transduced cells and therefore we hypothesize that the latent presence of CMV antigens in vivo will maintain the long-term presence of these T cells. These results demonstrate that redirection of virus specific T cells towards anti-leukemic reactivity can be an attractive strategy to use for clinical purposes.

Abstract# 821


Gene targeting is a powerful technique to introduce genetic change into the genome of eukaryotic cells. It is widely used to create defined mutations in murine embryonic stem cells and theoretically could be used to correct mutations in somatic cells. In this way gene targeting could be a powerful form of gene correction type therapy. Despite its potential, gene targeting has not been widely used in somatic cells because of its low efficiency. We report on a system based on the correction of a mutated GFP gene that allows the efficient stimulation of gene targeting in somatic cells. Using this system gene targeting is stimulated over 2000-fold by the introduction of a DNA double-stranded break in the target locus (DSB-GT). We find that the rate of DSB-GT can be increased by increasing the amount of repair substrate, the amount of homology between the gene target and repair substrate, and by increasing the frequency of double-stranded break creation. When we optimize conditions for DSB-GT we obtain targeting rates of 3.5%. Finally, we show that chimeric nucleases, protein fusions between zinc finger DNA binding domains and the type II endonuclease enzyme, can be used to greatly increase the rate of DSB-GT. We have attempted to combine the mobility and targeting functions of T cells with the potential, gene targeting has not been widely used in somatic cells because of its low efficiency. We report on a system based on the correction of a mutated GFP gene that allows the efficient stimulation of gene targeting in somatic cells. Using this system gene targeting is stimulated over 2000-fold by the introduction of a DNA double-stranded break in the target locus (DSB-GT). We find that the rate of DSB-GT can be increased by increasing the amount of repair substrate, the amount of homology between the gene target and repair substrate, and by increasing the frequency of double-stranded break creation. When we optimize conditions for DSB-GT we obtain targeting rates of 3.5%. Finally, we show that chimeric nucleases, protein fusions between zinc finger DNA binding domains and the type II endonuclease enzyme, can be used to greatly increase the rate of DSB-GT.

Abstract# 822

Direct Delivery of Adenoviral Vectors to Malignant Cells Using Tumor-Specific CTL. Patricia N. Yokota, Nicolas Charlet-Berguerand*, Barbara Salvedo*, Chio M. Rooney*, Malcolm K. Brenner. Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA.

A major barrier to successful gene therapy of disseminated malignancy is our inability to generate targeted vectors that can cross multiple tissue boundaries to reach all tumor deposits. Specific cytotoxic T lymphocytes (CTL), however, can be generated against tumor antigens, and in animal models and some human settings (Rooney et al Blood. 92:1549-55 1998) these CTLs are able to cross tissue barriers and to specifically recognize tumor cells. We have attempted to combine the mobility and targeting functions of T cells with the potential, gene targeting has not been widely used in somatic cells because of its low efficiency. We report on a system based on the correction of a mutated GFP gene that allows the efficient stimulation of gene targeting in somatic cells. Using this system gene targeting is stimulated over 2000-fold by the introduction of a DNA double-stranded break in the target locus (DSB-GT). We find that the rate of DSB-GT can be increased by increasing the amount of repair substrate, the amount of homology between the gene target and repair substrate, and by increasing the frequency of double-stranded break creation. When we optimize conditions for DSB-GT we obtain targeting rates of 3.5%. Finally, we show that chimeric nucleases, protein fusions between zinc finger DNA binding domains and the type II endonuclease enzyme, can be used to greatly increase the rate of DSB-GT.

Abstract# 823

Our efforts to correct murine models of human genetic diseases, chromosomal DNA as a result of vector integration in vivo is not known. As a first step toward elucidating the host chromosomal effects of integration in vivo, we attempted to isolate the whole rAAV proviral genomes together with flanking host chromosomal sequences using a yeast recombinase (lHTT) mouse model with fumarylacetoacetate hydrolase (FAH) deficiency. In the absence of NTBC drug administration, these mice accumulate toxic tyrosine metabolites that result in a cell autonomous lethal phenotype. Thus, genetically corrected hepatocytes have a selective advantage and can repopulate the liver. We constructed an FAH-expressing rAAV shuttle vector whose vector sequences are theoretically protected by the yeast recombinase. HTI mice were injected via the portal vein with particles of AAV-fAFAH, and in vivo selection (removal of NTBC) was started 6 weeks post-injection. Hepatocytes were isolated after an 8-week in vivo selection, transplanted into HTI mouse recipients (1x10^7 hepatocytes per mouse), selected again in vivo for 7 months, and then the livers were harvested for DNA extraction. The transplanted and second in vivo selection facilitated dilution of extrachromosomal genomes present in the liver samples. The whole proviral genomes were isolated from the liver samples together with adjoining cellular DNA with a standard plasmid rescue technique, and sequenced. To date, 12 proviral genomes were characterized and sequenced. The vector genomes were frequently deleted at both terminals and AAV-ITRs were completely deleted in some cases. rAAV integration commonly accompanied a host chromosomal DNA deletion (by 2, 4, 13, 27, 44, 58, 119, 216, and 2107 base pairs). A small insertion of unknown origin of up to 4 bp at junctions was sometimes observed. There was no significant homology between vector and cellular DNA sequences, while there was some microhomology of up to 4 bp between them. Integration sites were randomly distributed in chromosomes (chr. 1, 2, 4, 8, 9, 11, 15, 19) and there appeared to be no hot spot for vector integration. Five out of 12 integrations targeted within predicted or known mouse genes. Because our study relied on in vivo selection and ultimately transgene expression, we cannot establish if the integration sites were representative of all rAAV integration events. However, for the purposes of gene therapy, elucidating the structures of proviral genomes and the sites where the transgene inserts is an important parameter. This study is the first we are aware of that establishes the imperfect nature of rAAV vector integration in vivo.

Abstract# 824

Targeting CD19 with Genetically Modified EBV-Specific Human T Lymphocytes: The Role of the Antigen-Presenting Cell in Chimeric Receptor-Mediated T Cell Responses. Claudia Rossig*, Silvy Schr€orer*, Annette Baer*, Josef Vormoor, Cliona M. Rooney*, Malcolm K. Brenner. Department of Pediatric Hematology and Oncology. University Children's Hospital Muenster, Muenster, Germany; Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA.

Primary T cells expressing chimeric receptors specific for tumor antigens have considerable therapeutic potential. Their successful therapeutic use has been limited because most tumor cells lack the costimulatory molecules essential for the induction and maintenance of a T cell response. B cells latently infected with Epstein Barr virus (EBV-LCL) are known to be excellent antigen-presenting cells that are rich in costimulatory molecule expression. Cell surface expression of CD19 is shared between EBV-LCL and many B cell malignancies, including B cell precursor acute lymphoblastic leukemia (ALL). To investigate the role of costimulation for chimeric receptor-mediated T cell functionality, we generated EBV-specific cytotoxic T lymphocytes (CTL) from four healthy seropositive donors and transduced them with a retroviral vector encoding the CD19-specific chimeric receptor CD19-zeta. We then compared their capacity to respond to EBV-infected tumor cells and EBV-infected autologous and allogeneic B cells.

In conclusion, chimeric receptor-mediated recognition of autologous EBV-infected cells in the culture, 38% were transduced, even though these target cells are much more resistant than A549 cells to adenoviral vector transduction. Vector production decreased over 2-3 days, falling to 5-10% of the levels by day 4, but could be increased upon reinfection of the CTLs by fresh autologous tumor cells (EBV-LCL). The virus production decreased to 5-10% 4 days after the last stimulation. Directed delivery of adenoviral vectors to malignant cells using tumor-specific CTL may be a useful addition to cellular-based cancer immunotherapy.

Abstract# 825


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Recombinant adeno-associated virus (rAAV) vector is an attractive viral vector to treat genetic diseases. Although extrachromosomal vector genomes are the most predominant form of vector DNA, we and others have established that rAAV vector does integrate into the genome of host chromosomes of hepatocytes in vivo. Although the integration efficiency is normally very low even when very high doses are used (Nakai et al. J. Virol. 75:6699-6705, 2001), and in press), rAAV vector integration has raised the concern of insertional mutagenesis since: 1) development of hepatic tumors was reported in rAAV-treated mucopolysaccharidosis type VII mice (Donsante et al., Gene Ther. 17:1343-1346, 2001) and frequent chromosomal deletions were observed at rAAV integration sites in in vitro cultured cells (Miller et al. Nat. Genet. 30:147-148, 2002). However, the alteration of host