

distribution *in vivo*. At the cellular level, antibody-positive mice show reduced rhIDU distribution in hepatocytes, while distribution to tissue macrophages is maintained. *In vitro*, immunized serum reduced rhIDU uptake into human fibroblasts but increased uptake into murine macrophages. Our findings imply that the altered tissue distribution of rhIDU caused by anti-rhIDU antibodies is partly due to reduced uptake into fibroblasts and partly due to enhanced uptake into tissue macrophages. These results imply that functional immune assays of rhIDU uptake *in vitro* into fibroblasts may not completely predict the impact of the humoral immune response against enzyme replacement therapy.

431. Lung Antibody Factory for Passive Immunisation Against Influenza

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Influenza A is a major global health threat causing >500,000 deaths annually. Neither prior infections nor current vaccines provide lasting protection, mainly due to rapid antigenic evolution of the viral haemagglutinin (HA) protein. Broadly neutralising antibodies (bNAbs) isolated from vaccinated volunteers can provide passive immunity, but this approach is hindered by high antibody manufacturing costs and the relatively short half-life of antibody after delivery to the circulation. We hypothesise that using lentivirus pseudotyped with Sendai virus envelopes (rSIV.F/HN) to deliver genes encoding anti-influenza bNAbs to the lung could provide long-lasting passive immunity to widely divergent strains of influenza. We selected a novel bNAb that we isolated from vaccinated volunteers, namely T1-3B (V_H 1-69 germline family) that cross-reacts with multiple group 1 influenza A strains including H1 (A/PuertoRico/8/34 & A/Brisbane/59/2007), pandemic H1 (A/California/07/2009) and H5 (A/Vietnam/1203/2004). Delivery of rSIV.F/HN expressing *Gaussia* luciferase (GLux) reporter protein to the mouse lung ($1e7$ TU/mouse) resulted in persistent secretion of GLux into both bronchoalveolar lavage fluid (BALF) ($1,370,000$ RLU/ μ l; $p < 0.01$) and systemically into the serum ($1,000$ RLU/ μ l; $p < 0.01$), representing 18,000-fold, and 25-fold over background, respectively. We then generated rSIV.F/HN vectors expressing T1-3B antibody and showed detection of antibody in the serum (715 ng/ml; $p < 0.05$) for up to 28 days post intranasal delivery of rSIV.F/HN ($5e7$ TU/mouse). Animals treated with rSIV.F/HN expressing T13B were partially protected against a lethal challenge with either 1,000 or 10,000 TCID₅₀ (~10 and 100 LD₅₀) of A/PuertoRico/8/34 (H1N1) influenza. They experienced a ~2-3 days delay in symptoms and, significantly reduced weight-loss - less than 20% weight loss in 50% (1,000 TCID₅₀) and 20% (10,000 TCID₅₀) treated animals, while all control animals lost >20% weight irrespective of influenza dose ($p < 0.001$ and $p < 0.05$ for 1,000 and 10,000 TCID₅₀ respectively). One major advantage of rSIV.F/HN as a platform for bNAb passive immunization is the proven ability to achieve successful transgene expression after repeated lung vector administration (*Mol Ther* 18:1173, 2010; *Am J Respir Crit Care Med* 186:846, 2012), a major hurdle for all other viral vectors we have evaluated, including rAAV (*J Virol* 81:12360, 2007). Repeat administration of rSIV.F/HN vectors expressing alternative bNAbs, such as those that cross-react with both group 1 and 2 influenza, could provide a truly broad protection against a wide range of influenza strains. We speculate that during the next human influenza pandemic, prophylaxis provided by lung gene transfer may be feasible and more cost-effective and time-responsive than traditional vaccines or parenteral administration of therapeutic antibody.

432. Valproic Acid Treatment Enhances Hematopoietic Stem and Progenitor Cell Multipotency Ex Vivo for Enhanced Long-Term Engraftment of Gene-Modified Cells

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The promising field of genome editing in hematopoietic stem and progenitor (HSPC) for use in autologous and allogeneic transplantation therapies relies on being able to engraft the edited cells into the bone marrow and to have those engrafted cells produce all the hematopoietic lineages necessary for proper immune and red blood cell function. Depending on the cell source to be used in the editing process, the fraction of CD34+ HSPCs can be quite low - approximately 0.0005%, 0.01%, or 0.1% for mobilized peripheral blood (mPB), bone marrow aspirate (BM), or cord blood, respectively. The fraction of long-term repopulating true stem cells (LT-HSCs) within these CD34+ cell populations, capable of long-term reconstitution of the entire hematopoietic lineage after transplantation, is even lower (<1%). In addition, it has recently been shown that LT-HSCs derived from cord blood can be much less permissive to homology-directed repair (HDR)-driven gene correction which may be essential for some types of therapeutic genome editing.

Several groups have discovered small molecules that promote expansion of cord blood-derived CD34+ HSPCs *ex vivo* while maintaining the stemness of the HSPCs, however these effects have not been reported in HSPCs from mPB or BM. Here we show that the small-molecule epigenetic modifier valproic acid (VPA) improves HDR-mediated targeted integration (TI) in mPB and BM HSPCs. In addition to increasing the fraction of LT-HSCs with TI by up to 100-fold, VPA also dramatically increases the overall number of cells expressing LT-HSC markers (CD34+CD133+CD90+CD49f+ or CD34+CD38-CD45RA-CD90+CD49f+) by up to 500-fold. VPA-treated gene-edited HSCs differentiate normally *in vitro* and retain consistent levels of TI in both erythroid and myeloid lineages. These results further the development of genome-edited mPB and BM-derived HSPC therapies.

433. DNA Monoclonal Antibodies Target Influenza Virus In Vivo

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Despite promising innovations, influenza vaccines and antiviral drugs fail to provide full protection from seasonal infection, and provide little defense against novel and potentially pandemic viral strains. Broadly cross-protective monoclonal antibodies have been developed with the aim of providing protection against highly divergent influenza viruses. However, the utility of delivering purified protein antibody as therapy or prophylaxis against influenza is limited, especially in pandemic settings. Use of gene therapy to generate monoclonal antibodies *in vivo* provides a simplified, flexible, and relatively inexpensive alternative to protein antibody treatment.

In this study, we used intramuscular electroporation of plasmid DNA encoding immunoglobulin to express DNA monoclonal antibodies (DMAb) against influenza hemagglutinin (HA) surface protein in mice. Multiple aspects of plasmid construction, antibody design, and delivery were optimized to enhance expression of DMAb from muscle cells *in vivo*. We investigated multiple antibody clones, including the broadly-neutralizing anti-influenza-H1 antibody 5J8.