruses was the induction of cell fusion resulting in multinucleated giant cells (syncytia or polykaryons) when inoculated into naive cell cultures [11,19,21]. Two of these cattle retroviruses are the bovine syncytial virus (BSV), a 'foamy' or spumavirus [19], and bovine leukemia virus (BLV), an oncovirus [20]. The third bovine retrovirus morphologically resembles visna virus, 'a slow virus' that induces chronic, persistent disease in sheep with neurologic and pneu-

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monic involvement [11,22,23]. At the time, visna virus had only recently been determined to have an RNA genome and encode RT but was not yet classified within the retrovirus subfamilies [24,25]. The subfamily *Lentivirinae* was created to group pathogenic retroviruses with a slow disease induction and originally included visna virus and EIAV [26]. The members of the lentivirus subfamily are exogenous, non-oncogenic viruses that, in general, cause chronic multisystemic diseases, some with gradual and cumulative effects [2,3]. The visna virus-like bovine virus was recently characterized in greater detail by Gonda *et al.* [14], who adopted the name BIV because it structurally, immunologically, and genetically more closely resembled HIV-1 and simian immunodeficiency viruses (SIV), although the viral pathogenesis has not yet been fully characterized. BIV is one of the seven distinct classes of lentiviruses discovered to date (Table 2).

The chronicle of BIV began in the late 1960s, when Dr Cameron Seger, a veterinarian of the Louisiana State University Agricultural Center, was studying dairy cattle at the Southeast Louisiana Experiment Station at Franklinton, Louisiana. The dairy cows had persistent high white blood cell counts, a hematological finding often referred to as persistent lymphocytosis (PL) and associated with the development of bovine leukemia/lymphosarcoma [27]. It is interesting to note that the etiologic agent of enzootic bovine leukemia/lymphosarcoma, BLV, had not been clearly identified at the time that these studies began. The primary animal of importance in this history in 1969 was an 8-year-old Holstein cow (R-29) whose white blood cell counts were elevated and whose physical condition was steadily declining. After cow R-29 delivered a calf in 1970, she weakened and became severely emaciated. After all attempts to correct this situation failed, euthanasia and necropsy were performed. The tentative diagnosis of her condition was lymphosarcoma; however, none of the tumors usually associated with this diagnosis were observed in the postmortem gross examination.

For further evaluation and to determine the possible cause of the disease, tissue samples from cow R-29 were sent to Dr Van Der Maaten at the National Animal Disease Center. Generalized follicular hyperplasia of nodes and central nervous system lesions were noted in the histological examination of cow R-29's tissues. Dr Van Der Maaten isolated the polykaryon-inducing virus (BIV R-29) that resembles maedi, ovine progressive pneumonia, and sheep visna viruses from leukocytes and tissues in cocultures with bovine embryonic spleen (BESp) cells [11]. Hence, some early references to BIV have used this descriptive association [22,28].

When BIV R-29 was inoculated into colostrum-deprived young calves, these animals became infected and developed elevated leukocyte counts; the leukocytosis was attributed to an increased number of circulating lymphocytes. The mild lymphocytosis in these animals persisted for several months and lymphadenopathy was readily apparent in the subcutaneous lymph nodes. Histological examination of the enlarged lymph nodes revealed a follicular hyperplasia as observed in cow R-29. The enlarged lymphatic nodules consisted of masses of immature lymphocytes with a relative absence of blood. Virus could be rescued from the leukocytes of inoculated animals over several weeks. Nevertheless, the animals in this experiment were studied for less than a year and illthrift (steadily declining physical condition for which the most probable prognosis is death), as in cow R-29, was not observed.

Table 2. Clinical manifestations of lentivirus infections in natural hosts.

Lentivirus	Disease description
Ovine visna, maedi, and progressive pneumonia virus	Progressive lethal pneumonia, chronic encephalomyelitis, spasticity, paralysis, lymphadenopathy, mastitis, generalized wasting, opportunistic infections
Caprine arthritis encephalitis virus	Generalized wasting, chronic leukoencephalomyelitis, progressive arthritis, osteoporosis, paralysis
Equine infectious anemia virus	Fever, persistent viremia, hemolytic anemia, lymphoproliferation, immune-complex glomerulonephritis, bone-marrow depression, central nervous system lesions
Bovine immunodeficiency virus	Persistent lymphocytosis, lymphadenopathy, central nervous system lesions, weakness, emaciation
Feline immunodeficiency virus	Immunodeficiency-liké syndrome, generalized lymphadenopathy, leukopenia, fever, anemia, emaciation, opportunistic infections
Simian immunodeficiency virus	Immunodeficiency, neuropathologic changes, wasting, opportunistic infections
Human immunodeficiency virus	Immunodeficiency, lymphadenopathy, opportunistic infections, encephalopathy, emaciation, Kaposi's sarcoma, and other cancers

Adapted from Gonda, 1988 [82] with permission of the editors and Alan R. Liss, Inc., New York, New York, USA.

The reason for the lack of overt disease in the experimental animals is not known but could have been due to the short duration of the study. BIV was also isolated from two other cows from the same herd as R-29 [11].

Since BIV did not appear to be the intensely sought after agent of enzootic bovine leukemia/lymphosarcoma [17,18,20,27], it was put into low temperature storage and its biology went unstudied for nearly a decade and a half after its isolation. With the discovery that AIDS was caused by HIV-1, a retrovirus and a member of the lentivirus subfamily, there was a resurgence of interest in BIV, and lentiviruses in general. Initially, the primary reason for this response was to determine whether HIV-1 had its origins in domestic farm animals, which are known to harbor lentiviruses, and, secondarily, to develop animal lentivirus models that might be relevant to HIV-1 and AIDS research. In the years since HIV-1 was discovered, progress has been made in characterizing the biology of BIV.

Infection cycle of BIV

The retroviruses are protein-encapsulated RNA viruses that replicate via a DNA intermediate during a phase of their life cycle. Since BIV is an exogenous retrovirus, its replicative cycle (Fig. 1) is predicted to be similar to that of HIV-1 and other retroviruses [6,10,29,30], and studies of these agents have provided clues to the replication process of BIV. There are two major phases in the retrovirus infection cycle: entry and virus expression. Entry is initiated by the high-affinity association of virus envelope glycoprotein with a specific viral cell receptor. The attached virus is believed to penetrate the cell by receptor-mediated endocytosis or viral envelope–cell membrane fusion, although the exact mechanism is not known. Once the virus is uncoated, the single-stranded, genome-length RNA in the core is released into the cell cytoplasm where, via the action of the viral RT and cellular enzymes, it is reverse-transcribed into double-stranded viral DNA. The double-stranded viral DNA, known as the provirus, is transported to the nucleus where it integrates into the host chromosomes with the aid of the integrase protein (IN). Prior to integration, circular or linear proviral DNA can be found, and, in the case of lentiviruses, large quantities of these products may associate, unintegrated, with the high-molecular-weight host DNA. The virus is a permanent part of the cell, and the provirus is replicated every time the cell divides. The provirus can remain in the latent or 'unexpressed' state in an individual cell, although there is probably active replication occurring in infected cells somewhere in the body continuously.

In the productive phase of the BIV infection cycle, transcription of the provirus in the nucleus is believed to be initiated by various cellular and exogenous fac-

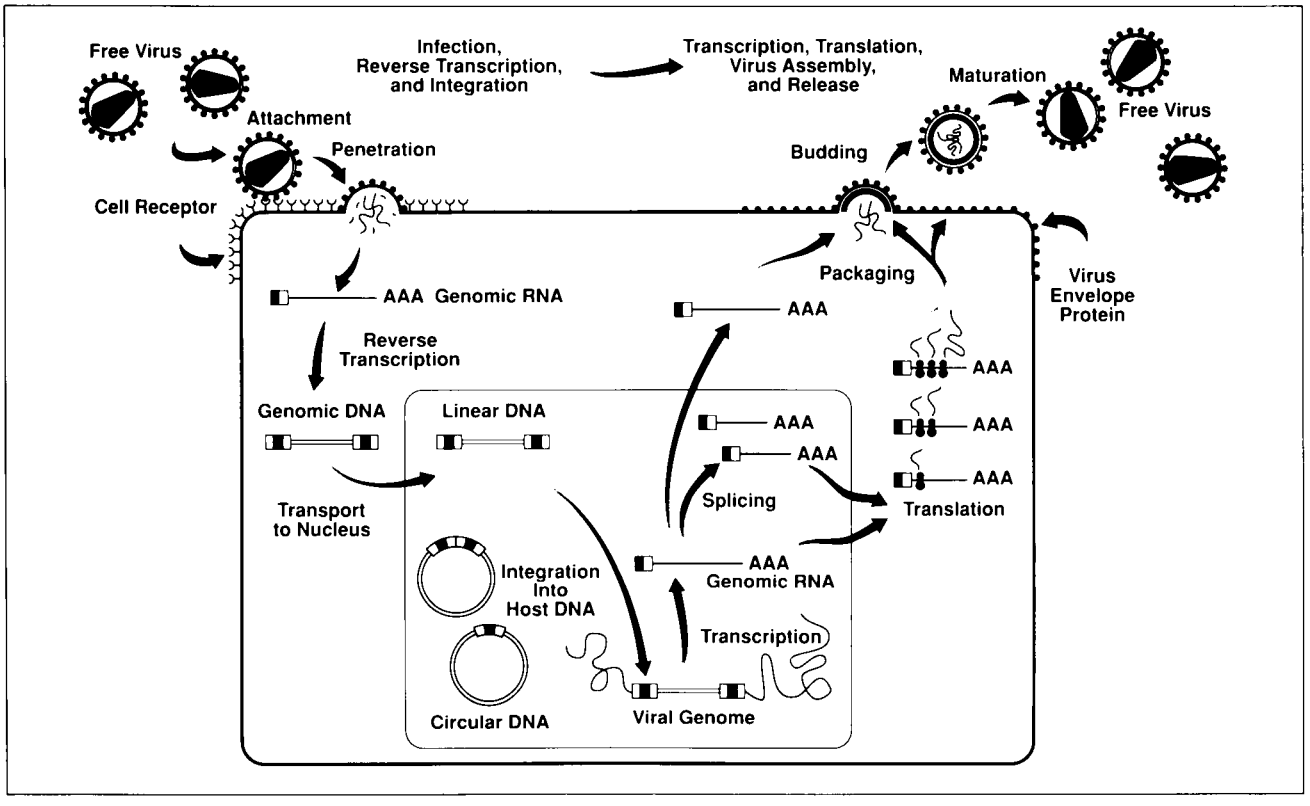

Fig. 1. Replicative cycle of bovine immunodeficiency virus (BIV). (Adapted from Gonda and Oberste [10] by courtesy of Marcel Dekker, Inc., New York, New York, USA.)

tors, and subsequently enhanced by the viral Tat protein. Splicing of the primary viral transcript into subgenomic messages and transport of viral mRNA to the cytoplasm is carried out by the interaction of the cellular splicing machinery and Rev. The genomic and subgenomic length transcripts are translated on ribosomes to produce the structural and accessory gene products. The viral structural proteins (precursors) for *gag*, *pol*, and *env* assemble into the virus particle at the plasma membrane and complex with the genomic-length viral RNA during the process of budding and release from the cell. Virus maturation imparts infectiousness to the virus particle and occurs after proteolytic processing of Gag-related precursors by the viral protease (PR). The mature extracellular virus is free to start the infectious cycle again. The emerging knowledge of components of BIV's replicative cycle will be discussed in more detail below.

In vivo and *in vitro* target cells for BIV replication

Lentiviruses infect cells of the immune system *in vivo*, primarily lymphocytes and monocytes/macrophages [2,31–39]. Even though some of the non-primate lentiviruses are capable of infecting and replicating in lymphocytes as well as monocytes/macrophages, they can also productively infect a variety of diploid and aneuploid adherent cells *in vitro*, frequently from heterologous species [40] (O.S. Andrésson *et al.*, submitted for publication). The specific cell receptors for non-primate lentiviruses are not known. In the case of HIV-1 and SIV, at least, the portal of entry is the CD4 molecule present on the surface of T-helper lymphocytes and monocyte/macrophages (reviewed in [41]). The establishment of CD4+ permanent lymphoid cell lines partially resistant to the cytopathic effects of HIV-1 and SIV permitted their large-scale *in vitro* production, which further facilitated their rapid characterization [42]. Such cells, susceptible to BIV infection and yet resistant to BIV's cytopathic effects, were not initially available.

BIV can be rescued from peripheral blood mononuclear cells (PBMC), lymph nodes, and spleens of infected animals by coculture with susceptible bovine cells [11,43,44] (D.Y. Pifat *et al.*, unpublished data). Specific amplification of BIV DNA in PBMC, lymph nodes, and spleen by polymerase chain reaction (PCR) and BIV-specific primers [44] and detection of BIV RNA in PBMC by *in situ* hybridization [43] from animals infected experimentally with BIV demonstrate the presence of BIV in these tissues. BIV antigen has also been localized in splenic mononuclear cells from BIV-infected rabbits that resemble cells of the macrophage lineage [44]. Moreover, several leukocyte adherent cell lines with a macrophage-like morphology have been isolated from the long-term culture of Ficoll–Hypaque-separated, mitogen-stimulated bovine peripheral blood leukocytes (PBL) cultured initially in the presence of interleukin (IL)-2; these cells (BLAC-20), as well as bovine PBL, have been used to replicate BIV [44] (M.A. Gonda *et al.*, unpublished data). All of the above suggest that BIV, like other lentiviruses, replicates in cells of the immune system [2]. The specific virus cell receptor and target cell for BIV replication remain to be characterized.

The original isolate of BIV (R-29) was rescued on BESp cells [11], but it, and progeny of molecularly cloned virus obtained from it [45], can be propagated in a variety of other embryonic bovine tissues, including lung, testes, thymus, kidney, synovial membranes, choroid plexus, and brain [14,45,46,47]. Replication in primary bovine spleen and lung cells is preferred as the virus grows to higher titers in these cells and they can be passaged numerous times in the uninfected state, although not indefinitely [14,48]. Established cell lines, including epithelial bovine trachea (EBTr), Madin Darby bovine kidney (MDBK), canine thymus (Cf2Th), canine osteosarcoma (D-17), and embryonic rabbit epithelium (EREp), have also been used successfully to propagate the virus [14,40,45–49]. Cells from heterologous species, including sheep and ferrets, have also been reported to be non-productively infected [40], indicating that the original R-29 isolate of BIV has a broader host range *in vitro* than most other lentiviruses. Although BIV has been reported to transform human cells *in vitro* [28], this finding has not been substantiated. Other studies suggest that BIV does not appear to infect human cells or humans [50,51].

Infection and replication *in vitro* results in the formation of syncytia due to cell fusion, as observed in other lentiviruses (Fig. 2); this event is usually terminal for the entire culture when primary cells are used. The addition of infected cells to uninfected cultures (1:4–1:100) is more efficient in passaging the virus infection, although cell-free virus can also be used. Induction of syncytia in cocultures used in virus rescues is indicative of virus replication but is not an absolute criterion for identification of BIV; BSV, BLV, and bovine herpesviruses also induce syncytia. The Cf2Th cell line is partially resistant to the cytopathic effects of the virus [40,46,47]; we have used this cell line for the large-scale propagation of BIV [46,47]. Virus presence can be measured in BIV-infected cell supernatants, using an RT assay with conditions optimal for HIV-1 [14,46,47,50,52], immunoblots of cell-free concentrated supernatants [14], an antigen-capture assay that uses monoclonal antibodies to BIV's major core antigen (p26) [53], competitive radioimmunoassays for BIV p26 [14], or electron microscopy. When using a poly(rA).oligo(dT_{12-18}) template-primer, BIV's RT has a primary preference for Mg^{2+} cation [14,46,47], although RT values in very low concentrations (1–20 μM) of Mn^{2+} are also indicative of virus presence but are not discriminatory

[47,50,53]. For example, BSV RT demonstrates a preference for Mn^{2+} over Mg^{2+}. The difference in cation preference between BIV and BSV is very important because BSV infections are nearly ubiquitous in US cattle and BSV is often co-rescued in cocultivations with BIV-infected PBL. In virus rescue experiments, RT assays using either Mg^{2+} or Mn^{2+} cations are dually performed to aid in virus identification.

Morphology of the virion

BIV has the typical lentivirus morphology when viewed in the electron microscope [10,11,14,22,40,44–47,54,55] (Fig. 3). In the budding process, the capsid proteins of the virus initially form a crescent beneath the plasma membrane. The electron-dense, crescent-shaped core is separated from the plasma membrane by a semi-electron-dense region (Fig. 3a). Intracytoplasmic virus-like particles have been observed in BIV-infected bovine cells [22]. Intracytoplasmic structures have also been observed using uncloned stocks of EIAV-infected equine fetal kidney cells [56] and visna virus-infected sheep choroid plexus cells (K. Nagashima and M.A. Gonda, unpublished data) and in recombinant baculovirus-infected insect cells that overexpress the BIV *gag* gene in the absence of any other viral genes [57]. They have not been observed in molecularly cloned BIV-infected cells. It has been suggested that the appearance of intracytoplasmic particles may result from the accumulation of uncleaved Gag precursor; this accumulation could be related to the expression of Gag precursors from truncated genomes in uncloned stocks of BIV and thus might not be typical of viral morphogenesis [57]. Free immature extracellular particles are released from the cell by budding; they are round, with a doughnut-shaped core and an electron-lucent center (Fig. 3b). The core in the mature virus particle condenses into a bar or cone shape after cleavage by the viral PR (Fig. 3c). The mature virus particle ranges in size from 110 to 130 nm. Spikes, indicative of envelope glycoproteins, are rarely observed on the outer surface of the viral envelope, even using procedures that have been used successfully for other lentiviruses [54,55]. This finding correlates well with the lack of envelope protein in most purified virion preparations [58]. HIV-1 and SIV are shown for comparison in Fig. 3 d f and 3 g i, respectively.

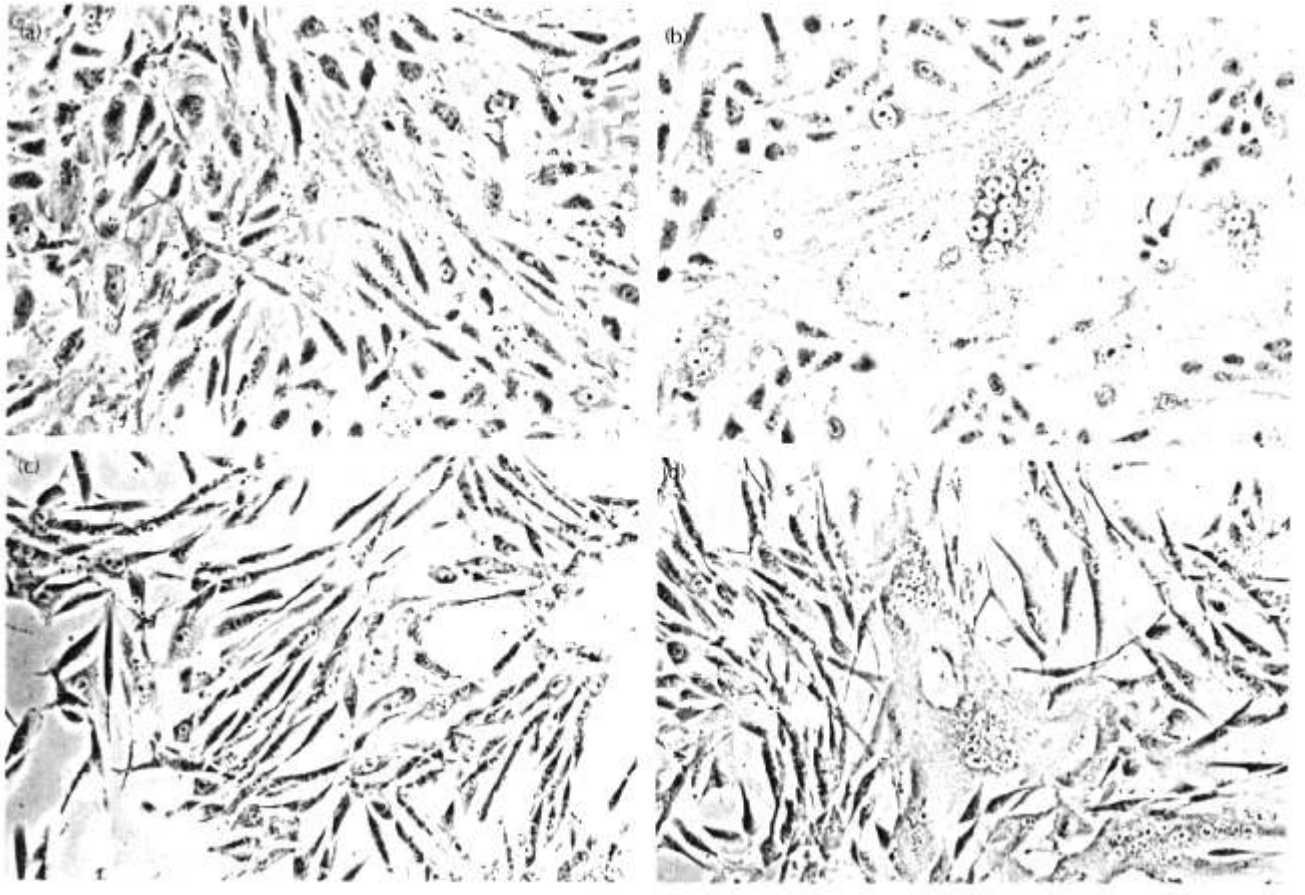

Fig. 2. Syncytia induction in productively infected cells. Phase contrast micrographs in (a) uninfected bovine embryonic spleen (BESp) cells; (b) BIV-infected BESp cells; (c) uninfected embryonic rabbit epithelium (EREp) cells; and (d) BIV-infected EREp cells. Magnifications of all micrographs are × 320. (Micrographs in (a) and (b) reprinted from Braun *et al.* [45] with permission of the editors and Academic Press, Inc., San Diego, California, USA.)

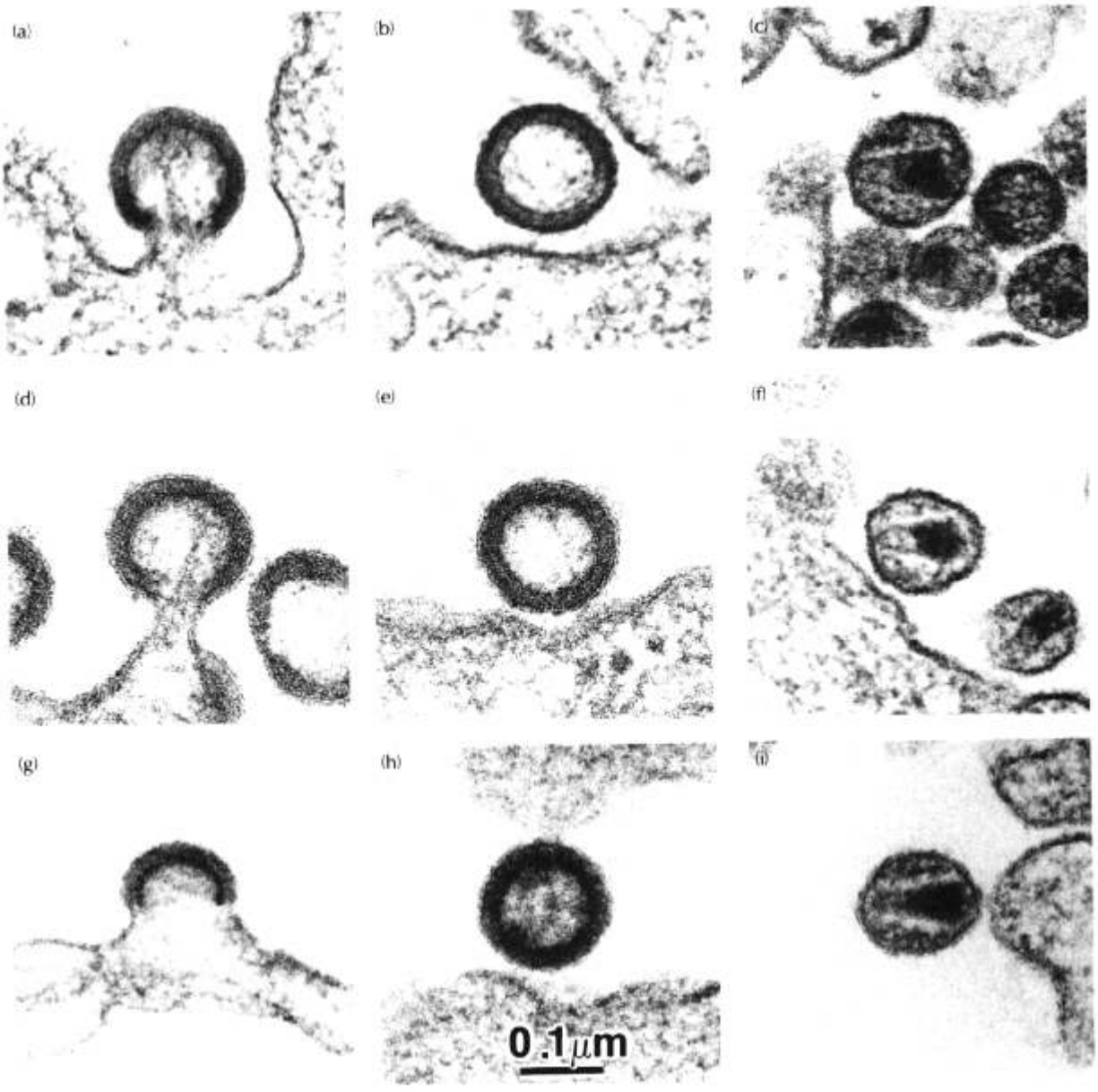

Fig. 3. Ultrastructure and morphogenesis of bovine immunodeficiency virus (BIV) (a–c), human immunodeficiency virus type 1 (HIV-1) (d–f), and simian immunodeficiency virus (SIV) (g–i). Early budding viruses (a, d and g). Free immature extracellular virions (b, e and h). Mature extracellular virions (c, f and i). Magnifications for the electron micrographs are all the same and are indicated by the micron bar in (h). (Adapted from Gonda and Oberste [10] by courtesy of Marcel Dekker, Inc., New York, New York, USA.)

Molecular characterization of BIV

Molecular cloning of infectious proviruses

An important step in understanding the molecular mechanisms of virus replication, persistence, and pathogenesis is the isolation of infectious proviral molecular clones and a determination of their 'genetic blueprint' by nucleotide sequencing. The first genomic clones of BIV, derived from progeny of parental R 29 virus, were reported in 1987 [14]. These clones were full or near full-length but were not functional upon transfection into susceptible cells. They were, however, useful in demonstrating the genetic uniqueness of BIV within the lentivirus subfamily and in developing BIV specific DNA probes to facilitate the cloning of additional proviruses. Two full length, infectious BIV proviruses (BIV106 and BIV127) with cellular flanking sequences were obtained subsequently [45]. The presence of flanking sequences indicates that integration has taken place. These clones have served as sources of donor DNA for gene regulation and expression studies, and genetic engineering of recombinant proteins for use in diagnostic assays [57, 61] (M.S. Oberste *et al.*, submitted for publication). Their progeny have also been used in experimental infections *in vivo* [43,44].

Nucleotide sequence and genome organization

Complete genetic maps of BIV106 and BIV127 were determined by DNA sequencing [59]. BIV127 is 8960 base pairs in the form of the proviral DNA. BIV106 is smaller because of deletions in *env* and the long terminal repeats (LTR). Nevertheless, BIV106 is fully functional *in vitro* and produces infectious virus in the supernatants, and progeny of BIV106 and BIV127 replicate in cattle and rabbits [44] (D.Y. Pifat *et al.*, unpublished data). The BIV genome (Fig. 4) contains the obligatory retroviral structural genes, *gag*, *pol* and *env*, flanked on the 5′ and 3′ termini by the LTR. In addition, at least five putative non-structural accessory gene open reading frames (ORF) are present in, or overlap, the region between the *pol* and *env* ORF. The region between *pol* and *env* is often referred to as the 'central region' and is the hallmark of the lentiviruses. The putative accessory genes of BIV are *vif* (viral infectivity factor), *tat* (*trans*-activator of transcription), *rev* (regulator of expression of virion proteins), *W*, and *Y*. The genome locations and other conserved traits in predicted products of the BIV *vif*, *tat*, *rev*, *W*, and *Y* ORF suggest that they are probably analogous to the *vif*, *tat*, *rev*, *vpr*, and *vpu* or *vpx* genes of primate lentiviruses [10,59,60,62]. There is no separate *nef* (negative factor) ORF, as is present in the primate lentiviruses, in the genome of BIV. In terms of genomic organization, BIV is the most complex non-primate lentivirus, containing at least eight putative genes, and is rivaled only by HIV-1, HIV-2, and some SIV that contain nine genes [10]. The genomic organization of HIV-1 is shown for comparison in Fig. 4. The structural and accessory genes of BIV will be discussed in more detail below.

Characterization of BIV transcripts

The transcriptional pattern of BIV has been studied by Northern blot using gene-specific probes and cDNA cloning of viral mRNA from BIV-infected cells [60] (M.S. Oberste *et al.*, submitted for publication; L.A. Pallansch *et al.*, unpublished data). As anticipated by the complexity of the BIV genome, the BIV transcriptional pattern is also intricate (Figs 5 and 6). By Northern blot analyses (Fig. 5), there are five size classes of BIV-specific viral RNA of 8.5, 4.1, 3.8, 1.7, and 1.4 kb in infected cells; the smaller bands of 1.7 and 1.4 kb may harbor more than one type of gene transcript [60] (M.A. Gonda *et al.*, unpublished data). A transcriptional map for the BIV genome derived from Northern blot analyses and cDNA cloning experiments [60] (M.S. Oberste *et al.*, submitted for publication; L.A. Pallansch *et al.*, unpublished data) is shown in Fig. 6. By analogy with other lentiviruses, the genome-length viral RNA (8.5 kb) is the primary transcript and is used for translation of both Gag and Gag–Pol precursors [29,57,60] (J.K. Battles *et al.*, submitted for publica-

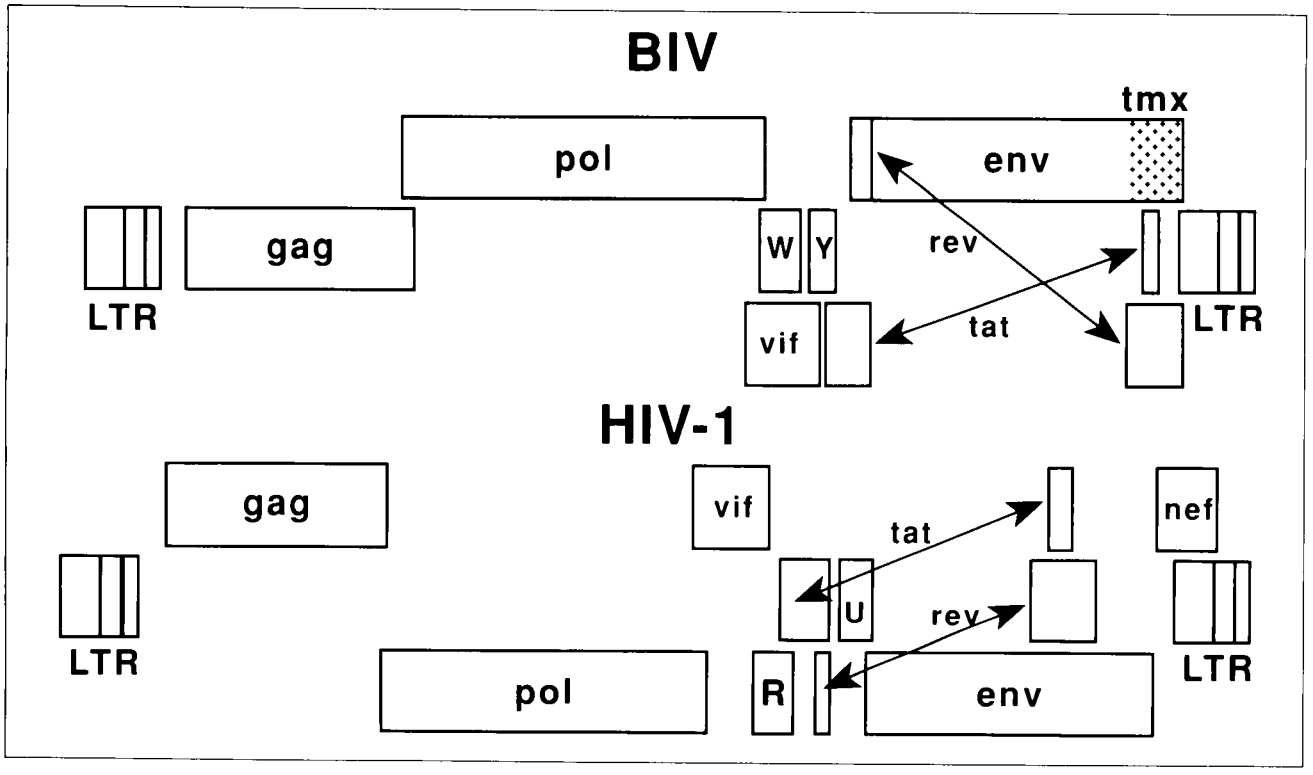

Fig. 4. Comparative genomic organization of bovine immunodeficiency virus (BIV) and human immunodeficiency virus type 1 (HIV-1). The major structural open reading frames (ORF) (*gag*, *pol* and *env*) are shown as well as those corresponding to the non-structural regulatory genes (*vif*, *tat*, *rev*, *vpu* (U), *vpr* (R), *nef*, *W* and *Y*) found in either BIV or HIV-1. The location of the putative *tmx* gene is shown by the shading in the *env* ORF in the genome of BIV. (Adapted from Gonda *et al.* [46] with permission of the editors and Elsevier Science Publishers B.V., Amsterdam, The Netherlands.)

tion). The *vif* and *env* mRNA (4.1 and 3.8 kb, respectively) appear to arise from the primary transcript by a single splicing event in which the untranslated common leader sequence present in all BIV viral mRNA is joined to the 3′ end of the genome by deletion of intervening RNA; the splice acceptor for the *env* mRNA is known [60]. Both *tat* and *rev* mRNA (1.7 and 1.4 kb, respectively) are derived from the primary transcript by multiple splicing events and comprise three exons, including the untranslated common leader sequence and two coding exons [60] (M.S. Oberste *et al.*, submitted for publication; L.A. Pallansch *et al.*, unpublished data). Interestingly, the first BIV *rev*-coding exon is in the *env* ORF (Fig. 4); there is a similar scenario for the first coding exon of *rev* in visna virus [63] and caprine arthritis-encephalitis virus (CAEV) [64]. By mapping the splice junctions for the *W* ORF, a discrete cDNA in which the first initiator methionine is in the *W* ORF has been found (M.A. Gonda *et al.*, unpublished data). This would make the putative *W* ORF mRNA approximately the same size as that of *vif*. It is also possible that *W*, like *tat* and *rev*, may contain another intron and be multiply-spliced (Fig. 6). No mRNA or cDNA clone for the *Y* ORF has been observed to date. Although the BIV genome does not contain a discrete ORF for a *nef* gene, as discussed above, multiple cDNA clones (*tmx*) in which the common leader sequence is joined to the 3′ end of the genome using the same splice acceptor site as *rev* coding exon 2 have been obtained (Figs 4 and 6). These clones initiate translation from the first AUG 14 nucleotides from the splice junction in frame with the translation of the 3′ terminus of the *env* ORF (M.A. Gonda *et al.*, unpublished data). It would be interesting to speculate some analogy between BIV *tmx* and the *nef* genes found in primates. Further study is required to ascertain the biological significance of the *tmx* viral transcript.

Structural components of the virion

As in all retroviruses, the mature extracellular virus particle contains two copies of a single-stranded RNA genome of positive polarity in its nucleoid and can be separated into two major compartments: the viral envelope and the core (Fig. 7). The sizes of the structural gene products were predicted originally from translations of the nucleotide sequence of identified ORF in the genome of BIV127 [59], and have proven particularly useful in putatively identifying BIV-specific proteins in serologic studies of infected animals [48,49]. However, the PR cleavage products of BIV Gag proteins and processing of Env proteins are more complex than anticipated from studies with HIV-1 and other lentiviruses [10,58] (J.K. Battles *et al.*, submitted for publication). There are several smaller Gag and Env polypeptides that co-migrate in gel electrophoresis (J.K. Battles *et al.*, submitted for publication). More recently, the sizes of BIV Gag and Env proteins have been derived experimentally, using gene-specific expression in insect and bacterial cells and by immunological studies using gene-specific and monospecific sera to BIV and HIV-1 subunits [53,57,58] (J.K. Battles *et al.*, submitted for publication). The predicted and observed sizes, where known, of BIV structural gene products are presented for comparison in Table 3.

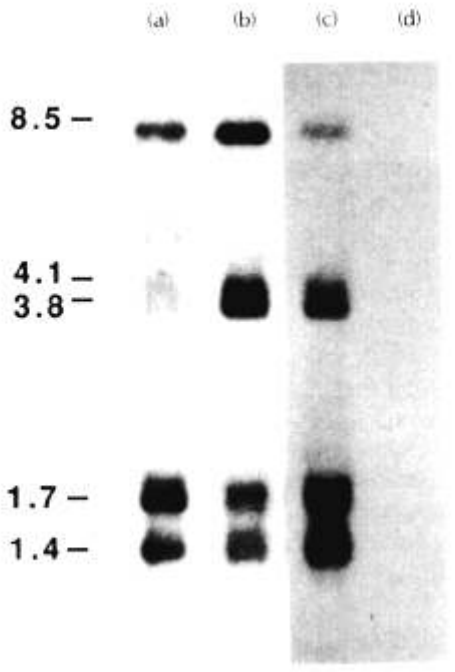

Fig. 5. Northern blot analysis of bovine immunodeficiency virus (BIV) transcripts. (a) BIV 106-infected; (b) BIV 127-infected; (c) BIV parental (R-29)-infected; and uninfected (d) BLAC-20 cells. A ^{32}P-labeled probe representing the entire BIV proviral sequence was used to detect the viral RNA. (Adapted from Oberste *et al.* [60] with permission of the American Society for Microbiology, Washington, DC, USA.)

The viral proteins in BIV virions are named using the standard nomenclature proposed for retroviruses by Leis *et al.* [65]. The viral envelope is derived from the plasma membrane of the infected cell during the process of budding and contains, inserted into it, the surface (SU) and transmembrane (TM) viral envelope glycoproteins (gp100 and gp45, respectively) [58]. The viral core comprises both Gag and Gag-Pol polyproteins in disproportional amounts (Gag:Gag Pol, 20:1). The Gag precursor (Pr53^{gag}) and Gag-Pol polyprotein precursor (pPr170$^{gag\text{-}pol}$) are further cleaved in the mature particle into the functionally defined subunits by the PR present in the Pol portion of the Gag Pol polyprotein. The major Gag subunits are matrix (MA), capsid (CA), and nucleocapsid (NC) (p16, p26, and p13, respectively) [14,57,59] (J.K. Battles *et al.*, submitted for publication). The demonstrated serologic cross-reactivity between the BIV, EIAV, and HIV-1 CA proteins [14,51,57,66] (J.K. Battles *et al.*, submitted for publication) and BIV and HIV-1 NC proteins (J.K. Battles *et al.*, submitted for publication) indicates the strong conservation of Gag epitopes between BIV and HIV-1. There are several unusual cleavage products of the Gag precursor, yield-

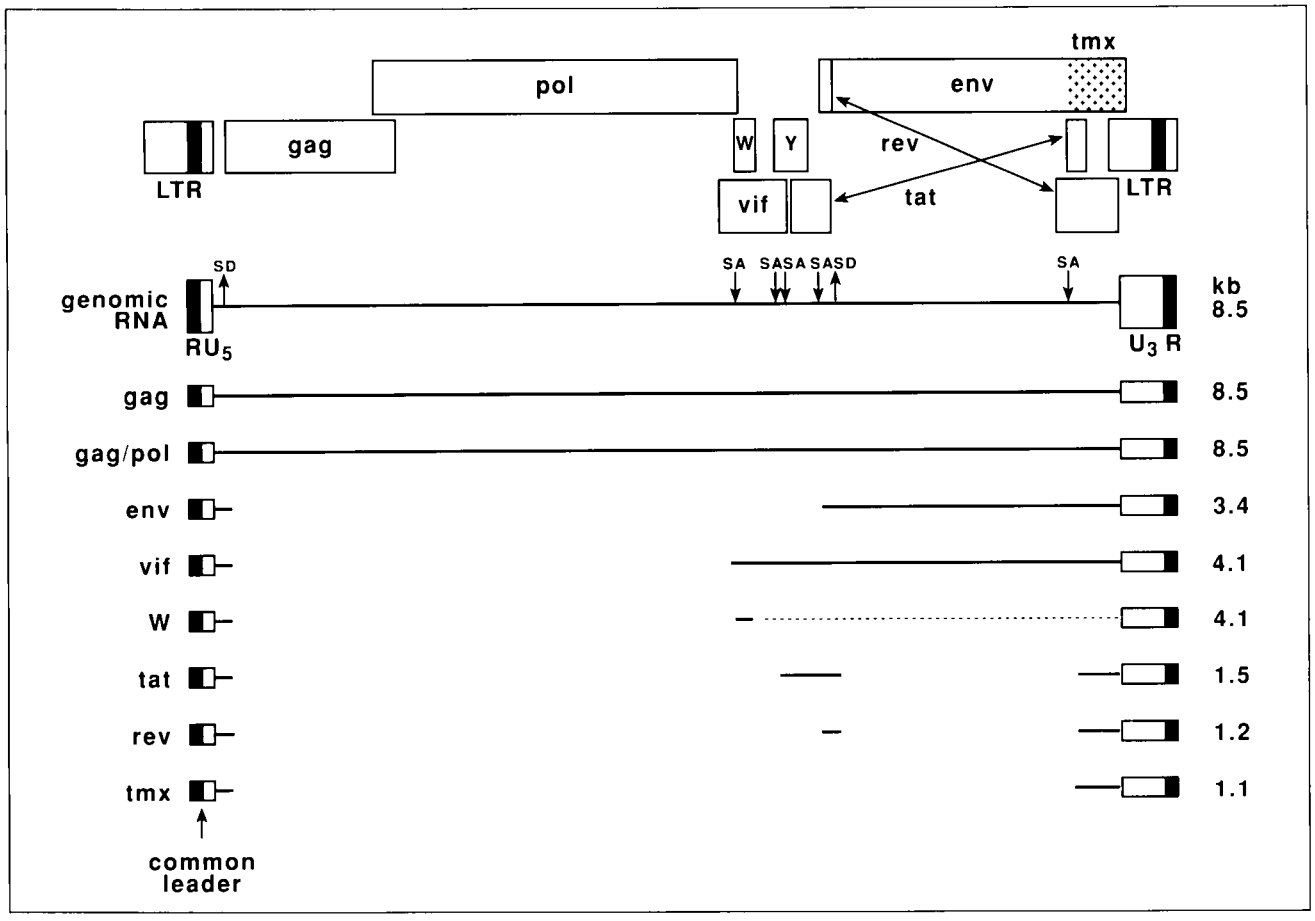

Fig. 6. Transcriptional map of bovine immunodeficiency virus (BIV).

ing chimeric molecules with CA and NC epitopes and smaller peptides with p26 CA epitopes; these products are often recognized in immunoblots with antisera from experimentally or naturally infected animals, although their function in the virus is unknown [48,49] (J.K. Battles *et al.*, submitted for publication). The MA domain in the amino-terminus of the Gag precursor inserts into the plasma membrane during the initial stages of virus particle morphogenesis; the p16 MA protein remains associated with the viral envelope during virus maturation and separation of the core from the viral envelope, and thus should be considered part of the viral envelope in the mature particle (Fig. 7), as suggested for HIV-1 [10]. Interestingly, the BIV MA protein is not myristylated, as it is in HIV-1 and SIV [10,57] (J.K. Battles *et al.*, submitted for publication). The Pol subunits are PR, RT, and IN; the Pol-derived proteins of BIV have not been serologically identified.

Non-structural/accessory genes and LTR

The non-structural/accessory genes and LTR are known to play important roles in regulating viral gene expression, replication, and pathogenesis of HIV-1 (reviewed in [10,30,67–71]). Although structural maps have been deduced for the BIV LTR [59], compared with HIV-1 less is known of the function of the non-structural/accessory genes of BIV. Nevertheless, the presence of at least five genes in or overlapping the central region of the genome of BIV and its complex transcription pattern suggest that these genes are important in the infection cycle of the virus and that some could have functions similar to those of their primate lentivirus analogs [59,60,62] (M.S. Oberste *et al.*, submitted for publication). In contrast to the structural gene products, the accessory proteins of BIV are not found in virions. The predicted and observed sizes of the BIV accessory gene products, where known, are shown in Table 4.

Most lentiviruses encode factors that *trans*-activate expression from the viral LTR [68,69,72–75]. *tat* is one of the accessory gene loci important in positively regulating viral transcription. HIV-1 Tat activates transcription from the LTR and increases the steady-state level of viral RNA in infected cells by interacting with viral RNA at the TAR (*trans*-activation region); this region is a conserved secondary structure near the 5′ end of all HIV-1 viral mRNA [68,69,71]. The overall organization of the BIV LTR is similar to that of other retroviruses; it contains the mandatory U3-R-U5 elements and signal sequences for the initiation, enhancement, and termination of transcription. Several transcription factor binding sequences that are known to have a marked up-regulating effect on LTR-directed gene expression in cultured cells have also been identified [59,76]. A

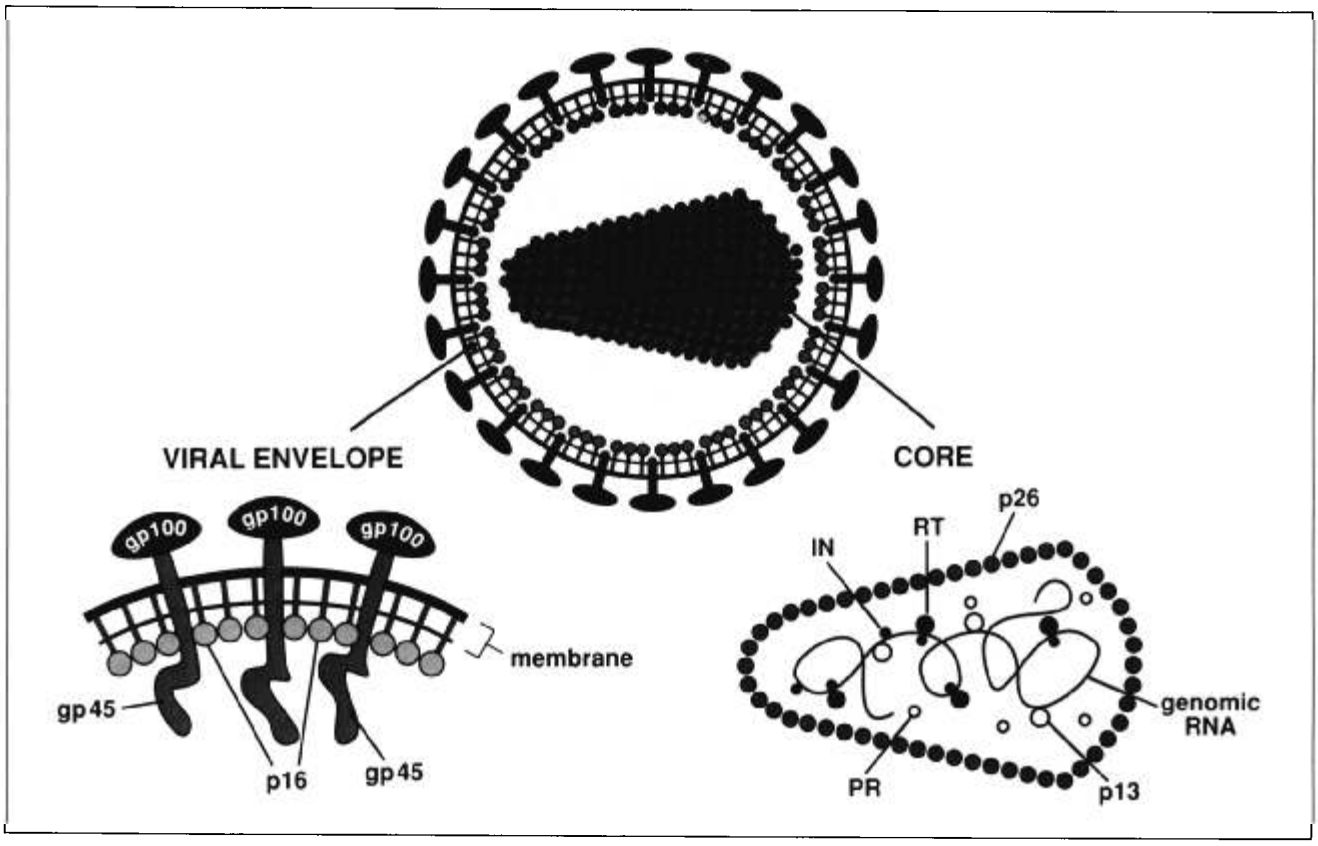

Fig. 7. Schematic representation of the bovine immunodeficiency virus (BIV) virion. The putative location of structural gene products found in the viral envelope and core are shown.

Table 3. Bovine immunodeficiency virus (BIV) structural proteins*.

Gene	Protein location	Protein	Molecular weight (kD) Predicted	Observed
gag	nt316–1743	Gag precursor	53.4	53
	aa1–126	MA	14.6	16
	127–360	CA	26.2	26
	361–476	NC	12.7	13†
pol	–	Gag–Pol polyprotein precursor	168.0	170
	nt1581–4739	Pol polyprotein	120.7	ND
	aa51–143	PR	10.6	ND
	144–724	RT	72.2	ND
	775–1053	IN	32.1	ND
env	nt5415–8126	Env precursor	102.3‡	145
	aa1–555	SU	62.1‡	100
	556–905	TM	40.2‡	45

*Predictions of BIV structural proteins are from Garvey *et al.* [59]. †The actual size of the NC protein may be smaller than both the predicted and the observed values in Table 3, since it is known that small flanking peptides are commonly removed by lentiviral proteases [10] (J.K. Battles *et al.*, submitted for publication). ‡Unglycosylated backbone. nt, nucleotide; aa, amino acid; ND, not determined.

Table 4. Putative bovine immunodeficiency virus accessory gene proteins*.

Gene	Protein location	Protein	Molecular weight (kD) Predicted	Observed
vif	nt4601–5194	Vif	22.8	ND
W	nt4729–4890	Vpw	6.6	ND
Y	nt5089–5328	Vpy	9.5	ND
tat†	nt5228–5521	Tat (exon 1)	11.7	14
rev	nt5415–5540	Rev (exon 1)	20.7‡	23
	nt7637–8068	Rev (exon 2)	ND	ND

*From Garvey *et al.* [59]. †Two forms of *tat* cDNA have been found, one in which only the first coding exon (103 amino acids) is used and one in which there are two coding exons (the first 98 amino acids from the first coding exon and 10 from the second), as observed in HIV-1 (L.A. Pallansch *et al.*, unpublished data). ‡Calculation for predicted molecular weight was obtained from translation of *rev*-coding exons 1 and 2 in *rev* cDNA (M.S. Oberste *et al.*, submitted for publication). nt, nucleotide; ND, not determined.

putative *tat* gene was recognized in the central region of the BIV genome, based on location and sequence similarity with known lentivirus *tat* genes and their products (Fig. 4) [59]. The structure of the BIV *tat* mRNA is similar to HIV-1 *tat* mRNA: the two *tat*-coding exons are joined by multiple splicing of the primary transcript (Figs 4 and 6) [59] (L.A. Pallansch *et al.*, unpublished data). A recent study, using BIV LTR-reporter genes transfected into uninfected and infected cells, determined that BIV encodes a factor(s) that enhances expression from the viral LTR; this virus-spe-

cific *trans*-activation presumably occurs through the action of a Tat-like protein encoded by the putative BIV *tat* gene [61]. The predicted molecular size of BIV Tat is 12 kD; however, consistent with a high isoelectric point (11), a protein of 14 kD has been observed in BIV-infected cells using a Tat-specific antisera (Table 4). Both cellular and heterologous viral factors play a role in regulating gene expression from the BIV LTR [61,77], as in HIV-1 and visna virus [76,78]. Although most lentiviruses are known to encode a nuclear *trans*-activator of transcription, it will be interesting to learn whether the putative Tat protein of BIV also acts via an RNA target sequence as in HIV-1.

Another accessory gene locus in BIV is similar to that identified as *rev* in the HIV-1 and visna virus genomes [10,59,60,63] (M.S. Oberste *et al.*, submitted for publication). HIV-1 Rev is a potent *trans*-activator that temporally regulates the post-transcriptional splicing and transport of viral mRNA from the nucleus to the cytoplasm; it is a phosphorylated protein that localizes to the nucleus and, particularly, to the nucleoli of expressing cells [69–71]. The presumed BIV *rev* mRNA is also multiply-spliced (Fig. 5) [60] (M.S. Oberste *et al.*, submitted for publication). The predicted size of the *rev* product is 21 kD; however, its isoelectric point (10.3) suggests a slower mobility in gels, which in fact has been observed experimentally (Table 4). Using a BIV *rev* cDNA, Rev was expressed in bacteria and in a mammalian *in vitro* translation system. A 23-kD protein ($p23^{rev}$) was detected using antiserum to a synthetic Rev peptide predicted from the *rev* cDNA sequence. The $p23^{rev}$ was found to be phosphorylated and localized in the nucleus and nucleoli of BIV-infected cells (M.S. Oberste *et al.*, submitted for publication). The strong correlations between the BIV and HIV-1 Rev proteins suggest that similarities will also be found in function.

Much less is known about the remaining three BIV accessory gene loci: *vif*, *W*, and *Y*. In HIV-1 and other lentiviruses, the *vif* ORF is located in the central region of the genome overlapping the 3′ end of the *pol* gene. Although there is little similarity in overall amino-acid alignments in the lentivirus Vif proteins analyzed, there are two well-conserved motifs [SL(I/V)X_4YX$_9$Y and SLQXLA] in the majority [62]. The BIV *vif* ORF is predicted to encode a protein of 23 kD (Table 4). The *W* and *Y* ORF, reminiscent of the *vpr*, *vpu*, and *vpx* genes of the primate lentiviruses, are predicted to encode proteins of 7 and 10 kD, respectively (Table 4).

Genomic diversity

To date there are only three reports of complete or partial proviral clones of BIV; they were derived from the progeny of BIV R-29 [14,45,66]. Nucleotide sequences for the infectious BIV106 and BIV127 proviral molecular clones derived by Braun *et al.* [45] have been reported [59]. Sequence comparisons between BIV106 and BIV127 indicate that there is genomic variability (1.7%) within a single isolate when all deletions and substitutions are considered; 75% of the substitutions occur in the SU-coding region of the *env* gene. It will be interesting to observe the amount of genetic variation in the BIV106 and BIV127 genotypes after *in vivo* passage [44] and to determine and compare the nucleotide sequences of new field isolates of BIV (D.Y. Pifat *et al.*, unpublished data). Analysis of these data will facilitate a more detailed map of conserved and variable regions in *env* and other genes, and permit studies to determine the biological significance of this variability.

Evolutionary relationships

At the time of the discovery of HIV-1, its relationship to other retroviruses was unclear. Based on the morphology of the virus particle and conserved sequences in the *pol* gene of molecular clones of HIV-1 and visna virus detected in hybridization experiments, it was determined that HIV-1 was more closely related to ovine visna virus than to HTLV-I and HTLV-II, the oncogenic human retroviruses [12]. The lentivirus relationship of HIV-1 was strengthened in additional studies with EIAV and CAEV, the only other known lentiviruses at the time, and suggested that HIV-1 was a unique member of the lentivirus family [13]. The finding that the AIDS virus was a lentivirus heightened the search for its origins and led to the discovery or characterization of several new members of the lentivirus group, including a variety of SIV, BIV, and the feline immunodeficiency virus (FIV) [14,79–81]. The lentiviruses are a diverse group of retroviruses in terms of their genome organization, nucleotide sequences, and pathogenesis. The diversity is more than likely a result of species-specific adaptation, since the infections occur in genetically distinct hosts. Despite this diversity, this group is characterized by certain common traits [2,3].

In determining evolutionary relationships for viruses with a substantial amount of genomic diversity, it is most effective to use sequences that are more invariable in character. One such highly conserved region in retroviruses is the amino-terminus of the RT domain of the *pol* gene translation [13,14,59]. Phylogenetic trees for this region (90 amino acids) depict the evolution of the retrovirus family members from a hypothetical ancestor [10,13,14,59,82,83]. The trees were rooted artificially, so that the retroviruses whose RT have a preference for the Mn^{2+} cation were the outgroup taxa (Fig. 8). There are two main branches, one leading to the oncoviruses and one to the lentiviruses, among the viruses whose RT have a preference for Mg^{2+}. BIV clusters with the lentiviruses, which is in keeping with the biological data [14]. The branching order of the lentiviruses has BIV diverging first from an ancestor that gave rise to the lentiviruses. Although BIV appears to be more closely related to EIAV, calculations of the

evolutionary distances between lentiviruses in this phylogenetic tree indicate that BIV is relatively equidistant from all of the lentiviruses. More importantly, BIV is a distinct member of the lentivirus subfamily. Sequence comparisons from other regions of the genomes of lentiviruses give alternate protein-dependent tree topographies, often showing a closer relationship between BIV and the primate lentiviruses (for example, Tat).

Seroprevalence in naturally infected cattle

Millions of cattle are produced in the world every year, but only a very small sample has been analyzed for BIV infection. Initially, the lack of adequate sources and methods to produce BIV antigen in large quantities hindered the development of diagnostic assays to determine the seroprevalence of BIV infections in cattle. The development of cell lines persistently infected with BIV has enabled the large-scale propagation of virus [40,46–49,53] for use in enzyme-linked immunosorbent assays (ELISA) and immunoblot (Western blot) assays. Primary cultures of bovine lung have also been used, but problems inherent in the use of primary cells for the long-term, large-scale propagation of virus have been noted [48]. More recently, recombinant antigens to Gag and Env proteins have been reported [57,58,66] (J.K. Battles *et al.*, submitted for publication) and have been very useful in developing sensitive confirmatory assays of the seroprevalence of BIV. An example of immunoblots using whole virus and recombinant Gag and Env proteins is shown in Fig. 9.

Immunoblots of purified BIV virions and immunofluorescence of BIV-infected cells were first used to determine the immunological relationship of BIV to other lentiviruses and to assess virus production in BIV-infected cells [14]. These two techniques have more recently been applied to the screening of beef and dairy herds in the United States [48,49,84,85]. The results from randomly selected samples from dairy and beef herds primarily located in the south and southwest suggest a seroprevalence for BIV of approximately 4% [84,85]; the prevalences within individual herds were considerably higher [49,84].

While substantial information can be obtained using immunofluorescence and Western blot assays, a more useful assay for large-scale screening of animals, in terms of speed and labor investment, is an antigen-based ELISA that uses purified whole virions or recombinant proteins as target antigen [57,86] (G. Luther *et al.*, unpublished data). Luther *et al.* investigated the seroprevalence of BIV in selected dairy and beef herds in Louisiana with whole virus ELISA and confirmatory Western blots that use whole virus or recombinant Gag and Env proteins. They found a seroprevalence of 40% in beef herds and 64% in dairy herds (ranges, 5–53% in beef and 37–82% in dairy herds); there were no attempts to correlate seroprevalence with the health status of these animals. However, BIV-positive animals were frequently also found to be BLV-positive. A BLV- and BIV-positive herd with PL in Colorado was also screened; 21 and 52% of animals were positive for BIV and BLV, respectively; no correlation between PL and BIV infection was found [86]. Using recombinant Gag antigen [57], the seroprevalence of BIV infections in two Mississippi dairy herds was determined to be 50.2% by ELISA [87]. Although the prevalence has not been calculated, randomly selected bovine serum samples from other areas of the eastern and northeastern United States have rarely been positive (M.A. Gonda *et al.*, unpublished data). These data support the suggestion that BIV is more prevalent in southern than in northern regions of the United States.

The worldwide prevalence of BIV is not known, but serologic screening of bovine herds in the Netherlands using a combination of immunofluorescence, Western blots, and radioimmunoprecipitation assays [53] suggests that BIV infections are in the order of 1.4%. However, the reactions of field sera to BIV R-29 virus were weak, suggesting that a variant of BIV may be responsible for these results. In a small sample of bovine sera in Switzerland tested by Western blot using recombinant Gag protein [57], a few animals were also found positive, but again the reactions were weaker than that of bovine sera from the United States (H. Lutz, personal communication, 1992). These results indicate that BIV is present in Europe as well as the United States, and that European strains may be distinct from those in the United States. The significance of BIV prevalence to the health of herds remains to be determined. The presence of BIV in species closely related to domestic cattle has not been tested. Thus, it is not known whether BIV originated in cattle populations or was acquired from some other species of *Bovidae*.

Experimental infections

Cattle

Experimental BIV infections in cattle have received the most attention [11,43,48,49,53]. Intravenous inoculation using cell-associated and cell-free virus from tissue culture sources or blood from infected animals is the most common medium for initiating infections [11,43,49] (D.Y. Pifat *et al.*, unpublished data). Infected cells have also been inoculated into subcutaneous lymph nodes [53]. Newborn, young, and older (> 1 year) animals can be infected. Virus can be rescued from PBMC of infected animals as early as 2 weeks. Within 2–4 weeks, serum antibodies to BIV p26 can be detected; antibodies to envelope proteins appear shortly thereafter [43,48,49]. Virus rescues from animals infected longer than 16 weeks have proven more difficult [11], even though animals naturally or

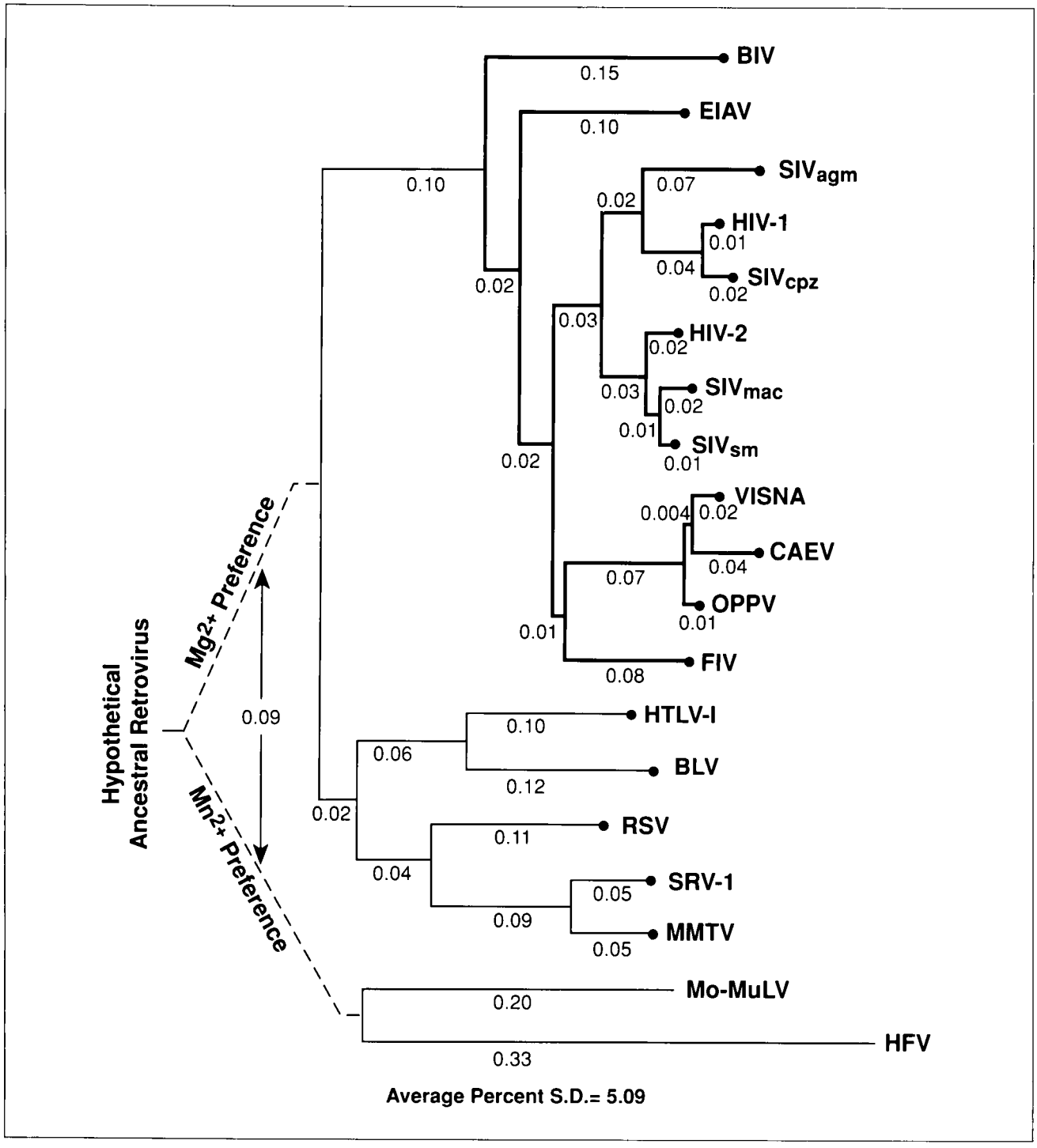

Fig. 8. Evolutionary relationship of bovine immunodeficiency virus (BIV). Fitch-Margoliash phylogenetic tree of retroviral relationships based on the highly conserved 90 amino acids from the reverse transcriptase (RT) domain of the *pol* gene as described [13,14,59,82]. Retroviruses used are visna virus, ovine progressive pneumonia virus (OPPV), caprine arthritis-encephalitis virus (CAEV), simian immunodeficiency viruses (SIV_{sm}, SIV_{cpz}, SIV_{mac}, SIV_{agm}), HIV-2, HIV-1, equine infectious anemia virus (EIAV), bovine immunodeficiency virus (BIV), feline immunodeficiency virus (FIV), HTLV-I, bovine leukemia virus (BLV), Rous sarcoma virus (RSV), simian AIDS retrovirus (SRV-1), mouse mammary tumor virus (MMTV), Moloney murine leukemia virus (Mo-MuLV), and human foamy (or spuma-) virus (HFV). Sequences were obtained from Garvey *et al.* [59] or Genbank (Los Alamos, New Mexico, USA). Branch lengths are in units of -logM, where M is the frequency of matching residues. The tree was rooted with HFV and Mo-MuLV as the outgroup taxons because they consistently had the lowest alignment scores and because their RT preferentially use Mn^{2+} cations as cofactor. The average standard deviation of the tree was 5.09%.

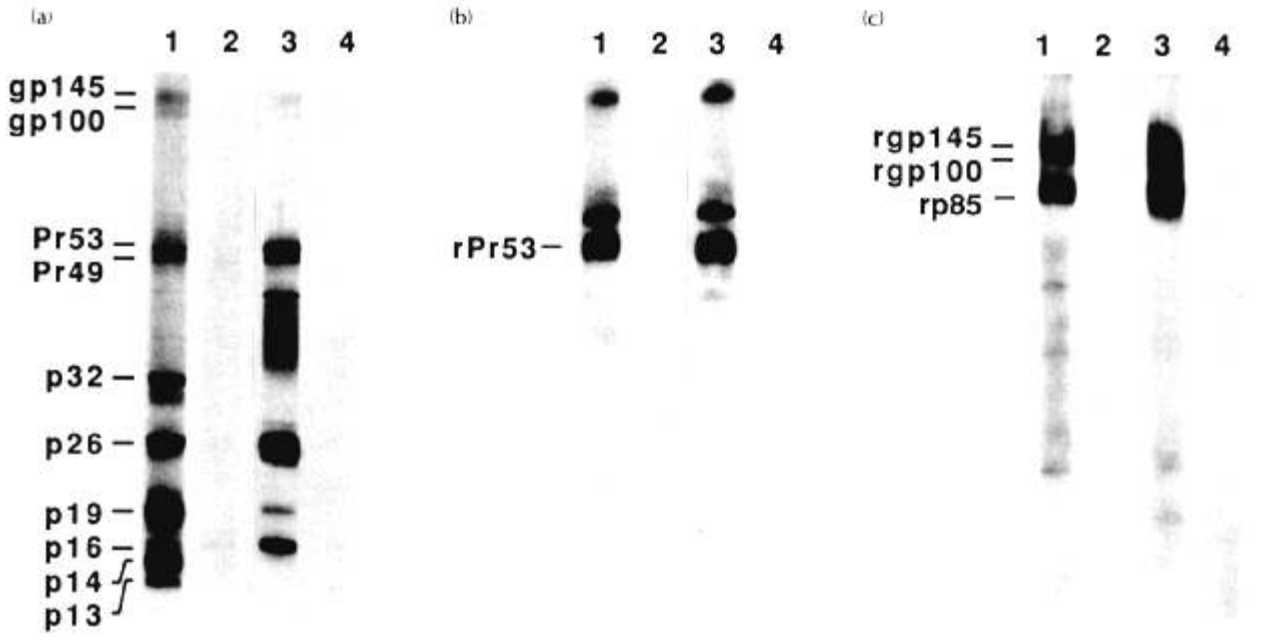

Fig. 9. Western blots of sera from bovine immunodeficiency virus (BIV)-infected cattle and rabbits. The antigens are whole virus (a) and recombinant Gag precursor, $Pr53^{gag}$ [57] (b) and recombinant Env proteins [58] (c) made in the baculovirus-insect cell expression system. Serum from a naturally infected cow (lane 1); serum from a negative control cow (lane 2); serum from an experimentally infected rabbit (lane 3), and pre-inoculum serum from a rabbit used as a negative control (lane 4). Proteins recognized in (a) are gp145 (Env precursor) and gp100 (SU glycoprotein). Gag proteins recognized in (a) are Pr53 (Gag precursor), Pr49 (truncated Gag precursor from Gag–Pol polyprotein), p26 (CA), p16 (MA), and p13 (NC). Additional BIV-specific proteins whose gene derivations have not been identified are p32, p19, and p14. Proteins recognized in (b) are rPr53 (recombinant Gag precursor). Proteins recognized in (c) are rgp145 and rgp100 complex [recombinant Env precursor and recombinant surface (SU) glycoprotein], and rp85 (unglycosylated Env precursor). Viral proteins are designated on the left of (a), (b), and (c). [The blots in (a) are reproduced from Pifat *et al.* [44] with permission of the American Society for Microbiology, Washington, DC, USA.]

experimentally infected with BIV and followed for longer periods still contain high titers of serum antibodies to BIV [48,49] (D.Y. Pifat *et al.*, unpublished data; G. Luther *et al.*, unpublished data).

The initial work describing the early pathogenic effects after experimental infection of calves with BIV R-29 [11], elaborated above, was recently corroborated in more detail [43]. The animals in this new study were observed up to 6 weeks. Virus was recovered by 2 weeks and all animals had seroconverted to BIV by 6 weeks postinoculation. Levels of BIV replication in the animals appeared to be low, since only 0.03% of the PBMC were expressing detectable levels of viral RNA by *in situ* hybridization; and the level of RNA detected *in vivo* was one-tenth the level of BIV RNA detected in infected tissue-cultured cells. The major hematological effect that could be directly attributed to BIV upon physical examination was an increase in the numbers of circulating lymphocytes. Postmortem examination of these young calves at 5–6 weeks revealed gross (swollen nodes) as well as histopathologic changes (lymphoid follicular hyperplasia) in lymph nodes, hemal nodes, spleen, tonsil, Peyer's patches, and gut-associated lymphoid tissue that appeared to be related to BIV infection.

Sheep and goats

Both sheep and goats have recently been inoculated with BIV-infected cells [48]. Antibodies to BIV p26 in both infected sheep and goats were detected as early as 2 weeks postinoculation, and antibodies to several other viral polypeptides, including envelope, developed 9 months postinfection and were still positive a year after initial exposure. None of the animals was reported to have developed disease, and virus could not be rescued from the infected blood. The persistence of antibodies to BIV was used as presumptive evidence that the animals were infected. Since virus rescues were unsuccessful and detection of viral DNA was not attempted, it is not clear whether these animals were infected productively or whether an immunizing dose of viral antigen was administered.

Rabbits

The chronic infection of New Zealand white rabbits can be reproducibly induced by a single intraperitoneal inoculation of 10^7 BIV-infected cells [44,46, 47]. In one study, the persistent infection with molecularly-cloned and parental BIV R-29 stocks was monitored serologically for up to 2 years [44]. The virus infection resulted in a rapid (within 2 weeks of inoculation), high-titered (ELISA titers as high as 1:25 000), and sustained (up to 22 months) humoral response, and the reliable rescue of virus from immune organs. Antibodies to both Gag and Env epitopes were detected. BIV could be rescued by cocultivation from spleen, PBL, and lymph nodes of infected animals, and viral DNA in immune tissues was confirmed by PCR amplification of BIV-specific se-

quences. Viral antigen was also found in mononuclear cells with a macrophage-like morphology in the spleen by immunohistochemical staining using monospecific Gag sera. The results of serologic, virus rescue, and PCR assays indicated that all animals became infected. Postmortem examination revealed an enlargement of lymph nodes and spleens in infected animals terminated at 5 and 7 months postinoculation, but no consistent histopathology was observed in the tissues of infected animals and differential and total leukocyte counts remained within normal ranges. Although 10^7 BIV-infected cells appear to be the most effective infectious dose, 10^4 BIV-infected cells introduced intraperitoneally have also been used to produce infections in young rabbits [44]. Another report of short-term BIV infections in rabbits has corroborated these findings and further demonstrated that minced spleen taken from BIV-infected rabbits 1 month postinoculation can be used to serially passage BIV infections *in vivo* [88]. The studies in rabbits suggest that BIV infection can be adapted to cells of the immune system and in some respects resembles the early infection of cattle with BIV and humans with HIV-1.

Rodents

Mice, rats, and guinea pigs have been inoculated intraperitoneally with BIV-infected cells and monitored serologically for up to 1 year to determine whether they can be infected with BIV. Neither the mice nor the rats seroconverted to BIV. In contrast, guinea pigs weakly seroconverted to BIV, although virus could not be rescued (D.Y. Pifat *et al.*, unpublished data). Transgenic mice containing the entire BIV proviral genome have been made (L.A. Pallansch *et al.*, unpublished data). Preliminary observations suggest that these animals respond immunologically to virus expression and there is notable pathology, as observed in HIV-1 proviral mice [89]. Further characterization of these transgenic mice carrying the BIV provirus may yield important insights into the pathogenesis of BIV and other lentiviruses.

Pathogenesis

The transient leukocytosis and lymphadenopathy seen early in BIV infections of bovines are characteristic of the early events observed in several of the immunosuppressive lentivirus infections [90–92]. The clinical and pathologic findings in cow R-29, which was 8 years old, were greater than observed in the two well-documented short-term experimental studies with young calves [11,43]. It is not known how long cow R-29 was infected with BIV before severe illthrift became apparent. It is also not known whether cow R-29 was dually infected by BLV, which causes PL, since diagnostics for BLV were not available at the time. Thus, little is known currently about the long-term pathogenic effects of BIV infections. Since BIV infections are not acute in the early stages, it could take several years before BIV pathogenesis becomes readily apparent, as in most HIV infections. It should also be noted that all experimental infections performed to date have been made with BIV R-29 or molecularly-cloned virus from BIV R-29 stocks and animals not subjected to natural conditions and pathogens. It may be that the original isolate of BIV lost its virulence, or that non-virulent viruses were selected during extensive tissue culture in BESp and other fibroblastic cells. Virulence may also depend on cofactors naturally present in cattle populations, such as BLV, which is highly prevalent in animals also infected by BIV (G. Luther *et al.*, unpublished data). A synergistic effect between BIV and BLV may be necessary to enhance lymphoproliferative disease and illthrift as seen in cow R-29.

Several things need to be accomplished to better determine the pathogenesis of BIV. First, there is a need for concurrent and retrospective serologic studies of BIV infections where clinical information on the health status and maintenance of animals is well documented. Second, new field isolates of BIV are needed. Preferably, these should be obtained from animals in which substantial disease is noted. To date this has been difficult, perhaps because of the use of BESp or other fibroblastic cell lines in which pathogenic strains of BIV might not grow well. It is well known that the speed of virus replication influences the pathogenicity of lentiviruses [3]. A better understanding of the accessory genes and LTR of BIV could be helpful in this context. Third, the exact target cell (and viral cell receptor) for BIV infection in the immune system needs to be more clearly identified; these cells should be used to propagate the virus *in vitro*. Again, this has been difficult because of the lack of established bovine lymphoid cell lines and the difficulty in propagating PBMC. Fourth, it should be determined whether BIV infections of the immune system cause dysfunction that leads to immunosuppression. A diminution of lymphocyte transformation responses to mitogens in BIV-infected calves has been noted, suggesting that there are immunologic abnormalities in BIV-infected cattle [93]. Additional studies on the effects of BIV on the immune system of infected animals are needed to determine whether BIV has immunosuppressive properties. Finally, the role of cofactors in disease induction should be studied.

Possible modes of transmission

BIV can be transmitted experimentally by the injection of infected whole blood and cell-free and cell-associated virus [11,43,44,48,49] (D.Y. Pifat *et al.*, unpublished data). Lentiviruses, in general, are spread by the exchange of body fluids. Casual contact does not appear to play a role in the infectious transfer. The natural route of BIV infection has not been studied

thoroughly, but several findings on the seroprevalence of BIV permit speculation. First, BIV infections are more prevalent in the southern United States. When a herd tests positive, many of the animals within that herd are also positive. BIV infection is more prevalent in dairy than beef herds. It is speculated that the reuse of contaminated needles used in multiple routine vaccinations and bleedings, the practice of communal sharing of colostrum by calves, and failure to cleanse instruments used in invasive procedures, such as dehorning, may all contribute to BIV spread. Whether insects play a role, as in EIAV transmission [94], is not known; however, a negative control bovine, pastured with animals experimentally infected with BIV, became infected after 16 months' contact (G. Luther, personal communication). Experimental transmission via insect bites and invasive practices used in herd management and health monitoring need to be investigated further.

Concluding remarks

BIV is a lentivirus with structural, antigenic, and genetic features in common with HIV-1, and is an example of a complex retrovirus whose genome contains at least eight genes. Substantial advances have been made in understanding the molecular biology of BIV; these were made possible by the isolation and sequencing of infectious proviral molecular clones [45,59]. Moreover, these infectious clones have provided the media and 'blueprint' for the development of much-needed reagents to probe the infection of animals and determine the molecular mechanisms regulating BIV replication and pathogenesis [43,44,57,58,60] (M.S. Oberste *et al.*, submitted for publication). Lympadenopathy and lymphocytosis, the early pathogenic changes in cattle infected by BIV, appear to parallel some of the effects of HIV in man; however, an AIDS-like disease is not known to be produced [11,43]. The long-term effects of natural and experimental BIV infections in cattle remain to be determined.

The development of animal models for HIV-1 and AIDS is needed to provide valuable tools for *in vivo* analysis of candidate anti-HIV drugs and vaccines prior to their clinical evaluation in man. While the SIV infection in macaques appears to be the 'gold standard' [95], no single animal retrovirus model will be sufficient for all aspects of HIV-1 and AIDS research (reviewed in [96,97]). The ultimate selection of the most appropriate animal retrovirus model will depend on the parameters of the study design, including the gene function being targeted. Because HIV-1 is an exogenous virus that shares many of the features of lentivirus biology, the lentiviruses offer the most promising models. The replication in immune cells, induction of chronic, life-long infections in spite of a strong host immune response, shared antigenic and genetic features, and availability of infectious proviral molecular clones with a similar genomic complexity with HIV-1 all suggest that BIV infection of both cattle and rabbits and production of transgenic mice could provide useful animal models for testing innovative antiviral strategies, if targeted to parts of the BIV infection cycle similar to those in HIV-1. Already, studies have shown that both zidovudine [98] and dideoxyinosine [99], two potent anti-HIV drugs, and a rationally designed C_2 symmetric HIV-1 PR inhibitor [100] are effective in suppressing BIV replication *in vitro* using drug concentrations shown to be effective against HIV-1 (G.J. Tobin and M.A. Gonda, unpublished data). The size of rabbits makes them ideal for both antiviral and vaccine work; cattle, in contrast, will be most useful for vaccine studies because their size prohibits their use in *in vivo* antiviral work. Through the comparative study of lentiviral infections in their natural and surrogate hosts, a better understanding of virus–host interactions that are needed to initiate or promote pathogenesis will be obtained. These findings will be of use in developing strategies to stop infection or abrogate disease relevant to HIV-1 and AIDS.

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