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Review article

Association of *BoLA-DRB3* with bovine leukemia virus

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The major histocompatibility complex (MHC) of cattle is known as *BoLA* and located on chromosome 23. It was found that *BoLA* shows significant differences in genome organization with human MHC but shares similar protein structures and functions. Among the entire *BoLA class II subregion*, *BoLA-DRB3* is the most polymorphic and functional gene that has been intensively studied its correlation with various cattle infection diseases and economic traits. In particularly, *BoLA-DRB3* polymorphism has been linked to resistance or susceptibility of bovine leukemia virus (BLV) infection outcome, transmission and disease progression. BLV leads to enzootic bovine leucosis which is the most common neoplastic disease in cattle and leads to severe financial loss in industry worldwide. As a result, genetic selection of BLV-resistant animals, as well as the preferential culling of BLV-susceptible animals, based on *BolA-DRB3* polymorphism, serves as a promising strategy for the control of BLV spreading and disease risk estimation, especially in the time there are no vaccines and treatment available. In this review, we outline the *BoLA* system in protein and genetic levels and focus particularly on the literatures of association between *BoLA-DRB3* polymorphism with BLV.

キーワード：*BoLA-DRB3* polymorphism, BLV, disease susceptibility, association study, disease control

I. Introduction

The major histocompatibility complex (MHC) serves a central role in adaptive immune response in all vertebrate animals. The research on cattle MHC is benefited from a general appreciation for the origin of immunogenetics and the discoveries that led to the elucidation of the mouse H-2 complex and the human leukocyte antigen (HLA) system[1]. The discovery of the cattle MHC is attributed to Amorena[2], and Spooner and their co-worker[3]. The genetic region they and others defined using serological reagents produced by skin transplants and alloinmunization was named the bovine leukocyte antigen (BoLA) system[4]. BoLA appears to be organized in a similar way to HLA, but still some notable differences were found. Besides, BoLA has been linked to the susceptibility of diseases and appears to affect economic traits such as milk yield, growth and reproduction, which are not often measured in human[5]. Thus, it is of interest to investigate the relationship between BoLA with these diseases and traits. Among the *BoLA class II*

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subregion, *BoLA-DRB3* is the most polymorphic and well-studied gene that relates with cattle diseases. In this review, we summarized current knowledge of BoLA and emphasize on the part of the association between *BoLA-DRB3* and the major disease, bovine leukemia virus (BLV), in cattle.

II. Molecular features of BoLA

The protein structure of MHC class I and class II are basically conserved in different animal species. Most of current knowledge of MHC structures are based on HLA molecules which have been determined by X-ray crystallography[6,7]. A decade ago, the structure of BoLA class I has been solved and shown that it bound to an immunodominant epitope demonstrating unconventional presentation to T cell receptors which is extremely rare in other species[8]. The BoLA class I molecule is consisted of an α-chain that contains a transmembrane domain, and a β-chain derived from microglobulin. MHC class II is composed of an α-chain and a β-chain, and both chains have a transmembrane domain. The peptide binding groove of BoLA class I molecule is built from the α-chain; and for BoLA class II, it is constituted from both the α-chain and the β-chain. The structure of peptide binding groove of MHC molecule shapes the size of bound peptide. In BoLA class I, the length of bound peptide is usually restricted as 9–10 residues because of the closed structure at both ends of the peptide binding groove[9,10]. In contrast, BoLA class II has an binding groove with open-ends allowing accommodate a larger peptide ranging from 13–25 residues in length[11]. The bound peptide species of BoLA molecules are governed by the properties of peptide binding pockets of MHC binding groove, i.e. the geometry, charge distribution, and hydrophobicity[12].

III. Genomic organization of BoLA

BoLA has been mapped to the bovine chromosome 23 (ch23) and this organization differentiates *BoLA* from the MHC of humans and rodents where the *MHC* genes are located on chromosome 6 (*HLA*) and 17 (*H-2*), respectively[13]. The *BoLA* region includes approximately 154 predicted functional genes which could be categorized into three classes: class I, II and III[14]. *BoLA class I* covers the region on ch23 from 770 Kb to 1650 Kb, including classical class I gene *BoLA-A* and *BoLA-B* that are located 200 Kb far from each other which *BoLA-A* is closer to telomeric side of ch23[15]. In cattle, none of these classical *class I* genes identified is consistently expressed, and haplotypes differ from one to another in both the gene number and composition. However, in human, three classical, polymorphic genes (*HLA-A*, *-B* and *-C*) are each present on all haplotypes[16]. *BoLA class III* locates on the centromeric side of *BoLA class I* and is constituted by genes related to immunological functions or inflammation, such as the complement factors BF and C4, steroid 21-hydroxylase (*CYP 21*), TNF α (*TNFA*), and heat shock protein 70 (*HSP 70*)[16]. There is a major difference between *BoLA* with *HLA* in the class II subregion. The organization of *BoLA class II* subregion lies near the centromere of Chromosome 23[16] and differs from human and mice that it splits into two regions: *class IIa* and *class IIb* separated by at least 15cM[17] where *class IIb* subregion locates in the centrimeric side to the *class IIa* subregion. *BoLA class IIa* includes *DR* locus and *DQ* locus which *BoLA-DRA* is monomorphic. By contrast, there are three genes that encode for the β chain of the DR (DRB) molecule of which *BoLA-DRB1* is a pseudogene and *BoLA-DRB2* is barely expressed, whereas the *BoLA-DRB3* locus is the most polymorphic and strongly expressed gene from this group. In human, the *HLA-DRB* locus contains four copies of the *HLA-DRB* gene: *HLA-DRB1* (coding), *DRB5* (coding), *DRB6* (non-coding) and *DRB9* (non-coding). Among them, *HLA-DRB1* is the most polymorphic gene whereas *DRB6* and *DRB9* are non-coding gene. In *HLA* system, multiple *DQ* genes have been identified but only one DQ molecule is expressed[18]. On the country, cattle individuals could carry either a single copy of *DQA* and *DQB* genes or

duplicated haplotypes[19]. It is interesting to note that unlike in human genome, there are no *DP* genes in cattle, but here is one pair of *DY* genes that is absent in human. The *BoLA* class IIb subregion encodes *DMA*, *DMB*, *LMP2*, *LMP7*, and *TAP* genes, which are involved in antigen processing and transportation[20]. However, the function of other genes within class IIb, such as *DYA*, *DYB*, *DIB*, *DOB* and *DNA* is currently unclear[20].

IV. Diversity of *BoLA-DRB3*

In BoLA system, *BoLA-DRB3* is the most polymorphic gene which 384 alleles have been registered in the Immuno Polymorphism Database (IPD)-MHC database (https://www.ebi.ac.uk/ipd/mhc/group/BoLA/, accessed on 18 January 2022) and is associated with plenty of diseases. Thus, the diversity and distribution of *BoLA-DRB3* in different cattle breed and geographic locations are especially of interest to cattle breeders and veterinary geneticists to design breeding strategies for increasing the number of disease resistance offspring. There is over a billion of bovine worldwide (https://www.fas.usda.gov/data/livestock-and-poultry-world-markets-and-trade). The documented *BoLA-DRB3* diversity in different cattle breeds have been summarized in table 1. Although the allele diversity is varied in different location, the major alleles *DRB3 *001:01*, **011:01* and **015:01* are shared in Holstein cows. Nevertheless, a great allele distribution is observed between different cattle breeds. For example, the most abundant allele in Bolivian Nellore is *DRB3*028:01*; in Sudanese Kenana is *DRB3*024:01* while Japanese Jersey is *DRB3**045:01 which are all rarely found in Holstein cows. The observation suggests the importance in investigating allele divergency in different cattle breeds. In fact, there are over 1,000 recognized breeds of cattle worldwide and most of their *BoLA-DRB3* diversity has not been investigated yet[21].

Table 1 *BoLA-DRB3* diversity in different cattle breeds

Breed		cattle No.	Heard region	Number of different BoLA-DRB3 alleles	Major alleles (frequencies >10%)	Reference
Holstein	Argentinean	424	4	33	**001:01, *015:01, *011:01*	[52]
	Bolivian	159	2	23	**015:01, *009:02, *011:01, *010:01, *006:01*	[52]
	Chilean	113	4	21	**015:01, *001:01, *011:01*	[52]
	Japanese	102	-	18	**011:01, *012:01, *015:01, *001:01*	[53]
	Paraguayan	127	5	26	**001:01, *015:01, *011:01*	[52]
	Peruvian	133	2	20	**015:01, *011:01, *001:01*	[52]
	Vietnamese	81	-	27	**001:01, *012:01, *015:01, *027:03*	[54]
Other breed	Bolivian Gir	55	2	19	**015:01, *014:01:01*	[55]
	Bolivian Nelore	58	2	26	**028:01, *022:01*	[55]
	Chilean Black Angus	50	4	26	**002:01, *001:01, *011:01, *032:01*	[56]
	Chilean Hereford	25	3	15	**016:01, *020:01:02*	[56]
	Chilean Red Angus	50	5	29	**018:01, *001:01*	[56]
	Hartón del Valle Creole	33	-	24	**011:01*	[57]
	Japanese Black	100	-	23	**010:01, *016:01, *011:01*	[53]
	Japanese Jersey	69	-	14	**045:01, *002:01, *020:06, *025:02*	[53]
	Japanese Shorthorn	50	-	20	**012:01, *003:01, *009:01*	[53]
	Myanmar Pyer Sein	82	6	58	-	[58]
	Myanmar Shwe Ni	35	6	43	-	[58]
	Nellore x Brahman	98	1	33	**022:01, *009:02*	[55]
	Philippine Brahman	118	6	58	**030:01*	[58]
	Philippine Native	241	6	71	**003:01*	[58]
	Sudanese Baggara	57	2	46	-	[59]
	Sudanese Butana	26	3	33	**021:01*	[59]
	Sudanese Kenana	30	2	33	**024:01*	[59]
	Yacumeño Creole	56	-	35	**007:01*	[57]

V. Association of *BoLA-DRB3* with cattle economic traits and diseases

The polymorphic region, exon 2, of *BoLA-DRB3* encodes the peptide binding groove. The polymorphism in this region is therefore associated with the variability in the immune responsiveness in different individuals to particular infectious diseases and economic traits such as reproduction[22], colostrum and milk microbiota[23], milk quality and production rate[5]. For infection disease, *BoLA-DRB3* relates with mastitis[24,25], tick-borne disease[26,27], foot and mouth disease (FMD)[28], bovine herpesvirus 1 (BoHV-1)[29], bovine papillomavirus-induced bladder cancer[30], but not relates with the bovine tuberculosis (bTB) susceptibility[31]. The association studies of *BoLA-DRB3* and economic traits and disease are summarized in Table 2. Besides those infection diseases mentioned above, the most intensively studied topic is the relationship of *BoLA-DRB3* with bovine leukemia virus (BLV).

Table 2 Association of *BoLA-DRB3* with economic traits and diseases

Trait/ disease	BoLA	Effect	Ref.
Viral infection			
BLV-related	summarized in table 3		
bovine herpesvirus 1 infection	*37	susceptibility	[29]
Bovine Papillomavirus Infection/ bladder tumor	011:01	resistance	[30]
Foot and mouth disease	Hae III A	susceptibility	[60]
	Hae III C	resistance	[60]
Bacterial infection			
Mastitis	012:01	susceptibility	[61]
	015:01	susceptibility	[61]
	001:01	resistance	[61]
	011:01	resistance	[61]
	027:03	resistance	[61]
Staphylococci	001:01	susceptibility	[25]
	011:01	resistance	[25]
Escherichia coli	015:01	susceptibility	[25]
	011:01	resistance	[25]
fusobacteriosis	*03	resistance	[62]
	*22	resistance	[62]
	*16	susceptibility	[62]
	*23	susceptibility	[62]
	*24	susceptibility	[62]
Parasites			
Anaplasma marginale	*14	resistance	[26]
	*41	resistance	[26]
	*2	susceptibility	[26]
Babesia bovis	*14	resistance	[26]
	*3	susceptibility	[26]
	*16	susceptibility	[26]
Babesia bigemina	*10	resistance	[26]
	*51	resistance	[26]
	*20	susceptibility	[26]
Boophilus microplus	*18	resistance	[27]
	*20	resistance	[27]
	*27	resistance	[27]
Production traits			
milk yield	*36	decreased	[5]
fat yield	*36	increased	[5]
fat percentage	*36	increased	[5]
	*19	increased	[5]
protein percentage	*33	increased	[5]

VI. Association of *BoLA-DRB3* with BLV (PVL, infection, PL, lymphoma)

1) Characteristics of BLV

BLV is a retrovirus that closely relates with human immunodeficiency virus type- 1 (HTLV-1). BLV decreases conception rate and milk production in cow and causes enzootic bovine leucosis (EBL) which is the most common neoplastic disease in cattle[32]. After BLV infection, approximately 70% of infected cattle remain asymptomatic, 30% of infected animals develop persistence lymphocytosis (PL) characterized by proliferation of non-malignant polyclonal B-cell and approximately 1- 5% of infected individuals develop B-cell leukemia/lymphoma after a long latency period[32,33]. As a member of retrovirus, the genome copies of BLV could integrate into host genome called as provirus and lead to lifelong infection. The proviral load (PVL) is associated with viral transmission risk and infection outcome and thus is recognized as a marker for developing strategies against BLV spreading or monitoring disease progression[34-39]. As the vaccine and treatment for BLV infection have not been developed yet, alternative strategies of BLV controlling are required.

2) Genetic association of *BoLA-DRB3* with BLV

Host genetics polymorphism at *BoLA-DRB3* has been shown the relationship with susceptibility to PVL or BLV-induced symptom including persistence lymphocytosis and lymphoma in different countries and breeds of cattle (Table 3). Although *BoLA-DRB3* were genotyping through various methods in different researches, only the results from PCR sequence-based typing, which has the highest resolution were summarized in this review. In general, *DRB3*009:02* is the PVL resistance allele and *DRB3*011:01* is lymphoma resistance allele in most of these association studies. Furthermore, the effect of resistance allele is dominant over susceptibility allele (68). However, besides alleles *DRB3*009:02* and *DRB3*011:01*, other resistance or susceptibility alleles are varied in different cattle breeds and geographic locations, potentially due to the variation of allele frequency in different studies. Thus, it is worth to study the association between *BoLA-DRB3* and BLV in different region and cattle breeds. In Japan, the lymphoma and PVL associated alleles are well characterized in both Holstein and Japanese Black cattle. In Holstein cows, *DRB3*009:02* (Odds ratio (OR)=0.04) is the strongest PVL resistance allele followed by *DRB3*002:01* (OR=0.12) and *DRB3*014:01:01* (OR=0.31); while *DRB3*012:01* (OR=2.51) is a susceptibility allele. For lymphoma in Holstein cows, *DRB3*009:02* (Odds ratio (OR)= 0.07) is the strongest allele followed by *DRB3*014:01:01* (OR=0.36), *DRB3*011:01* (OR=0.40) and *DRB3*010:01* (OR=0.43). The PVL association study in Japanese Black cattle shows that *DRB3*009:02* is the strongest resistance allele followed by *DRB3*011:01;* while *DRB3*016:01* is a susceptibility allele. For the lymphoma related allele in Japanese Black cattle, *DRB3*011:01* (OR=0.19) is the resistance allele; while *DRB3*005:02* (OR=11.26) is the strongest susceptibility allele followed by *DRB3*016:01* (OR=2.95). It should be noticed that most of current conclusions were based on association studies which may vary due to sample collection, cattle breed, and cattle geography location. Consequently, future biological studies on the mechanisms of how the polymorphism of *BoLA-DRB3* affects diseases susceptibility are encouraged and will contribute to understanding the disease susceptibility strength to each *BoLA-DRB3* allele.

Recent researches further shown that BLV infectivity in cattle with PVL-resistance *BoLA-DRB3* allele is lower and has decreased horizontal[35] and vertical[34] transmission potential. Furthermore, we found that *BoLA-DRB3* polymorphism is associated with the PVL in milk that imply the potential mechanism how *BoLA-DRB3* affects BLV transmission in cattle[40]. Besides the effect from specific alleles, it has been found that individuals with *BoLA-DRB3* heterozygote have lower risk of both carrying high PVL and developing

Table 3 Association studies of *BoLA-DRB3* with BLV-related traits

Trait	Breed	cattle No.	heard region	effect	*BoLA-DRB3*	references
Proviral load	Japanese Ho	910	nationwide	resistance	*002:01, 009:02, 014:01:01*	[63]
				susceptibility	*012:01, 015:01*	
	Japanese Ho	611	nationwide	resistance	*002:01, 009:02, 014:01:01*	[64]
				susceptibility	*012:01*	
	Argentinean Ho	230	7	resistance	*009:02, 017:01*	[65]
				susceptibility	*015:01, 015:03*	
	Argentinean Ho	107	1	resistance	*002:01*	[66]
				susceptibility	*012:01, 015:01*	
	Argentinean Ho and Ho X J	832	16	resistance	*009:02*	[67]
				susceptibility	*010:01, 012:01*	
	Vietnamese Ho Friesian	162	Northrn Vietnam	resistance	-	[54]
				susceptibility	*012:01*	
	Japanese Black	187	1	resistance	*009:02, 011:01*	[68]
				susceptibility	*016:01*	
	Harton del Valle	100	not available	resistance	*009:02, 011:01*	[69]
				susceptibility	*020:01:02, 027:03*	
					010:02, 016:01, 017:01	
Persistent lymphocytosis	Iranian Ho	190	3	resistance	*032:02*	[70]
				susceptibility	*001:01, 011:01, 042:01*	
Lymphoma	Iranian Ho	190	3	resistance	*001:01, 011:01*	[70]
				susceptibility	*009:01, 018:02, 032:02*	
	Japanese Ho	471	nationwide	resistance	*009:02, 010:01, 011:01, 014:01:01*	[64]
				susceptibility	-	
	Japanese Black	333	nationwide	resistance	*011:01*	[71]
				susceptibility	*005:02, 016:01*	

Ho= Holstein

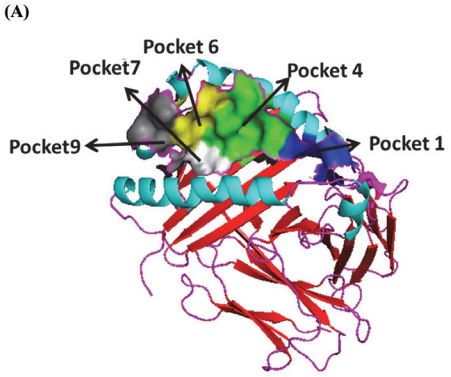
Ho X J = Holstein X Jersey

lymphoma irrespective of cattle breeds[41]. Therefore *BoLA-DRB3* is an ideal target that could be applied in disease risk estimation, or breeding management and selection to resist BLV-induced damage.

3) Antigen presentation and immune response on BLV-related disease

It is believed that the amino acid sequences within the highly polymorphic peptide binding region of BoLA-DR*β* (encoded by *BoLA-DRB3*) influence the binding and presentation of viral peptides to immune system. There are five binding pockets of BoLA-DR*β*, namely as pockets 1, 4, 6, 7, and 9, that responsible for the binding with the amino acids of peptide antigen at the corresponding positions[42]. These binding pockets restrict the species of bound peptide and thus influence the susceptibility to particular diseases[42-44]. It has been reported that amino acids 70 and 71 at pocket 4 of ovine leukocyte antigen (OLA) DRB1 relate with BLV-induced lymphoma in sheep[45]. Animals with pocket 4 resistance motif strongly expressed IFN-*γ*, the marker of Th1 response, which effectively inhibits viruses[46]. In contrast, individuals with susceptibility type motif strongly expressed IL-2, implying the Th2 response activation, which less efficiently inhibits BLV. These observations suggest the role of binding pocket polymorphisms in the determination of immune responsiveness and BLV-related disease susceptibility[46]. Besides, we recently found that in addition to amino acids 70 and 71 at pocket 4, amino acids 9, 11, 13, 26, 30, 47, 57, 70, 71, 74, 78, and 86 that covered all binding pockets 1, 4, 6, 7, and 9, were associated with BLV-induced lymphoma susceptibility in Japanese Black cattle (Figure 1A). Specifically, pocket 1: 86V; pocket 4: 13G, 70R, 71R, 74E, 78V; pocket 6: 30H; pocket7: 47F, 71R; pocket9: 9Q, 57S are lymphoma resistance. In contrast, pocket 1: 86G; pocket 4: 13K, 70E, 71K, 74A, 78Y; pocket6: 11T, 30Y; pocket 7: 47Y, 71K; pocket9: 9E, 57D are lymphoma susceptibility (Figure 1B). These results could contribute to the prediction of lymphoma risk of BLV-infected cattle with *BoLA-DRB3* rare alleles, which could not be evaluated in allele association studies owing to their low frequencies.

The electrostatic charge of protein affects protein-protein interactions[47]. In the case of MHC, the charge of MHC binding pocket influences peptide binding preference and thus affects susceptibility to different diseases. For instance, HLA-DQ2 molecule with positively charged binding pockets elevates its capacity to accommodate gluten-derived peptides which are rich in prolines and negatively charged glutamates[48]. Similarly, *HLA-DRB1* polymorphisms lead to differential electrostatic charges of binding pocket 9 and are thus related to the susceptibility to primary sclerosing cholangitis[49]. In the same line, we found two lymphoma susceptibility molecules, *DRB3*005:02* and *DRB3*016:01*, were neutrally charged in binding pocket 9; whereas the resistance BoLA-DR *β* molecule, *DRB3*011:01*, carries a positive charge. This electrostatic charge variation may allow the



(B)

Pocket 1		Pocket 4							Pocket 6		Pocket 7					Pocket 9		
85	86	13	26	28	70	71	74	78	11	30	28	47	61	67	71	9	37	57
G	M	S	F	D	E	K	E	V	S	Y	D	F	W	F	K	E	T	D
V	V	R	Y	E	R	A	A	Y	H	C	E	Y	L	I	A	Q	F	V
	G	G	L	N	D	E	N		Y	S	N		C	L	E		Y	S
	F	K			Q	R	S		C	H				T	R		L	A
							Y		A								N	
									T								R	
									L									

Figure 1

Susceptibility amino acid of BoLA-DR*β* peptide binding pocket to BLV-induced lymphoma. (A) Peptide binding pockets of BoLA-DR*β*. Protein structure of BoLA-DR*β* was generated using PyMOL 2.4 (Schrodinger LLC, New York, NY, USA). (B) Susceptibility of amino acid of BoLA-DR peptide binding pocket to BLV-induced lymphoma in Japanese Black cattle. Resistance amino acid was marked in blue. Susceptibility amino acid was marked in red.

recognition of different peptide antigens and thus exert a differential immune reaction against BLV-induced lymphoma. Although we have demonstrated that the charge of binding pockets associates with BLV-induced lymphoma the relationship between charges and BLV PVL still needs further investigation as it was known that lymphoma and PVL are associated with differential *BoLA-DRB3* polymorphism. Besides, it is worth to study binding pocket properties other than charge e.g. hydrophobicity[50], size or structure[51] that known for influencing peptide interaction.

VII. Perspective

BLV is a major risk causing severe financial damage in cattle industry. Plenty of researches show that polymorphism of *BoLA-DRB3* relates with BLV susceptibility. Thus, *BoLA-DRB3* or its protein BoLA-DRβ is ideal target in cattle for BLV-related disease risk estimation. In fact, the correlation of *BoLA-DRB3* polymorphism with BLV perinatal transmission has been demonstrated, strongly supporting the applicability of breeding strategy based on *BoLA-DRB3* to cope with BLV. In addition to vertical transmission, cattle carrying PVL resistance allele are not sources of BLV spreading and thus could act as biological barriers to prevent BLV horizontal transmission. Consequently, *BoLA-DRB3* polymorphism could be used in farm management to avoid BLV transmission. In fact, our lab has experimentally applied *BoLA-DRB3* polymorphism in cattle management in farms. By inserting cattle with PVL resistance allele as biological barriers between BLV infected and uninfected cattle and removing the high PVL cattle (high risk BLV transmitter) from farm, the overall BLV infection rate in farms are successfully decreased (unpublished data). Thus, based on these observations, BLV-related *BoLA-DRB3* alleles have potential to be applied in cattle breeding selection or farm management to reduce the damage-caused by BLV infection.

The vaccine and treatment for BLV infection are still unavailable. As *BoLA-DRB3* influences specific antigen peptide presentation and the efficiency is varied in different alleles, studying the polymorphism of *BoLA-DRB3* and the structure of BoLA-DRβ may help the development of vaccine against BLV. The binding pocket properties of each BoLA-DRβ molecule e.g. the polarity, hydrophobicity, charge of amino acids in binding pockets, confer the affinity of bound peptide and the following anti-BLV ability. The strong peptide binding affinity tends to trigger the cellular immunity that known for efficient antivirus and anti-tumor ability. So far, only the electrostatics charge is investigated with its association with BLV-induced lymphoma. Other properties and in-depth mutational and protein conformational studies are needed to gain more insights into the interaction between BoLA-DRβ and peptide that will contribute to vaccine development.

Disclosures

none to declare.

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