



**HAL**  
open science

## Pathobiology of bovine leukemia virus

I Schwartz, D Lévy

► **To cite this version:**

I Schwartz, D Lévy. Pathobiology of bovine leukemia virus. *Veterinary Research*, 1994, 25 (6), pp.521-536. hal-00902257

**HAL Id: hal-00902257**

**<https://hal.science/hal-00902257>**

Submitted on 11 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Review article

## Pathobiology of bovine leukemia virus

I Schwartz, D Lévy \*

*URA-INRA d'Immuno-Pathologie Cellulaire et Moléculaire, École Nationale Vétérinaire d'Alfort,  
7, avenue du Général-de-Gaulle, 94704 Maisons-Alfort cedex, France*

(Received 16 March 1994; accepted 25 July 1994)

**Summary** — Bovine leukemia virus (BLV) is a retrovirus similar to the human T-cell leukemia virus (HTLV). Most BLV infected animals (70%) develop a B-cell lymphoproliferative syndrome with altered productive traits and 1 to 5% die with B-cell lymphosarcomas. Although BLV infection is world-wide, western European countries have almost eradicated it by slaughtering the seropositive animals. BLV infection remains endemic in many countries including the United States and prophylactic strategies involving recombinant vaccine vectors, genetically modified BLV and transgenic animals resistant to the infection are under study.

### **BLV / animal models / leukosis**

**Résumé** — **Pathobiologie du virus de la leucose bovine enzootique.** *Le virus de la leucose bovine enzootique (BLV) est un rétrovirus semblable au virus leucémogène humain HTLV. La plupart (70%) des bovins infectés par ce virus développent une prolifération lymphocytaire B accompagnée d'une diminution des performances zootechniques, et 1 à 5% des animaux meurent de lymphosarcome B. Bien que l'infection par le BLV soit mondialement répandue, les nations de l'Europe occidentale l'ont pratiquement éradiquée par abattage systématique des animaux séro-positifs. L'infection par le BLV reste cependant endémique dans de nombreux pays, comme les États-Unis. Des stratégies de prophylaxie faisant appel à la vaccination avec des vecteurs vaccine recombinants pour la protéine d'enveloppe, à des provirus BLV génétiquement modifiés et à des animaux transgéniques résistants sont en cours d'étude.*

### **BLV / modèles animaux / leucémie**

---

\* Correspondence and reprints

<b>INTRODUCTION</b> .....	523
<b>HISTORICAL BACKGROUND</b> .....	523
<b>BLV PATHOGENICITY</b> .....	524
<b>BLV TRANSMISSION</b> .....	525
<i>Natural transmission</i> .....	525
<i>Experimental transmission</i> .....	525
<b>SANITARY AND ECONOMIC IMPACT OF BLV INFECTION</b> .....	526
<b>PREVALENCE OF BLV INFECTION IN THE WORLD</b> .....	526
<i>In France</i> .....	526
<i>In Europe</i> .....	527
<i>In North America</i> .....	527
<i>In Africa</i> .....	527
<b>BLV VIRUS BIOLOGY</b> .....	527
<i>Viral structure</i> .....	527
Viral genome .....	527
Viral mRNAs .....	528
Viral structural proteins .....	528
Gag, pol and prt orf products .....	528
Env orf products .....	529
<i>Regulation of BLV expression</i> .....	529
Activation of BLV expression by the Tax protein .....	529
Regulation of BLV expression by the Rex protein .....	530
RIII and GIV proteins .....	530
Lymphocyte activation and viral synthesis .....	530
<i>BLV and cell transformation</i> .....	531

**PROPHYLAXIS OF BLV INFECTION AND PATHOGENICITY** ..... 532

*Vaccination with env recombinant vaccine*..... 532

*Vaccination with a non-pathogenic BLV*..... 532

*Selection of naturally resistant animals* ..... 532

*Construction of resistant transgenic animals*..... 533

**CONCLUSION**..... 533

**REFERENCES** ..... 534

**INTRODUCTION**

The bovine leukemia virus (BLV) is a cattle retrovirus responsible for bovine enzootic leukemia. Its genetic structure, sequence and pathogenicity present many similarities to those of the human T-cell leukemia viruses (HTLV-1 and 2). Seventy percent of the infected cattle present a chronic increase in the number of circulating B lymphocytes, which can lead to a haematological status called persistent lymphocytosis, and 1 to 5% of the infected cattle develop a B-cell lymphosarcoma (Burny *et al*, 1988). BLV infection involves a large number of cattle around the world and the prevalence is high on the American continent, in Australia, Africa, and Asia. As herd contamination follows the introduction of an infected animal, many European countries have decided to eradicate the infection by applying strict sanitary measures. Nowadays lymphosarcoma cases are rarely found in most western European countries.

Except for the rare lymphosarcoma lesions, the impact of BLV on bovine health and productivity is not well known and still raises many questions. Our purpose here is to review the epidemiological, pathological

and molecular knowledge about BLV, and to stress the biological aspects which provide us with an original and powerful model for studying and fighting retroviral diseases.

**HISTORICAL BACKGROUND**

The first reported case of bovine leukemia was described in 1871 in the Klaipeda area, Lithuania, and mentioned a cow with superficial lymph node hypertrophy and splenomegaly (Burny *et al*, 1980). Thereafter other cases were reported, indicating that the disease was propagating west and it eventually reached the USA. In 1917, Kenneth showed that the disease was mediated by a contagious agent (Burny *et al*, 1980). In 1969, Miller *et al* discovered that lymphocytes of cows with persistent lymphocytosis produced viral particles that were visible by electron microscopy after *in vitro* culture (Miller *et al*, 1969). In 1976, Kettmann *et al* showed that BLV particles are RNA exogenous viruses and carry a RNA reverse transcriptase complex; this finding allowed the classification of BLV amongst the oncogenic retroviruses (Kettmann *et al*, 1976).

## BLV PATHOGENICITY

BLV induces a persistent and chronic infection that affects essentially the B lymphocyte population (Kettmann *et al*, 1980; Levy *et al*, 1987). The infection can be transmitted by the cellular components of infected bovine blood, by infected cultured cells, or by free viral particles produced in cell culture (Burny *et al*, 1980). Viraemia is only detectable during the first 2 weeks of infection (Portetelle *et al*, 1978). Thereafter the expression of viral antigens is difficult to evidence but it can sometimes be detected in the intrafollicular and margin zones of lymph nodes using immunocytochemistry (Heeney *et al*, 1992). Cattle develop a serological response to capsid and envelope proteins 2–8 weeks after inoculation (Burny *et al*, 1980). These antibodies are lifelong and show important level variations that probably relate to the physiological and immune status of the animal. Antibodies to viral regulatory proteins (the Tax and Rex proteins) can also be detected in about 20% of infected cattle (Powers *et al*, 1991).

In the years following infection, 70% of infected animals show an increase in the B/T lymphocyte ratio in the blood, with a seemingly normal total number of lymphocytes (4 000 to 10 000/mm<sup>3</sup>) (Lewin, 1989). Among these animals, half develop a persistent lymphocytosis, which corresponds to a stable increase in the number of circulating lymphocytes, sometimes reaching up to 100 000/mm<sup>3</sup> (Lewin, 1989). The integration of the BLV genome is found in 30% of the circulating lymphocytes and concerns many clones of lymphocytes (Kettmann *et al*, 1980). The expansion of the lymphocyte population results from the polyclonal proliferation of mature B lymphocytes which are cytologically and karyotypically normal (Grimaldi *et al*, 1983). Most of these lymphocytes carry the CD5, CD11b and CD11c markers (Letesson *et al*, 1991). In mice, it seems that the CD5<sup>+</sup> B-cells constitute a B-

lymphocyte population of a different lineage from the CD5<sup>-</sup> B-cells that could be involved in the synthesis of autoantibodies (Hayaçawa *et al*, 1985). This type of lymphocyte is also found in chronic lymphoid leukemia in humans (De la Hera *et al*, 1988). Are CD5<sup>+</sup> B-lymphocytes the preferential target for BLV infection? Using fluorescence activated cell sorting, we showed that BLV is found in CD8<sup>+</sup> T-lymphocytes, monocytes, granulocytes, and mostly in B-lymphocytes, with no preference for the CD5<sup>+</sup> B-cell population (Schwartz *et al*, 1994). The parameters involved in the sensitivity of the CD5<sup>+</sup> B-lymphocytes to the lymphoproliferative potential of BLV remain to be determined.

The ultimate and rare expression of BLV infection is the emergence of a multicentric lymphosarcoma that occurs 1–8 years after infection, in about 1–5% of the animals (Burny *et al*, 1980; Lewin, 1989). Two-thirds of the animals with tumors have had a persistent lymphocytosis (Burny *et al*, 1980). The tumors may affect 1 or several superficial and/or deep lymph nodes (Burny *et al*, 1980). In a single animal, tumors are mostly monoclonal for BLV integration and the virus does not seem to display preferential integration sites among tumors from different animals (Kettmann *et al*, 1983). According to some authors, the tumor cells are pre-B-lymphocytes that are characterized by the lack of expression of the immunoglobulin light chain (Van den Broecke *et al*, 1988); for others, they are mature B-lymphocytes showing an isotypic commutation in the immunoglobulin gene (Heeney and Valli, 1990). Most often, the CD5 marker is expressed on the tumor cells (Depelchin *et al*, 1989; Letesson *et al*, 1991). This tumoral form of BLV only affects adult cattle that are over 2 years of age, with an incidence peak around the age of 5–8 years (Lewin, 1989). This has to be distinguished from the bovine leukemia sporadic cases that affect animals under 1 year of age and that are not associated with BLV infection.

## BLV TRANSMISSION

### *Natural transmission*

In natural conditions, only cattle, zebu, buffalo and capybaras have been found to be infected (Burny *et al*, 1980).

The virus is transmitted by infected lymphocytes. About 1 500 lymphocytes from a cow with persistent lymphocytosis (0.1  $\mu$ l of blood) are sufficient to infect another animal (Mammerickx *et al*, 1988). Most of the time, contamination is iatrogenic and occurs when the animals are manipulated without the respect of hygiene recommendations for injections, horning, tattooing or rectal examinations (Burridge and Thurmond, 1981). In some areas, transmission by biting insects such as *Tabanides* has been documented (Oshima *et al*, 1981). Bronchial secretions containing infected lymphocytes can be a source of infection especially when the animals are maintained in crowded housing (Burridge and Thurmond, 1981). BLV does not seem to be sexually transmissible (Valikhov *et al*, 1984). Calves can be infected during the second half of gestation, mainly when the mother is in lymphocytosis (Burridge and Thurmond, 1981). Although potentially virulent, the colostrum is a minor source of infection because the anti-BLV antibodies it contains protect it (Van der Maaten *et al*, 1981).

### *Experimental transmission*

Sheep have often been preferentially used for experimental infection with BLV. Indeed, sheep are highly sensitive to BLV infection with infected bovine lymphocytes or with viral particles (Mammerickx *et al*, 1988). Sheep develop lymphosarcomas in more than 90% of the cases in the 6 months to 7 years following infection, depending on the

viral load at the time of infection (Mammerickx *et al*, 1988). It seems that the higher sensitivity of sheep to BLV pathogenicity is related to a higher permissiveness of ovine cells to viral replication (Burny *et al*, 1980). Ovine intraspecies transmission is extremely rare in natural conditions and are cases of *in utero* or accidental transmission (Burny *et al*, 1980).

Goats are sensitive to BLV infection, but only 2 out of 24 infected animals developed lymphoid tumors in 10 years (Burny *et al*, 1980).

BLV infection in rabbits is an interesting experimental model as rabbits develop an immunodeficiency syndrome 45–763 d after infection (Altanerova *et al*, 1989). After experimental infection, antibodies to capsid protein appear in 3 weeks and the integrated provirus is detectable in peripheral blood leukocytes (Altanerova *et al*, 1989).

In chicken infected at birth, leukemia development was observed in 4 chickens out of 88 and BLV genome could be detected in the tumoral cells although there was no detectable seroconversion (Altanerova *et al*, 1990).

Other species, such as rat, pig, rhesus monkey and chimpanzee have been found to develop antibodies following experimental infection, but the integration of BLV provirus in host cells was not investigated (Burny *et al*, 1980). Therefore, one does not know whether the seroconversion represents an immune response to a replicative infection or a serological reaction to the introduction of viral antigens.

According to serological studies, humans do not seem to be sensitive to BLV infection (Oshima *et al*, 1981). However, human cells are sensitive to BLV infection *in vitro* (Derse and Martarano, 1990). Besides, hybridization with BLV-derived genomic sequences was found in 3 cases of human myeloma (Slavikova *et al*, 1987), and antibodies to BLV capsid proteins were found in

cases of multiple sclerosis (Clausen *et al*, 1990). However we should be careful in interpreting these results because different viruses can show crossed genetic and serological reactivities (Maruyama *et al*, 1989; Battles *et al*, 1992). More extensive epidemiological studies should be undertaken in order to definitively rule out the risk of infection for consumers and professionals.

### **SANITARY AND ECONOMIC IMPACT OF BLV INFECTION**

BLV is not very pathogenic or infectious in natural conditions. Only minor economical losses are related to BLV-induced tumors. However, the average dairy production of animals with persistent lymphocytosis is 3–10% lower than that of the herd and it can therefore lead to early culling (Brenner *et al*, 1989; Da *et al*, 1993). Moreover, cattle with lymphocytosis seem less able to get rid of some herd infections (Brenner *et al*, 1989), and losses linked to carcass removal when tumors are discovered can be dramatic at the herd level. In the United States, the economic loss due to BLV infection was estimated to 86 million dollars in 1992 (Da *et al*, 1993).

Considering the transmission mode of the virus, it is theoretically possible to eradicate the infection using sanitary measures. Denmark (1959) and Germany (1963) began a sanitary fight against BLV infection (Burny *et al*, 1980). In 1981, France had to proceed similarly for the sake of EEC commercial exchanges, although seizures for lymphosarcoma were only 2.5 per 100 000. Anyhow, south-western and north-eastern France were particularly affected regions, with 25.4% of seropositive animals (55% of the herds) in the Landes area. The tumoral form of bovine leukemia was declared as a "legally contagious reputed disease" in the 8 May 1981 decree. This implies the systematic culling and removal

of carcasses with tumoral lesions and the marking of seropositive animals with culling for consumption within 6 months. At that time, farms were also offered a quality label "livestock free of enzootic bovine leukemia". In 1988, measures against BLV were intensified with a systematic screening for seropositive animals. In order to encourage breeders to get rid of their seropositive animals, financial grants were given as an allowance of 2 450 French francs per animal culled within the month following its identification. Overall these measures cost 394 million francs in 1988. This eradication program was expensive but, in the long run, it was necessary in order to facilitate the sale of French cattle on the European market.

### **PREVALENCE OF BLV INFECTION IN THE WORLD**

Commercial exchanges of reproductive cattle led to the spread of the infection around the world. However, the seroepidemiological status of the infection remains unknown in a large number of countries. Moreover, regarding the way the virus is transmitted, the infection unevenly affects different regions within a country, and different farms within a region.

#### ***In France***

The mean infection rate was 0.075% in 1992, whereas it was 2% in 1981 (*Bulletin Épidémiologique Vétérinaire*, 2078, June 1993, Ministère de l'Agriculture et de la Pêche, Paris, France). However, great variations remain between regions. In 1992, the infection rates of the eastern and south-western French livestock were over 5%. Therefore, even though the BLV sanitary situation concerning BLV has very much improved since 1981, French cattle live-

stock is not free of enzootic bovine leukemia yet. In 1992, 10 928 infected cattle were culled during the seroepidemiological screenings and only 2 animals showed lymphosarcoma lesions (*Bulletin Épidémiologique Vétérinaire, 2078, June 1993, Ministère de l'Agriculture et de la Pêche, Paris, France*).

### **In Europe**

Several European countries established fighting programs against BLV. BLV infection has been eradicated in Belgium, Ireland, Luxembourg and Norway and is on its way to eradication in other western European countries. It does exist as an enzootic form in eastern European countries such as Albania, Bulgaria, Yugoslavia and Poland, especially in the Baltic countries (Latvia, Estonia, Lithuania) (*Santé Animale Mondiale en 1992, Office International des Epizooties, Paris, France*).

### **In North America and Australia**

United States, Canada and Australia are strongly affected by BLV infection. In USA and Canada, 66% of the dairy herds (10.2% of the animals) and 14% of the meat herds (1.2% of the animals) are infected by the virus and there are important variations between states or provinces (Da *et al*, 1993). In North America, bovine leukemia cases do not have to be declared.

In Queensland, Australia, the prevalence of the infection in the dairy livestock is 13.7% (*Santé Animale Mondiale en 1992, Office International des Epizooties, Paris, France*). A sanitary program has been established. In New Zealand, 2.4% of the herds are infected (*Santé Animale Mondiale en 1992, Office International des Epizooties, Paris, France*).

### **In Africa**

Some African countries have estimated the BLV infection rate and came up with a rather high prevalence: 37% in West Africa (Walrand *et al*, 1986), 10% in Guinea, 20% in Zimbabwe, and 50% in some great farms in Malawi (*Santé Animale Mondiale en 1992, Office International des Epizooties, Paris, France*).

BLV infection is thus widely spread around the world. Prophylaxis by simple sanitary measures based on culling of the seropositive animals allowed a dramatic reduction in the infection rate in western Europe. However, when the infection is endemic and affects a majority of animals, culling is not applicable and other protection measures have to be considered.

## **BLV VIRUS BIOLOGY**

BLV is a type C retrovirus which belongs to the Oncovirinae family. BLV is very similar to human retroviruses HTLV-1 and -2 in terms of its genetic structure, sequence and regulation of expression.

### **Viral structure**

#### **Viral genome**

The complete sequence of the integrated BLV is 8 714 nucleotides long (Sagata *et al*, 1985). Like all retroviruses, the extremities of the integrated BLV present a sequence called LTR (long terminal repeat) which is composed of 3 consecutive regions named U3, R and U5. The U3 region is the most 5' region and includes transcriptional regulatory sequences. The viral mRNAs are initiated at the first base of the R region. At least 7 alternately spliced RNAs have been identified as well as 8 open reading frames



(orf) designated as *gag*, *prt*, *pol*, *env*, *tax*, *rex*, *RIII* and *GIV* (Alexandersen *et al*, 1993). The 3' U3 region includes the polyadenylation signals and the 3' extremity of the R region corresponds to the polyadenylation site (fig 1).

### Viral mRNAs

Transcription of BLV genome leads to at least 7 viral mRNAs obtained by alternate splicing (fig 1). The unspliced mRNA encodes for the capsid proteins, the reverse transcriptase and the viral protease. A monospliced viral RNA codes for the envelope glycoproteins, and a doubly spliced RNA codes for 2 transcription regulatory proteins called Tax and Rex. The Tax protein stimulates the initiation of transcription, whereas the Rex protein favors the stabilization and the processing of the monospliced and the unspliced viral mRNAs and thus regulates the synthesis of the viral structure proteins. Two other mRNAs present orf encoding for 2 novel proteins whose role have yet to be defined, the RIII and GIV proteins.

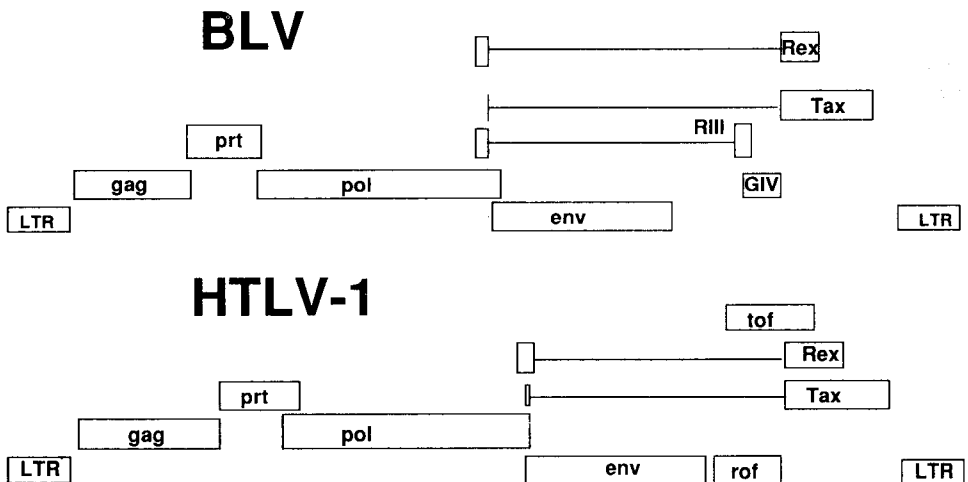
### Viral structural proteins

BLV viral particles are constituted of 2 viral RNAs and of structural proteins that include the capsid proteins, the envelope proteins, the reverse transcriptase and the viral protease encoded from the *gag*, *env*, *pol* and *prt* orf respectively.

### Gag, pol and prt orf products

The capsid proteins, reverse transcriptase and protease are obtained after cleavage of 3 different protein precursors initiated from a common AUG located at the 5' end of the *gag* region. These 3 precursors are made from 3 different reading frames, and are the result of a 1 or 2 nucleotide ribosomal shift during translation (Hatfield *et al*, 1989).

The proteins encoded by the *gag* gene are involved in the formation of the viral capsid (Yoshinaka *et al*, 1986). A 45 kDa precursor is first synthesized and is subsequently cleaved by the viral protease in the mature proteins p15, p24 and p12. The p24 is the main capsid protein. The p12 constitutes the nucleocapsid and is associated with 2 viral RNAs. The p15 is a myristilated



**Fig 1.** Genomic organization of bovine leukemia virus (BLV) and human T-cell leukemia virus type 1 (HTLV-1). The rectangles correspond to open reading frames.

protein and is associated both to the capsid proteins and to the lipid bilayer of the viral membrane (Yoshinaka *et al*, 1986).

The mature *pol* product (70 kDa) is produced from a 145 kDa precursor. It presents a RNA-dependent DNA polymerase activity (reverse transcriptase) at its N-terminal portion and an RNAase H activity at its C-terminal portion. The *pol* product also has an integrase activity (Burny *et al*, 1980).

The *prt* is synthesized from a 65 kDa precursor which leads after cleavage to a 14 kDa dimeric aspartic protease. It performs the cleavage of the *gag*, *prt*, *pol* and *env* precursors (Katoh *et al*, 1989a).

#### *Env orf products*

The mature gp51 and gp30 glycoproteins are obtained from the cleavage of a 72 kDa glycosylated precursor by the viral protease. Gp51 is found at the surface of the viral membrane and ensures the recognition of the cellular viral receptor (Vonèche *et al*, 1992a). The N-terminal segment of gp30 is inserted in an oblique orientation into the lipid bilayer and anchors the gp51/gp30 complex in the viral envelope and the infected cell membrane (Vonèche *et al*, 1992b). The gp30/gp51 association helps membrane fusion during infection of the host cell and syncytia formation (Voneche *et al*, 1992a).

The gp30 intracytoplasmic portion presents a motif (tyrosine X X leucine), which is found in the signal transduction molecules of the T cell receptor ( $\zeta$  chain) and of the B cell receptor (Ig $\alpha$  and Ig $\beta$  chains). The stimulation of a chimeric molecule made of the CD8 extracytoplasmic portion and the gp30 intracytoplasmic domain with anti-CD8 antibodies induces the phosphorylation of cellular proteins and a calcium influx into the cell (Alber *et al*, 1993). Thus, gp30 may have a signalling function in the infected cell and anti-BLV envelope antibodies may be able to stimulate gp30 and dysregulate the infected cell proliferation.

Finally, unlike lentiviruses, envelope proteins, the BLV gp30 and gp51 are well conserved among the different variants studied. The comparison between the env nucleotide sequences of 7 European, American and Japanese BLV isolates showed a variability lower than 6% (Mamoun *et al*, 1990) whereas the nucleotide variability reaches around 66% between the env proteins of HIV-1 isolates (Goudsmit *et al*, 1991).

#### **Regulation of BLV expression**

The 3' portion of the BLV genome, formerly called the pX region, codes for at least 4 proteins, the Tax, Rex, RIII and GIV proteins; it is actually well documented that the Tax and Rex proteins participate in the regulation of BLV expression.

#### **Activation of BLV expression by the Tax protein**

BLV Tax protein is a 34 kDa nuclear phosphoprotein which transactivates the initiation of RNA synthesis from the U3 region of the viral LTR (Katoh *et al*, 1989b). The U3 region includes three 21 base pair (bp) elements that are responsive to Tax transactivation (Derse, 1987). These 3 elements are strongly homologous and include an 8-bp core, which is homologous to the cAMP-responsive element (CRE). A mutation in the CRE in the BLV LTR abolishes transactivation by the Tax protein (Katoh *et al*, 1989b). In addition, a cell nuclear factor called CRE-binding-protein-2 (CREB2) has been shown to bind to the 21 bp regions and to be implicated in the transactivation of the BLV LTR (Willems *et al*, 1992b).

The U3 region of HTLV-1 also includes three 21 bp repeats centered on CRE, which are homologous to that found in the BLV LTR; these are essential for HTLV-1 transactivation by the HTLV-1 Tax protein as well

as for promoter basal activity (Jeang *et al*, 1988). Neither of the HTLV-1 or BLV Tax proteins are bound directly to DNA; this implies that cellular nuclear factors are needed to mediate transactivation (Jeang *et al*, 1988). In the case of HTLV-1, the Tax protein makes a nuclear complex with CREB and with activating transcription factor-2 (ATF-2) and increases CREB/ATF-2 affinity for CRE in the 21 bp units but not for the other CREs, indicating that the CRE flanking nucleotides are important in Tax transactivation activity (Franklin *et al*, 1993). The CRE flanking nucleotides may also govern the specificity of the Tax proteins for their respective LTR as the Tax protein of BLV is unable to transactivate HTLV-1 LTR and *vice versa* (Derse, 1987).

#### **Regulation of BLV expression by the Rex protein**

Rex is a 18 kDa nuclear phosphoprotein which is involved in the post-transcriptional control of BLV expression. The Rex protein allows the accumulation of unspliced or monospliced viral RNAs, probably by enforcing their stability (Derse, 1988). A 250-bp sequence located between the polyadenylation signal and the polyadenylation site in the 3' R region of the LTR, is necessary for Rex function (Derse, 1988). This sequence, called Rex responsive element (RRE), as well as its secondary structure, present great homologies with the equivalent HTLV-1 structure. Interestingly the HTLV-1 Rex protein can replace the BLV Rex protein and *vice versa* (Felber *et al*, 1989). In the absence of Rex protein, only doubly spliced RNAs are synthesized, which implies that the expression of virion proteins is conditioned by Rex protein.

#### **RIII and GIV proteins**

The study of BLV mRNA differential splicing reveals the existence of 2 mRNAs coding

for 2 novel proteins, the RIII and GIV proteins (Alexandersen *et al*, 1993). The expression level of the GIV RNA correlates with the development of persistent lymphocytosis. However these 2 proteins have not been detected *in vivo* and their role is still under study. Using expression vectors for GIV and RIII in cell transfection experiments, it could be shown that GIV weakly transactivates BLV LTR activity and potentiates Rex protein function whereas RIII inhibits Rex protein function (S Alexandersen, Workshop on the Pathogenesis of Animal Retrovirus, La Rochelle, France, 1993).

#### **Lymphocyte activation and viral synthesis**

*In vivo* mRNAs coding for Tax and/or Rex proteins are easily detectable by the highly sensitive technique of reverse transcription followed by polymerase chain reaction (RT-PCR) (Haas *et al*, 1992). Unspliced or monospliced viral mRNAs are absent or weakly expressed, a finding that fits with the paucity of virus expressing cells detected by immunocytochemistry (Jensen *et al*, 1990). BLV infection is therefore rarely replicative *in vivo* and the mechanisms that regulate this viral latency are still elusive. However a non-immunoglobulin plasma factor absent from serum has been shown to participate in the inhibition of the viral synthesis *in vivo* (Gupta *et al*, 1984).

The culture of infected lymphocytes from sheep or cattle with lymphocytosis unblocks the viral synthesis after 6 h incubation at 37°C (Cornil *et al*, 1988). In our laboratory, we have shown that BLV structural protein synthesis is strongly stimulated following the activation of T lymphocytes by lectins such as concanavalin A or phytohaemagglutinin A (Djilali *et al*, 1987; Cornil *et al*, 1988). This stimulating effect is mimicked by the supernatant of activated lymphocyte cultures which indicates that cytokines are implicated in the stimulation of viral synthe-

sis (Cornil *et al*, 1988). The identity of the cytokine(s), as well as the cell type responsible for virion synthesis (B cells, CD8<sup>+</sup> T-cells, monocytes or granulocytes) are unknown (Djilali *et al*, 1987).

### **BLV and cell transformation**

The mechanism by which BLV leads to tumoral development has not yet been elucidated. No rearrangement of any known oncogene (*c-myc*, *c-myb*, *c-erbA*, *c-erbB*, *c-src*, *c-Ha-ras*, *c-fos* and *c-sis*) could be evidenced in BLV-induced tumors (Kettmann *et al*, 1983). Moreover, BLV does not show any clear preferential integration site between tumors, a finding which does not support the insertional mutagenesis hypothesis (Kettmann *et al*, 1983). However, a number of experimental observations indicate that BLV Tax protein, as well as HTLV-1 Tax protein, can act as oncogenes. Transfection of BLV Tax together with the *Ha-ras* oncogene renders rat embryo fibroblasts tumorigenic in nude mice (Willems *et al*, 1990). Moreover, mRNAs coding for Tax (and/or Rex) are detectable in animals with persistent lymphocytosis or with tumors whereas mRNAs coding for structural proteins are not (Haas *et al*, 1992). The transactivating protein Tax is therefore the most probable viral element involved in lymphocyte transformation. However, its expression is not always encountered in advanced tumoral stages (Sagata *et al*, 1985); Tax could therefore play a role in the initiation of tumorigenesis but does not seem to be essential in the maintenance of the tumoral stage.

In the case of transformation with HTLV-1, a number of cellular genes transactivated by Tax have been identified, such as the interleukin-2 receptor (Inoue *et al*, 1986), the granulocyte/macrophage-colony stimulating factor (Nimer *et al*, 1989), vimentin

(Lilienbaum *et al*, 1990), the transforming growth factor  $\beta$  (Kim *et al*, 1990). The transactivation of cellular genes is proposed in order to explain the lymphocyte perturbations induced by HTLV-1. The BLV Tax protein can transactivate the *c-fos* and somatostatin gene promoters (Kato *et al*, 1989b). Recent studies have also shown that the Tax transactivating domain (a zinc finger region) is different from the domain involved in cell transformation (Willems *et al*, 1992a). Therefore, the way in which BLV Tax protein leads to oncogenesis may involve a function other than transactivation. For instance, viral antigens, such as the SV40 T antigen (Fanning and Knippers, 1992), the adenoviral E1A antigen (Whyte *et al*, 1989) and human papillomavirus E7 (Dyson *et al*, 1989), bind to cellular proteins and modulate or abrogate their functions. The cellular proteins that physically associate with Tax during cell transformation are actively sought and may reveal the existence of new molecules involved in cell transformation.

Although Tax is most probably involved in the development of BLV-induced lymphoproliferative syndrome, other viral proteins namely Rex, GIV, RIII and even env proteins, are also likely to play a role in BLV pathogenicity. The Rex protein of BLV may disturb cellular mRNAs stability, as the Rex of HTLV-1 increases the IL-2 receptor mRNA half-life (White *et al*, 1991). The env protein may transduce intracellular signals after stimulation by anti-env antibodies (Alber *et al*, 1993). The possible role of RIII and GIV proteins in BLV pathogenicity must be investigated, especially for GIV whose mRNA level is high during persistent lymphocytosis (Alexandersen *et al*, 1993). Therefore, lymphocyte dysregulation induced by BLV infection potentially implicates several viral molecules and a lot has still to be done in order to delineate the specific role of each of them.

## PROPHYLAXIS OF BLV INFECTION AND PATHOGENICITY

As mentioned above, it is important to develop fighting strategies against BLV in countries where the spread of the infection is too high to undertake a sanitary prophylaxis. Moreover, BLV represents a good animal model for studying the prophylaxis of retrovirus diseases. Indeed, its complex genetic structure is analogous to the one of primate retroviruses, and its pathogenicity in sheep is reproducible, frequent and easily detectable within delays suitable to experimentation. Classical vaccination strategies have been tested. Innovative strategies *via* molecular targeting of the viral regulatory proteins are under study and are discussed below.

### ***Vaccination with env recombinant vaccine***

The humoral immune response to BLV is protecting since serum immunoglobulins of infected sheep prevent infection of healthy sheep in an intradermal challenge with infected lymphocytes (Kono *et al*, 1986). A *env* recombinant vaccine vector was constructed and tested by a Belgian team (Portetelle *et al*, 1991, 1993). The protection was tested in sheep with an intravenous challenge of infected lymphocytes 6 weeks after vaccinal boost. The protecting vector codes for the gp51 and gp30 proteins. On the other hand, immunization with vectors coding for gp51 or gp30 alone does not induce neutralizing antibodies and is not protective. The immune memory induced by this vaccine remains to be studied.

### ***Vaccination with a non-pathogenic BLV***

This strategy relies on the construction of infectious, immunogenic, but non-pathogenic

BLVs. BLV mutants with deletions in various genes have been constructed (Willems *et al*, 1993). Since BLV proviruses injected intradermally in cationic liposomes are infectious in sheep, the infectious and pathogenic potentials of mutant proviruses could be tested (Willems *et al*, 1993). The infectious potential is altered for *gag*, *pol* and *env* mutated proviruses but not for mutants in RIII and GIV. The pathogenic potential of RIII and GIV mutants remains to be established. In case these mutants are not pathogenic, they may be used as infectious but protective vectors against a wild type virus.

For the same purpose, a chimeric provirus made of BLV *gag*, *pol* and *env* genes under the control of heterologous LTRs derived from the murine spleen necrosis virus was constructed and turned out to be replicative *in vitro* (Temin, 1993). The infectivity and protection conferred by this chimeric virus devoid of regulatory proteins remain to be tested.

### ***Selection of naturally resistant animals***

Immunogenetic studies have shown that class-1 major histocompatibility complex alleles are associated with resistance or sensitivity to the development of a persistent lymphocytosis, depending on the race of the cattle. For instance, in Holstein herds, a relative sensitivity to persistent lymphocytosis is associated with the BoLA w8-1 allele (Lewin *et al*, 1988). On the other hand, in Shorthorn herds, it was found to be associated with the BoLA DA 12-3 allele (Lewin, 1986). However the development of persistent lymphocytosis is strongly associated with the DRB2 and DRB3 alleles of the class-2 major histocompatibility complex (Van Eijk *et al*, 1992; Xu *et al*, 1993). These reports indicate that major histocompatibility complex genes are associated with sensitivity to BLV, but more extensive

studies are needed to find the genes directly responsible. Information on these genes may have agronomic applications shedding light on genetic resistance to retroviral infections and/or cancer development.

### **Construction of resistant transgenic animals**

Today transgenesis in cattle is possible. Constructing transgenic animals that express molecules aimed at conferring resistance to BLV is conceivable. In this perspective, a ribozyme that cleaves the Tax/Rex coding RNA has been constructed (Cantor *et al*, 1993). Ribozymes are complementary RNA sequences to a target RNA and are made of a hammer-shaped region endowed with a catalytic activity. An efficient ribozyme to the RNA coding for BLV Tax and Rex proteins was shown to reduce the reverse transcriptase activity by 92% in a BLV-infected bat lung cell line (Cantor *et al*, 1993). When expressed constitutively in transgenic animals, such a ribozyme would prevent the accumulation of Tax and Rex coding RNAs from the very beginning of infection and would thus interfere with Tax transactivation. Consequently, the virus infection of the neighboring sensitive cells would be considerably slowed down or even abrogated and the transgenic animals may then control the infection and resist to the virus pathogenicity.

In a similar perspective, transdominant mutants for HTLV-1 Tax and Rex proteins have also been obtained (Rimsky *et al*, 1989; Gitlin *et al*, 1991). These mutants are inactive in transactivation and they inhibit wild type Tax and Rex transactivation, probably by interfering with their transport to the nucleus. Isolation of such BLV Tax and Rex mutants could be very interesting for constructing transgenic BLV-resistant animals. Transgenic cattle would constitutively express transdominant mutant Tax and Rex

proteins, which would quench the wild type Tax and Rex proteins, thereby blocking viral replication. These cattle would then be resistant to the propagation and pathogenicity of the virus.

It is clear that such transgenic animals would only be experimental animals that would help to evaluate the efficiency of these "gene prophylaxis" molecules. Beside the cost of these transgenic constructions, their introduction to the field is not easy. It is necessary to keep in mind the potential risks inherent to exogenous gene expression in animals, because the sanitary and zootechnical consequences are difficult to predict. Nevertheless, their introduction in some American farms where the infection is widespread could be seriously considered.

### **CONCLUSION**

BLV infection is widespread throughout the world and it significantly affects cattle zootechnical performances without any dramatic health damage. However, we still do not know whether BLV potentiates other herd infection symptomatology, especially in the USA and in Africa where BLV infection is endemic. In any case, it would be interesting to propose a cheap prophylaxis related to the BLV-associated loss of income. For the time being, vaccination strategies are still under trial and are not yet applicable on a large scale.

Furthermore, BLV infection in sheep turns out to be an excellent experimental model for studying the complex primate retroviruses, as no equivalent model can be proposed with the murine or avian retroviruses whose genomic structures are much simpler. BLV pathogenicity is reproducible and readily detectable in sheep, an animal easily amenable to experimentation. It is also a good model for analyzing the molecular events involved in cell transformation and tumor progression. However many ques-

tions remain open. Why are only CD5+ B sensitive to the lymphoproliferative potential of BLV *in vivo*? What is the real impact of the Tax protein on lymphocyte dysregulation? With which molecules does it interact? The answers to these many questions will allow us to unveil the cellular molecules that are essential for the control of lymphocyte homeostasis.

## ACKNOWLEDGMENT

The authors are especially grateful to B Schwartz for critical reading of the manuscript.

## REFERENCES

- Alber G, Kim KM, Weiser P, Reisterer C, Carsetti R, Reth M (1993) Molecular mimicry of the antigen receptor signalling motif by transmembrane proteins of the Epstein-Barr virus and the bovine leukemia virus. *Current Biology* 3, 333-338
- Alexandersen S, Carpenter S, Christensen J *et al* (1993) Identification of alternatively spliced mRNAs encoding potential new regulatory proteins in cattle infected with bovine leukemia virus. *J Virol* 67, 39-52
- Altanerova V, Ban J, Altaner C (1989) Induction of immune deficiency syndrome in rabbits by bovine leukemia virus. *AIDS* 3, 755-758
- Altanerova V, Ban J, Kettmann R, Altaner C (1990) Induction of leukemia in chicken by bovine leukemia virus due to insertional mutagenesis. *Arch Geschwulstforsch* 60, 89-96
- Battles JK, Hu MY, Rasmussen L, Tobin GJ, Gonda MA (1992) Immunological characterization of the *gag* gene products of bovine immunodeficiency virus. *J Virol* 66, 6868-6877
- Brenner J, Van Haam M, Savir D, Trainin Z (1989) The implication of BLV infection in the productivity, reproductive capacity and survival rate of a dairy cow. *Vet Immunol Immunopathol* 22, 299-305
- Burny A, Bruck C, Chantrenne H *et al* (1980) *Bovine Leukemia Virus: Molecular Biology and Epidemiology*, Raven Press, New York
- Burny A, Cleuter Y, Kettmann R *et al* (1988) Bovine leukemia: facts and hypotheses derived from the study of an infectious cancer. *Adv Vet Sci Comp Med* 32, 149-170
- Burridge MJ, Thurmond MC (1981) An overview of modes of transmission of bovine leukemia virus. *In: Proc 85th Ann Meet US Anim Hlth Ass*, 165-169
- Cantor GH, McElwain TF, Birkebak TA, Palmer GH (1993) Ribozyme cleaves *rex/tax* mRNA and inhibits bovine leukemia virus expression. *Proc Natl Acad Sci USA* 90, 10932-10936
- Clausen J, Hoff-Jorgensen R, Rasmusen HB (1990) Antibody reactivity against animal retrovirus in multiple sclerosis. *Acta Neurol Scand* 81, 223-228
- Cornil I, Delon P, Parodi AL, Lévy D. (1988) T-B-cell cooperation for bovine leukemia virus expression in ovine lymphocytes. *Leukemia* 2, 313-317
- Da Y, Shanks RD, Stewart J, Lewin HA (1993) Milk and fat yields decline in bovine leukemia virus-infected Holstein cattle with persistent lymphocytosis. *Proc Natl Acad Sci USA* 90, 6538-6541
- De la Hera A, Alvarez Mon M, Sanchez Madrid F, Martinez C, Durantez A (1988) Co-expression of Mac-1 and p150,95 on CD5+ B cells. Structural and functional characterization in a human chronic lymphocytic leukemia. *Eur J Immunol* 18, 1131-1134
- Depelchin A, Letesson JJ, Lostrie Trussart N, Mamerickx M, Portetelle D, Burny A (1989) Bovine leukemia virus (BLV)-infected B-cells express a marker similar to the CD5 T cell marker. *Immunol Lett* 20, 69-76
- Derse D (1987) Bovine leukemia virus transcription is controlled by a virus encoded *trans*-acting factor and by *cis*-acting response elements. *J Virol* 61, 2462-2471
- Derse D (1988) *Trans*-acting regulation of bovine leukemia virus mRNA processing. *J Virol* 62, 1115-1119
- Derse D, Martarano L (1990) Construction of a recombinant bovine leukemia virus vector for analysis of virus infectivity. *J Virol* 64, 401-405
- Djilali S, Parodi AL, Lévy D (1987) Bovine leukemia virus replicates in sheep B-lymphocytes under a T-cell released factor. *Eur J Cancer Clin Oncol* 23, 81-85
- Dyson N, Howley PM, Mungel P, Harlow E (1989) The human papilloma virus 16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 341, 934-937
- Fanning E, Knippers R (1992) Structure and function of simian virus 40 large tumor antigen. *Ann Rev Biochem* 61, 55-85
- Felber BK, Derse D, Athanassopoulos M, Campbell M, Pavlakis GN (1989) Cross activation of the *rex* proteins of HTLV-1 and BLV and of the *rev* protein of HIV-1 and non-reciprocal interactions with their RNA responsive elements. *New Biologist* 1, 318-330
- Franklin AA, Kubik MF, Uittenboogaard MN *et al* (1993) Transactivation by the human T-cell leukemia virus Tax protein is mediated through enhanced binding of activating transcription factor-2 (ATF-2) ATF-2 response and cAMP element-binding protein (CREB). *J Biol Chem* 268, 21225-21231

- Gitlin SD, Lindholm PF, Marriott SJ, Brady JN (1991) Transdominant human T-cell lymphotropic virus type I TAX1 mutant that fails to localize to the nucleus. *J Virol* 65, 2612-2621
- Goudsmit J, Bac, NKT, Nara PL (1991) Genomic diversity and antigenic variation of HIV-1: links between pathogenesis, epidemiology and vaccine development. *FASEB J* 5, 2427-2436
- Grimaldi MG, Poli G, Sartoelli P, Caldora C, Oldani L, Locatelli A (1983) Karyotype analysis of lymphocytes from cattle at different stages of bovine leukemia virus infection. *Br Vet J* 139, 240-246
- Gupta P, Kashmiri S, Ferrer J (1984) Transcriptional control of the bovine leukemia virus genome: role and characterization of a non-immunoglobulin plasma protein from bovine leukemia virus infected cattle. *J Virol* 50, 267-270
- Haas L, Divers T, Casey W (1992) Bovine Leukemia Virus gene expression *in vivo*. *J Virol* 66, 6223-6225
- Hatfield D, Feng Y, Lee B, Rein A, Levin J, Oroszlan S (1989) Chromatographic analysis of the aminoacyl tRNAs which are required for the translation of codon at and around the ribosomal frameshift sites of HIV, HTLV-1 and BLV. *Virology* 173, 736-754
- Hayakawa K, Hardy RR, Herzenberg LA (1985) Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J Exp Med* 161, 1554-1568
- Heeney JL, Valli VEO (1990) Transformed phenotype of enzootic bovine lymphoma reflects differentiation linked leukemogenesis. *Lab Invest* 62, 339-346
- Heeney JL, Valli PJS, Jacobs RM, Valli VEO (1992) Evidence for bovine leukemia virus infection of peripheral blood monocytes and limited antigen expression in bovine lymphoid tissue. *Lab Invest* 66, 608-617
- Inoue J, Seik, M, Taniguchi T, Tsuru S, Yoshida M (1986) Induction of interleukin 2 receptor gene expression by p40x encoded by human T-cell leukemia virus type 1. *EMBO J* 5, 2883-2888
- Jiang QT, Boros I, Brady J, Radanovich M, Khoury G (1988) Characterization of cellular factors that interact with the human T-Cell Leukemia Virus Type 1 p40X responsive 21 base-pair sequence. *J Virol* 62, 4499-4509
- Jensen WA, Sheehy SE, Fox MH, Davis WC, Cockerell GL (1990) *In vitro* expression of bovine leukemia virus in isolated B lymphocytes of cattle and sheep. *Vet Immunol Immunopathol* 26, 333-342
- Katoh I, Ikawa Y, Yoshinaka Y (1989a) Retroviruses protease characterized as a dimeric aspartic proteinase. *J Virol* 63, 2226-2232
- Katoh I, Yoshinaka Y, Ikawa Y (1989b) Bovine leukemia virus trans-activator p38tax activates heterologous promoters with a common sequence known as a cAMP-responsive element or the binding site of a cellular transcription factor ATF. *EMBO J* 8, 497-503
- Kettmann R, Portetelle D, Mammerickx M *et al* (1976) Bovine leukemia virus: an exogenous RNA oncogenic virus. *Proc Natl Acad Sci USA* 73, 1014-1018
- Kettmann R, Cleuter Y, Mammerickx M *et al* (1980) Genomic integration of bovine leukemia virus: comparison of persistent lymphocytosis with lymph node tumor form of enzootic bovine leukosis. *Proc Natl Acad Sci USA* 77, 2577-2581
- Kettmann R, Deschamps J, Claustriaux JJ, Palm, R, Burny A (1983) Chromosome integration domain for bovine leukemia provirus in tumors. *J Virol* 47, 146-150
- Kim SJ, Kehrl JH, Burton J *et al* (1990) Transactivation of the transforming growth factor beta 1 (TGF-beta 1) gene by human T lymphotropic virus type 1 tax: a potential mechanism for the increased production of TGF-beta 1 in adult T cell leukemia. *J Exp Med* 172, 121-129
- Kono Y, Arai K, Sentsui H, Matsukawa S, Itohara S (1986) Protection against bovine leukemia virus infection in sheep by active and passive immunization. *Jpn J Vet Sci* 48, 117-125
- Letesson JJ, Van den Broecke A, Marbaix Cleuter Y *et al* (1991) FACS analysis of bovine leukemia virus (BLV)-infected cell lines with monoclonal antibodies (mAbs) to B cells and to monocytes/macrophages. *Vet Immunol Immunopathol* 27, 207-213
- Lévy D, Kettmann R, Marchand P, Djilali S, Parodi AL (1987) Selective tropism of bovine leukemia virus (BLV) for surface immunoglobulin-bearing ovine lymphocytes. *Leukemia* 1, 463-465
- Lewin HA (1986) Evidence for BoLA-linked resistance and susceptibility to clinical progression of bovine leukemia virus infection. *Anim Genet* 17, 197-207
- Lewin HA, Wu MC, Stewart JA, Nolan TJ (1988) Association between BoLA and subclinical bovine leukemia virus infection in a herd of Holstein-Friesian cows. *Immunogenetics* 27, 338-344
- Lewin HA (1989) Disease resistance and immune response genes in cattle: strategies for their detection and evidence of their existence. *J Dairy Sci* 72, 1334-1348
- Lilienbaum A, Duc Dodon M, Alexandre C, Gazzolo L, Paulin D (1990) Effect of human T-cell leukemia virus type I tax protein on activation of the human vimentin gene. *J Virol* 64, 256-263
- Mammerickx M, Palm R, Portetelle D, Burny A (1988) Experimental transmission of leukosis in sheep: latency period of the tumoral disease. *Leukemia* 2, 103-107
- Mamoun RZ, Morisson M, Rebeyrotte N *et al* (1990) Sequence variability of bovine leukemia Virus *env* gene and its relevance to the structure and antigenicity of the glycoproteins. *J Virol* 64, 4180-4188
- Maruyama K, Fukushima T, Mochizuki S (1989) Cross-reactive antibodies to BLV and HTLV in bovine and human hosts with retrovirus infection. *Vet Immunol Immunopathol* 22, 265-273



- Miller JM, Miller LD, Olson C, Gillette KG (1969) Virus-like particles in phytohemagglutinin, stimulated lymphocyte cultures with reference to bovine lymphosarcoma. *J Natl Cancer Inst* 43, 1297-1305
- Nimer SD, Gasson JC, Hu K *et al* (1989) Activation of the GM-CSF promoter by HTLV-I and -II tax proteins. *Oncogene* 4, 671-676
- Oshima K, Okada K, Nomakunai S, Yoneyma Y, Sato B, Takahashi I (1981) Evidence of horizontal transmission of bovine leukemia virus due to blood-sucking tabanid flies. *Jpn J Vet Sci* 43, 79-81
- Portetelle D, Bruck C, Burny A, Dekegel D, Mammertickx M, Urbain J (1978) Detection of complement dependent lytic antibodies in sera from bovine leukemia virus infected animals. *Vet Res* 9, 667-674
- Portetelle D, Limbach K, Burny A *et al* (1991) Recombinant vaccine virus expression of the bovine leukaemia virus envelope gene and protection of immunized sheep against infection. *Vaccine* 9, 194-200
- Portetelle D, Callebaut I, Bex F, Burny A (1993) Vaccination against animal retroviruses. In: *Progress in Vaccinology. Vol 4: Veterinary Vaccines*. Pandey, Hoglund, Prasad, Springer Verlag, Berlin, 87-138
- Powers MA, Grossman D, Kidd LC, Radke K (1991) Episodic occurrence of antibodies against the bovine leukemia virus rex protein during the course of infection in sheep. *J Virol* 65, 4959-4965
- Rimsky L, Duc Dodon M, Dixon EP, Greene WC (1989) Trans-dominant inactivation of HTLV-1 and HIV-1 gene expression by mutation of the HTLV-1 Rex transactivator. *Nature (Lond)* 341, 453-456
- Sagata N, Yasunuga T, Tsuzuku J, Ohishi K, Ogawa Y, Ikawa Y (1985) Complete nucleotide sequence of the genome of bovine leukemia virus: its evolutionary relationship to other retroviruses. *Proc Natl Acad Sci USA* 82, 677-681
- Schwartz I, Bensaid A, Polack B, Perrin B, Berthelemy M, Lévy D (1994) *In vivo* leukocyte tropism of bovine leukemia virus in sheep and cattle. *J Virol* 68, 4589-4596
- Slavikova K, Kettmann R, Reinerova M, Zajac V, Portetelle D, Burny A (1987) Provirus integration of bovine leukemia virus into DNA of infected human myeloma cells. *Neoplasma* 34, 653-657
- Temin HM (1993) A proposal or a new approach to a preventive vaccine against human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 90, 4419-4420
- Valikhov AF, Burba LG, Shishkov VP (1984) Virological examination of semen, milk and blood in BLV infected cattle. In: *5th Int Symp on Bovine Leukemia Virus*, Tübingen (OC Straub, ed) Martinus Nijhoff, The Hague
- Van den Broecke A, Cleuter Y, Chen G *et al* (1988) Even transcriptionally competent proviruses are silent in bovine leukemia virus induced sheep tumor cells. *Proc Natl Acad Sci USA* 85, 9263-9267
- Van der Maaten MJ, Miller JM, Scherr MJ (1981) Effect of colostrum antibodies on Bovine Leukemia Virus infection of neonatal calves. *Am J Vet Res* 42, 1498-1500
- Van Eijk MJT, Stewart JA, Beever J, Fernando RL, Lewin HA (1992) Development of persistent lymphocytosis in cattle is closely associated with BRB2. *Immunogenetics* 37, 64-68
- Voneche V, Callebaut I, Kettmann R, Brasseur R, Burny A, Portetelle D (1992a) The 19-29 amino acid fragment of GP51 adopts an amphiphilic structure and plays a key role in the fusion events induced by bovine leukemia virus. *J Biol Chem* 267, 15193-15197
- Vonèche V, Portetelle D, Kettmann R *et al* (1992b) Fusogenic segments of bovine leukemia virus and simian immunodeficiency virus are interchangeable and mediate fusion by means of oblique insertion in the lipid bilayer of their target cells. *Proc Natl Acad Sci USA* 89, 3810-3814
- Walrand F, Fumoux F, Roelants G, Parodi AL, Lévy D (1986) Incidence of bovine leukemia virus specific antibodies in West African cattle. *Int J Cancer* 37, 619-621
- White KN, Nosaka T, Kanamori H, Hatanaka M, Honjo T (1991) The nucleolar localisation signal of the HTLV-1 protein p27-rax is important for stabilisation of IL-2 receptor. *Biochem Biophys* 175, 98-103
- Whyte P, Williamson NM, Harlow E (1989) Cellular targets for transformation by the adenovirus E1A proteins. *Cell* 56, 67-75
- Willems L, Heremans H, Chen G *et al* (1990) Cooperation between bovine leukaemia virus transactivator protein and Ha-ras oncogene product in cellular transformation. *EMBO J* 9, 1577-1581
- Willems L, Grimonpont C, Heremans H *et al* (1992a) Mutations in the bovine leukemia virus tax protein can abrogate the LTR directed transactivating activity without concomitant loss of transforming potential. *Proc Natl Acad Sci USA* 89, 3957-3961
- Willems L, Kettmann R, Chen G *et al* (1992b) A cyclic AMP responsive DNA binding protein (CREB2) is a cellular transactivator of the bovine leukemia virus long terminal repeat. *J Virol* 66, 766-772
- Willems L, Kettmann R, Dequied F *et al* (1993) *In vivo* infection of sheep by bovine leukemia virus mutants. *J Virol* 67, 4078-4085
- Xu A, Van Eijk MJT, Park C, Lewin HA (1993) Polymorphism in BoLA-DRB3 exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukemia virus. *J Immunol* 151, 6977-6985
- Yoshinaka Y, Katoh I, Copelan TD, Smythers GW, Oroszian S (1986) Bovine leukemia virus protease: purification, chemical analysis, and *in vitro* processing of gag precursor polypeptides. *J Virol* 57, 826-832