2141

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Quantitative Analysis of Microchimerism With Y-Chromosome–Specific PCR In Canine Small Bowel Transplantation

T. Okuda, N. Ichikawa, Y. Zhu, H.J. Chun, A.J. Demestris, M.A. Nalesnik, B. Rudert, M. Trucco, T.E. Starzl, and N. Murase

PASSENGER leukocytes, normal constituents of whole organs, migrate after transplantation and produce microchimerism, which is suggested to be essential for sustained survival of allografts.[1] Canines have been widely used as a more clinically relevant outbred large animal transplantation model, rather than inbred rodent, and provided important information to directly improve the results of clinical transplantation. However, the analysis of microchimerism in this species is hampered by the lack of reagents. In this study, we demonstrate recently developed quantitative PCR analysis of chimerism in dog samples using primers specific for the sex determining region-Y (SRY) gene. Profitability of this method to determine the level of chimerism after organ transplantation was also shown in samples obtained from sex-mismatched canine small bowel transplantation (SBTx).

METHODS

Dog SRY sequence was cloned by PCR amplification of male dog lymphocytes using human primers; SRY-1F and SRY-2R,[2] and the primer set for the dog Y-chromosome PCR was obtained; 5′-CGTCAGACGACCCATGAA-3′ and 5′-CTCGGTGCATGGC CTGTA-3′ (product size; 188 bps). PCR was performed with 1.5 μg genomic DNA in a 50 μL total reaction mixture containing 1.25 U Taq DNA polymerase, 0.25 μmol/L primers, 0.2 mmol/L dNTP, and 10 × PCR buffer (500 mmol KCl, 20 mmol/L $MgCl_2$, 100 mmol/L tris HCl, 0.1% gelatin, adjusted to pH 8.4). Variable PCR conditions examined in this study included; denature; 94 to 95°C for 1 minute, annealing; 55 to 62°C for 45 to 60 seconds and extension; 72°C for 45 to 60 seconds with 25 to 35 cycles using DNA Thermal Cycler 480 (Perkin Elmer Cetus, Norwalk, Conn). After PCR amplication, DNAs were transferred onto nylon membranes, and southern blotting was performed using ^{32} P-labeled probe for dog SRY. After 3-hour exposure, radioactivity was measured by PhosphorImager (Molecular Dynamics, Sunnyvale, Calif) as previously described.[3] Small bowel transplantation was performed in dogs according to procedures previously described.[4] The male and female adult hound dogs (Harlan Sprague Dawley, Indianapolis, In) weighing 21 to 23 kg were used as donors and recipients, respectively.

RESULTS

DNAs were prepared from peripheral blood lymphocytes of normal male and female dogs, and mixed at various ratios ranging from 1:0 to $1:10^6$. Using these samples, appropriate PCR products were gained under denature; 94°C for 1 minute annealing; 60°C for 1 minute and extension; 72°C for 1 minute with 33 cycles with first denature; 94°C for 3 minutes, last extension; 72°C for 8 minutes. Under this condition, radioactivities after southern hybridization of samples with known male DNA concentrations showed nearly linear increase according to male DNA concentration. Clear correlation between male DNA concentration and radioactivity was achieved, and a standard curve was created for semiquantitation of male DNA concentration. It was possible to detect male DNA concentration as low as 0.0001%.

After SBTx female recipients were treated from FK 506 (Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan) at daily oral dosages between 0.125 and 0.5 mg/kg (bid). Concentrations of donor male DNA in the peripheral blood was >15% for the first 2 weeks, and quickly declined to <0.01% at 6 weeks (n = 1). To modulate the quality of chimerism after SBTx by eliminating mature lymphocytes in intestinal allografts and adding immature bone marrow cells, female recipients received ex vivo irradiated (7.5 Gy) intestinal allografts and unfractionated bone marrow (5×10^8 cells/kg on day 0) from male donors. Male DNA in recipient blood of these modulated recipients was nearly 15% early after transplantation, and was continuously detected for the first 6 weeks at level of 8% to 15% (n = 2).

CONCLUSIONS

The quantitative PCR method targeting the dog SRY gene provides a useful tool in analyzing the level of microchimerism after canine organ transplantation.

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From the Thomas E. Starzl Transplantation Institute, Departments of Surgery, Pathology, and Pediatrics, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

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Address reprint requests to Dr T. Okuda, Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15216.

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