Pathobiology of bovine leukemia virus

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Review article

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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

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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^3) (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^3 (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 $CD5^+$ B-cells constitute a B-lymphocyte population of a different lineage from the $CD5^-$ B-cells that could be involved in the synthesis of autoantibodies (Hayakawa *et al*, 1985). This type of lymphocyte is also found in chronic lymphoid leukemia in humans (De la Hera *et al*, 1988). Are $CD5^+$ B-lymphocytes the preferential target for BLV infection? Using fluorescence activated cell sorting, we showed that BLV is found in $CD8^+$ T-lymphocytes, monocytes, granulocytes, and mostly in B-lymphocytes, with no preference for the $CD5^+$ B-cell population (Schwartz *et al*, 1994). The parameters involved in the sensitivity of the $CD5^+$ 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, zebus, buffalos 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 µl 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 livestocks 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

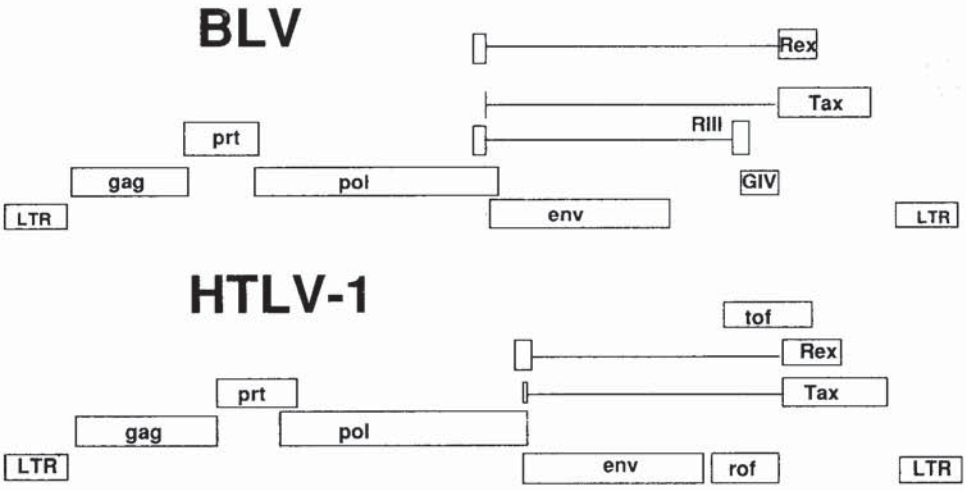

Fig 1. Genomic organization of bovine leukemia virus (BLV) and human T-cell leukemia virus type 1 (HTLV-1). The rectangles correspond to open reading frames.

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 syncitia 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 (ζ chain) and of the B cell receptor (Igα and Igβ 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, $CD8^+$ 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 β (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 (Katoh *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 infectiosity 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 symptomatologies, 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.

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