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Sequence Analysis of Goat Major Histocompatibility Complex Class I Genes

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ABSTRACT

The polymorphism of major histocompatibility complex (MHC) class I genes has been often involved in the resistance/susceptibility to a variety of infectious and parasitic diseases. In this work, the complete sequence of the coding region of a major histocompatibility complex (MHC) class I gene in goats (*Cahi-N*01701,* GenBank accession no. EF569216) is reported. The length of the corresponding open reading frame was 1,077 bp encoding a mature protein of 337 amino acids. Sequencing of additional clones allowed us to identify a second locus (*Cahi-NC4*50301,* GenBank accession no. EF569217) that, after performing a Bayesian phylogenetic analysis, happened to cluster with a bovine nonclassical MHC class I gene. Nonclassical MHC class I molecules display low levels of polymorphism and fulfill an important immunoregulatory role in the placenta to inhibit maternal rejection. This initial description of the gene content of the goat MHC class I region will contribute to the characterization, in this ruminant species, of one of the most important genetic factors in the elicitation of innate and adaptive immune responses against pathogens.
Key words: goat, major histocompatibility complex, classical and nonclassical MHC class I genes

Name of Sequence

Goat major histocompatibility complex class I *Cahi-N*01701* and *Cahi-NC4*50301* alleles.

GenBank Accession Nos.

EF569216 and EF569217.

Species

Capra hircus.

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Origin of the Clones and Sequencing Method

Total RNA was extracted from either liver or blood of 4 Murciano-Granadina goats according to Amills et al. (2002, 2003). Complementary DNA was synthesized with the ThermoScript kit (Invitrogen S.A., Barcelona, Spain). A 12-μL reaction containing 1 μL of oligo dT primer (50 μ*M*), 1 μL of total RNA (1 to 2 μg/μL), 2 μL of dNTP, 10 m*M*, and diethyl pyrocarbonate-treated water was incubated at 65°C for 5 min and kept on ice. A master mix containing 4 μL of 5× cDNA synthesis buffer, 1 μL of dithiothreitol, 1 μL of RNaseOUT (40 U/μL, Invitrogen), 1 μL of diethyl pyrocarbonate-treated water, and 1 μL of ThermoScript reverse transcriptase was added to each reaction. Each reverse transcription (**RT**) reaction was incubated at 50°C for 1 h and terminated by incubating at 85°C for 5 min; RT reactions were stored at –20°C until use.

Two primers were designed to amplify 1.3 kb of the goat major histocompatibility (**MHC**) class I gene/s (**PCR1**): mhcI-5′, 5′- ATG GGG CCG CGA ACC CTC CT-3′ and mhcI-3′ 5′-GAT GGA GAC GGC CCA GCC C-3′. The thermal profile consisted of 31 cycles of 94°C for 90 s, 57°C for 2 min, and 72°C for 4 min. The composition of the amplification reaction was 1.5 m*M* $MgCl_2$, 100 μ*M* of each dNTP, 0.5 μ*M* of each primer, 6 μL of RT reaction, and 1.25 U of *Taq* DNA polymerase (Ecogen s.r.l., Barcelona, Spain) in a final 50-μL volume. For some technical reason, PCR1 was successful in amplifying class I sequences in only 1 of 4 of the analyzed individuals. As a consequence, a second amplification protocol (**PCR2**) was devised based on employing primers mhcI-5′ and mhcI-3I, 5′-GGC CCA GCA CCT CAG GGT GAC-3′, which is more internal than mhcI-3′. This PCR protocol amplified 0.69 kb of the coding region and was more robust and efficient than PCR1 (Figure 1), likely because mhcI-3′ is complementary to the 3′ untranslated region that is highly divergent among class I loci. The conditions of PCR2 were as previously described for PCR1 but the number of cycles was 36. Amplicons (PCR1 from 1 individual and PCR2 from 3 individuals) were purified with the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany), cloned in the pCR

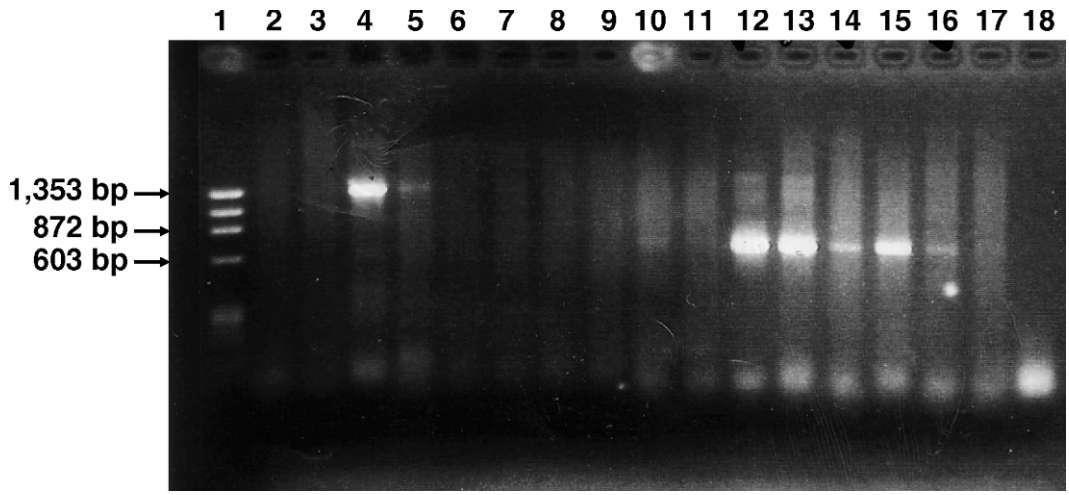

Figure 1. Comparison of the efficiency of 2 PCR reactions (PCR1 vs. PCR2) when amplifying major histocompatibility (MHC) class I sequences in 8 goats (the number of cycles was 36 in both cases). Lane 1 = Marker IX (Roche Diagnostics SL, Barcelona, Spain): 1,353, 1,078, 872, 603, 310, 282/271, 234, 194, 118, and 72 bp; lanes 2 to 9 = PCR1 (25% of successfully amplified samples); lanes 10 to 17, PCR2 (75% of successfully amplified samples); lane 18 = negative control.

2.1-TOPO vector (Invitrogen S.A.) and sequenced forward and reverse following the protocols described by Amills et al. (2003). Fifteen clones were sequenced forward and reverse. We only considered as valid MHC class I alleles those represented by good quality sequences obtained in at least 3 independent clones. More specifically, sequences *Cahi-N*01701* and *Cahi-NC4*50301* were represented by 3 and 4 clones, respectively. A Bayesian phylogenetic analysis was performed with the MrBayes 3.1 software (Ronquist and Huelsenbeck, 2003). Caprine (GenBank accession no. EF569216 and EF569217), ovine (GenBank accession no. AJ874673 to AJ874682), and bovine (GenBank accession no. DQ140357 to DQ140360, L02833, X80933, AY188805, AB259012, DQ140371, and DQ140374) MHC class I sequences were analyzed by using a pig sequence (GenBank accession no. DQ104338) as an outgroup. A model was used with 6 different substitution rates and the flat priors defined in the program to describe nucleotide frequencies, proportion of invariant sites, and the α-parameter (Ronquist and Huelsenbeck 2003). The phylogenetic tree was visualized with TreeView (Page, 1996).

Comments

The MHC class I molecules play a fundamental role in the innate and adaptive immune response by presenting self- and pathogen-derived peptides to $CD8^+$ T cells as well as by regulating natural killer cell function (Janeway et al., 2004). The MHC class I molecules comprise several structural domains including the highly polymorphic α_1 and α_2 domains, mostly involved in peptide binding, the immunoglobulin-like α_3 domain, which pairs with β-microglobulin, and the transmembrane and cytoplasmic domains (Janeway et al., 2004). In cattle, sheep, and goats, resistance and susceptibility to a variety of infectious and parasitic diseases such as mastitis (Vage et al., 1992; Schukken et al., 1994), bovine leukemia virus infection (Lewin et al., 1999), caprine arthritis-encephalitis (Ruff and Lazary, 1988; Ruff et al., 1993), and trichostrongilosis (Douch and Outteridge, 1989) have been associated with the polymorphism of the MHC class I genes. These features reinforce the interest in identifying the number of class I genes expressed in these ruminant species and in characterizing their levels of genetic variation.

The genomic organization of the MHC class I region is very complex and its gene content differs amongst mammalian species. In cattle, there is evidence of the existence of at least 6 expressed classical MHC class I genes (Ellis, 2004), and 62 alleles have been validated and stored in the IPD-MHC database (http://www.ebi.ac.uk/ipd/mhc/bola/index.html). A minimum of 4 nonclassical MHC class I genes have also been defined in cattle, being highly expressed in the trophoblast (Davies et al., 2006). The analysis of ovine class I sequences suggests the existence of at least 4 transcribed class I loci, 2 of which are highly expressed at the mRNA level (Miltiadou et al., 2005; Dukkipati et al., 2006). In goats, the amount of information about MHC class I genes is very sparse because several serological and isoelectric focusing specificities have been defined but the identities of the loci encoding them are unknown (Nesse and Ruff, 1989; Joosten et al., 1993). In this work, the complete coding region of a goat MHC class I gene (Gen-

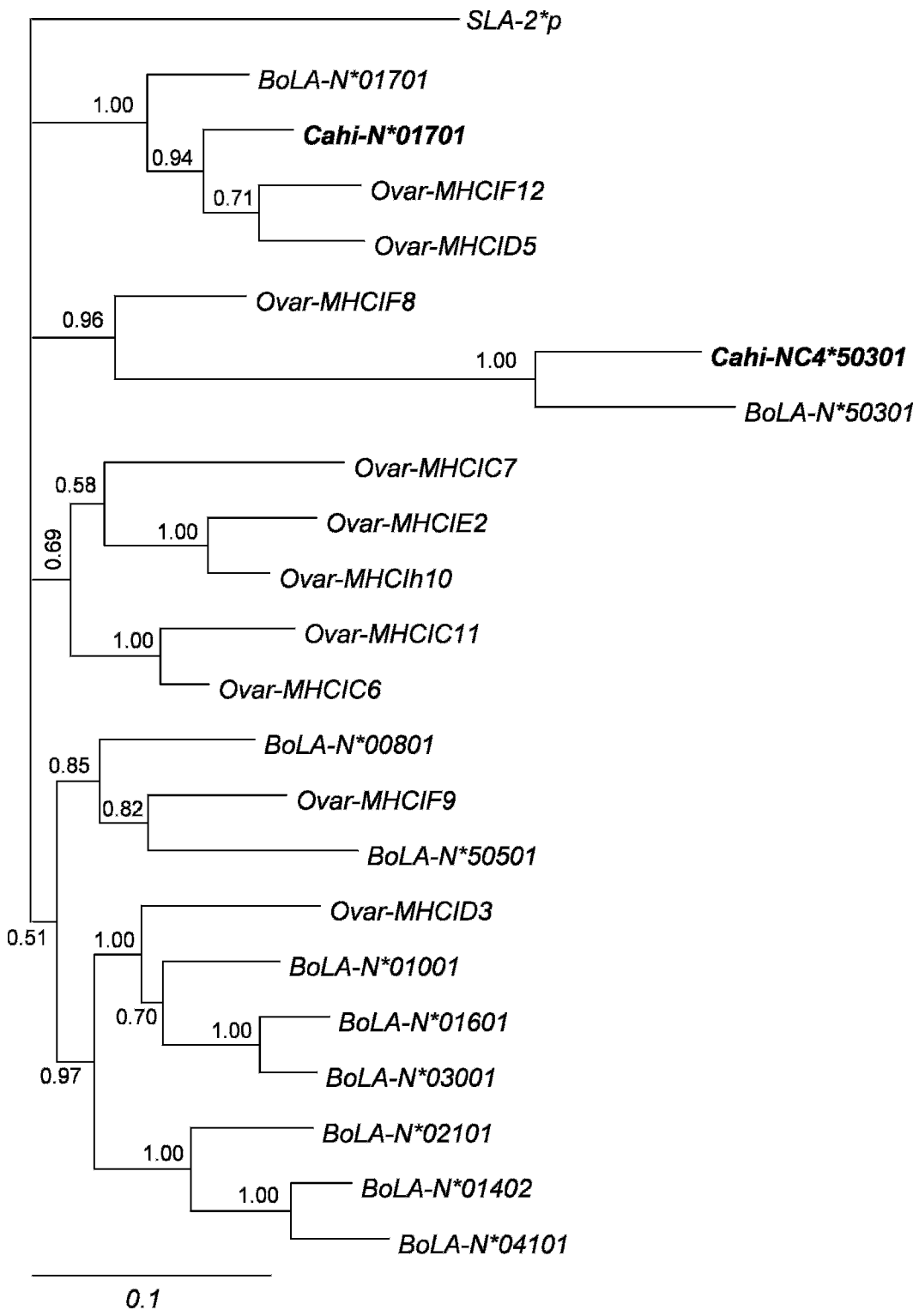

Figure 2. Bayesian phylogenetic tree of ovine (Ovar), bovine (BoLA), and caprine (Cahi) major histocompatibility (MHC) class I sequences. Pig SLA-2*p sequence with GenBank accession no. DQA104338 was used as an outgroup.

Bank accession no. EF569216) was sequenced. The coding region comprised 1,077 bp that, once translated, yielded a mature protein of 337 AA (the leader peptide was 21 AA long). BlastN analysis of this goat MHC class I sequence revealed high nucleotide identity with other bovine (GenBank accession no. DQ140360, *BoLA-N*01701* allele, identity: 93%) and ovine (GenBank accession no. AJ874680, identity: 93%) MHC class I sequences. The complete description of the domain organization of the relevant goat class I molecule has been annotated in the GenBank entry EF569216. The transmembrane and cytoplasmic domains were 35 and 28 AA long, as previously observed in diverse ovine class I sequences. This goat class I sequence was named *Cahi-N*01701,* given its high nucleotide identity with the corresponding bovine allele and in accordance with the MHC class I nomenclature rules cited by Davies et al. (2006). Moreover, another caprine class I cDNA (GenBank accession no. EF569217) was partially sequenced, and displayed 89% nucleotide identity with *BoLA-NC4*50301* allele (GenBank accession no. DQ140374). Following the criteria mentioned above, this goat sequence was named as *Cahi-NC4*50301.* These 2 caprine MHC class I sequences have not been confirmed in other individuals because a procedure for typing class I genes is currently unavailable in goats.

Goat class I sequences and other artiodactyla sequences were subjected to a Bayesian phylogenetic analysis, taking into consideration a 571-bp region shared by all of them (Figure 2). Goat sequence *Cahi-N*01701* grouped with ovine sequences D5 and F12, which correspond to a highly expressed classical MHC class I locus (Miltiadou et al., 2005). Conversely, goat sequence *Cahi-NC4*50301* clustered with *BoLA-NC4*50301* allele, which corresponds to a nonclassical MHC class I gene expressed in the bovine trophoblast (Davies et al., 2006). Nonclassical MHC class I genes are distinguished by their low level of polymorphism and they have been implicated in the protection of syncytiotrophoblast cells from natural killer cell lysis during pregnancy (Ishitani et al., 2006) as well as in the crosstalk between intestinal epithelial cells and other cells from the innate and adaptive immune system (Shao et al., 2005).

As a whole, these results are the first description of caprine classical and nonclassical MHC class I genes. This new insight should be considered as a first step to characterize the gene content and variability of the goat class I region with the long-term goal of dissecting the genetic factors that govern the immune response in this livestock species.

REFERENCES

Amills, M., N. Jiménez, D. Villalba, M. Tor, E. Molina, D. Cubiló, C. Marcos, A. Francesch, A. Sànchez, and J. Estany. 2003. Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. Poult. Sci. 82:1485–1493.

Amills, M., V. Ramiya, J. Norimine, C. A. Olmstead, and H. A. Lewin. 2002. Reduced IL-2 and IL-4 mRNA expression in CD4+ T cells from bovine leukemia virus-infected cows with persistent lymphocytosis. Virology 304:1–9.

Davies, C. J., J. A. Eldridge, P. J. Fisher, and D. H. Schlafer. 2006. Evidence for expression of both classical and non-classical major histocompatibility complex class I genes in bovine trophoblast cells. Am. J. Reprod. Immunol. 55:188–200.

Douch, P. G., and P. M. Outteridge. 1989. The relationship between ovine lymphocyte antigens and parasitological and production parameters in Romney sheep. Int. J. Parasitol. 19:35–41.

Dukkipati, V. S., H. T. Blair, D. J. Garrick, and A. Murray. 2006. "Ovar-Mhc" - Ovine major histocompatibility complex: Structure and gene polymorphisms. Genet. Mol. Res. 5:581–608.

Ellis, S. 2004. The cattle major histocompatibility complex: Is it unique? Vet. Immunol. Immunopathol. 102:1–8.

Ishitani, A., N. Sageshima, and K. Hatake. 2006. The involvement of HLA-E and -F in pregnancy. J. Reprod. Immunol. 69:101–113.

Janeway, J., P. Travers, M. Walport, and M. Shlomchik. 2004. Immunobiology. 6th ed. Garland Science, Oxford, UK.

Joosten, I., G. Ruff, M. F. Sanders, and E. J. Hensen. 1993. Use of isoelectric focusing to define major histocompatibility complex class I polymorphism in goats. Anim. Genet. 24:47–51.

Lewin, H. A., G. C. Russell, and E. J. Glass. 1999. Comparative organization and function of the major histocompatibility complex of domesticated cattle. Immunol. Rev. 167:145–158.

Miltiadou, D., K. T. Ballingall, S. A. Ellis, G. C. Russell, and D. J. McKeever. 2005. Haplotype characterization of transcribed ovine major histocompatibility complex (MHC) class I genes. Immunogenetics 57:499–509.

Nesse, L. L., and G. Ruff. 1989. A comparison of lymphocyte antigen specificities in Norwegian and Swiss goats. Anim. Genet. 20:71–77.

Page, R. D. 1996. Tree View: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12:357–358.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Ruff, G., and S. Lazary. 1988. Evidence for linkage between the caprine leucocyte antigen (CLA) system and susceptibility to CAE virus-induced arthritis in goats. Immunogenetics 28:303–309.

Ruff, G., J. G. Regli, and S. Lazary. 1993. Occurrence of caprine leucocyte class I and II antigens in Saanen goats affected by caprine arthritis (CAE). Eur. J. Immunogenet. 20:285–288.

Schukken, Y. H., B. A. Mallard, J. C. Dekkers, K. E. Leslie, and M. J. Stear. 1994. Genetic impact on the risk of intramammary infection following *Staphylococcus aureus* challenge. J. Dairy Sci. 77:639–647.

Shao, L., O. Kamalu, and L. Mayer. 2005. Non-classical MHC class I molecules on intestinal epithelial cells: Mediators of mucosal crosstalk. Immunol. Rev. 206:160–176.

Vage, D. I., F. Lingaas, R. L. Spooner, E. F. Arnet, and O. Lie. 1992. A study on association between mastitis and serologically defined class I bovine lymphocyte antigens (BoLA-A) in Norwegian cows. Anim. Genet. 23:533–536.