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Abstract

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Grant Number: 2P01HL053750-060002
PI Name: RUSSELL, DAVID
PI Email:
PI Title:
Project Title: Stem cell transduction by foamy virus vectors

Abstract: The major objective of this proposal is to develop viral vectors based on human foamy virus (HFV) for use in gene therapy, especially applications requiring the genetic modification of hematopoietic stem cells. HFV is a retrovirus that offers several potent advantages as a vector system, including a wide host range, improved transduction of non-dividing cells, large packaging capacity, resistance to serum inactivation, and lack of pathogenicity. These properties, along with the ability of HFV vectors to efficiently transduce hematopoietic cells, suggests that they may overcome some of the barriers that have inhibited stem cell transduction by other types of viral vectors. Recently, methods for the production of high titer, helper- free HFV vector stocks have been developed, making possible definitive preclinical studies with this novel vector system. In this proposal HFV vectors with an expanded packaging capacity and improved safety features will be constructed by removing all non-essential cis-actin viral sequences. HFV packaging constructs will be designed that optimize the expression of essential trans-acting viral functions and these will be incorporated into stable packaging cell lines. These improved HFV vectors will be used to transduce primary hematopoietic progenitors from mice, non-human primates and humans. Irradiated mice will be transplanted with congenic marrow cells transduced ex vivo, and the transduction rates of long-term repopulating stem cells will be determined by transgene expression assays and an analysis of vector pro-virus copy numbers. Similar autologous transplantation experiments will be performed in baboons as a non-human primate model, and human hematopoietic cells will be investigated by transplanting transduced human cells into immunodeficient NOD/SCID mice. A variety of experimental variables will be evaluated, including a range of vector MOIs, different reporter genes (alkaline phosphatase, neo, and GFP), several internal promoters, and various transduction conditions, with the goal of developing methods that result in high level, long-term transgene expression in the reconstituted hematopoietic system. Globin expression constructs will be incorporated into optimized HFV vectors. It is hoped that these experiments will lead to effective genetic tretments for the many diseases that require efficient gene transfer into hematopoietic stem cells for therapeutic effect, including the hemoglobinopathies.

Thesaurus Terms:
Retroviridae, biotechnology, cell transplantation, genetic transduction, globin, hematopoietic stem cell, transfection vector
gene expression, gene therapy, sickle cell anemia, technology /technique development
SCID mouse, baboon, bone marrow transplantation, tissue /cell culture

Institution:	UNIVERSITY OF WASHINGTON 3935 UNIVERSITY WAY NE SEATTLE, WA 98195
Fiscal Year:	1999
Department:	
Project Start:	
Project End:	
ICD:	NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
IRG:	

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Abstract

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Grant Number:	1R01DK055759-01
PI Name:	RUSSELL, DAVID W.
PI Email:	drussell@u.washington.edu
PI Title:	ASSISTANT PROFESSOR
Project Title:	GENE TARGETING BY VIRAL VECTORS

Abstract: Most current gene therapy approaches are based on a "gene addition" strategy, where a functional transgene cassette is delivered to cells and expressed from episomal or randomly integrated molecules. A potentially more powerful approach would be to correct mutations at their normal chromosomal locations. The major advantages of this "gene correction" approach include properly regulated gene expression and the removal of dominant disease-causing mutations. Until recently, therapeutic gene correction was felt to be beyond the scope of genetic technologies. The applicant's recent demonstration that adeno-associated virus (AAV) vectors can be used to introduce specific genetic modifications into homologous human chromosomal loci at high frequencies (nearly 1 percent of normal human fibroblasts) suggests that this strategy may ultimately be efficient enough to allow for therapeutic gene correction. The experiments described in this proposal will explore several aspects of gene correction by AAV vectors, in an attempt to optimize gene targeting rates and improve our understanding of the mechanisms involved. AAV vectors will be used to introduce specific modifications into both normal human genes (such as HPRT) and engineered chromosomal target sites containing mutant marker genes introduced by retroviral vectors. Targeted chromosomal loci will be analyzed by sequencing recovered plasmids containing corrected genes to completely define the structure of the targeted loci. Different types of mutations (insertions, deletions and mismatches) will be corrected and the length of homology between the vector and chromosomal target locus will be adjusted to optimize vector design. Cellular factors important for the gene targeting reaction will be analyzed by varying cell division rates, treating cells with agents that induce DNA repair functions, and measuring gene correction rates in cells with mutations in DNA repair genes. Transgenic and mutant mouse models will be used to study AAV- mediated gene targeting in vivo, including gene correction at beta-glucuronidase and beta-galactosidase genes, which will be assayed by histochemical staining of tissue sections and sequencing of corrected loci recovered as plasmids in bacteria. These experiments should help define the basic biology of gene targeting by AAV vectors, and determine its potential for scientific and therapeutic applications.

Thesaurus Terms:

gene targeting, transfection vector
DNA repair, cell cycle, gene mutation, genetic mapping
adeno associated virus group, histochemistry /cytochemistry, human genetic material tag, laboratory mouse, tissue /cell culture, transgenic animal

Institution:	UNIVERSITY OF WASHINGTON 3935 UNIVERSITY WAY NE SEATTLE, WA 98195
Fiscal Year:	1999
Department:	MEDICINE
Project Start:	15-JUN-1999
Project End:	31-MAR-2004
ICD:	NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
IRG:	MEDB