ASSOCIATION STUDY OF SNP OF THE TNF-ALPHA GENE WITH BOVINE LEUKOSIS AND EVALUATION OF ITS FUNCTIONAL SIGNIFICANCE

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SUMMARY

Motivation: The bovine leukemia virus (BLV) is an infectious agent that has taken enormous economic tolls in cattle breeding worldwide. Efficient search of the molecular-genetic markers of susceptibility to this infectious disease is required for effective breeding. The TNF-alpha protein plays an important defensive role during the early stages of the disease. The aim was to analyze the association of polymorphism of the TNF-alpha gene with bovine leucosis susceptibility and to evaluate its possible functional contribution to the pathogenesis of the disease.

Results: A single nucleotide polymorphism (SNP), G to A substitution in the first intron of the TNF-alpha gene (G4389A), was detected in black-spotted cattle. The occurrence frequency of the variant A of this polymorphism was significantly higher among the BLV carriers and animals at the terminal stage of leucosis than in the healthy controls.

The region containing the SNP G4389A comprised the potential sites for the transcriptional factor binding (NF-kB, E2F/DP1, SAP1). The DNA region was highly homologous and consequently conserved in bovine, human mouse and rat. Hence, a cause of the TNF-alpha gene association with bovine leucosis may be alteration of DNA interaction with transcription factor due to the nucleotide substitution at the G4389A.

INTRODUCTION

The bovine leukemia virus (BLV) is an infectious agent causing great economic losses. The data in the literature indicate that the certain cattle breeds are more susceptible to the BLV infection than others. However, to combat leucosis and to provide effective breeding for resistance to BLV infection, search of the molecular-genetic markers of susceptibility to the bovine leucosis is needed. The TNF-alpha gene can be involved in leucosis susceptibility. It is known that TNF-alpha performs an important defensive function in the early phase of BLV infection. When exogenous TNA-alpha is added to BLV infected cells *in vitro*, the expression of viral antigens is strongly suppressed (Meirom *et al*., 1997). TNF(-/-) mice are rendered more susceptible to an infection with BLV (Muller *et al*., 2003). The mean mRNA expression level for TNF-alpha is considerably higher in the spontaneously proliferating peripheral blood mononuclear cells (PBMCs) derived from BLV-infected cattle than in non-spontaneously proliferating PBMCs from normal cattle (Konnai *et al*., 2006).

Detection of the association between the gene polymorphism under study and a disease does not, as yet, mean that the gene polymorphism is the direct cause of disease. Other neighbor polymorphisms may perhaps be causative. To define the putative involvement of polymorphism in the emergence of disease, an evaluation of its functional significance is another requirement. The current bioinformatics tools allow predicting the potential functional significance of any DNA region.

Here our aim is to analyze the association between the polymorphism (G4389A) of the first intron of the TNF-alpha gene with bovine leucosis and to evaluate the possible contribution of the polymorphism to the pathogenesis of the disease.

METHODS AND ALGORITHMS

Based on serological and hematological assays, 5 groups of black-spotted cattle were differentiated: with terminal leucosis (N = 11), persistent lymphocytosis (N = 14), and BLV-carriers (N = 20) from the cattle farm "Novospasskoe", also BLV-carriers (N = 63) and healthy (BLV-free) controls (N = 28) from the cattle farm "Tulinskoe". The groups of BLV-carriers from the two farms did not significantly differ by genotype and allele frequencies. For this reason, the two groups were pooled as one BLV-carrier group (N = 83). Allele-specific PCR was utilized for DNA genotyping. To recognize the potential binding sites for the transcriptional factors (TF), we applied the SITECON method, based on analysis of the physicochemical and conformational DNA properties (Oshchepkov *et al.*, 2004).

RESULTS

In black-spotted cattle widely bred in Russia, single nucleotide polymorphism in the first intron of the TNF-alpha gene was detected. The polymorphism resulted from G to A substitution at position 4389 (GenBank accession number Z14137).

The results of analysis of the association between the SNP G4389A of the TNF-alpha gene with BLV-carriers and various stages of leucosis are summarized in Table 1. Variant A at position 4389 occurred significantly more frequently in BLV-carriers and in those with leucosis at its terminal stage, as compared with the healthy controls. The variant A frequency in cattle with persistent lymphocytosis was also higher than in the controls; however, the differences did not reach statistical significance.

Table 1. Genotype and allele frequencies of the SNP G4389A of the TNF-alpha gene in BLV-carriers, and at the two leucosis stages

	Genotype frequencies, n (%)			Allele frequencies, n (%)	
	G/G	G/A	A/A	G	A
BLV-carriers (N=83)[1]	58 (69.9 %)	24 (28.9 %)	1 (1.2 %)	140 (84.3 %)	26 (15.7 %)
Persistent lymphocytosis (N = 14)[2]	12 (85.7 %)	2 (14.3 %)	0 (0.0 %)	26 (92.9 %)	2 (7.1 %)
Terminal leucosis (N = 11)[3]	7 (63.6 %)	4 (36.4 %)	0 (0.0 %)	18 (81.8 %)	4 (18.2 %)
Healthy controls (N = 28)	27 (96.5 %)	1 (3.5 %)	0 (0.0 %)	55 (98.3 %)	1 (1.7 %)

[1] Differences from the controls are significant for the genotypes ($\chi^2 = 8.2$, $p < 0.02$) and for the alleles ($\chi^2 = 6.3$, $p < 0.02$); [2] Differences from the controls are insignificant; [3] Differences from the controls are significant for the genotypes ($\chi^2 = 4.9$, $p < 0.03$) and for the alleles ($\chi^2 = 4.6$, $p < 0.03$).

Using the SITECON method for the recognition of the TF binding sites, we analyzed a DNA region of 100 bp of the first intron of the TNF-alpha gene containing the SNP G4389A. Recognition of the binding sites was performed for TF of about 200 types. It was found that the 100 bp region contained a considerable number of potential TF binding sites. The G to A substitution had the strongest effect on the context-dependent

conformational and physicochemical DNA properties significant for the interaction with the NF-kB, E2F/DP1, SAP1 factors (Fig. 1). Multiple alignment of the bovine, human, mouse, and rat DNA fragments of the TNF-alpha gene demonstrated that the motif containing G4389A is highly conserved in these four species (Fig. 2).

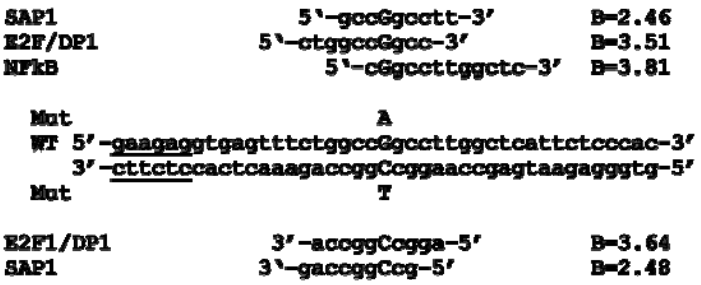

Figure 1. Results of context analysis of the intronic DNA fragment containing of SNP G4389A of the TNF-alpha gene. A rectangle outlines the bovine TNF-alpha gene sequence. The sequence that corresponds to the end of the first exon is underlined. The alternative nucleotides that occur at position 4389 are denoted by capital letters. The sequence regions of the bovine TNF-alpha gene that is homologous to the potential TF binding sites NF-kB, E2F/DP1, and SAP1 in direct orientation are above the gene sequence, those found in the reverse orientation are under it. B is the ratio of the scores for the two context similarities of the potential TF binding sites for the normal and rare alleles.

Figure 2. Multiple alignments of the bovine (GenBank accession number Z14137), human (AY066019), mouse (M20155) and rat (D00475) DNA fragments of the first intron of the TNF-alpha gene. The first exon is underlined. The identical nucleotides are shaded. SNP G4389A is in bold.

DISCUSSION

The obtained data indicate that the variant A of SNP G4389A affects the cattle susceptibility to infection with the BLV and also the clinical pattern of leucosis. There was no significant association between the polymorphism under study and persistent lymphocytosis presumably because of the small sample size.

The data on context DNA analysis for the first intron of the TNF-alpha gene and the high homology of the bovine nucleotide sequence comprising polymorphism with the same sequences in the three mammalian species demonstrate that the SNP G4389A occurs most likely in the functionally significant TF binding site or in a composite element. It is known that the NF-kB TF can regulate the expression of the human MICA (Molinero *et al*., 2004), and BCL3 (Ge *et al*., 2003) genes by binding to the specific sequence in their introns. Possibly, change in the ability of NF-kB to bind to the first intron of the A variant carriers may affect on the expression of the TNF-alpha gene and, as a consequence, modify antiviral immunity. Other alternatives may be the influence on the binding of the heteromeric E2F1/DP1 TF that regulates cell cycle or on the binding of the SAP1 TF that alters the expression of the *c-fos* oncogene. It should be stipulated that we detected an association between the SNP G4389A and BLV susceptibility at the early stage of the disease (BLV-carriers). This lends credibility to our suggestion that precisely NF-kB binding may be the cause of the association.

It should be noted that in human, mouse and rat, adenine is at position homologous to SNP G4389A, i.e. the rare variant that is associated with cattle susceptibility to infection with BLV and bovine leucosis. Search in the dbSNP database also detected no SNPs in a region 10 bp upstream and 10 bp downstream from SNP G4389A in human, mouse and rat. This was expected because in the closely related species as human and orangutan scanning on the long arm of the X chromosome also did not reveal shared SNPs between the species (Miller, Kwok, 2001). BLV is a model for studying of human leukemias caused by the closely related human T-cell leukemia virus type 1 (HTLV-1) (Johnson *et al*., 2001). There are reports indicating that BLV has potential infectivity and oncogenicity for humans (Johnson, Griswold, 1996, among other). Our current data suggests that the TNF-alpha gene is promising for the identification of hereditary predisposition to leukemia in human.

It may be concluded that the variant A of the TNF-alpha gene is associated with cattle susceptibility to infection with BLV and bovine leucosis. The current study has demonstrated this for black-spotted cattle. A mechanism underlying this association may possibly be the effect of polymorphism on the interaction between DNA and the NF-kB, E2F/DP1, and SAP1 transcriptional factors. This ultimately results in altered expression of the TNF-alpha gene.

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