other two, using a replication competent VSV or replication defective HIV vector, were done in an enhanced BSL2 environment. Results indicate that the two methodologies using replication competent Ebola virus are slightly more sensitive than with pseudotyped VSV or HIV vector. Ebola expressing EGFP was found the method of choice in the BSL4 laboratory since it can be performed in 48 hours (instead of 6 days for immunoplaque assay) and does not depend on the sensitivity of an antibody/antigen reaction. Overall, each protocol offers advantages and inconveniences that will be discussed.

James Wilson had equity in Targeted Genetics at the moment of the study.

605. Long-Term Protection Afforded by a Single Administration of an Ad-Based Anti-*Y. pestis* Vaccine Against a Lethal Respiratory Challenge with *Y. pestis*

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The aerosol form of the bacterium *Yersinia pestis* causes the pneumonic plague, a virulent and rapidly fatal disease that develops within days post-infection. In the context of a bioterror threat, an ideal anti-plague vaccine should elicit rapid, robust and long-acting protective immunity after a single dose. At present, no plague vaccines are available for use in the USA. One candidate for developing a subunit vaccine is the *Yersinia pestis* V antigen, a protein that influences the function of the *Yersinia* outer membrane proteins (Yops) virulence factors and has local anti-inflammatory effects in the host. Although protective immunity against *Y. pestis* challenge can be induced with recombinant V antigen, this immunization regimen requires multiple administrations and recent evidence suggests that long-term protection against *Y. pestis* requires strong cellular immune responses that are not stimulated by protein-based vaccines. Based on the knowledge that adenovirus (Ad)-based vaccine platforms rapidly induce humoral and cellular immune responses against expressed transgenes, we tested the hypothesis that a single administration of a replication-defective Ad gene transfer vector encoding the *Y. pestis* V antigen (AdsecV) could stimulate long-lasting protective immune responses, including cellular immune responses, without a requirement for repeat vaccine administration. To test the efficacy of the vaccine, mice immunized with a single administration of 10^{11} pu AdsecV were challenged intranasally 4 wk or 6 months after vaccination with doses of *Y. pestis* CO92 up to 10^6 cfu. At four wk post-administration, control mice that received saline (naive) or 10^{11} pu AdNull, a control vector, died within 3 to 5 days after challenge. In contrast, 100% of AdsecV-immunized mice (10/10) survived the intranasal challenge with 10^4 cfu and 8/10 and 9/10 of mice survived after infection with 10^5 and 10^6 cfu *Y. pestis* CO92, respectively (p<0.0001 for all doses). When mice immunized with a single dose of AdsecV were challenged 6 months post-administration, 100% of animals (10/10) survived intranasal infection with 10^4 cfu and 9/10 survived intranasal infection with 10^6 cfu *Y. pestis* CO92 (p<0.0001 for each dose). None of the control animals (naive or immunized with 10^{11} pu AdNull) survived the challenge. Protection was correlated with high anti-V antibody titers and, importantly, V- specific T cell responses (measured by ELISPOT). Consistent with the concept that cellular immune responses were relevant to protection, immunization of CD8+ T cell deficient mice (*Cd8a^{tm1Mak}*) with AdsecV did not protect any mice (0/10) from an intranasal infection with *Y. pestis* CO92 4 wk after immunization. These data suggest that AdsecV is a promising candidate as an effective single dose vaccine against the plague and that the cellular immune responses evoked by the vaccine play an important role in protection against respiratory infection with *Y. pestis.*

606. Identification of a 45-aa Domain of the F12-Vif Mutant Possessing Anti-HIV Activity

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Our previous results have demonstrated that T-cell lines and primary T lymphocytes transduced with a Tat-dependent HIV-based lentiviral vectors expressing the mutant isoform of the *vif* gene, F12-*vif,* are protected from HIV-1 infection. F12-Vif is a 192-aa natural variant polypeptide owing 14 unique amino acid substitutions. The substitutions are randomly scattered along the entire sequence with the exception of a 5-aa cluster located at positions 127, 128, and 130-132. None of the 14 aa substitutions is present in the SOCS box that recruits the E3 ubiquitin ligase responsible of APOBEC3G (AP3G) degradation during HIV infection. In line with this notion, we have shown that the antiviral function of F12-Vif is not due to a dominant negative feature of the mutant in regards to the Vif-mediated degradation of AP3G rather to some other unknown means. Therefore, in the effort to elucidate the F12-Vif mechanism of action, we started to identify the protein domain of F12-Vif responsible of HIV-1 inhibition. To this end, we have constructed three chimeric genes (Chim1, Chim2 and Chim3) composed by wild-type and F12-*vif* regions. T cell lines and cord blood derived CD4+ T lymphocytes were transduced with the lentiviral vectors expressing the chimeric genes and then challenged with both X4 and R5 HIV-1 strains. We show that 45 amino acids in the C-terminal domain of the F12-Vif mutant are sufficient to exert anti-viral effect in transduced cells. In contrast to F12-Vif, Chim3 does not allow the rescue of the replication of a *vif*-deficient HIV-1 in the context of either X4 or R5 tropism in non permissive cells. This specific feature renders Chim3 a truly dominant negative protein more suitable than F12-Vif for an anti-HIV gene therapy approach.

607. Novel Adeno-Associated Viruses as Vaccine Carriers for HIV-1: Evaluation in Non-Human Primates

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Adeno-associated virus (AAV) has been widely studied as a vector for gene therapy applications. Many of the features that make AAV an attractive vector for gene therapy, such as non-pathogenicity, wide tropism and the ability to infect non-proliferating cells, low vector immunogenicity, also make it a potential candidate as a vector for vaccine development. Three groups of *Cynomolgus macaque* (n=5/group) were intramuscularly (im) immunized with AAV2, AAV2/7 or AAV2/8 mixture, expressing HIV-1W61Dgp140 (Env), Gag-Nef (GN2) and HIV-1 RT (RT3) at a dose of 1E+12 GC per vector per animal. Peripheral blood mononuclear cells (PBMCs) were collected at baseline, and weeks 2, 4, 8, 14, 16, 20 and 24 after immunization. T cell mediated immune response was assessed over time by IFNγ ELISPOT and intracellular cytokine staining (ICS) and B cell mediated immune response by Gag specific antibodies (abs) detection. The AAV2/7 and AAV2/8 groups displayed a diverse T cell response towards Gag, RT, Nef but not Env. Unlike the AAV27 and AAV2/8 groups, AAV2 group displayed a strong response only towards Env and negligible response towards Gag, RT and Nef. One animal from the AAV2/8 group mounted a strong response towards Nef. ICS data suggest that this response was $CD8^+$ T cell mediated. The T cell responses were remarkably stable during the duration of this experiment which currently is 24 weeks.

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