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Functional analysis of a single nucleotide polymorphism in a potential binding site for GATA transcription factors in the ovine interleukin 2 gene

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Abstract

The transcription factor GATA-3 is one regulator of Th1/Th2 differentiation. In sheep, we recently discovered a putative GATA-binding site (WGATAR) in the second intron of the Th1-cytokine gene interleukin 2 (*IL2*), showing a single nucleotide polymorphism (G/C). As genetic variations in cytokine genes are thought to regulate cytokine production, we studied the significance of this polymorphism for *IL2* transcription. Sheep with different *IL2* genotypes were identified by single-strand conformation polymorphism (SSCP)-analysis and *IL2* transcription levels in peripheral blood mononuclear cells (PBMC) isolated from these animals were compared. For this purpose, transcription of *IL2* mRNA was quantified by real-time polymerase chain reaction in unstimulated PBMC and in PBMC incubated for 4 h in the presence of concanavalin A (ConA) or phorbol 12-myristate 13-acetate plus ionomycin (PMA/I). Compared to unstimulated cells, stimulation with ConA and PMA/I increased the *IL2* mRNA transcription in average by 300- and 20-fold, respectively. Nevertheless, no significant differences in *IL2* transcription between the genotypes could be detected. These findings were confirmed by band shift studies using different oligonucleotides containing variations of the potential binding motif, which showed no differences in the gel mobility after incubation with nuclear extract containing GATA-3. The obtained results argue against an impact of this polymorphism on the *IL2* transcription and the genetic disease resistance in sheep.

Keywords: Sheep; Interleukin 2; GATA-3; Single nucleotide polymorphism; *IL2* transcription; Band shift assay

Despite some evidence that the classical Th1/Th2 paradigm is oversimplified (Brown et al., 1998), the divergence of the T helper cell subsets (Th1 and Th2) during a developing immune response is believed to be critical for the outcome of infections (Abbas et al., 1996). Human Th1 cells preferentially develop during

Abbreviations: SSCP, single-strand conformation polymorphism; SEM, standard error of mean

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infections by intracellular bacteria, protozoa and viruses, whereas Th2 cells predominate during helminthic infections (Romagnani, 1994). The latter can also be observed in sheep after challenge with the nematode *Haemonchus contortus* (Gill et al., 2000): in lambs bred for resistance to *H. contortus*, lower abomasal worm burdens are paralleled by significantly stronger Th2-responses in comparison to random-bred lambs with higher abomasal worm burdens. Similarly, sheep that are genetically resistant to *Trichostrongylus coumbriformis* mount a Th2-type cytokine response following natural exposure to the nematode (Pernthaner et al., 1997). Although genetic resistance in sheep is controlled by polarization of T helper subsets like in other species, the underlying molecular mechanisms remain to be elucidated.

In man and mice, the transcription factor GATA-3 appears to play a dominant role in regulating Th1 and Th2 development, as ectopic expression of GATA-3 in developing Th1 cells induces Th2 cytokines and inhibits Th1 cytokines (Lee et al., 2000). A GATA-binding site in the second intron of the murine interleukin 4 gene differs in its enhancer activity after sequence alteration (Henkel and Brown, 1994). No information has yet been published about the presence of GATA-sites in Th1 cytokine genes, including interleukin 2 (*IL2*), and their possible role. We recently identified a WGATAR (W = A or T, R = A or G) motif, a potential target site for GATA-3 as described by Merika and Orkin (1993), in the second intron of the ovine, caprine and bovine *IL2* by sequence analysis (GenBank accession numbers AF287479 (ovine), AF535145 (caprine) and AF535144 (bovine), position 646–651 in the ovine sequence). Comparison of the ruminant sequences with the second intron of *IL2* from man, pig and chicken revealed a high conservation of this motif between these species (Fig. 1), pointing to a potential function. Interestingly, we observed a nucleotide transition (G/C) within this motif at nucleotide position 647 (Fig. 1, sequence ''OA'', underlined) in one sheep each of Romanov and Grey Horned Heath sheep. Polymorphisms in cytokine genes might regulate cytokine production (for review, see Warlé et al., 2003), as shown in human *IL2* for the −330 promoter polymorphism (Hoffmann et al., 2001; Matesanz et al., 2004). The aim of this study was to develop a single-strand conformation polymorphism (SSCP)-analysis method to facilitate the screening of sheep for the G/C-transition regarding the potential binding motif and to investigate its functional impact by comparing *IL2* transcription levels in PBMC isolated from sheep with different *IL2* genotypes (*GG*, *CC*, *CG*). Additionally, we tested the significance of the nucleotide transition for interactions of GATA-3 with the binding motif by band shift studies. Results of this study should indicate whether the observed polymorphism might be valuable as a genetic marker for disease resistance in sheep.

For SSCP analysis, a 603-bp PCR fragment containing the G/C-transition in the second intron of ovine *IL2* was amplified with the primers 5′-AACTTCTACATGCCCAAGGTT-3′ (forward) and 5′-GGTTAAATAATCTGCCCTAGG-3′ (reverse) for

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          629                                     670
OA        aaaaagaacatcaagtttgataatg.ggcttctgaaaaatggc
CH        aaaaagaacatcaagtttgataatg.ggcttctgaaaaatggc
BT        aaaaagaatctcaagtttgataatg.ggtttctgaaaaatggc
SC        aa....aac..caagtttgataataaggcgtctgaaaaatgcc
HS        ..gaa.aacc.caagtttgataatgaagcctct.attaa.a.a
GG        ttcttaaacattaac.aagataatga.....atgatattactt
Consensus ------aa----aa---wgataat--------t-a--------
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Fig. 1. Genomic sequence of *IL2*, intron 2, containing a putative GATA-binding motif (bold typed). Cross-species alignment of consensus sequences from sheep (*Ovis aries*, OA), goat (*Capra hircus*, CH), cattle (*Bos taurus*, BT), man (*Homo sapiens*, HS), pig (*Sus scrofa*, SC) and chicken (*Gallus gallus*, GG). Dots indicate absence of bases in comparison to the other sequences. Where the sequences of all four species are identical, consensus is indicated. Lack of consensus is marked by dashes. The position of the nucleotide transition G/C within the putative GATA-binding motif identified in Romanov and Grey Horned Heath sheep is underlined within the sequence ''OA''. GenBank accession numbers: AF287479 (OA), AF535145 (CH), AF535144 (BT), X00695.1 (HS), AB041935.1 (SC) and AJ224516.1 (GG). Nucleotide positions refer to sequence AF287479.