Therapeutic approaches involving gene transfer to various hematopoietic cell types are currently being investigated as treatments for cancer and genetic diseases. In many instances, canine neoplastic diseases and genetic diseases share genotypic and phenotypic similarities to their human counterparts and thus represent excellent large animal models to study treatments of these diseases. In both human and mouse studies the cell surface expression of the sialomucin CD34 has been exploited to isolate cells enriched for hematopoietic progenitor cell activity for bone marrow transplant (BMT) studies and as targets in gene transfer experiments. To perform comparable studies in dogs, we have taken advantage of the availability of dogs that have a naturally occurring immunodeficiency disease, X-linked severe combined immunodeficiency (XSCID), and the use of an anti-canine CD34 monoclonal antibody.

Initial studies were aimed at the isolation and characterization of highly enriched populations of canine pediatric CD34+ cells. Bone marrow from two to three week old dogs contained up to 20% CD34+ cells and this percentage dropped sharply with age. Magnetic bead separation technology was used to isolate cell populations that were >95% CD34+. Non-ablative BMT using highly enriched populations of normal pediatric CD34+ cells transplanted into XSCID dogs led to the reversal of the XSCID phenotype. When transplanted after sub-lethal pre-transplant conditioning pediatric CD34+ cells gave rise to both myeloid and lymphoid lineages in the periphery of one recipient up to 10 months post-BMT.

A murine MSCV-based retroviral vector, containing the enhanced green fluorescent protein (EGFP) cDNA, was used to optimize conditions for infection of canine CD34+ cells. A two day transduction protocol using the CH-296 recombinant human fibronectin fragment, centrifugation, together with human IL-6 (huIL-6), canine stem cell factor, human flt-3 ligand, and media from phytohemagglutinin-stimulated canine peripheral blood mononuclear cells (PBMC), produced the highest percentage of EGFP expressing cells (average of 11%).

CD34+ cells from normal dogs were transduced in this way and introduced into littermate XSCID puppies before 2 weeks of age. The percent transduction of CD34+ cells varied from 7% to 14%. Following transplantation of the transduced cells, donor T-lymphocytes appeared in the periphery by two months post-bone marrow transplant (BMT). PCR analysis of post-BMT PBMC DNA indicated donor/recipient chimerism that persisted for the duration of the experiments. EGFP PCR analysis of post-BMT PBMC DNA and fluorescence microscopy documented peripheral EGFP expressing cells in some dogs which when stained with an anti-canine CD3 antibody, were found to be peripheral blood (PB) CD3+ T-lymphocytes. The level of post-BMT EGFP expression of T-lymphocytes varied from 0% to 28%. Sorting of EGFP+ and EGFP- PB T-lymphocytes from 1 dog at 2 and 3.5 months post-BMT, followed by EGFP PCR analysis, showed no evidence of vector shutdown. EGFP expression in PB granulocytes was not seen. These marking experiments demonstrated that the engraftment of ex vivo transduced CD34+ cells is possible and suggests that the MSCV retroviral vector may be particularly well suited to long-term in vivo expression of retrovirally introduced transgenes.

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