Understanding Trypanosome Lytic Factor biogenesis through human serum, tissue culture, and murine models

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African Trypanosomiasis is a disease caused by the African Trypanosoma family of bloodborne parasites. Cattle are susceptible to trypanosomiasis, while humans and some non-human primates are protected against most species of trypanosomes due to an immunity complex called Trypanosome Lytic Factor (TLF). TLF is a specialized High-Density Lipoprotein (HDL), that carries a lytic cation channel-forming protein (Apolipoprotein-L1 (APOL1)), and a ligand (Haptoglobin-related protein (HPR)), which binds to parasite receptors increasing uptake into the parasite. There are two TLF HDL complexes in blood: TLF1 (~500 kDa) and TLF2 (~1,200 kDa). How these TLFs are assembled is unknown. We have used human serum, tissue cell culture, and transgenic murine models to better understand how and where TLFs are assembled and how to translate that information to resistant transgenic cattle model. Generation of transgenic cattle constitutively expressing genes that encode for TLF components would reduce animal disease, as well as the reservoir for human disease.

To fully characterize all possible TLF species, we used anti-APOL1 affinity chromatography and size exclusion chromatography by FPLC. Both TLF1 and TLF2 were isolated, as well as a third complex of 180 kDa. Immunoprecipitation confirmed APOL1 and APOA-I were on the same complex, and activity assays based on normalized APOL1 concentration showed this complex lyses trypanosomes equivalently to TLF1, indicating the presence of HPR. We propose that this TLF complex, TLF3, is a nascent HDL made in hepatocytes based on its size, density, and predicted liver gene expression. These data inform us that liver specific promoters could be used to drive the expression of *APOL1* and *HPR* in transgenic cattle. We have generated many germine transgenic murine models to test different promoters for the expression of *HPR* and *APOL1*. However, we find that the Ubiquitin promoter (not liver specific promoters) drives the highest expression of both *HPR* and *APOL1,* which is key for sustained and robust protection against trypanosome challenges.

To revisit the biogenesis of TLF3 we turned to *in vitro* studies of human hepatocyte cell lines, HepG2. We find co-assembly of APOL1 and APOA-I by size fraction, and plan to affinity purify APOL1 complexes secreted by HepG2 cells to evaluate their protein composition by mass spectrometry and the trypanosome lytic capacity of the complexes. We hypothesize that all three proteins are assembled together in/on the hepatocyte with minimal but sufficient lipids to generate a nascent HDL. Thereafter, the TLF3 complex is released into the blood and matures into TLF1 by accumulating lipids and potentially more APOL1 from peripheral tissues. By understanding TLF biogenesis, we can use the appropriate promoters in transgenic cattle models to generate cattle resistant to trypanosomiasis.