configurations (monoclonal antibody [MAb] and immunoadhesin [IA]) were subcloned under TBG and CMV promoters for liver and muscle expression, respectively, and the resulting transgenes were packaged into AAV2/8. RAG KO mice were chosen as a model for this study to mitigate immunogenic effects of expression of non-cognate antibodies. A dose of $1x10^{11}$ GC per mouse ($5x10^{12}$ GC/kg) of each of the four vectors was injected into the right and left gastrocnemius muscles of RAG KO mice (n = 5 per group). At day 28 post IM administration, serum expression levels for VRC01 IA and MAb reached 64 ± 12 and 29 ± 6 μg/ml, respectively. At the same time point, expression levels for PG9 IA and MAb were 67 ± 19 and 100 ± 22 μg/ml, respectively. For the IV route of administration, a dose of $1x10^{11}$ GC per mouse ($5x10^{12}$ GC/kg) for each vector was injected via the tail vein (n=5 per group). At day 28 post administration, serum expression levels of VRC01 IA and MAb reached 916 ± 139 and 126 ± 8 μg/ml, respectively. Nasal lavage studies showed that approximately 1% of the circulating VRC01 MAB distributed to mucosal surfaces. Expression levels for PG9 IA and MAb at the same time point were 376 ± 56 and 201 ± 52 μg/ml, respectively. Similar expression levels persisted to day 56 post-administration for both routes of delivery. The biology of passively delivered VRC01 and PG9 antibodies was verified by testing the serum samples from AAV2/8 injected mice against a characteristic panel of 19 multiclade HIV isolates in validated cell based neutralization assays. Both PI delivery routes achieved sufficient and sustainable systemic levels of NAbs thus confirming the feasibility of the use of PI as a disease prophylactic.

324. DNA Vaccination To Generate Chikungunya Virus-Specific Immunity

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Chikungunya virus (CHIKV) is an emerging mosquito borne alphavirus indigenous to tropical Africa and Asia, which has caused massive outbreaks of endemic and epidemic fever for at least half a century. Unfortunately, recent evidence suggests that CHIKV, which infects humans via the mosquito vector Aedes species, can be transmitted by other carriers, raising concern of continued pathogen spread outside of its original host. CHIKV is no longer confined to the developing world as it has begun to branch into the boundaries of neighboring regions. As a result, the NIAID has designated CHIKV as a Category C pathogen alongside the influenza and SARS-CoV viruses. Despite the emergence of CHIKV, no licensed vaccines or therapeutics are currently available for preventing or treating CHIKV infection. Realization of the potential severity of CHIKV-induced disease is exigent; for example, if used as a biological weapon, the world economy could be severely crippled. If enough members of the armed forces were to become infected during a military deployment, a military operation could be significantly affected. Efforts to monitor the disease will only provide minimal warning in a global society. Accordingly, steps should be taken to prevent the morbidity and mortality associated with a CHIKV pandemic. In an effort to address this important need, we isolated a new CHIKV virus from an acutely infected human patient and developed a defined viral challenge stock in mice and neutralization assay. We then constructed a synthetic DNA vaccine that expresses the component of the CHIKV envelope glycoprotein. Following electroporation (EP) immunization of the vaccine, we observed induction of robust antigen-specific cellular and humoral immune responses individually capable of providing protection against CHIKV challenge in mice. Furthermore, vaccine studies in Rhesus Macaques demonstrated strong neutralizing antibody (Nab) responses which mimicked those induced in convalescent human patient sera. These data suggest a protective role for neutralizing antibodies against CHIKV disease and support further study of envelope-based CHIKV DNA vaccines.

325. Lentiviral Gene Therapy Against HIV-1 Using a Novel TRIM21-Cyclophilin A Fusion Restriction Factor

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Despite advances in drug treatment for HIV-1, there are still many associated side effects including cost, toxicity and the emergence of viral escape mutants. However gene therapy offers potential to provide long term treatment after a single intervention. Some primates are resistant to HIV due to the expression of TRIM5-Cyclophilin A (TRIM5CypA) fusion proteins. These restriction factors have arisen twice independently in primate evolution by retrotransposition of CypA into the TRIM5 gene with no evidence of mutagenic escape. We are investigating using a humanised TRIM5CypA protein delivered by lentiviral vectors to provide protection to susceptible cell populations. TRIM5CypA inhibited HIV-1 vectors up to 100 fold compared to control populations. Furthermore, potent restriction of replication competent HIV-1 and a survival advantage was seen in TRIM5CypA expressing cell lines and primary T cells. However, TRIM5CypA expression interfered with the restrictive abilities of endogenous TRIM5α, rescuing MLV infection. An alternative restriction factor, TRIM21CypA, has been developed by replacing the RING, B-Box and coiled coil domains of TRIM5 with the corresponding domains of the related human TRIM21. TRIM21CypA provides 100 fold restriction of HIV-1 equal to that of TRIM5Cyp, but importantly does not disrupt TRIM5α retroviral restriction. In addition, neither TRIM5CypA nor TRIM21CypA expression affected the anti-viral activity of endogenous TRIM21. We propose that these TRIMCyp restriction factors could form the basis of a novel treatment strategy of HIV-1 by intracellular immunization of susceptible cell populations using lentiviral vectors, with TRIM21CypA having advantages over the TRIM5CypA protein due to the maintenance of endogenous TRIM protein function.

326. HCV Inhibition by a Multicistronic Anti-HCV miRNA AAV Vector In Vitro and In Vivo

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The successful treatment of HCV requires a combination of multiple drugs, due to the genetic diversity of the virus and its propensity to mutate. RNA interference (RNAi), the sequence specific post transcriptional mechanism of gene silencing, provides a means for achieving this. We are exploiting the endogenous miRNA 17 92 cluster as a scaffold for the expression of multiple exogenous miRNAs that are complementary to the HCV genome. The miRNAs are delivered to cells using recombinant AAV (rAAV) vectors and are expressed in hepatocytes, the site of HCV replication. Previously, we created an rAAV vector encoding four active miRNAs, and demonstrated up to 98% inhibition of bona fide HCVcc replication in the Huh-7.5 cells (Yang et al; Hepatology 2010). We have now constructed a more potent rAAV vector (rAAV-Cluster 5) that expresses five active anti-