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

Chimeric Nucleases Stimulate Gene Targeting in Human Cells. Matthew Porteus, David Baltimore*. *Biology, California Institute of Technology, Pasadena, CA, USA.*

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 create or repair mutations in somatic cells. In this way gene targeting could be a powerful form of gene correction type gene 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 study of gene targeting in somatic cells. Using this system we show that 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 endonuclease domain of the FokI restriction enzyme, can stimulate gene targeting in the genome of human somatic cells by several-thousand fold. Our data provides a paradigm for the use of gene targeting as a form of gene therapy for monogenic diseases.

Abstract# 822

Direct Delivery of Adenoviral Vectors to Malignant Cells Using Tumor-Specific CTL. Patricia N. Yotnda, Nicolas Charlet-Berguerand*, Barbara Salvodo*, Clio 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 (CTLs), 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 capacity of adenoviral vectors to efficiently transduce tumor cells and deliver oncolytic genes. As proof of principle, we genetically modified Epstein Barr Virus (EBV)specific-CTLs (currently used for the treatment of EBV associated malignancies) to make them able to locally produce a therapeutic or lytic adenovirus when specifically activated by their target cells. The intent was to see whether these CTL would produce vector only on activation once they had encountered their tumor target, and to discover if the vector they produced would have functional activity. EBV specific CTLs were transduced with a Moloney based retrovirus encoding a CD40L promoter driving the E1 adenovirus gene. The CD40L promoter is strictly regulated by T cell activation and is functional only for a brief period during the T cell activation process. The CTL were also transduced by an E1 deficient chimeric Ad5F35 vector that we have previously shown is effective at transducing hemopoietic lineage cells that lack the conventional coxsackie adenovirus receptor (Yotnda et al, Hum Gene Ther.12:659-70 2001). We used Ad5/f35-GFP or Ad5/f35-TK to transduce the E1-CTLs (103 viral particles per cells). Twenty-four hours after transduction, 40% of the E1-CTL expressed the Ad transgene. When these CTLs were co-cultured with A549 cells, which are highly sensitive to adenoviral transduction, less than 5% were transgene positive, indicating low baseline production of active adenovector by CTL "producer" cells. When autologous, but not allogeneic, EBV-infected B cells (EBV-LCL) were added to the cultures, >95% of A549 target indicator cells were transduced by day 4 of culture, indicating increased vector production following T cell activation. Of the autologous EBV-LCL 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 peak values by day 4, but could be increased upon restimulation 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# 823

Host Chromosomal Effects of Recombinant Adeno-Associated Virus Vector Integration In Vivo. Hiroyuki Nakai,[1] Eugenio Montini*,[2] Sally Fuess*,[1] Theresa A. Storm*,[1] Markus Grompe*,[2] Mark A. Kay*.[1] *[1]Departments of Pediatrics and Genetics, Stanford University School of Medicine, Stanford, CA, USA; [2]Departments of Moleuclar and Medical Genetics and Pediatrics, Oregon Health Science University, Portland, OR, USA.*

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 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: 6969-6976, 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 2) 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 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 hereditary tyrosinemia type I (HTI) 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 then retrieved in bacteria. HTI mice were injected via the portal vein with $3x10^{11}$ particles of AAV-FAH, 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 ($1x10^{6}$ hepatocytes per mouse), selected again in vivo for 7 months, and then the livers were harvested for DNA extraction. The transplantation 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, 21, 27, 44, 58, 119, 216, 274 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 these integration 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 expressible 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*,[1] Sibylle Pscherer*,[1] Annette Baer*,[1] Josef Vormoor,[1] Cliona M. Rooney*,[2] Malcolm K. Brenner,[2] Heribert Juergens*.[1] *[1]Department of Pediatric Hematology and Oncology, University Children's Hospital Muenster, Muenster, Germany; [2]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 CD19+ tumor cells and to EBV-transformed CD19+ B cells. While nontransduced CTL were EBV-specific and HLA-restricted, lysing 36-78% autologous EBV-LCL in the absence of significant cytotoxicity against mismatched EBV or tumor targets, CD19-transduced CTL lysed both autologous (29-74%) and mismatched allogeneic EBV-LCL (46-73%) as well as the CD19+ Reh leukemia cell line (37-44%) and primary ALL blasts (30-47%), but not CD19-negative K-562 tumor cells (0-19%). Whereas lysis of mismatched LCL and Reh cells was not significantly inhibited by HLA class I and II antibodies, up to 58% (Reh) and 49% (LCL) inhibition of lysis was obtained by preincubation with anti-CD19 monoclonal antibody, confirming a chimeric receptor-mediated, non-MHC-restricted mechanism of recognition. Incubation of a clonal population of CD19-transduced CTL with CD19+ tumor targets failed to elicit a proliferative response, as demonstrated by [3H]thymidine incorporation. However, following coincubation with CD19+ mismatched allogeneic LCL, [3H]thymidine uptake was 54-80% of that obtained by native receptor stimulation using autologous LCL. In contrast, the failure of tumor cells to induce T cell proliferation could not be overcome by CD28 costimulation provided by antibody crosslinking. Hence, CD19-zeta-expressing CTL could not be maintained in culture for longer than three weeks when stimulated with CD19+ tumor cells even in the presence of immobilized anti-CD28 antibody. By contrast, co-culture with irradiated mismatched allogeneic LCL promoted expansion not significantly different from that observed with autologous LCL. Thus, chimeric receptor-mediated T cell proliferation and expansion are significantly enhanced when the target antigen is expressed in the cellular microenvironment of a professional antigen-presenting cell. By delivering abundant and diverse costimulatory signals along with the chimeric receptor signal, adoptively transferred CD19-zeta-expressing EBV-CTL may induce long-lasting immune control of B cell precursor leukemia. As the modified cells maintain their native specificity for EBV, this strategy may be exceptionally useful for patients recovering from allogeneic stem cell transplantation, thus preventing both EBV-associated lymphoproliferative disease and leukemia relapse.