abundance in the muscles as early as 3 days after electroporation, and were observed through out our study to day 21. But only a few DC cells were present at day 14 and day 21. Based on those observations, we hypothesize that the infiltrating macrophages are responsible for cross-presentation of antigen from transfected muscle fibers to T cells. Indeed, we observed T cells activation in the draining LN by chemokines and receptors cDNA array, with enhanced expression of CXCR-1, CXCR-2, CCR-1, CCR-3, CCR-4, CCR-7, CCR-8, CCR-9 and Cmkbr1l2 genes. CD4+ and CD8+ T cells were observed to appear in the muscle at as early as day 5, peak between day 7 and day 14, but disappear at day 21 by immunohistochemistry analysis. Full recovery of the muscle fibers was found at day 28 after electroporation, with center nucleated regenerated muscle cells and eliminated inflammatory cell infiltrates.

552. Single-Chain Antibodies for Viral-Based Passive Immuno-Therapy for Prion Disease: Linear Epitope Mapping and Clearance from Thalamic Injection

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Transmissible spongiform encephalopathies (TSE) are characterized by the conversion of a normal cellular protein (PrPc) to an abnormal disease causing conformation, designated PrPsc. Efforts to use antibodies to alter the rate of conversion of PrPc to PrPsc suggest it to be a plausible therapeutic strategy. Notably, during the normal progression of disease the host organism mounts no detectable immune response to alter the process of PrPsc accumulation. This is hypothesized to be due to the low antigenicity of PrPc and host tolerance to a self-polypeptide. We intend to circumvent the lack of a host-based immune response to impede the conversion of PrPc using novel PrPc-specific single-chain variable fragment (scFv) antibodies that will be utilized in a viral-based passive immuno-therapy. Several PrPc-specific antibodies have been obtained from a naïve human combinatorial phage display library. Epitope mapping has been performed on our scFvs using an ELISA based peptide array for linear epitope determination. We have also investigated the clearance of scFvs from a thalamic injection and determined the elimination half-life ($T_{1/2}$).

553. Silencing HBV Gene Expression with Pol II and Pol III Promoter-Derived Short Hairpin RNA

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Chronic infection with hepatitis B virus (HBV) occurs in approximately 6% of the world's population and is a major risk factor for cirrhosis and hepatocellular carcinoma (HCC). Current therapy for persistent HBV infection, including interferon alpha and nucleoside analogues, is often unsuccessful and developing effective HBV treatment remains an important global medical priority. The virus has a compact genome with limited sequence plasticity that makes it a good target for therapy that is based on nucleic acid hybridization. Thus, exploiting the RNA interference (RNAi) pathway to knock down HBV gene expression is a promising novel approach to treating HBV infection. To induce HBV gene silencing, synthetic siRNA duplexes or short hairpin RNA (shRNA) molecules derived from Pol III promoters have typically been employed to induce RNAi. Using these approaches, silencing is not targeted to the liver and potentially harmful non specific extrahepatic effects may occur. Pol II promoters, which are capable of precise transcription control, may be applied to effect more controlled silencing. The objective of this work was to assess the anti HBV silencing efficiency of transiently expressed short-hairpin RNAs (shRNA) regulated by a Pol II promoter and to compare their efficacy to equivalent Pol III-derived transcripts. A panel of 6 shRNAs regulated by U6 (Pol III) or CMV (Pol II) promoters were designed to target 3 regions of the conserved HBV *X* open reading frame. The HepG2.2.15 cell line, which constitutively produces HBV, and Huh7 human hepatoma cell line were transfected with plasmids encoding Pol II or Pol III shRNA expression cassettes. Huh7 cells were also cotransfected with pCH3091 or pCH3091-GFP HBV target vectors. pCH3091 is a plasmid that encodes all HBV sequences, and in pCH3091-GFP, the preS2-S region has been substituted with a sequence encoding the Enhanced Green Fluorescent Protein (EGFP). The effects of the shRNAs on HBV gene transcription were determined by measuring HBsAg, HBeAg, HBV transcripts and EGFP. shRNAs that are derived from both Pol II and Pol III promoters were capable of significant inhibition of HBV gene expression, although Pol III-derived shRNAs were stronger inducers of HBV gene silencing (95% vs 70% inhibition). To improve precisely controlled silencing, refinements in the design of Pol II cassettes that regulate strand bias and limit compromising effects of extra 5' and 3' sequences are being investigated.

554. A Candidate SARS-Associated Coronavirus Vaccine Elicits Broad Immunity in Monkeys

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The causative agent of Severe Acute Respiratory Syndrome (SARS) has been identified as a new type of coronavirus. Here we have examined the ability of adenoviral gene transfer of codon-optimized SARS-CoV strain Urbani structural antigens spike (S) protein S1 fragment, membrane protein (M), and nucleocapsid protein (N) to induce broad immunity in the non-human primate. Six rhesus macaques were immunized intramuscularly with a combination of the three Ad5-SARS-CoV vectors and boosted on day 28. The vaccinated animals all had antibody responses against S1 and T-cell responses against the antigens. Importantly, all six vaccinated animals showed strong neutralizing antibody responses to SARS-CoV infection in vitro. These results demonstrate that an adenoviral-based vaccine can induce strong SARS-CoV-specific immune responses in the monkey, and hold promise for development of a protective vaccine against the SARS etiologic agent.