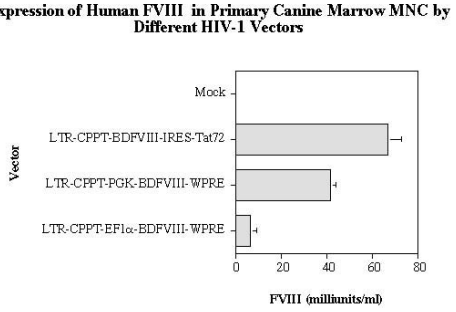
[Srinivasakumar and Schuening (2000) J. Virol. 74:6659-6668]. The promoters expressed transgenes at high levels in hematopoietic cells, as has been previously shown [Srinivasakumar et. al., (2002). J. Virol. 76:7334-7342]. Vector stocks for each of the above vectors were prepared by transient transfection of 293T cells [Srinivasakumar (2002) Methods Mol. Med. 69:275-302] and their titers were determined by real-time quantitative PCR of genomic DNA isolated from vector infected indicator cells. Each vector stock was then used for infection of primary canine marrow MNC by three spin-infections over 2 days on CH296-coated 6-well plates. The medium was replaced at the end of the transduction procedure. Conditioned medium was harvested 3-days after transduction and assayed for functional FVIII activity using the Coamatic Factor VIII kit (Chromogenix, Monza, Italy). Measured factor VIII activity in conditioned medium was normalized to input vector titer. The results, shown in the figure below, indicate that vector-derived FVIII could be detected in the supernatants of canine marrow MNCs transduced with any of the three HIV-1 vectors. Highest levels of functional FVIII were obtained with the Tat-encoding bicistronic HIV-1 vector. These results demonstrate that canine marrow MNC support expression and secretion of functional human FVIII. Further experiments are warranted to determine if the levels of FVIII observed in vitro will translate into therapeutic benefit in vivo in canine models of hemophilia A.



826. Transduction of Human Hematopoietic Stem Cells by RD114-TR-Pseudotyped Lentiviral Vectors

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HIV-1-derived lentiviral vectors are efficiently pseudotyped by a chimeric envelope (RD114-TR) encoding the extracellular and transmembrane domains of the FLV RD114 glycoprotein fused to cytoplasmic tail (TR) of the MLV 4070A amphotropic glycoprotein. RD114-TR pseudotyped vectors may be concentrated by centrifugation, are resistant to complement inactivation, and are of particular interest for both ex vivo and in vivo gene therapy applications. We carried out a comparative analysis of VSV-G and RD114-TR-pseudotyped lentiviral vectors in transducing human cord blood-derived CD34+ hematopoietic stem/progenitor cells. Transduction efficiency was comparatively analysed in CD34+ cells in liquid culture, in the progeny of CD34+ clonogenic progenitors in semi-solid culture, and in the progeny of CD34+ repopulating stem cells after xeno-transplantation in NOD-SCID mice. In all cases, RD114-TR-pseudotyped vectors transduced hematopoietic cells at lower m.o.i., resulting in lower toxicity and more efficient stable transduction at comparable vector copy number per genome. Potential changes in CD34+ cells transcription profile and phenotype upon transduction with RD114-TR or VSV-G-pseudotyped vectors was investigated by Affymetrix Gene Chips microarray analysis. We found no significant difference in gene expression patterns between mock-RD114-TR and VSV-G-transduced cells. Our study show that the biology of repopulating hematopoietic stem cells and their progeny is not affected by transduction with RD114-TR-pseudotyped lentiviral vectors.

827. Oncogenesis Following Delivery of Lentiviral Vectors to Fetal and Neonatal Mice

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Gene therapy by use of integrating vectors carrying therapeutic transgene sequences offers the potential for a permanent cure of genetic diseases due to the ability of these vectors to integrate in a stable manner into the patients' chromosomes. Since three cases of T-cell leukaemia have been identified after retrovirus gene therapy for X-linked severe combined immune deficiency as being associated with the integrating vector used for gene therapy the need for animal models to test for vector safety has become of paramount importance. Our previous work has shown that a high frequency of hepatocellular carcinomas has occurred following in utero and neonatal injection with certain lentivirus vectors. It has been hypothesized that the woodchuck post regulatory element (WPRE) carried by the vectors used in this study could be implicated in the tumour development process. Our recent study using novel vectors with mutations in the WPRE shows that mice treated with these vectors still develop liver tumours. In this report we discuss these findings and preliminary data to support an alternative cause for tumorigenesis. We also discuss the fetal and neonatal system as a novel and sensitive in vivo model to test the effects and safety of integrating vectors under consideration for clinical applications.

828. Promoter Strength Versus Transactivation Potential: Creating a Safer Integrating Vector

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The ability of retroviral and lentiviral vectors to permanently integrate into the host genome makes them attractive tools for gene therapy applications which require long-term expression. However, the benefits of permanent integration are counterbalanced by two areas of weakness: 1) the difficulty in obtaining high-level transgene expression which persists over time and 2) the potential for insertional mutagenesis and transactivation of host genes. The recent development of leukemia-like disease in SCID-X1 clinical trial patients, as well as the development of tumors in several animal models receiving retroviral gene therapy, demonstrates the problems associated with strong vector promoters. In order to create a safer integrating vector, it would be beneficial to identify promoters which drive high levels of transcription of the linked transgene but which