Chapter 6

Major Histocompatibility Complex-Associated Resistance to Infectious Diseases: The Case of Bovine Leukemia Virus Infection

Silvina Elena Gutiérrez, Eduardo Néstor Esteban, Claudia María Lützelschwab and Marcela Alicia Juliarena

Additional information is available at the end of the chapter

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Abstract

The major histocompatibility complex (MHC) is a polymorphic gene cluster of about 150 genes, present in all vertebrates. Many of these genes contribute to immunity. Particularly, MHC-encoded class I and class II molecules, which are typically highly polymorphic and polygenic, are central in defining the specificity of the adaptive immune response. Among the diversity of genes associated with disease resistance, MHC genes are particularly interesting as they are associated with resistance and susceptibility to a wide range of diseases, some of which produce important economic losses in livestock. Enzootic bovine leukosis is an infectious disease caused by the retrovirus bovine leukemia virus (BLV), with an important economic impact, mainly in dairy herds. In this chapter, MHC-associated genetic resistance to BLV is revised. Certain alleles of the bovine MHC (*BoLA*) class II locus have been found strongly associated with resistance to viral dissemination. Genetic selection of resistant animals emerges as a natural strategy for the control of infectious diseases, especially when there is no other alternative of control or prevention, as vaccines. Founded on this knowledge, a BLV control program based on selection of genetically resistant cattle was designed. The proof of concept indicates that this strategy is feasible to implement in dairy herds.

Keywords: major histocompatibility complex, BoLA, genetic resistance, infectious diseases, bovine leukemia virus, control, veterinary, livestock

1. Introduction

The immune system has evolved in vertebrates to protect them from invading pathogens. To attain this objective, it compromises an enormous variety of cells and molecules that interact with each other in a complex network to recognize, counteract, and, if properly regulated, eliminate the pathogen. The major histocompatibility complex (MHC), which is found to occur in all mammalian species, plays a central role in the development and function of the immune system. Genes encoding the MHC are highly polymorphic, and numerous associations between allelic variants and immune responsiveness and disease resistance are well documented. Hence, the MHC genes are attractive as candidate genes involved in susceptibility/resistance to various diseases.

Breeding for improved disease resistance has emerged as a major challenge for animal geneticists. The benefits of successfully improving the resistance of animals to an infectious disease are manifold, including animal welfare, increased efficiency and productivity, and hence a reduced environmental footprint, reduced reliance on other disease-control measures, and improved public perception [1].

Enzootic bovine leucosis is an endemic disease in many countries, causing important economic impact in the dairy industry. The fine characterization of the resistance phenotype, the strong association between certain MHC class II alleles with resistance, and the absence of preventive or therapeutic measures against the disease make the genetic selection of resistant animals a feasible approach to control bovine leukemia virus (BLV) infection.

2. The major histocompatibility complex

Vertebrates have the capacity to recognize, destroy, and develop immunological memory to invading microorganisms through the activation of cells and molecules of their immune system. In order to achieve these ends, the two arms of the immune system (i.e., the innate and the acquired immunity) have to interact with each other. The innate immune system comprises mainly cells from the myeloid lineage that recognize common structures on a broad spectra of microorganisms, known as the pathogen-associated molecular patterns (PAMPs) through their pathogen recognition receptors (PRRs). Some of these cells, like macrophages and dendritic cells, are also involved in the activation of the adaptive immune system, by capturing and processing the antigens and acting as antigen presenting cells (APC) for T lymphocytes.

The effector cells of the adaptive immune system, consisting of B and both helper T lymphocytes (LTH) and cytotoxic T lymphocytes (CTL), recognize a very large variety of self and nonself antigens in a more specific manner by their antigen receptors (BCR and TCR, respectively). The BCR can bind directly to free or soluble native antigen, while the TCR requires the protein antigen to be processed into small peptides. These peptides have to be associated within the endoplasmic reticulum with molecules encoded by a single genetic locus containing many polymorphic genes, the MHC. The assembled MHC molecule (mMHC)-peptide

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complex is then transported to and expressed on the surface of the APCs, where the antigen-loaded mMHC interacts with the TCR and initiates the activation of T lymphocytes [2]. The CTL and the LTH recognize antigen in the context of two different mMHCs: class I and class II, respectively. The class II mMHCs have a restricted expression, mainly on professional APCs, and expose peptides mainly derived from captured extracellular antigens for the recognition by the LTH. The class I mMHCs are displayed on the surface of every nucleated cell of the organism, presenting peptides essentially originated from intracellular proteins (i.e., intracellular microorganisms or cell-derived proteins) to be recognized by the CTLs. The class I mMHC does not only play a fundamental role in the recognition of foreign intracellular antigens but also in inducing self-tolerance and alloreactive immune responses.

2.1. Structure of class I and class II MHC molecules

Most of the current knowledge about these molecules arose from pioneer studies on the rejection of normal and malignant transplanted tissues in mice and rabbits [3–6]. The evidence produced by these studies indicated that the destruction of the grafted tissue was determined upon the existence of inherited antigenic differences between transplant and host, leading to the discovery of the mMHC and, later on, their genetic complexity.

The mMHCs have different domain organizations but similar structure. The variations are concentrated in three to four discrete hypervariable regions in the extracellular domains, while the rest of the molecule is highly conserved. The X-ray crystallography of the proteins demonstrated that the class I mMHC is a heterodimer consisting of a transmembrane α chain non-covalently linked to a small non-transmembrane chain, called β2-microglobulin (β2m). Besides a transmembrane and an intracellular region, the α chain has three extracellular globular domains (α1, α2, and α3). The α1 and α2 domains of the α chain form a groove that accommodates an 8- to 10-mer antigenic peptide [7, 8]. The class II mMHC is a heterodimer composed of an α and a β chain, both with an intracellular, a transmembrane, and two extracellular domains (**Figure 1A**). The pairing of the α1 and β1 domains form an antigen-binding groove with open ends, allowing the allocation for a larger peptide (14-mer or more) extending out of both sides of the groove [9] (**Figure 1B**).

The polygenic and polymorphic features of the MHC grant these molecules with an enormous capability for antigen presentation. A single individual co-expresses several mMHCs from a large pool of alleles within a population. Moreover, each mMHC molecule can associate with a great amount of similar peptides, expanding even more the breadth of MHC-regulated immune responses to pathogens. The polymorphic residues in the mMHC antigen-binding groove are responsible for the different peptide specificities of the different alleles. The class I molecules require an allele-specific peptide length and a defined peptide motif that includes two anchor amino acids or residues with closely related side chains, which interact with both ends of the antigen-binding site of that particular mMHC [10]. The class II mMHCs have less stringency for size but also have allele-specific peptide motifs (or anchor residues) that reach into pockets within and on the sides of the groove of the class II mMHC [11] (**Figure 1B**). Moreover, conserved residues within the class II peptide-binding groove induce a conformational change, forcing bound peptides into a twisted configuration and exposing sites for external interactions [12].

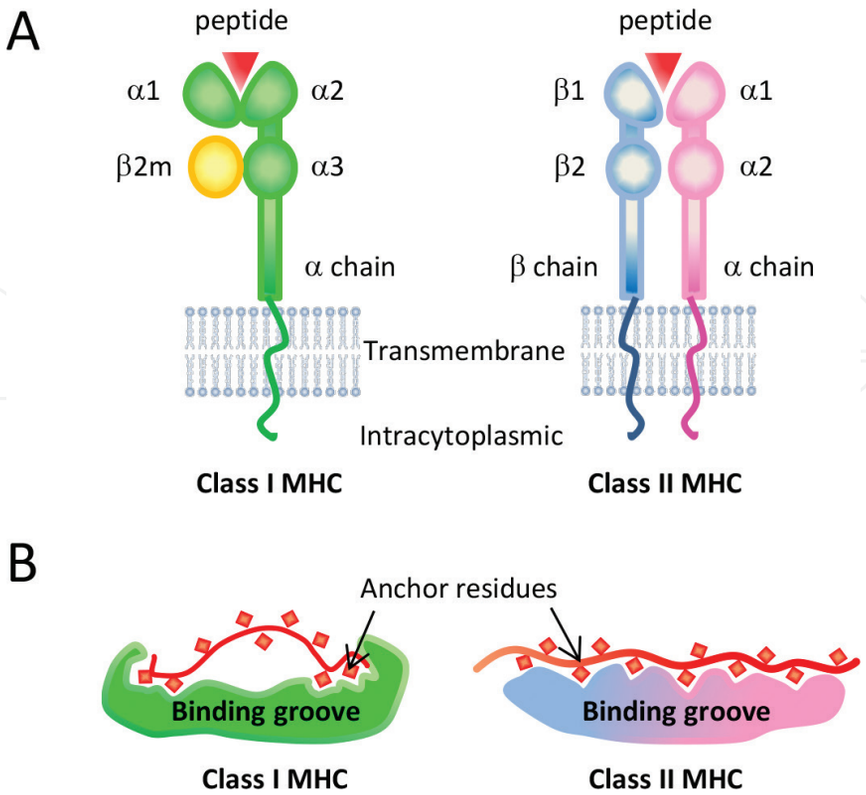

Figure 1. Structure of MHC molecules and its binding sites. Panel A: schematic representation of class I and class II mMHCs. Panel B. MHC peptide-binding sites. Amino acid positioning within the peptide-binding grooves of class I and class II MHC proteins is shown. Squares represent individual amino acids of the antigenic peptides binding in the groove of each class I and class II mMHC.

2.2. Organization of the Major Histocompatibility Complex

Since its first description in mice and humans [5, 13], the study of the MHC genetic architecture has expanded substantially, with the discovery and characterization of many class I and class II genes in different vertebrate species, except for jawless fish [14]. The collective name given to the proteins encoded by MHC genes depends on the species, except for mice and chickens, in which they were first described as transplantation antigens and maintain their original nomenclature H-2 and B, respectively. Hence, in humans, they are called human leukocyte antigen (HLA); in swine, SLA; in ovine, OLA; in equine, ELA; in dogs, DLA; in bovine, BoLA; and so on. The genetic structure of the MHC is best known for HLA and is relatively conserved among other mammalian species [15].

The HLA complex covers about 4 Mb of the short arm of chromosome 6 and contains three major regions with the confirmed presence of more than 260 loci, including over 160 protein-coding genes [16, 17].

The HLA locus is divided into three closely linked regions: class I, class II, and class III. The first two regions contain genes that control the specific immune response (so-called "classical" MHC genes), and the class III region, enclosing about 75 genes, encodes a variety of different proteins, some related to the innate immunity [18].

The class I region contains three classical genes, *HLA-A*, *HLA-B*, and *HLA-C*, and three nonclassical genes: *HLA-E*, *HLA-F*, and *HLA-G* [19] (**Figure 2**). Each of the classical HLA is a single functional gene, encoding a class I mMHC α chain. The gene coding for the β2m is located outside the MHC, on chromosome 15. *HLA-H*, *HLA-J*, *HLA-K*, and *HLA-L* are nonfunctional pseudogenes, closely related in nucleotide sequence to the class I functional genes [18].

The class II HLA cluster comprises three classical class II genes: *HLA-DP*, *HLA-DQ*, and *HLA-DR*, each encoding one α and one or two β chains; three nonclassical, non-polymorphic class II genes; *HLA-DM, HLA-DN*, and *HLA-DO*; and some pseudogenes [19] (**Figure 2**). These nonclassical class II genes are not expressed on the cell surface, but form heterotetrameric complexes involved in catalytic peptide exchange and loading onto classical class II molecules [20, 21].

The class III region is located between the class I and class II regions and contains genes coding for molecules with diverse function. Among the most prominent are the complement factor genes coding for factors C2, C4, and B; genes coding for cytokines belonging to the tumor necrosis factor (TNF) superfamily, TNF-α, lymphotoxin-α and lymphotoxin-β, which are involved in various inflammatory pathways; heat shock protein genes; and many other genes encoding proteins not related to the immune system [19].

2.3. The major histocompatibility complex in cattle

In cattle, the first evidence for the existence of a MHC system was found by lymphocyte immunizations and by the generation of monospecific antilymphocyte antisera against skin grafts, followed by studies on the inheritance of the antigens they detected [22, 23]. As in the human, the *BoLA* locus is highly complex and contains about 154 predicted functional genes spanning about 4 centimorgan on chromosome 23 [24]. **Figure 2** shows the BoLA organization

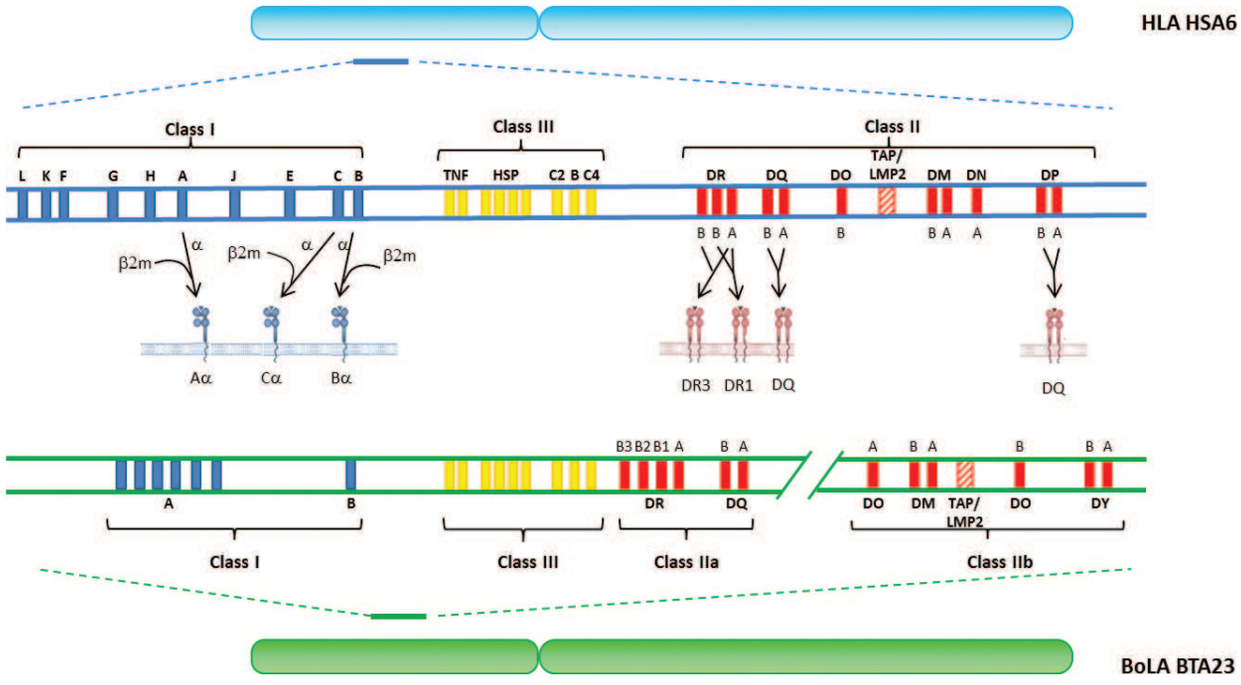

Figure 2. An abridge map of the genetic organization of HLA and BoLA.

compared to its human counterpart. Compared to the HLA system, the BoLA system differs in gene arrangement without compromising its functions. Class I genes are clustered in two regions: *BoLA-A* and *BoLA-B*. Only the *A* locus seems to be functional with at least six putative classical class I genes, named *genes 1–6* [25]. To date, 96 *BoLA-A* alleles are listed on the Immuno Polymorphism Database (IPD)-MHC database [26], and 29 different haplotypes have been identified, expressing between one and three combinations of these classical class I genes [27, 28]. The nonclassical BoLA class I genes include *NC1*, *NC2*, and *NC3*, encoding molecules with a relevant role during reproduction in dairy cows [29].

The main difference between BoLA and HLA gene organization is that in cattle the class II gene cluster is divided into two subregions, separated about 15 cM apart from each other: the class IIa, located near the class I/III regions, and the class IIb, resulting from a transposition, located close to the centromere on autosome 23 [30, 31]. This feature is shared by various ruminant species [32].

The subregion IIa incorporates the gene clusters *DR* and *DQ* but lack a *DP* gene [27]. This subregion expresses one DR molecule and one or two DQ molecules per haplotype [33]. Bovines have one monomorphic *DRA* gene. By contrast, there are three genes that encode for the β chain of the DR (*DRB*) molecule of which *DRB1* is a pseudogene and *DRB2* is poorly expressed, leaving the *DRB3* locus as the most polymorphic and strongly expressed gene from this group. To date, 130 alleles have been identified using different approaches in various breeds of cattle (listed in IPD-MHC database) [34].

The *DQ* cluster comprises five *DQA* (*DQA 1–5*) and five *DQB* (*DQB 1–5*) genes, which have arisen from gene duplication. From this cluster, at least 61 *BoLA DQA* and 81 *BoLA DQB* alleles have been described and listed in the IPD-MHC database, and 56 different haplotypes were described in Japanese Black and Holstein cattle [35]. The high number of different alleles, both on the *DR* and *DQ* loci, suggests that DR and DQ molecules complement each other for the presentation of a broad spectrum of antigens in cattle.

The BoLA class IIb locus is divided into two regions. The region known as "extended class II" region contains some genes involved in antigen processing and transport, i.e., LMP complex (low molecular mass polypeptide, *LMP2*, *LMP7*) and *TAP* genes (transporter associated with antigen processing: TAP2.1, TAP1, and TAP2) and also some non-MHC genes like *H2B* (histone H2B-like), among others [36–38].The second region is known as "classical class II" region and encloses genes of unknown function and unique to ruminants: *DSB (DRβ-like)*, *DYA*, and *DYB*. The *DYA* and *DYB* genes encode for proteins of 253 and 259 amino acids, respectively. DY molecule has been shown to be expressed by a subpopulation of afferent lymph dendritic cells, suggesting its involvement in the prominent antigen processing and presentation capability of these cells [39–41].

2.4. Association of the major histocompatibility complex to disease susceptibility and resistance

Although age, stress, and physiologic status are important factors influencing the outcome of infection, evidence for genetic control has been observed in many animal species. Based on data registered in the Domestic Animal Diversity Information System (DAD-IS) 2015 report [42], 483 mammalian breed populations are recorded as having resistance or tolerance to

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specific diseases or parasites, among which 236 correspond to breeds of cattle, 94 breeds of sheep, 56 breeds of chicken, 54 breeds of goats, and 36 breeds of pigs.

Not unexpectedly, the high degree of genetic polymorphism in the MHC has been associated with health status, vaccine responsiveness, and production traits in cattle [43–47]. Examples of some studies for which there is documented evidence of MHC association with resistance or susceptibility to disease include mastitis in cattle and sheep [44, 48], tick-borne disease [49–51], dermatophilosis in cattle [52], enzootic bovine leucosis in cattle and sheep [53–56], neosporosis in cattle [57], theileriosis in cattle [58], gastrointestinal parasites in sheep [59, 60], diarrhea in pigs [61, 62], Marek's disease in chicken [63, 64], coccidiosis in chicken [65], and coronavirus resistance in chickens [66], among others.

3. Enzootic bovine leukosis and the bovine leukemia virus

Enzootic bovine leukosis is one of the most frequent neoplastic diseases of cattle caused by an exogenous retrovirus designated BLV. BLV is the type species of the genus *Deltaretrovirus* in the Retroviridae family. This genus also includes pathogenic viruses from human and nonhuman primates (human and simian T-cell leukemia viruses) that share biological and molecular characteristics with BLV [67].

BLV infection is globally distributed in cattle-raising countries. An assessment of BLV infection in US dairy operations in 2007 showed that 83.9 % of them were seropositive for BLV [68]. A national study of BLV infection in Canada in 1980 showed that 40 % of its dairy herds and 11 % of its beef herds were infected [69]. On the other hand, BLV control programs have been established in member countries of the European Union since the 1980s, resulting in seroprevalence between 0.5 and 1.5 % in some countries, while others such as Belgium, Denmark, Germany, Estonia, Spain, France, Ireland, Austria, Finland, Sweden, the United Kingdom, and few others are considered officially free by the European Community [70–72].

In natural conditions BLV only infects cattle, zebus, buffalos, and capybaras, but other species such as sheep, goats, and rabbits can be experimentally infected [73]. Although both beef and dairy breeds are equally susceptible to BLV infection [74], the impact is higher in dairy herds, mainly because of differential management practices.

The major target of the virus is the B lymphocyte [75]. Although evidence of infection of other peripheral blood cell subpopulations has been reported [76], these results have not been confirmed by others. Soon after infection of a cell, the viral RNA is copied into DNA by the virus-encoded reverse transcriptase. The provirus then integrates into the cellular DNA at random sites, and the infection persists for the whole life of the animal, despite the presence of neutralizing and other antiviral antibodies.

3.1. Pathological and clinical features associated to BLV

BLV infection is characterized by the "iceberg principle," typical of many viral diseases. While the majority (approximately 70 %) of infected cattle remain asymptomatic, one third of infected cattle develop a permanent increase in the number of B lymphocytes termed persistent

lymphocytosis (PL), which is considered a benign condition. The tip of the iceberg is represented by those animals that develop the neoplastic disease, which is usually less than 5 % of the infected cattle. The accumulation of transformed lymphocytes in one or more organs after a long latency period of 1–8 years leads to a multicentric lymphosarcoma. This condition is typically observed in cattle older than 3 years of age. In two thirds of the animals, the development of tumors is preceded by a phase of PL. Lesions can be localized in almost any organ, but the abomasum, heart, visceral and peripheral lymph nodes, spleen, uterus, and kidneys are most frequently affected. Lesions can be observed as white firm tumor masses or as a diffuse tissue infiltrate in any organ. Clinical signs are variable and depend on the affected organ, the speed of grow of tumors, and the degree of dissemination of the neoplastic process. In most cases the course of the illness is subacute to chronic, initiated by a marked loss of weight and appetite, and weakness. Clinical signs most often observed are decreased milk production, lymphadenopathy, and posterior paresis. Once the clinical signs of the illness are evident, the course is rapid and invariably culminate in death [77].

3.2. Transmission of BLV and economic impact of the infection

As cell-free virus is rarely detected in vivo, most susceptible cattle become infected by exposure to infected lymphocytes. Vertical transmission may occur in utero but is infrequent. The main biologic fluids that contain sufficient infected lymphocytes to transmit the infection are the blood, colostrum, and milk. Other fluids such as saliva, semen, urine, and nasal secretions, while potentially infectious, have not been demonstrated to transmit the infection in natural conditions [78].

Under general or standard production conditions, the risk of horizontal transmission is augmented by management practices or procedures involving blood transfer such as gouge dehorning, ear tagging or tattooing, blood extraction, and rectal palpation, using shared or not properly disinfected instruments [78]. This risk is augmented when contaminating blood comes from cattle with persistent lymphocytosis [79]. The use of natural service (i.e., bulls) to breed heifers was also identified as a risk factor for augmented prevalence of BLV infection compared to artificial insemination [80].

Evidence has been reported on the role of bloodsucking insects, such as stable flies, horn flies, and tabanids in the transmission of BLV [81]. Furthermore, the lack of insect control program has been recognized as a risk factor for BLV infection [82, 83]. In warm regions, the animals may be exposed to a high density of hematophagous insects that continuously fed on them; hence, the control of bloodsucking insects by pesticides has been reported to prevent the transmission in a model farm [84]. Both colostrum and milk from BLV-positive cows contain infected lymphocytes, and evidence exists for the transmission of BLV to calves by feeding bulk milk, a common practice in dairy herds [78, 85]. The rate of transmission attributable to this route has been estimated to be 6–16 % under natural conditions. On the other hand, feeding colostrum from infected dams, which contains high titers of antiviral antibodies, seems to have a protective role, being the susceptibility of calves dependent on the presence of specific antibodies obtained from the dam's colostrums and the age of the calf [78, 86, 87].

Enzootic bovine leucosis causes significant economic losses. The most obvious economic losses are due to culling or death due to lymphosarcoma, shortening of lifespan, and loss of

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production potential. Other indirect losses are related to the costs of control and eradication programs and restrictions in the international trade of cattle and their by-products. Annual economic losses to the US dairy industry associated with BLV are estimated to be $285 million for producers and $240 million for consumers [80]. The effects of subclinical BLV infection on milk production, reproductive performance, longevity, and culling rate are variable. Using data from the National Animal Health Monitoring System's 1996 dairy herd study, it was found that herds with test-positive cows produced 218 kg less milk per cow/per year than those with no test-positive cows [88].

There is no treatment for BLV infection or its associated disease. The possibility of a vaccine for protection against BLV has been explored (reviewed in [89]). A BLV vaccine would have to be noninfectious and non-oncogenic and should not interfere with the serological tests commonly used to detect infection [77].

3.3. Phenotypes associated with resistance and susceptibility in BLV infection

Studies from our laboratory have gone further into the characterization of BLV-infected, hematologically normal cattle (i.e., those animals that do not develop PL). Based on the proviral load in the peripheral blood and antibodies against BLV major proteins, we could describe two defined phenotypes in BLV-infected cattle. Proviral load was determined in DNA from peripheral blood leukocytes by a very sensitive nested PCR or a semiquantitative PCR [90], while antibody titers were measured against the BLV major antigens by ELISA [90, 91].

One group of animals is characterized by high proviral load (HPL) in peripheral blood (>100,000 BLV proviral copies/μg of DNA) and high antibody titers against the envelope protein of BLV of 51 kDa (BLVgp51). On the other hand, the remaining non-PL animals harbor an exiguous number of infected lymphocytes in the peripheral blood, almost undetectable by the molecular methods currently used (PCR and real-time PCR); we have termed this group low proviral load (LPL) cattle. These cattle develop low titers of antiviral antibodies against BLVgp51, while antibodies against BLVp24, the main core protein of BLV, are undetectable in the majority of LPL cattle or developed at very low titers. The follow-up of HPL and LPL cattle showed that each phenotype was maintained throughout, at least, a 1.5-year period. The characterization of PL cattle in terms of proviral load and titers of antibodies against BLV showed no significant differences with non-PL HPL cattle. Cattle with LPL profile represented about 60 % of hematologically normal cattle and 40 % of all BLV-infected cattle [92]. **Table 1** shows the main parameters that characterize both HPL and LPL infection phenotypes.

As virtually all cattle infected with BLV will continuously have antibodies against the virion proteins in their serum, serologic tests are commonly used for diagnosis of BLV infection in cattle > 6 months old. PL cattle and non-PL HPL cattle have high titers of antibodies against the major viral antigens, and then, they are easily classified as positive by means of the majority of serological tests in the market (mainly ELISAs or immunodiffusion tests). On the other hand, antibody titers against BLVgp51 are lower in most LPL animals compared to HPL cattle, and antibodies against BLVp24 are at very low titers or undetectable in most serum samples

BLV infection phenotype	Hematological status	Proviral load[a]	Antibody titer	
			BLVgp51	BLVp24
HPL	PL	≥100,000	400 to ≥ 6400	50–800
HPL	Non PL	≥100,000	400 to ≥ 6400	Seronegative to 400
LPL	Non PL	≤100	2–1600	Seronegative to 50

Proviral load was determined by semiquantitative PCR in DNA extracted from the peripheral blood. Antibody titers against BLVgp51 and BLV p24 were determined in plasma samples by ELISA.
[a] Proviral copies/μg DNA
Adapted from Juliarena et al. [92]

Table 1. Characterization of HPL and LPL phenotypes in terms of proviral load in the peripheral blood, hematological status, and antibody titer against BLVgp51 and BLVp24.

from LPL cattle. Hence, sensitivity of serological tests is decisive in their capacity to detect LPL animals. We have consistently detected LPL animals with a highly sensitive blocking ELISA developed in our lab, designated ELISA 108. This method detects antibodies against an immunodominant conformational epitope of the BLVgp51 at low titers. Other serological methods using BLVgp51 as antigen may also detect BLV-infected LPL animals, provided that they are sensitive enough. This is not the case of agar gel immunodiffusion (AGID) test commercialized in Argentina. On the other hand, serological tests using BLVp24 as antigen would not be able to detect LPL cattle in most cases.

We inoculated sheep with blood from LPL cattle to give further evidence of infection. This was an easily available alternative for the amplification and study of the BLV strain [93]. As the number of infected lymphocytes in peripheral blood from LPL cattle is extremely low, it was necessary to inoculate a large volume of blood to obtain the minimal quantity of infected lymphocytes to transmit the infection. The minimal dose necessary to infect sheep is about 926 BLV-infected lymphocytes [94]. One microliter of blood from a HPL cow was enough to infect lambs. In contrast, blood from LPL cattle also infected lambs, but 100 ml of blood was necessary to infect the ovine host. In order to determine if differences in the observed phenotypes could be attributable to differences at the nucleotide level between strains, a fragment of the *env* gene was amplified from infected lambs by nested PCR and sequenced. No mutations in the *env* gene that could be attributable to the passage from cow to lamb were detected. When comparing a 400-bp sequence from the *env* gene from six LPL and six HPL strains, no mutations were found that could be associated with any particular phenotype, strengthening the hypothesis that the development of each phenotype could be associated primarily with some genetic or epigenetic property of the host [95].

As LPL cattle maintained their phenotype for prolonged periods of time, without developing any hematologic or pathologic condition, it is proposed that these animals are naturally resistant to BLV replication.

3.4. Association of BLV phenotypes to polymorphisms at the major histocompatibility complex

Early observations on the aggregation of lymphoma/leukemia or PL in certain BLV-infected families, but not in others, suggested that host genetic factors were involved in the development

of these conditions [96]. There were also indications that host genetic factors influenced the susceptibility to one condition or the other independently [97].

Studies on the relationship between BLV infection and bovine MHC (BoLA) revealed an association between serologically determined *BoLA-A* class I antigens and resistance and susceptibility to B-cell lymphocytosis in Shorthorn cattle [98], Holstein [99, 100], and other breeds [101]. However, these associations were relatively weak at the population level, and different *BoLA-A* alleles had significant effects in different breeds [102]. Subsequently, it was shown that resistance and susceptibility to PL map more closely to MHC class II *BoLA-DRB3* gene than to *BoLA-A* locus [56, 103]. A peptide motif named ER, which is present in *BoLA-DRB3* alleles **11*, **23*, and **28*, was associated with resistance to PL in BLV-infected cattle. Resistance appears to be dependent upon the presence of the polar amino acids Glu-Arg at positions 70–71 within a highly polymorphic segment of the peptide-binding region [56]. This contributes to the putative peptide-binding specificity of the molecule at the β1 domain [11]. It is believed that the allelic differences influence the binding and orientation of viral peptides [104], thus determining allele specificity differences in the spectrum of peptides presented to the immune system. These differences may have important consequences for infection resistance [105]. Furthermore, it was shown that *BoLA-DRB3* alleles encoding Glu, Arg, and Val at positions 74, 77, and 78 of the BoLA-DRβ chain, respectively, might be related to tumor development resistance [106]. Moreover, evidence was presented that BLV-infected cattle selected for the presence of the *DRB3*11* allele carry less infected lymphocytes than other infected animals [107].

Based on these previous reports found in literature about the association of *BoLA* polymorphisms with PL development or the number of BLV-infected lymphocytes in peripheral blood up to 1998, we decided to evaluate the association between the BLV infection phenotypes described above and *BoLA-DRB3* genotype. Various molecular methods were used for the genotyping of *BoLA-DRB3* exon 2 (the only one expressed from *BoLA-DRB3* gene). In a first instance, we used the PCR-restriction fragment length polymorphism (PCR-RFLP) method, which can differentiate only 57 different alleles (designated by the nomenclature based on this method). The PCR-sequence specific oligonucleotides polymorphisms (PCR-SSOP) is an alternative method which is more specific, allowing the identification of 104 alleles from the 130 described by sequencing, and defines the alleles according to the nomenclature adopted by the International Society for Animal Genetics [34].

Xu et al. [56] described a correlation between *BoLA-DRB3*11* allele and PL in Holstein cattle. The allele **11* (identifiable by PCR-RFLP) can be differentiated into two variants: *BoLA-DRB3*0901* and *BoLA-DRB3*0902* by using PCR-SSOP or by sequencing. By studying the genotype of 230 BLV-infected cattle belonging to seven dairy herds, we found that allele *BoLA-DRB3*11* was significantly associated with the LPL phenotype (odds ratio (OR) = 5.82; $p < 0.0001$); however, the subtype *BoLA-DRB3*0902*, which is the most prevalent subtype of allele *11 in our population, showed a stronger association with the LPL phenotype (OR = 8.24; $p < 0.0001$) than allele **11* itself. Allele *BoLA-DRB3*1701* also showed significant association with LPL profile (OR = 3.46; $p < 0.0055$). The HPL phenotype was significantly associated (OR = 0.36; $p < 0.0005$) with only one allele: *BoLA-DRB3*1501* (allele **16* as determined by PCR-RFLP). According to these associations, the DRB3 alleles were assigned to three categories: resistant (R) if the allele

was associated with LPL phenotype, susceptible (S) if the allele was associated with HPL phenotype, and neutral (N) if the allele was not associated with any phenotype [53].

It is concluded that the host genetic background influences BLV infection phenotype development and that allele *BoLA-DRB3*0902* appears to be, up to now, the best candidate marker of BLV resistance in Argentinean Holstein cattle. The penetrance of *BoLA-DRB*0902* allele for the LPL phenotype is notably high compared to the penetrance of MHC alleles in other systems which, in general, is much lower (about 10 %). More than 80 % of cattle carrying the *BoLA-DRB3*0902* allele develop LPL when naturally or experimentally infected with BLV [53, 108, 109]. However, only one third of LPL cattle harbors the *BoLA-DRB3*0902* allele [53]. This finding suggests that other genetic or epigenetic factors might be involved in the regulation of BLV proviral load.

3.5. Influence of resistance-associated alleles on specific immune response and infectivity of LPL cattle

Cattle with LPL phenotype were grouped according to the presence of alleles associated to the LPL phenotype (i.e., R alleles **0902* and **1701*) in their genotype. **Table 2** shows the average anti-BLVgp51 antibody titer for each group and the resulting classification of cattle according to the level of antibody response against the main envelope glycoprotein of BLV. The R alleles *BoLA-DRB3*0902* and *BoLA-DRB3*1701* were significantly associated with low antibody titers against both BLVgp51 and BLVp24 [110]. Important differences were observed in the infectivity of the blood from cattle in each group, as determined by the sheep bioassay. While all lambs inoculated with blood from LPL cattle not harboring any allele R or harboring one R allele *(*0902* or **1701*) in heterozygosis were infected, only one from six lambs inoculated with 100 ml of blood from LPL cattle with R/R genotype acquired the infection. From these results, it seems that LPL cattle carrying two R alleles have an increased ability to restrict BLV replication [108].

It is unlikely that BLV-infected LPL cattle would be a source of infection for BLV-free animals, as large volumes of blood are never exchanged between animals under usual management practices and natural breeding in dairy farms.

***BoLA-DRB3* genotype**	**n**	**BLVgp51 antibody titer[a]**	**Classification[b]**	**Infectivity[c]**
Without allele R	54	662.3	Medium or high responders	6/6
Allele R in heterozygosis	34	344.3	Medium or low responders	4/4
Allele R in homozygosis	9	275.0	Extremely low responders	1/6

[a] Average
[b] Classification was done according to the level of antibody titer against BLVgp51.
[c] Infectivity was assessed by inoculating 100 ml of blood in lambs. Number of lambs infected/number of lambs inoculated

Table 2. Grouping of BLV-infected cattle with LPL phenotype, according to the presence of alleles associated with resistance (R) in their genotype.

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3.6. Strategies to control BLV infection

BLV control and eradication programs based on culling infected animals have been accepted and implemented successfully in several western European countries. In dairy farms with low prevalence of BLV infection, the cost of implementing these programs is less than the total cost caused by BLV infection [111]. However, in herds where the prevalence of infection is high, the feasibility of implementation of these programs depends on the official compensation to producers for the removal of BLV-positive animals. In Argentina, not only there is no official BLV control program, but official apathy since the 1970s has been the best ally for the lush spread of BLV in all dairy regions, including Patagonia. By 1995, the failure of the voluntary control program of BLV proposed by the National Authority in Animal Heath (Resolution 337/94 from SENASA) was the indicator that Argentina had lost the opportunity to reduce the spread of BLV by means of serological testing and culling and to protect the scarce herds and regions that were still free of BLV.

To limit the spread of BLV, infected cattle are often eliminated on the basis of their risk of transmitting the infection to other cattle. Among BLV-infected cattle, those with PL are considered the most efficient transmitters because they harbor a high percentage of infected lymphocytes in peripheral blood [112] and consequently have HPL [107]. Furthermore, although not all PL animals develop lymphosarcoma, in approximately two thirds of cases, neoplasia is preceded by PL. The application of control plans based on hematological tests and elimination of PL animals has failed to control not only BLV infection but also the associated lymphosarcoma. In 1959, Denmark implemented an eradication program based on the occurrence of clinical lymphosarcoma and the identification of PL cattle by using the Bendixen hematological keys [113]. Affected herds were quarantined, and indemnity was offered to induce owners to have slaughtered their entire herd. This herd-slaughter policy was continued until 1982. However, when the serological tests were introduced, some herds which were classified as leucosis-free based on the hematological keys were found to be infected [77]. One of the major causes of this failure was probably due to the presence of HPL animals that do not present PL. These non-PL HPL animals are as efficient as PL cattle to transmit the BLV, and it is likely that the animals that develop lymphosarcoma without a previous stage of PL also belong to this group of cattle. Therefore, the corrective management based in classification of animals according to HPL and LPL phenotypes should be safer that the plan based on traditional hematological classification (PL and non-PL).

4. Genetic selection of resistant animals

The genetic selection of resistant animals would be an efficient strategy for controlling the spread of BLV in the host and, therefore, to reduce the occurrence of clinical lymphosarcoma. We have shown that, in the Holstein breed, the *BoLA-DRB3*0902* allele is the best molecular marker for the selection of resistant animals. The *BoLA-DRB3* gene is highly polymorphic, with 130 alleles described so far. The allele frequency of the **0902* allele is relatively high (5–10 %) at the population level; therefore, it is possible to easily increase its frequency by directed crossbreeding or artificial insemination. At the herd level, it is essential that these

resistant animals disrupt the transmission of BLV to uninfected animals. Hence, the effectiveness of this procedure would depend on the accuracy of the premise that BLV-infected LPL cattle harboring *BoLA-DRB3*0902* marker do not transmit the virus to BLV-negative cattle under normal breeding conditions in commercial dairy herds.

4.1. The proof of concept

In order to test this premise, an experiment was carried out in a commercial dairy herd located in a subtropical region of Argentina, having extreme environmental conditions (experimental dairy herd). In the experimental dairy herd, BLV-free and BLV-infected LPL-*BoLA-DRB3*0902* animals cohabited for 20 months. This period included two summers during which BLV-infected and BLV-uninfected cattle were exposed to heat stress and to a high density and varied population of bloodsucking insects that continuously fed on the animals. As is usual in any commercial dairy herd, the cattle population was dynamic, and necessary replacements were made according to production needs and milk production requirements, which were within expected parameters in the region. Nevertheless, only previously characterized animals (BLV-free or BLV-infected LPL-*BoLA-DRB3*0902* animals) were introduced to the experimental herd. Normal management practices and production requirements were maintained in the herd. We examined the spread of BLV in the experimental herd and also in other four local commercial herds in which no selection of cattle was made. The incidence rates observed in these four herds were between 0.06 and 0.17 cases per 100 cattle-days. The observed incidence rate explains the increase of prevalence registered in the region from 2002–2003 to 2010–2011 [114, 115]. In contrast, in the experimental herd, no new BLV-infected animals were detected, and as a corollary, the incidence rate was zero. These results indicate that LPL-*BoLA-DRB3*0902* cattle do indeed disrupt the BLV transmission chain and that selection of cattle carrying this allele represents a promising approach to control the virus [116].

The results presented are the base of a BLV control strategy based on genetic selection of resistant animals, using *BoLA-DRB3*0902* allele as molecular selection marker. The idea is to gradually replace HPL cattle in high-prevalence herds with *BoLA-DRB3.2*0902* harboring cattle. The expected increase in herd resistance to BLV would block the transmission of BLV, thereby counteracting the main epidemiological advantage of BLV, i.e., its transmission.

4.2. Influence of resistance and susceptibility-associated alleles on other health and productive traits

Due to the relevant role of MHC genes in the immune response, a potential risk in expanding or segregating *BoLA*-selected populations of cattle is that it might increase susceptibility to other common viruses. A special concern is raised by the strong association found between *BoLA-DRB3*0902* and *BoLA-DRB3*1701* and low antibody titer against major BLV structural proteins. This phenomenon, which may depend on host genetic factors, might influence the host response to other viruses requiring, unlike BLV, strong and long-lasting humoral immune response in order to prevent infection. Therefore, we determined the association between antibody titer against three widespread bovine viruses and *BoLA-DRB3* gene polymorphism. These three viruses, the bovine viral diarrhea virus (BVDV), the

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bovine herpesvirus type 1 (BHV-1), and the foot and mouth disease virus (FMDV), which are spread in most or several countries all over the world, cause diseases and syndromes that negatively affect the economic performance of the dairy and beef cattle industries. Protection against these three viruses is achieved when high titers of neutralizing antibodies are raised. No association was found between neutralizing antibody titers against FMDV, BVDV, or BHV-1 and polymorphism of the *BoLA-DRB3* gene [110]. Therefore, increasing *BoLA*-selected BLV-resistant cattle or segregating *BoLA* alleles associated with BLV susceptibility would not affect the resistance or susceptibility to BVDV, BHV-1, or FMDV infection. Further studies involving other parameters, such as the persistence or viral load status concerning FMDV, BHV-1, and BVDV, and their association to *BoLA-DRB3* polymorphism, are needed. These parameters would probably represent a better indicator of resistance against the aforementioned viruses.

Preliminary data obtained from endemic areas showed that there is no association between the allele *BoLA-DRB3*0902* and infection with BVDV, bovine herpesvirus type 4 (BoHV-4), and *Neospora caninum* [117]. A recent study also showed no association of the aforementioned allele with infection by *Mycobacterium bovis (M. bovis)*, although this observation should be confirmed in a more extensive study. Moreover, it was shown that the ability of cattle carrying resistance-associated marker to control BLV and to progress to LPL phenotype was not altered by *M. bovis* coinfection [118].

Alleles *BoLA-DRB3*0902* (or *11 as determined by PCR-RFLP) and *BoLA-DRB3*1701* (or *12 as determined by PCR-RFLP) are associated with resistance to intramammary infection and higher production traits [119, 120]. Thus, expanding the population of cattle harboring these alleles in order to control BLV infection would also increase resistance to mastitis. When targeting BLV control by means of genetic selection, animals carrying alleles associated to susceptibility, such as *BoLA-DRB3*1501*, are not desired. This allele did not show association with antibody titer against FMDV, BHV-1, or BVDV [110] nor it affected productive traits [45]. *BoLA-DRB3*1501* has been previously associated with high somatic cell score (SCS) in milk [44, 121]. Thus, if we are to decrease the frequency of the *BoLA-DRB3*1501* allele in order to control BLV infection, the SCS of the cattle population would additionally be reduced. It has been reported that BLV infection is frequently associated with an increase in SCS [122–124]. It is conceivable that this association derives from host genetic susceptibility to both conditions, probably conferred by *BoLA-DRB3*1501* allele.

Up to date, the presence of *BoLA-DRB3*0902* allele in the genotype of Holstein cattle neither has it been associated with susceptibility to any infectious disease nor with negative effects on production or reproductive traits.

4.3. Concluding remarks

We have proposed a BLV control and eradication program based on genetic selection. This plan is based on two main premises: (1) the development of LPL phenotype (BLV-resistant cattle) can be predicted by a marker (*BoLA-DRB3*0902*) that is transmitted to the progeny by controlled crossbreeding, and (2) BLV-infected cattle with LPL phenotype do not transmit the virus.

The *BoLA-DRB3*0902* heterozygous animals comply these two premises. This marker has a penetrance of 82 % and its pattern of inheritance is codominant. *BoLA-DRB3*0902* animals with LPL phenotype did not transmit the infection at herd level in natural conditions, and under experimental conditions, it was required to inoculate a large volume of blood to infect sheep. Finally, up the date, the *BoLA-DRB3*0902* allele has not been associated with susceptibility to other infectious agents, nor has it affected production or reproduction traits.

The BLV control and eradication program has been designed in two steps: as the first step, HPL cattle should be replaced by cattle harboring the *BoLA-DRB3*0902* marker. Once culling of HPL animals has been completed, as the second step, LPL cattle should be replaced by BLV-negative cattle. The selected animals for the replacement in the first step should carry the *BoLA-DRB3*0902* allele in heterozygosis with other alleles not associated with any BLV phenotype [53]. Finally, it should be mentioned that a potential risk exists in expanding or segregating *BoLA* genotype-selected populations, which might increase susceptibility to other infectious agents.

5. Conclusions

Among the diversity of genes associated to disease resistance, MHC genes are particularly interesting as they are associated with resistance and susceptibility to a wide range of diseases. Some of these diseases produce important economic losses in livestock. Genetic selection of resistant animals emerges as a natural strategy for the control of infectious diseases, especially when there is no other alternative of control or prevention, as vaccines.

The control of BLV infection based on genetic selection of resistant cattle is presented as a model that may potentially be replicated in other infectious diseases affecting livestock.

Author details

Silvina Elena Gutiérrez[1]*, Eduardo Néstor Esteban[1, 3], Claudia María Lützelschwab[1] and Marcela Alicia Juliarena[1, 2]

*Address all correspondence to: segutier@vet.unicen.edu.ar

1 Department of Animal Health and Preventive Medicine, Veterinary Research Center of Tandil (CIVETAN), CIC-CONICET, Faculty of Veterinary Science, National University of the Center of Buenos Aires Province, Tandil, Argentina

2 Department of Animal Production, Veterinary Research Center of Tandil (CIVETAN), CIC-CONICET, Faculty of Veterinary Science, National University of the Center of Buenos Aires Province, Tandil, Argentina

3 Director of BIOALPINA Program (GENIAL/COTANA), Colonia Alpina, Santiago del Estero, Argentina

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