Online Proceedings

POSTER SESSION 4

MGH 241, Easel 158

4:15 PM to 5:45 PM

Alteration of Homing Endonuclease Recognition Site to Target Latent HBV Infection

Kevin Tze Chi (Kevin) Kwong, Junior, Biochemistry
Mentor: Nick Weber, Laboratory Medicine
Mentor: Keith Jerome, Laboratory Medicine

There are currently an estimated 350 million people worldwide chronically infected with the Hepatitis B virus (HBV). These individuals, over the course of their life, are at an increased risk of developing liver cancer. While antiviral drugs can suppress HBV replication, latent HBV viruses, lying dormant but still potent in the hosts' cells, so far have proven difficult to eradicate. Homing endonucleases (HE), enzymes that recognize long DNA sequences and induce DNA double strand breaks, could be a means to target and inactivate latent HBV. In particular, we aim to modify the recognition site of the WT I-GzeII homing endonuclease specific to the HBV genome and introduce DNA double-strand breaks. By exploiting the error prone nature of non-homologous end joining, the cellular process to repair double-strand breaks, these modified HEs will have a mutagenic effect on target sequences. Repeated HE activity will eventually cripple the replicative ability of latent viruses. To selectively modify the structure of homing endonucleases, a variant library of the I-GzeII gene is generated with randomized bases corresponding to the amino acid residues that interact with the DNA substrate. Variants that encoded for active HEs with the appropriate recognition site are selected for by In Vitro Compartmentalization (IVC). The selected variants will then be run through bacterial selection and in vitro cleavage assays to further select for structurally stable variants that function in vitro. The final step is to test the HE product on an in vitro HBV cell line. Through our research, we hope to prove that the target of I-GzeII recognition sites can be effectively altered and that latent viruses can be targeted and inactivated through mutagenesis by homing endonucleases. Hopefully, our research will be the first step to a more permanent cure for HBV infections and help to reduce incidences of liver cancer.

POSTER SESSION 4

MGH 241, Easel 132

4:15 PM to 5:45 PM

Exploring the Behavior of T Cell Responses Against Malaria Liver-Stage Antigens

Zachary Billman, Senior, Biochemistry
Mentor: Sean Murphy, Laboratory Medicine

Pre-erythrocytic malaria vaccines may need to target numerous sporozoite and/or liver-stage proteins to be effective. If the protective antigens could be identified, multi-component vaccines could be produced. Using a high-throughput T cell screening, our lab recently identified a $CD8^+$ response against the *Plasmodium yoelii* L3 ribosomal protein in sporozoite-immunized BALB/c mice. Unlike the $CD8^+$ T cell response against the circumsporozoite protein (CSP) that increases with each parasite exposure in our system, the L3-specific response is not boosted by repeated exposures to attenuated *P. yoelii* sporozoites. Our lab has also shown that L3-specific cells have no cell intrinsic defects that counteract their re-expansion or function but rather that broad antisporozoite immune responses in secondary or later exposures eliminate expression of L3, thereby preventing any opportunity for activation of memory L3-specific $CD8^+$ T cells. This T cell outcome following immunization may be emblematic of other T cells with liver-stage targets as well. In my project, we are studying the L3-specific T cell response in other murine parasite species (*P. berghei*) and other mouse strains (C57BL/6) by immunizing BALB/c and C57BL/6 mice with both species of *Plasmodium* and monitoring L3-specific $CD8^+$ T cell responses via IFNγ ELISPOT to determine if this phenomenon is conserved in related models. Further, we are testing whether or not heterologous immunization of BALB/c mice with radio-attenuated *P. berghei* and *P. yoelii* followed by genetically-attenuated *P. yoelii* sporozoites bypasses the anti-CSP response and allows for boosting of late liver-stage antigens. If this occurs, such approaches could potentially yield more protective outcomes in human challenge studies and lead to more effective malaria vaccines in the future.