RESISTANCE AND SUSCEPTIBILITY TO PERSISTENT LYMPHOCYTOSIS IN RUSSIAN CATTLE BREEDS BY DISTRIBUTION OF BOLA-DRB3 ALLELES

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INTRODUCTION

Leukemia of cattle is a chronic tumor disease caused by bovine leukemia virus (BLV). BLV is an oncogenic retrovirus, which generates B-cell lymphocytosis, leukemia and lymphosarcoma of sheep and cattle. Persistent lymphocytosis (PL) is generated in 30 % of cows with BLV infection. PL is a subclinical stage of BLV infection. Development of PL is under the genetic control of the host. Alleles of *BoLA-DRB3* influence the subclinical development of BLV infection (Lewin *et al*., 1999). The spectrum of *BoLA–DRB3* alleles associated with resistance *(DRB3.2*11, *23, *28)* and susceptibility *(DRB3.2*8, *16, *22, *24)* to PL in Holstein-Friesian breed (Xu *et al*., 1993) was confirmed in Black Pied breed (Sulimova *et al*., 1995). Resistance was dominant in contrast with susceptibility, which was inherited as a complicated recessive Mendelian trait.

Short concrete amino acid sequences (or motives) in the protein sequences of class II antigens were revealed, which mediated the influence of definite alleles on PL development. The presence of the polar amino acid sequence *Glu-Arg* (ER) in positions 70-71 of β-1 domain indicates the connection of the *BoLA-DRB3* allele, coding the amino acid sequence, with resistance to leukemia (Xu *et al*., 1993). These positions of DR alleles are involved in the binding and corresponding location of virus peptide in the peptide binding site (PBS) and in T-cell recognition (Stern *et al*., 1994). Motive *Val-Asp-Thr-Tyr* (VDTY) in positions 75-78 determines the association with susceptibility to leukemia. The motive is identical to the short amino acid sequence of reverse transcriptase of BLV, that evidence molecular mimicry in the system "parasite-host". Homology of virus peptide with amino acid sequence of class II antigen of MHC, probably, causes reduced ability to recognize virus peptide in the carriers of *BoLA-DRB3* alleles, which code motive VDTY, or motive VDTV (*Val-Asp-Thr-Val*) with less prolonged homology to virus protein (Xu *et al*., 1993).

The goal of our study was to perform comparative analysis of associations of PL with alleles of BoLA-DRB3 in Ayrshire and Black Pied breeds.

MATERIALS AND METHODS

Selection of animal samples for the study. Cows of age from 3 to 12 years were included in the studied samples of Ayrshire (n = 101) and Black Pied (n = 85) cattle breeds from Moscow region farms. In these farms, healthy cows and animals infected with BLV (virus-carriers (PL-) and PL cows) were studied. Animals, infected by BLV, were detected in reaction of immune diffusion (RID) with glicoproteid BLV antigen.

Extraction of DNA and typing of BoLA-DRB3 alleles. Samples of DNA were isolated from blood of cows using kit of reagents DIA***tom***™ DNA prep ("Biokom", Moscow).
Alleles of gene *BoLA-DRB3* were studied by polymerase chain reaction with subsequent restriction fragment length polymorphism analysis (PCR-RFLP). PCR was performed with primers HLO30, HLO31 and HLO32 (Van Eijk *et al*., 1992). Alleles of gene *BoLA-DRB3* were identified with restriction analysis of PCR-product (284 bp. or 281 bp for alleles with deletion of codon 65) in standard conditions with endonucleases *Rsa*I, *Hae*III and *Xho*II ("Promega"). We used nomenclature of *BoLA-DRB3* alleles identified by PCR-RFLP, proposed earlier (Van EiJk *et al*., 1992).

Statistical analysis. Profiles of allele frequencies of *BoLA-DRB3* in the ill with PL, virus-carriers and healthy animals were compared by χ^2- criterion. We calculated the estimate of relative disease risk (RR) to detect the probability of PL development for cows with concrete *BoLA - DRB3* allele in their genotypes or concrete genotype by *BoLA - DRB3.* We used formula : $RR = F_{PL}*(1 - F_{PL- BLV-}) / F_{PL- BLV-}*(1 - F_{PL})$, where F is the frequency of concrete genotype. Significance of RR values was estimated by exact probability by Fisher's method. For distinct groups estimates of the sample inbreeding coefficient (F) were determined.

Table 1. Relative risk of PL disease for cows of Ayrshire and Black Pied breeds with different genotypes according to the amino acid motives in positions 70-71 and 75-78, coded by *BoLA-DRB3* alleles

Genotypes by motives 75-78(70-71)	Ayrshire breed						Black Pied breed		
	PL	PL-BLV-	RR	PL	PL-BLV+	RR	PL	PL BLV-	RR
	n=40	n=37		n=40	n=24		n=28	n=22	
VDTY/X	21	16	1.45 [B]	21	3	7.74 [E]	24	13	4.14 [B]
VDTY/ VDTY	12	5	2.74 [B]	12	2	4.71 [F]	7	4	1.50 [B]
VDTY/ VDTV	1	3	0.29 [B]	1	0	Nd [B]	10	1	11.7 [F]
VDTV/X	6	3	2.01 [B]	6	1	4.06 [B]	13	6	2.34 [B]
VDRV/X	14	12	0.89 [B]	14	13	0.63 [B]	7	14	0.16 [F]
VDRV (ER+)/X	10	9	1.04 [B]	10	13	0.32 [F]	2	8	0.13 [F]
VDRV (ER-)/X	4	3	1.26 [B]	4	1	2.52 [B]	5	6	0.44 [B]
7* [A]/X	17	21	0.51 [B]	17	20	0.13 [D]	0	3	0 [B]
7*/7*	6	1	6.35 [B]	6	8	0.53 [B]	0	0	Nd [B]
7*/VDTY	0	6	Nd [CE]	0	2	0 [B]	0	1	0 [B]
7*/VDTV	5	3	2.50 [B]	5	1	3.29 [B]	0	0	Nd [B]

[A] - Amino acid motif of allele *BoLA-DRB3.2*7* is given separately because it carries deletion of codon 65 and its antigen has changed conformation. [B, D, E , F]- exact Fisher's probability : $P > 0.05$, $0.01 < P < 0.01$, $P = 0.001$, $0.01 < P < 0.05$, respectively. [C] - Nd (not determined) values of RR because the genotype was absent in the sick cows.

RESULTS

An association of *BoLA-DRB3* alleles with resistance to leukemia in Ayrshire and Black Pied cattle breeds. We observed preferentially horizontal virus transmission in Black Pied breed and substantial proportion of vertical virus transmission (before development of immune competence) in the herd of Ayrshire breed. In two breeds, we detected *BoLA-DRB3* genotypes mediating significant susceptibility of cows to PL : genotypes coding VDTY/VDTV (in Black Pied breed) and VDTY/X and VDTY/VDTY (pos. 75-78) (in Ayrshire breed (Table 1). We observed significant resistance of cows with genotypes coding BoLA-DRB3.2*7/X (in Ayrshire breed) and ER/X (pos. 70-71) (in two breeds) (table 1). Distribution of values of heterozygosity by *BoLA-DRB3* alleles and by crucial motives which they code indicate high homozygosity of ill cows in two breeds especially prominent in respect of motives (table 2). Heterozygosity level can be considered as unspecific factor of resistance to PL, which is especially important, when BLV transmission occurs before development of immune competence and usual genetic resistance mechanisms to PL do not operate.

Table 2. Distribution of values of heterozygosity level in cows of Ayrshire and Black Pied breeds with different PL status

H	Ayrshire breed			Black Pied breed		
	PL n=40	PL-BLV+ n=24	PL-BLV- n=37	PL n=28	PL-BLV+ n=10	PL-BLV- n=22
	By *BoLA-DRB3* alleles					
Ho	0,725	0,750	0,810	0,929	1,000	0,946
He	0,854	0,638	0,813	0,842	0,875	0,911
F	0,151	-0,176	0,004	-0,104	-0,143	-0,038
	By antigen motives in positions 75 - 78, coded by *BoLA-DRB3* alleles					
Ho	0,475	0,630	0,700	0,550	0,670	0,700
He	0,703	0,600	0,682	0,600	0,678	0,685
F	0,324	-0,050	-0,026	0,084	0,011	-0,021

Ho – observed level of heterozygosity, He – expected level of heterozygosity, F – inbreeding sample coefficient.

CONCLUSION

The influence of amino acid motives in β1 domain of the antigen coded by *BoLA-DRB3* alleles in resistance and susceptibility to PL was confirmed : ER (positions 70-71) and VDTY and VDTV (75-78), respectively. Susceptibility to PL was demonstrated for the cows with genotypes, coding VDTY/VDTV motives in Black Pied breed (RR = 11,67, p=0.014) and with VDTY/ X and VDTY/VDTY — in Ayrshire breed (RR= 7.74, p = 0.001, RR = 4,71, p = 0,022, respectively).

Heterozygosity level by *BoLA-DRB3* alleles and by amino acid motives (in positions 75 – 78), which are coded by them, was demonstrated to be an unspecific factor of resistance to PL. The influence of this factor was shown to be the most prominent in the cases of vertical BLV transmission (in Ayrshire breed).

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