Identifying the Degree of Major Histocompatibility Complex Matching in Genetically Unrelated Dogs With the Use of Microsatellite Markers

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ABSTRACT

Background. The dog has served as an important experimental model for biomedical research such as transplantation and developing immunosuppressive agents. Although major histocompatibility complex (MHC) in dogs is a dominant factor of graft rejection, it has not been well investigated in dogs compared with human. For that reason, imprecise cross-matching or time-consuming sequence-based typing methods have generally been used to choose specific donor and recipient pairs. Investigation of matching distribution of MHC in dogs with the use of simple and accurate methods would be beneficial for biomedical researchers. The aim of this study was to identify the diversity of dog leukocyte antigen (DLA) types in genetically unrelated dogs by means of microsatellite markers.

Methods. Thirty-three Beagle and Shih-Tzu dogs, which were negative in cross-matching, were chosen. The genomic DNA was isolated from peripheral blood leukocytes, and highly polymorphic short tandem repeats located in MHC class I and II were amplified with the use of specific primers.

Results. Among all of the dogs, MHC matching groups, including class I full match–class II full match (M-M), class I full match–class II haplo match (M-H), class I haplo match–class II full match (H-M), class I haplo match–class II haplo match (H-H) groups, were ~1.55%, 0.39%, 1.94%, and 6.59%, respectively. MHC class I nonmatch–class II nonmatch (U-U) groups were 58.14% of the total dogs.

Conclusions. Because differences of histocompatibility between donor and recipient leads to various allograft rejections, knowledge of the distribution of MHC matching in unrelated dogs would be helpful in designing studies and to get more accurate results from experiments using dog transplantation models.

CANINE models are important for preclinical transplantation research [1]. Although small animal models such as mice and rats are easy to control and use for the initial evaluation, the experimental results using rodents have many limitations to translating directly for human medicine owing to inherent differences [2–4]. Developing a new immunosuppressant or clinical protocol requires the use of reliable animal models, and canine models have contributed for outstanding development in the transplantation medicine fields [4,5].

Disparities in various polymorphic systems, mainly MHC, are the most crucial factor of rejection against transplants [6]. The technical progress of MHC matching of experimental animals is needed, but, unfortunately, canine-specific monoclonal antibodies for serologic typing have not been available, and sequence-based typing methods of MHC alleles are time

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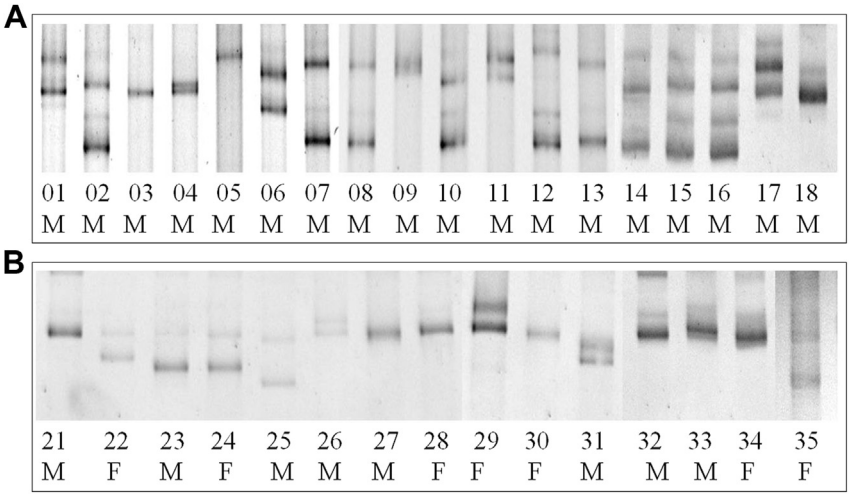

Fig 1. Polymerase chain reaction results of MHC class I microsatellite loci with the use of specific primers in (**A**) beagle and (**B**) shih-tzu dogs. Numbers indicate each dog. Abbreviations: M, male; F, female.

consuming [7,8]. In an earlier study, the use of highly polymorphic microsatellite short tandem repeats were identified by Wagner et al [1,7,9].

However, information on the degree of MHC matching in genetically unrelated dogs is unknown, and investigation of MHC matching in dogs would be helpful for researchers in designing studies using canine models. Our goal was to investigate the diversity of MHC matching in unrelated dogs with the use of highly polymorphic microsatellite markers.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee (Kangwon National Universitiy, Korea). A total of 33 dogs, 18 beagles (all male) and 15 shih-tzus (8 male and 7 female), were chosen randomly for this study. Serologic complement-dependent cytotoxic cross-matching and blood typing were performed as previously described [10].

For these experiments, DNA was isolated from blood leukocytes with the use of a DNA extraction kit (Intron, Sungnam, Korea) according to the manufacturer's recommendation. Primer sequences of microsatellite markers of dog leukocyte antigen (DLA) class I, FH2200, were forward 5′-GGCATGATCGTGGAGTCCC-3′ and reverse 5′-CCCACCCCAGTTGTCCTATT-3′. The primer sequences for highly polymorphic loci of DLA class II, FH2202, were forward 5′-GTTGAGTGGTTGCCTTTAGC-3′ and reverse 5′-CAGGATCTTCATATGTCACC-3′ [7] Polymerase chain reaction (PCR) was performed with the use of PCR master mix (2× Topsample Dyemix; Enzynomics, Daejeon, Korea) with genomic DNA, distilled water, and each specific primer. We performed PCR examination with the use of a Tprofessional standard 96 gradient machine (Biometra, Goettingen, Germany) with the following protocols: denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, annealing temperature (59°C for FH2200 and 60°C for FH2202) for 30 seconds and 72°C for 45 seconds, and a final extension step at 72°C for 10 minutes. The PCR products were detected with the use of 6% Trisacetate-EDTA polyacrylamide gel electrophoresis. The samples were loaded into the wells with the use of electrophoresis equipment (EPS 2A200; Amersham Biosciences) for 5 hours 50 minutes (FH2200) and 4 hours 30 minutes (FH2202) using 100 V. One or 2 bands of DLA were visualized by means of ethidium bromide staining with the use of a Geldoc machine (Gbox EF2; Syngene, Cambrige, United

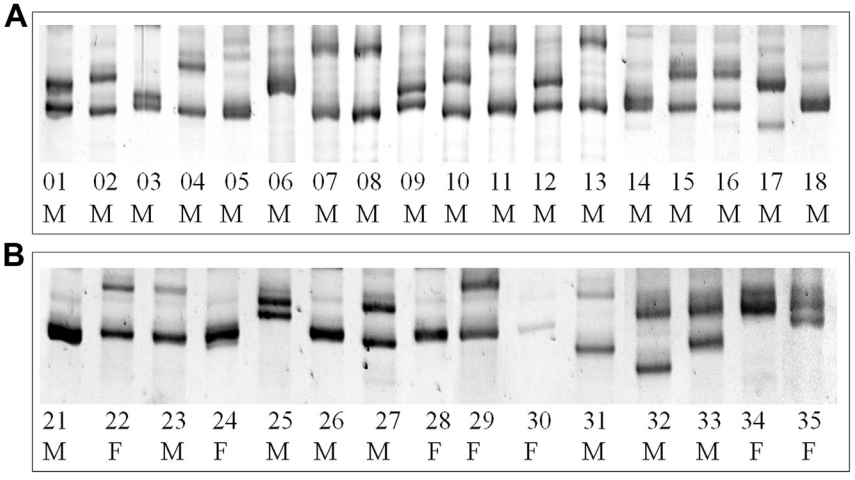

Fig 2. Polymerase chain reaction results of MHC class II microsatellite loci with the use of specific primers in (**A**) beagle and (**B**) shih-tzu dogs. Numbers indicate each dog. Abbreviations: M, male; F, female.