either pre-existing T-cell immunity to Ad11 or, most likely, T-cell cross-reactivity between Ad serotypes; ii) the use of HD vectors did not prevent induction of anti Ad T cells in naïve animals, and the incoming Ad particles activated memory T cell responses in *in vitro* human studies; iii) we could not detect strong antigen specific immune responses in our experiments. Then, these studies support the idea that cellular immunity, directed to the Ad capsid epitopes, is an important problem to be solved for antigen specific vaccination/ immunotherapy purposes.

1092. Adenovirus-Based Vaccine Prevents Pneumonia in Ferrets Challenged with the SARS Coronavirus

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The human severe acute respiratory syndrome coronavirus (SARS-CoV) is the etiologic agent responsible for the spread of the acute respiratory syndrome in Asia and Canada that claimed several human lives in 2003. A ferret model of SARS-CoV infection was used to evaluate the efficacy of an adenovirus-based vaccine. Ferrets infected with a clinical isolate of SARS-CoV via intranasal inhalation developed a syndrome similar to that observed in humans that consisted of fever and clinical signs of toxicity. Virus replicated in lung and was recovered in nasal washes. Patchy but widely disseminated bronchopneumonia developed characterized by infiltration of neutrophils and monocytes. Animals were vaccinated with recombinant adenoviruses expressing the Spike protein of SARS-CoV prior to challenge with SARS-CoV. The most impressive therapeutic results were obtained when the animals were primed with a human adenovirus expressing Spike and boosted with a serologically distinct adenovirus from chimpanzee also expressing the Spike antigen. Following challenge with SARS-CoV the acute fever was lessened and clinical sequalae were diminished. Viral loads in lung and nasal washes were down 6 logs as compared to animals vaccinated with a control vector. There was no evidence of gross lung pathology at necropsy and histological findings were limited to mild focal inflammation in alveolar septa and around some small airways. The same prime boost strategy was also effective in rhesus macaques and resulted in SARS-CoV neutralizing antibody titers of up to 1/2560 and γ-INF positive T cells reaching 12,000 SFC per million PBMCs as assessed by ELISPOT. These data indicate that a heterologous adenovirus-based prime boost vaccine strategy could stimulate a strong immunity that may be needed for complete protection against SARS-CoV infection.

1093. An Adenovirus-Retrotransposon Hybrid Vector Achieves Stable Transformation of Quiescent and Primary Human Somatic Cells

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Adenoviral vectors (AdVs) have particular advantages for use as in vivo gene transfer vehicles, including a broad host range, the ability to infect both dividing and non-dividing cells, and ease of high-titer purification. However, AdVs rarely integrate into the host genome, and so the proportion of transduced cells decreases with each cell division. It is therefore desirable to develop a vector that can both integrate into the host genome to achieve sustained gene expression and be easily grown to titers sufficient for clinical use.

We have developed a hybrid adenoviral vector, designated A/RT, for delivery of a newly isolated human L1 retrotransposon element, L1RP. After delivery via the first-stage helper-dependent AdV, the retrotransposon element can mediate permanent integration of transgenes in the transduced cells. The L1RP element itself exhibits one of the highest retrotransposition frequencies observed to date. Delivery of an optimized PGK promoter-driven L1RP cassette in the context of the A/RT hybrid vector further enhanced the overall retrotransposition frequency to 15-30% of AdV-transduced cells as measured by reconstitution of an intron-disrupted GFP marker gene, corresponding to efficiencies up to 30-fold higher than those previously reported with plasmid-based delivery. Hybrid vector-mediated retrotransposition frequency was observed to correlate with increasing multiplicity of infection, and increased over a period of 6 days following transduction, thereafter remaining stable at the same levels for >8 weeks. Retrotransposition events were also detected in differentiated human somatic cells, including hepatocytes and fibroblasts, following hybrid vector transduction in primary culture, and in the liver parenchyma following in vivo administration. Furthermore, retrotransposition leading to stable long-term transgene expression was achieved in quiescent cells in G1 phase stationary culture. A/RT hybrid vector systems may thus be useful as a vehicle for both efficient delivery and long-term stable transduction of therapeutic genes to both dividing and non-dividing cells.

1094. A Novel Replication Competent, but Packaging Deficient Adenoviral(Ad) Vector [E1+, 100K-]hGAA for High Level hGAA Expression and Reduced Toxicity in Gene Therapy of Glycogen Storage Disease II (GSD-II)

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GSD-II is a recessive lysosomal storage disorder in which lack of human acid-alpha glucosidase(hGAA) activity results in massive accumulations of glycogen in cardiac and skeletal muscles. Recombinant hGAA enzyme replacement therapy(ERT) is currently in clinical trials, but in addition, gene therapy strategies are being explored to serve as an adjunct or alternative to ERT.

We have shown that hepatic targeting of a modified Ad vector expressing hGAA was able to correct the glycogen accumulation in multiple affected muscles in GAA-KO mice, by hepatic secretion of hGAA. In an effort to generate high-level hGAA expression while minimizing vector dosage, we set out to build a replication competent but packaging deficient Ad vector expressing hGAA.

The Ad100K gene occupies about 10% of the entire viral genome confirming its importance in the wt Ad life cycle. The 100K protein is a late viral protein expressed after Ad genome replication. It has many important roles, including transport and trimerization of newly synthesized hexon subunits from the cytoplasm to the nucleus, and acting as a "scaffolding platform" for assembly of viral capsids. We hypothesized that an [E1+]Ad vector incorporating deletions within the 100K gene would result in a replication competent but a disabled vector that is unable to express 100K and incapable of hexon trimerization and transport. Previous studies by our group (Hodges et.al, 2001) has shown that the deletion of the 100K results in reduced late viral protein production and diminished liver toxicity when an [E1-, 100K-] vector was administrated *in vivo*. Based on these previous findings, we hypothesized that a replicating [E1+] Ad vector with a 100K deletion would increase the copy number of the therapeutic vector per target cell after transduction, with diminished toxicity associated with late viral protein production.