Bovine immunodeficiency virus : facts and questions

Catherine Belloc, Bruno Polack, I. Schwartz Cornil, J. Brownlie, Danielle Levy

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Review article

Bovine immunodeficiency virus: facts and questions

C Belloc [1], B Polack [1], I Schwartz-Cornil [1], J Brownlie [2], D Lévy [1]*

[1] *Unité de recherche associée d'immunopathologie cellulaire et moléculaire, Inra, École nationale vétérinaire d'Alfort, 7, avenue du Général-de-Gaulle, 94704 Maisons-Alfort cedex, France;*
[2] *Royal Veterinary College, London, UK*

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Summary — Bovine immunodeficiency virus (BIV) is a lentivirus whose serologic prevalence is worldwide. Little is known about its impact on animal health status, pathogenesis and mode of transmission. Understanding BIV biology implies isolation of new viral strains and long-term studies on experimentally-infected cows and surrogate hosts such as rabbits.

bovine immunodeficiency virus / cattle / review / lentivirus

Résumé — Le virus de l'immunodéficience bovine : faits et interrogations. *Le virus de l'immunodéficience bovine (BIV) est un lentivirus dont la séroprévalence est mondiale. Actuellement, on connaît mal ses conséquences en matière de santé animale ainsi que son pouvoir pathogène ou ses modes de transmission. Afin de répondre aux différentes questions concernant le BIV, il est nécessaire d'isoler de nouvelles souches virales et de réaliser un suivi prolongé d'animaux infectés expérimentalement.*

virus de l'immunodéficience bovine / bovin / revue générale / lentivirus

* Correspondence and reprints

INTRODUCTION

The bovine immunodeficiency virus (BIV) is a lentivirus and one of the three morphologically, biologically and genetically distinct syncytium-inducing retroviruses infecting bovines. Retroviruses are a family of RNA viruses that are pathogenic for both man and animals. Chronologically, BIV was the third animal lentivirus to be discovered among a group of eight, after equine infectious anemia virus (Vallée and Carré, 1904) and ovine visna-maedi virus (Sigurdssòn et al, 1960). However the role of BIV as a potential pathogen as well as its actual prevalence, especially in Europe, remain totally unknown. Our purpose here is to review the epidemiological, pathological and molecular knowledge concerning BIV.

HISTORICAL BACKGROUND

BIV was discovered in 1969 during the intensive search for the causative agent of enzootic bovine leukosis. A virus with the morphology of a lentivirus was isolated from R-29, a pregnant dairy cow with clinical signs suggesting bovine leukosis (Malmquist et al, 1969; Van der Maaten et al, 1972). It was first designated 'bovine visna-like virus' and remained unstudied until HIV was discovered, which generated renewed interest in the study of animal lentiviruses. BIV was further characterized using molecular biology techniques and two proviral infectious clones were sequenced: BIV R-29 106 and BIV R-29 127 (Gonda et al, 1987; Garvey et al, 1990). To date, all serologic information relating to the presence of a BIV infection has been obtained using the original R-29 isolate as the antigen. Only recently have new field isolates become available in the USA (Suarez et al, 1993).

A new bovine lentivirus, the Jembrana disease virus (JDV), associated with a severe acute syndrome in Bali cattle *(Bos javanicus),* has been found to be both antigenically and genetically closely related to BIV (Kertayadnya et al, 1993; Chadwick et al, 1995).

PREVALENCE OF BIV INFECTION

Serologic evidence for BIV infection has been reported in many countries around the

world following a non-uniform distribution. The prevalence of BIV infection is 4% in the southern and southwestern states of the USA (Black, 1989) and average frequencies of 64% and 40% have been reported in the Louisiana area within dairy and beef herds respectively. In Canada and the Netherlands, the prevalence is 5.5% and 1.4% (McNab et al, 1994; Horzinek et al, 1991). In France, we have found that 4% of selected cattle tested positive for BIV (Polack et al, 1996). We also observed a higher prevalence in dairy than in beef herds which may be the result of herd management practices and of the extended productive life of dairy relative to beef cattle. Seropositive cattle have also been detected in Great Britain (Brownlie et al, 1994; Howie et al, 1994), Switzerland, Australia (Forman et al, 1992), Costa-Rica, Venezuela (in Gonda et al, 1994) and Brazil (unpublished personal results). Since the European sera react weakly with the R-29 antigen, local isolates may be antigenically different from the American ones (Horzinek et al, 1991; Polack et al, 1996). Consequently, wild-type isolates are also needed in order to develop specific detection assays based on antibodies or nucleic acids.

CLINICAL AND PATHOLOGICAL FEATURES

Lentiviruses are responsible for a persistent lifelong infection despite inducing a strong immune response. The onset of the disease is preceded by a several-year incubation period. It affects multiple organs and is characterized by progressive debilitation, which, in the case of HIV, leads to death. The significance of BIV infection on the health status of field herds has not been clearly established due to the high turnover rate of production animals. It is not known whether BIV induces a specific syndrome or whether it renders animals more susceptible to other infectious agents. Nevertheless, cow R-29 had evidence of lymphocytosis, lymphadenopathy, central nervous system lesions and emaciation (Van der Maaten et al, 1972). Moreover, hematological changes, lymphadenopathy with follicular hyperplasia, skin lesions unresponsive to treatment and meningoencephalitis have been found in naturally- and experimentally-infected cattle (Braun et al, 1988; Martin et al, 1991; Carpenter et al, 1992; Onuma et al, 1992; Flaming et al, 1993; Gonda et al, 1994; Rovid et al, 1995). In these animals, the virus is transcriptionally active and can be isolated for many years after infection (Brownlie et al, 1994; Baron et al, 1995). Jembrana disease virus is highly pathogenic in *B javanicus* whereas it does not induce any disease in *B taurus*. The parameters determining this differential pathology remain to be discovered. Obviously, the BIV strains, bovine breed and the environment are factors that may influence susceptibility to BIV disease. Long-term studies are thus necessary to observe BIV pathogenicity since current opinions are based on a limited number of short-term experimental studies. Finally, lentiviruses exhibit variable virulence according to isolates. Isolation of new BIV variants is necessary in order to appreciate its pathogenesis.

HOST TROPISM

In vivo, bovines are the major naturally-infected animals. Antibodies against BIV have been reported in sheep and goats, however, without successful isolation of the virus or evidence of viral DNA (Whestone et al, 1991; Jacobs et al, 1994). Rabbits experimentally-infected with BIV exhibit alterations in their immune response (Onuma et al, 1990; Pifat et al, 1992; Van der Maaten and Whestone, 1992; Archambault et al, 1993; Hirai et al, 1994). The virus can be isolated from the peripheral blood mononuclear cells, spleen, lymph nodes and brain

throughout the life time of the infected animals. In transgenic mice, BIV induces a meningoencephalitis associated with an early 50% mortality (Gonda et al, 1994). Such neurological syndromes have been reported with HIV-1 transgenic mice (Leonard et al, 1988).

Mice, rats and guinea pigs are not susceptible to BIV infection (Gonda, 1992).

CELL TROPISM

In vitro, BIV is able to infect a broad range of adherent and suspension cells originating from different tissues and different animal species (Gonda et al, 1990; Gonda, 1992). In fibroblast-like cells, BIV induces a cytopathic effect characterized by syncytium formation. Through analogy with other lentiviruses, the in vivo targets of BIV infection are likely to be the immune cells of the lymphoid and monocyte/macrophage lineages. BIV provirus can be detected by polymerase chain reaction from the peripheral blood mononuclear cells, spleen, lymph nodes and brain (Pifat et al, 1992; Oberste et al, 1991). However, the cellular receptor for BIV has not yet been characterized.

BIV TRANSMISSION

BIV can be transmitted experimentally by the intravenous inoculation of infected material (blood, cell-free or cell-associated virus).

The natural transmission mode for BIV is unknown and is supposed to be analogous to other lentiviruses that are spread by exchange of body fluids. The virus has been observed in milk (Nash et al, 1995a) and bull semen (Nash et al, 1995b). At present, nothing is known about in utero transmission or the influence of age on an animal's susceptibility to infection.

VIRUS BIOLOGY

BIV infection cycle

BIV particles consist of two positive-sense single-stranded viral RNAs and have a structural capsid which envelops proteins.

BIV is an exogenous retrovirus with a replication cycle similar to that of other lentiviruses. Free virus particles attach to specific surface receptors and penetrate into the cell. The viral RNA is then released and converted by a viral reverse transcriptase into double-stranded DNA that is integrated into the cellular genome, a step that involves a viral integrase. This integrated provirus remains inactive unless appropriate cellular signals occur. When activated, genomic and subgenomic messages are translated in the cytoplasm. Viral particle assembly and RNA incorporation occur near the plasma membrane. Cellular machinery and viral proteins contribute to each step of this process.

Genomic organization

The BIV genome (proviral DNA) is 8 960 nucleotides long and contains the obligatory retroviral structural genes *gag*, *pol* and *env* flanked on the 5' and 3' termini by long terminal repeats (LTR) (fig 1). Each LTR is composed of three consecutive regions named U3, R and U5. The U3 region contains the transcriptional regulatory sequences. The first base of the R region is the site of the viral mRNAs transcription initiation. In addition, at least five non-structural open reading frames (ORF) are present in the 'central region' between and overlapping the *pol* and *env* ORFs (Garvey et al, 1990). These ORFs are designated *vif* (viral infectivity factor), *tat* (trans-activator of transcription), *rev* (regulator of virus expression) *vpw*, *vpy* and *tmx*. In terms of genomic organization, BIV is the most complex non-primate lentivirus. At least five alter-

nately-spliced BIV-specific mRNAs have been identified in infected cells by Northern blot analysis (Oberste et al, 1991).

Viral structural proteins

The Pr53 Gag precursor is synthesized first and is responsible for virus particle budding (Rasmussen et al, 1990). It is subsequently cleaved by a viral protease into the mature proteins p16 (matrix), p26 (capsid) and p7 (nucleocapsid) (Battles et al, 1992). The *pol* gene products are a protease, an RNA-dependent DNA polymerase (reverse transcriptase) and an integrase. They are derived by proteolytic cleavage from a Pr170 Gag-Pol precursor. A sequence in the *pol* region is associated with dUTPase activity in other lentiviruses such as equine infectious anemia virus, feline immunodeficiency virus and caprine arthritis encephalitis virus. It has an unknown function in the case of BIV (Garvey et al, 1990).

The virus envelope consists of a lipid bilayer derived from the plasma membrane of infected cells during the budding process. The gp100 (SU) and gp45 (TM) encoded by the *env* gene of BIV are inserted into this bilayer (Rasmussen et al, 1992).

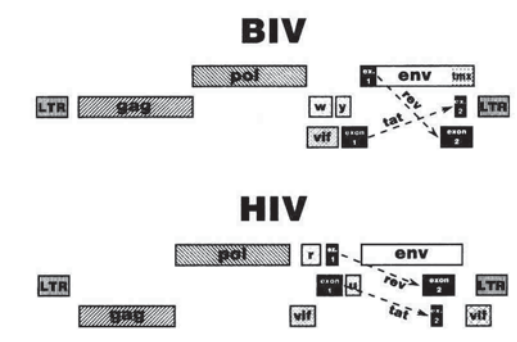

Fig 1. Genomic organization of bovine immunodeficiency virus (BIV) and human immunodeficiency virus 1 (HIV-1) proviral DNA. The rectangles correspond to open reading frames.

LTR and non-structural proteins

The U3 region of viral LTR contains transcription-factor binding sites (NFkB, Sp1, AP1, AP4) regulating viral replication and gene expression (Liu et al, 1992; Pallansch et al, 1992; Carpenter et al, 1993; Fong et al, 1995).

The Tat protein is found in the nucleus of infected cells where it transactivates viral LTR through a *trans*-activating region (TAR) resulting in an enhancement of virus expression. The Rev protein colocalizes with Tat in infected cells and presents the same positive effect on viral expression (Oberste et al, 1993).

The role of the other BIV accessory genes is presently unknown.

Genetic diversity

The two proviral molecular clones (BIV 106 and BIV127) were both derived from the original BIV R-29 field isolate. Their sequences exhibit a genomic variability of 1.7% with 75% of the substitutions occurring in the SU-coding region of the *env* gene (Garvey et al, 1990). DNA sequence analysis of new field isolates (Suarez et al, 1993) have demonstrated substantial genetic variation (7–8% nucleotide divergence in the conserved *pol* segment). This genetic diversity has been observed in all lentiviruses and probably plays a role in helping them to escape immune selection pressures and to new hosts. The characterization of this genetic diversity is critical for the development of diagnostic tests and the understanding of the viral pathogenic potential.

CONCLUSION

Bovine lentiviruses have been largely overlooked due to the absence of a test for

serodetection and their unknown pathogenicity. The recent availability of a diagnostic test based on the R-29 antigen provided evidence of seropositive animals all around the world. This leads to the following questions.

What is the real pathogenic potential of BIV?

The reported clinical manifestations are the following.

BIV-R29, initially isolated from a cow with a wasting condition, was able to induce in inoculated calves a lymphoproliferative reaction associated with neural disorders. In Florida (Suarez et al, 1993), two wild-type isolates were recovered from a group of cattle with the clinical manifestations of 'poor doers'. More recently, in Great Britain, some seropositive animals were found in a herd in which a few individuals exhibited a loss of weight and broncho-alveolar manifestations. However, evidence is lacking for a clear association between BIV infection and the reported clinical signs.

Lentiviruses, apart from BIV, have been reported to produce clinical syndromes characterized by a gradual and progressive debilitation, which in the case of HIV frequently leads to death. The incubation period varies, but in most cases, it takes several years for the disease to develop. Moreover, the devastating consequences of EIAV (equine infectious anemia virus), MVV (maedi visna virus), CAEV (caprine arthritis encephalitis virus) and HIV (human immunodeficiency virus) provide evidence for the pathogenesis of lentiviruses in general (Brownlie et al, 1994). By analogy, it would be exceptional if BIV were not to have a similar clinical syndrome, however it would be unrealistic to expect to see BIV pathogenesis before the completion of a three to five year incubation period.

Does BIV have variable virulence?

Due to the current low number of characterized BIV isolates, this question cannot be answered accurately. However, a spatial and temporal variation of lentiviruses in infected hosts has been well documented. After virus infection, some sort of virus-purifying selection occurs during the initial phases of replication in the newly-infected host, followed by rediversification into quasi-species. The progression to disease is then determined by complex interactions between the host, intercurrent factors, and the increasingly diverse viral population. It is not certain whether the burgeoning viral quasi-species as a whole or specific virulent members of the quasi-species are most critical to the progression of this disease. However, accumulating evidence from the oncovirus FeLV-FAIDS (Mullins et al, 1991) and from the simian lentiviruses SIV Pbj14 and SIV-Mac 239 (Dewhurst et al, 1990; Burns and Desrosiers, 1991) support the hypothesis that particular virulent variants are essential for disease induction. Therefore, by analogy, would it be safe to evaluate the pathogenicity of BIV after studying only one BIV isolate?

What is the distribution of BIV?

An understanding of BIV pathogenesis has been hampered in part by sparse information on the worldwide prevalence of BIV infection. At present, all of the serologic information on the presence of BIV infections has been obtained using the original R-29 isolate as antigen. Nonetheless, emerging data suggest that variants of BIV, which are antigenically distinct from BIV R-29 and only demonstrate immunologic cross-reactivity with the major capsid protein, are present in the eastern United States and Europe. This limited information emphasizes the need to isolate new BIV isolates in order to set up more specific diagnostic tests

and to obtain a more accurate estimate of BIV prevalence.

Is BIV a risk to human health?

Considerating BIV biology and phylogeny one may ask whether BIV enbodies a danger for human health. Several data support a negative answer. First, lentiviruses are highly specific in their host tropism. Moreover, injuries with contaminated material have been reported without inducing seroconversion. Finally, no cross-reactivity between sera from HIV-infected individuals and the BIV antigen was observed (Whetstone et al, 1992). In conclusion, there is currently no evidence to suggest the possibility of cross contamination.

Finally, BIV contains some peculiarities that emphasize the need to increase coordinated studies between practitioners and scientists in order to better understand the biology of this lentivirus.

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