Title:
Inducible Germline IgMs Bridge Trypanosome Lytic Factor Assembly and Parasite Recognition

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Summary
Trypanosomiasis is a devastating neglected tropical disease affecting livestock and humans. Humans are susceptible to two *Trypanosoma brucei* subspecies but protected from other trypanosomes by circulating high-density lipoprotein (HDL) complexes called trypanosome lytic factors (TLFs) 1 and 2. TLFs contain apolipoprotein L-1 contributing to lysis and haptoglobin-related protein (HPR), which can function as a ligand for a parasite receptor . TLF2 also uniquely contains non-covalently associated IgM antibodies, the role and origin of which remain unclear. Here, we show that these TLF2-associated IgMs interact with both HPR and alternate trypanosome surface proteins, including variant surface glycoprotein, likely facilitating complex biogenesis and TLF uptake into parasites. TLF2-IgMs are germline antibodies that, while present at basal concentrations in healthy individuals, are elicited by trypanosome infection in both murine models and human sleeping sickness patients. These data suggest that poly- and self-reactive germline antibodies such as TLF2-associated IgMs play a role in antimicrobial immunity.

Keywords: Trypanosome Lytic Factors, Variant Surface Glycoprotein, Haptoglobin Related Protein, Natural Germline Antibodies, Immunoglobulin M, Apolipoprotein L-1, Innate Immunity

Introduction

Humans, gorillas, and certain old-world monkeys are immune to infection by the fly-transmitted protozoan *Trypanosoma brucei brucei*. Immunity to *T. b. brucei* is due to a primate-specific innate defense mechanism mediated by a subset of circulating high-density lipoprotein (HDL) complexes called trypanosome lytic factors (TLFs) 1 and 2 (Rifkin, 1978, Hajduk et al., 1989, Raper et al., 1999). Humans can be infected by *T. b. rhodesiense* and *T. b. gambiense*, both of which have evolved mechanisms to block TLF-mediated innate immunity (Vanhamme et al., 2003, Stephens and Hajduk, 2011, Uzureau et al., 2013). Both TLFs contain the primate-specific ion channel-forming protein apolipoprotein L-1 (APOL1), which lyses the parasites by forming cation-selective pH-gated channels in the parasite membranes after endocytosis (Thomson and Finkelstein, 2015). To form open channels, APOL1 requires an initial membrane insertion step dependent on acidification in the endocytic compartments, followed by a neutralization step that likely occurs at the plasma membrane after endocytic recycling (Thomson and Finkelstein, 2015). Both TLFs also contain the primate-specific hemoglobin (HB) binding protein haptoglobin-related protein (HPR). HPR-HB complexes are endocytosed after binding the trypanosome haptoglobin-hemoglobin receptor (HPHBR) (Vanhollebeke et al., 2008), though this interaction can be inhibited by physiological concentrations of haptoglobin (HP)-HB complexes (Raper et al., 1996). Receptor-mediated endocytosis of TLF1 is dependent on the HPHBR, however, TLF2 endocytosis cannot be inhibited by HP-HB complexes and is therefore independent of the HPHBR (Raper et al., 1996). These biochemical experiments have also been supported by reverse genetics, wherein knockdown of the HPHBR increased *T. b. brucei* resistance to TLF1 by 10,000-fold, while resistance to TLF2 was increased by only 500-1000-fold (Bullard et al., 2012). While we cannot rule out the possibility of an interaction between TLF2 and the HPHBR, the data suggest that TLF2 has an alternative endocytosis mechanism and that TLF2 is the main lytic factor in normal human serum, as TLF1 activity is inhibited by physiologic HP-HB concentrations.

TLF2 is distinguished from TLF1 by the association of IgM antibodies with the TLF2 complex that may play a role in TLF2 endocytosis (Raper et al., 1999). Pentameric IgM antibodies typically bind with relatively low affinity and high avidity to their cognate antigen. These low-affinity TLF2-associated IgMs (TLF2-IgMs) are polyclonal and interact with the complex non-covalently (Raper et al., 1999). The exact stoichiometry is unclear, however, based on the sizes of the TLF1 (~500 kDa) and TLF2 (~1400 kDa) complexes, we hypothesize that each TLF2 is comprised of one TLF1 (~500 kDa) and one IgM (950 kDa). All healthy humans produce IgM antibodies as the first humoral response to infection. All healthy humans also produce IgM antibodies that do not undergo affinity maturation called natural or germline antibodies (Silosi et al., 2016). Germline antibodies are often associated with the recognition of self-

antigens, such as apoptotic debris and other damage associated molecular patterns (DAMPs), and do not require an infectious stimulus to be produced. TLF2-IgMs are present in every human plasma sample screened thus far, suggesting that they are germline antibodies that could simultaneously interact with both the TLF complex and with ubiquitous antigens.

Trypanosome parasites proliferate extracellularly in the bloodstream and interstitial spaces and are able to evade antibody-mediated adaptive responses by antigenic variation of their surface coat (Mugnier et al., 2015). Trypanosomes monoallelically express only one of 2000 alleles of the dominant coat protein, the variant surface glycoprotein (VSG). Mueller *et al*. clearly demonstrated that anti-VSG antibody specificities preexist in humans and mice that have never been exposed to the trypanosome (Muller et al., 1996). These germline anti-VSG antibodies also cross-react with – and are possibly raised against – DAMPs (Muller et al., 1996). Given that many germline antibodies target trypanosome surface proteins, and many germline antibodies are polyreactive, we hypothesized that TLF2-IgMs are germline antibodies that can interact with multiple trypanosome antigens with weak affinity. We report here an investigation into the role that TLF2-IgMs play in trypanosome immunity. We find that TLF2-IgMs interact with the TLF protein HPR and many trypanosome proteins, thus offering a route for TLF2 uptake and thereby providing primates with a second mechanism of targeting APOL1 for endocytic uptake by the parasite – one independent of the HPHBR.

Results

Coexpression of human HPR and APOL1 drives TLF2 formation in AID null mice—To biochemically query specific TLF component interactions, we designed a modular system which allows us to purify and assemble various TLF components from animal serum. We had previously reconstituted TLF1 in various mouse strains by transiently expressing the TLF proteins HPR and APOL1 by hydrodynamic gene delivery (HGD) (Kovacsics and Raper, 2014). While HGD has proven a useful tool for the study of TLF1, TLF2 formation has never been reported in any murine models. We hypothesize that the concentration of IgM antibodies available for TLF2 formation is too low in routinely used murine models to produce detectable levels of TLF2 after HGD. Since TLF2-IgMs are present in all analyzed human plasma samples, we hypothesized that TLF2-IgMs are germline antibodies. We therefore used HGD to express *HPR* and *APOL1* in mice that produce germline antibodies at higher concentrations than wild type mice due to knockout of activation-induced cytidine deaminase (AID), which mediates somatic hypermutation and class switch recombination (Robbiani et al., 2008). $AID^{-/-}$ mice can only produce immunoglobulins of the M class, many of which are autoreactive species. We then analyzed plasma from mice transfected by HGD by size exclusion chromatography to separate TLF2 (~1400 kDa) and TLF1 (~500 kDa). These columns are packed with a porous matrix resin that separates molecules based on differences in size, with the largest molecules eluting first (more information can be accessed at https://www.gelifesciences.com/en/us/solutions/protein-research/knowledge-center/protein-purification-methods/size-exclusion-chromatography#size-exclusion). Size exclusion chromatography of plasma from mice expressing human *APOL1* and *HPR* revealed both TLF2-like and TLF1-like HPR- and APOL1-containing complexes (Fig. 1*B*), though the TLF2-like complexes had smaller hydrodynamic radii than human TLF2 (Raper et al., 1999). TLF2 and TLF1 can also be separated by non-denaturing polyacrylamide (Weckerle et al., 2016) or agarose (Fig. S1) gel electrophoresis, which we used to characterize murine TLF2 complexes. Both the concentrated TLF2-containing fractions (lane 1) and the originating unfractionated plasma (lane 3) had APOL1 in the TLF2 range (Fig. 1*C*) and APOL1 was detected in the TLF1 range only in whole murine plasma (lane 3) (Fig. 1*C*). The TLF2-like complexes in ml 67-ml 67.5 of Figure 1*A* were immunoprecipitated (IP) using HPR as the target antigen, which precipitated both APOL1 and IgM (Fig. 1*D*), indicating that all three components were present in the same complex. Therefore, using HGD delivery in $AID^{-/-}$ animals we assemble TLF2 in mouse plasma.

TLF2-IgMs interact with HPR—As HGD-generated TLF2 contains germline IgM from mouse plasma, we sought to evaluate any interactions with TLF components: human HPR, human APOL1, and murine apolipoprotein A-I (APOA-I), the structural protein of HDLs. Germline antibodies often interact with low affinity to self-antigens, so we first asked whether TLF2-IgM was polyreactive and anti-self. For that we

used purified human TLF2 (Sup Fig. 3*A*-*C*) to probe membranes in Far western blot experiments and observed that TLF2-IgMs interact with various components of human and trypanosome cell lysates (Fig. S3). To then understand how such a polyreactive component would interact with the TLF proteins, we used our HGD mouse model to generate components of TLF and assess their individual interactions with IgM. To analyze the interaction between TLF2-IgM and native HDLs, we expressed either *HPR* or *APOL1* in AID$^{-/-}$ mice by HGD and analyzed the mouse plasma by size exclusion chromatography. Expression of *HPR* in AID$^{-/-}$ mice (Fig. 2*A*) resulted in the formation of an HPR-HDL-IgM immunocomplex that co-migrated with murine apolipoprotein A-I (APOA-I), the structural protein of HDLs, and eluted from the column prior to the bulk of the murine HDL complexes (i.e. shifted the migration pattern of APOA-1, Fig. 2*B*). The HPR-HDL-IgM complex was validated by Co-IP (Fig. 2*C*). Expression of *APOL1* in AID$^{-/-}$ mice (Fig. 2*D*) did not produce a detectable immunocomplex, and all of the APOL1 co-migrated with the bulk of the murine HDL and did not shift the migration pattern of APOA-I (Fig. 2*E*). Transfection by HGD did not significantly alter the total protein distribution in murine plasma (Fig. S4), and HGD of saline alone did not shift the migration pattern of APOA-I (Fig. 2*F*). These findings were validated through Far western experiments in which we used human TLF2-IgM to probe APOL1 HDLs or HPR HDLs derived from transfected mice (Fig. 2*G*). We found that TLF2-IgMs interacted with a human HPR-sized protein, but not human APOL1 (Fig. 2*G*). Unsurprisingly, due to the polyclonal IgM component, TLF2-IgMs can interact with many unidentified components of human and murine plasma, though the strongest detectable interaction was with the 45 kDa human HPR protein (Fig. 2*H*, panel d), which is associated with TLF *in vivo*. We conclude that an interaction between the TLF2-IgMs and HPR-bound HDL is required for TLF2 assembly *in vivo*, but that TLF2-IgMs can also interact with many other self-antigens with low affinity.

TLF2-IgMs purified from human plasma interact with trypanosome proteins—While both TLF1 and TLF2 have been identified as trypanosome lytic factors, previous work has demonstrated that TLF2 is far more relevant in the context of human disease (Raper et al., 1996). Given that TLF1 and 2 differ by a single component (IgM) and that the trypanosome surface is densely coated with VSG (a target for IgM), we and others (Vanhollebeke and Pays, 2010) hypothesized that human TLF2-IgMs could facilitate TLF2 uptake by trypanosomes via low-affinity interactions with trypanosome surface antigens. To investigate this *in vitro*, we first probed total trypanosome lysates with human TLF2-IgMs under native (dot blot) and denaturing (SDS-PAGE) conditions and observed interactions between TLF2-IgMs and many trypanosome antigens (Fig. 3*A* and *B*). Since the dominant trypanosome surface antigen, representing 10-20% of the cell's total protein and 99% of the cell surfeome (Shimogawa et al., 2015), is the ~60 kDa GPI-anchored VSG, we then investigated whether TLF2-IgMs could interact directly with VSG. To do so, we first isolated the GPI-anchored trypanosome proteins in their soluble form from three clones, each expressing a different VSG, by hypotonic lysis to enrich for VSG, as described previously (Cardoso de Almeida and Turner, 1983) (Fig. S5). Under native conditions, TLF2-IgMs interacted most strongly with the VSG3 preparation, while lower-affinity interactions were observed between TLF2-IgMs and VSGs 2 and 13 (Fig. 3*C*). VSGs have been classified into multiple groups based on the distribution of cysteines throughout the amino acid sequence (Carrington et al., 1991). Both VSG2 and 13 are class A VSGs, while VSG3 is a class B VSG that is highly structurally divergent (Pinger et al., 2018), suggesting that TLF2-IgMs may have some structural specificity, though this will require further investigation. Taken together, these data reveal that the polyclonal IgM component of TLF2, as it is present in the plasma of uninfected humans, can interact with VSG and other trypanosome proteins. Various kinetic experiments investigating the rate at which parasites are lysed by TLFs strongly suggest that TLF2 uptake occurs more rapidly than fluid phase endocytosis, despite the lack of an identified receptor (Vanhollebeke et al., 2007). We hypothesize that TLF2-bound VSG is internalized by the molecular sail mechanism by which antibody-bound VSGs are rapidly endocytosed (Engstler et al., 2007).

TLF2-IgM production increases substantially upon trypanosome infection in wildtype mice—Given that human TLF2-IgMs interact with trypanosome antigens, we hypothesized that trypanosome infection could increase the production of IgMs that could interact with TLF2 in wildtype mice. Swiss Webster and A/J mice were infected with *T. b. brucei* for 4 days prior to treatment with diminazene aceturate to cure

the infection. We then used HGD to express the TLF genes in the mice 12 days post-infection and analyzed the plasma by size exclusion chromatography at 14 days post infection (two days post-HGD with *HPR* and *APOL1*), during the peak of the IgM response to trypanosomes (Baral et al., 2007). Sizing the plasma revealed that the APOL1 protein migrated exclusively with the bulk HDL and did not shift the migration pattern of APOA-I in uninfected mice (Fig. 4*A*), while a significant portion of the APOL1 shifted to the fractions containing larger complexes in the infected and cured mice and co-migrated with bulk IgM (Fig. 4*A*). To further investigate whether the IgM elicited by trypanosome infection in mice is TLF2-IgM, we used plasma from another trypanosome-infected mouse to screen for potential infection-derived murine IgM interactions with human TLF proteins. Trypanosome infection-derived IgMs interacted with human HPR and another unidentified murine HDL protein (Fig. 4*B*, panel c), while IgM from uninfected mice does not recognize any HDL or TLF proteins (Fig. 4*B*, panel b). This may indicate that trypanosome infection increases the circulating TLF2-IgM pool by increasing the circulating amount of IgMs that can interact with the TLF protein HPR. As trypanosome infection has been shown to elicit a strong IgM response in mice (25), it is possible that a portion of these represent a unique population of TLF2-IgMs capable of being recruited into TLF2 complexes.

Trypanosome infection increases TLF2-IgM production in sleeping sickness patients—The production of TLF2-IgMs in mice was likely due to an increased concentration of circulating germline IgMs that can interact with TLF. This suggests that there is a threshold of (or binding quality of) germline IgM concentration that is required in order to form detectable quantities of TLF2. In humans, TLF2-IgMs are present regardless of infection status. We investigated whether trypanosome infection, akin to the mice, would also increase the concentration of circulating TLF2-IgMs in sleeping sickness patients. We found that trypanosome infection led to a roughly 50-fold increase in circulating total IgM concentration (Fig. 5*A*). We then analyzed the ratio of TLF2-associated APOL1 to TLF1-associated APOL1 and found that the ratio was substantially shifted towards TLF2 in infected individuals, while uninfected African or American control samples contained >95% TLF1 (Fig. 5*B*, Fig. S6). This suggests that, while only a small fraction of the circulating IgMs in healthy individuals are TLF2-IgMs, trypanosome infection increases that fraction by increasing the abundance of polyclonal trypanosome-reactive IgMs.

One hallmark of trypanosome infection is an increase in circulating type II interferons (Wu et al., 2017), which we confirmed in the study of this cohort of patient samples (Fig. 5*C*). Type II interferon signaling is known to upregulate *APOL1* mRNA expression in cell culture (Zhaorigetu et al., 2008), and human *T. b. gambiense* infection stimulates *APOL1* RNA upregulation (Ilboudo et al., 2012), suggesting that total APOL1 and thus TLF levels increase during trypanosome infection. This was demonstrably not the case, as we instead found that trypanosome infection did not significantly change the circulating APOL1 concentration relative to uninfected controls (Fig. 5*D*). The regulation of circulating APOL1 concentration is likely multifactorial, and therefore we investigated whether the total amount of HDL was affected by trypanosome infection, as it is reduced during many other infections (Feingold and Grunfeld, 2000). Indeed, the circulating HDL concentration (APOA-I protein) was reduced 5 to 6-fold in infected individuals relative to healthy controls (Fig 5*E*). These data reveal that trypanosome infection does not change the total amount of circulating TLF, defined by the total amount of circulating APOL1, but that it leads to a redistribution of the available APOL1 into TLF2 complexes by increasing the circulating levels of TLF2-IgMs.

Taking all of these data together, we interpret that TLF1 and IgM exist in equilibrium with TLF2 *in vivo*, and that the circulating concentration of TLF2 is entirely dependent on the amount of circulating TLF2-IgMs at any given time. Consistent with this hypothesis, purified human TLF2 samples reproducibly contain TLF1 (Fig. S1), likely due to a depletion of TLF2-IgMs from the sample during the stringent purification strategy. These data reveal that the interaction between germline IgMs and TLF1 is a low-affinity interaction that forms TLF2, and that this interaction likely plays a significant role in trypanosome immunity.

Discussion

Both TLF1 and TLF2 are trypanolytic protein complexes that harbor most of the same components, with the exception of the IgMs associated with TLF2 (Raper et al., 1999). Here, we have

investigated the properties of the TLF2-IgMs and the nature of their association with TLF. We hypothesize that TLF1 and TLF2 are the same protein complex that is IgM-free as TLF1 and IgM-associated as TLF2, and that TLF and IgM exist in equilibrium. The TLF-associated IgMs are likely germline antibodies, given that all tested humans have detectable levels of TLF2 (Fig. 5*B*) and AID$^{-/-}$ mice, which express high levels of germline IgM, produce TLF2-IgMs (Fig. 1 and 2). This hypothesis also stipulates that while the circulating concentration of TLF2-IgMs is high enough to mediate TLF2 formation in trypanosome-naïve humans, an increase in the production of TLF2-IgMs would subsequently lead to an increase in the circulating levels of TLF2. We have indeed observed that trypanosome infected mice (Fig. 4*A*) and humans (Fig. 5*B*) produce more TLF2 than their uninfected counterparts. In the context of a trypanosome infection, it is also possible that some of the antigen adapted IgMs produced by somatically hypermutated B cells could associate with TLF to form TLF2. However, we hypothesize that a significant portion of the TLF-IgM pool is of germline origin.

The data presented here indicate that TLF2-IgMs interact with the HPR component of TLF1 in order to form TLF2 (Fig. 2). HPR is a glycoprotein bound to HDL complexes via a retained signal peptide. This directed association ensures that the receptor binding face of HPR is consistently exposed to the external environment, thereby increasing the probability of binding the trypanosome HPHB receptor resulting in receptor-mediated uptake. TLF2 is taken up by trypanosomes independently of the HPHBR. We propose that the source of TLF2's HPHBR independence is due to the 950 kDa IgM antibody interacting with HPR and therefore blocking the interaction with the receptor. This HPR-IgM interaction is non-covalent and of low affinity, such that the IgM may dissociate at any time and reveal the receptor-binding domain, permitting HPHBR-mediated uptake. Bullard *et al.* found that ten thousand-fold more TLF1 was required to lyse parasites with decreased HPHBR expression as compared to wild type *T. b. brucei*, while only 100 fold more TLF2 was required (Bullard et al., 2012), suggesting that TLF2 uptake is partially dependent on the HPHBR. However, this model of TLF-IgM equilibrium may suggest that a fraction of the TLF in those purified TLF2 samples had disassociated from IgM, thereby forming TLF1, leading to HPHBR-mediated uptake. This is supported by our observation that purified TLF2 samples invariably contain free "dissociated" TLF1 (Fig. S1).

TLF2-IgMs are particularly promiscuous antibodies that interact with many self (Fig. S3 and Fig. 2) and non-self antigens (Fig. 3*B* and *C*). This remarkable polyreactivity is a classic characteristic of germline IgM antibodies. We hypothesize that the TLF2-specific trypanosome uptake pathway may involve this polyreactive property of TLF2-IgMs, whereby the decavalent IgMs could interact with both the TLF and the surface of the parasite simultaneously. Trypanosomes endocytose antibody-bound VSGs within 2 minutes of antibody binding in order to evade antibody-mediated immunity (Engstler et al., 2007). We therefore hypothesize that TLF2-bound VSG would lead to TLF-associated APOL1 uptake by this same mechanism. A TLF2 uptake mechanism that depends on anti-VSG germline antibodies is consistent with work from Mueller *et al*., who revealed that auto-reactive IgMs in humans and other mammals recognize VSG proteins from various trypanosome isolates (Muller et al., 1996). In fact, some of the murine antibodies analyzed by Mueller *et al*. were derived from germ-free mice, thereby formally revealing that foreign antigen stimulus-independent systems produce anti-VSG IgMs. VSG is not the sole potential TLF2-IgM target epitope, as TLF2-IgMs can interact with a wide range of trypanosome antigens (Fig. 3*B*). However, the fact that VSG exists in a roughly 10,000-fold molar excess relative to all other trypanosome surface proteins leads us to hypothesize that it is the most relevant surface protein with regard to TLF2 uptake. Under this assumption, it is possible that trypanosome infection-elicited TLF2-IgMs may afford TLFs with adaptive-like targeting potential by preferentially recognizing particular infection-specific VSG classes or variants.

Having two non-redundant mechanisms of APOL1 delivery to invading trypanosomes provides an immunological advantage. In normal human serum, while TLF2 uptake is unabated, TLF1-mediated APOL1 uptake is effectively inhibited by the physiological levels of circulating HP (Raper et al., 1996). HP is an acute phase response protein that prevents HB-induced toxicity and is induced by inflammatory cytokines (Levy et al., 2010). Therefore, in both the acute phase and healthy states, TLF2-mediated trypanosome immunity likely predominates. However, TLF1-mediated trypanosome immunity may play

a more prominent role when HP levels are low, such as in the case of chronic malaria infection. Malaria infection is associated with sharply decreased plasma HP levels as a result of cyclical hemolysis and subsequent HBHP complex clearance by macrophages, reviewed here (Rogerson, 2006). Human co-infection with malaria in the context of trypanosome infection is common, as trypanosome endemic regions typically overlap with the distribution of malaria in Sub-Saharan Africa (Onkoba et al., 2015). The estimated 200 million annual cases of malaria could select for an HPHBR-mediated trypanocidal effect in co-infected individuals. To clarify this explicitly, we hypothesize that TLF2 is the main lytic component of normal human serum and that the entry of TLF2 into the parasite is dependent on low-affinity interactions between the TLF2-IgMs and VSG, but that under conditions when circulating HP levels are low, TLF1 uptake will predominate.

Human-infective trypanosomes are resistant to TLF-mediated lysis. *T. b. rhodesiense* parasites express serum resistance associated protein (SRA) that binds to and inhibits the activity of human APOL1 (Vanhamme et al., 2003), while *T. b. gambiense* uses a combinatorial APOL1 resistance mechanism that is dependent on reduced APOL1 uptake via a mutated HPHBR and the Trypanosoma gambiense-specific glycoprotein (TgsGP), which is associated with the inhibition of APOL1 activity (Uzureau et al., 2013). However, human population studies (Cooper et al., 2017) and *in vivo* experiments using transgenic mice (Thomson et al., 2014) have revealed that the relationship between trypanosomes and human APOL1 is complicated. Human variants of APOL1 called G1 and G2 provide increased resistance to human-infective trypanosomes. The G2 variant of APOL1 has a decreased affinity for the SRA protein from *T. b. rhodesiense* (Thomson et al., 2014), and humans harboring the G2 allele are 5-fold less likely to be infected with *T. b. rhodesiense* parasites (Cooper et al., 2017). The G1 variant is associated with a 3-fold decreased probability of developing clinical symptoms during *T. b. gambiense* infection (Cooper et al., 2017), though the mechanism has not been characterized. Importantly, studies have revealed that human TLF can lyse *T. b. gambiense* parasites when TLF1-associated APOL1 uptake is increased by expressing the *T. b. brucei* HPHBR in the absence of HP (Uzureau et al., 2013). However, HP levels are elevated during trypanosome infections (Ngure et al., 1997), which prevents TLF1-mediated lytic activity (Raper et al., 1996). Thus, in individuals infected with African trypanosomes, we hypothesize that TLF2 would be the predominant lytic factor that prevents or controls the infection, and indeed we have observed that *T. b. rhodesiense* infected humans have markedly increased levels of circulating TLF2 relative to TLF1 (Fig. 5*B*). We interpret these data as a shift in the TLF1/TLF2 equilibrium towards TLF2 due to an increase in the production of IgM antibodies in response to the trypanosome infection. Taken together, these data suggest that the balance between trypanosome immunity, control, and active infection is determined by the host's APOL1 genotype and both the rate and ultimately the functional amount of APOL1 taken up by the parasites. We maintain that the field to this day does not have a full understanding of the structure and function of TLFs. Continued investigation of how these HDL complexes and their associated antibodies impact trypanosome immunity is crucial to understanding the relationship between trypanosomes and their primate hosts.

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Declaration of interests:
The authors declare no competing interests.

Figures legends

Figure 1. Co-Expression of Human *APOL1* and *HPR* Results in TLF2 Formation in AID$^{-/-}$ Mice. A. Schematic representation of the vector used to transiently induce TLF gene expression in AID$^{-/-}$ mice. The ubiquitin promoter (pink arrow) drives expression of both mammalian transgenes (HuAPOL1: green, HuHPR: orange) in order to induce gene expression in any cell type. A beta-globin intron (purple) and an SV40 polyadenylation signal (teal) flank all transgenes to increase mRNA stability. Beta-lactamase (yellow) is used for antibiotic selection during bacterial propagation in *E. coli* via an origin of replication (peach circle). B. Pooled plasma was obtained from two mice that were transiently expressing *APOL1* and *HPR*. The plasma (0.75 ml) was then separated by size on a Superose 6 size exclusion column (1.5 by 60 cm), and the fractions were analyzed by western blot. The earliest eluting TLF-containing fractions (≥1000 kDa) that coeluted with IgM were designated TLF2, while the TLF-containing fractions eluting after mL 69 were designated TLF1 (≤500 kDa). C. Anti-APOL1 western blot of pooled ml 67 and 67.5 (TLF2) from A, transfected murine plasma (TLF1 and TLF2), and normal human plasma (TLF1 and TLF2) after separation by native agarose gel electrophoresis. Lane 2 contains Ferritin and apoferrtin (Pharmacia) that migrates the same distance as human bulk HDL (denoted by **) that was used as a reference to compare relative migration distances across gels. A component of the marker interacts non-specifically with the secondary antibody at (*). D. Western blot analysis (IgM and APOL1) of a co-immunoprecipitation using an antibody recognizing human HPR (Sigma H6395) of pooled ml 67 and 67.5 from A.

Figure 2. TLF2-IgMs Interact with HPR. A. Schematic representation of the vector used to transiently induce *HPR* expression in AID$^{-/-}$ mice. The labeling is analogous to the labeling in Fig 1*A*. B. Western blots of size fractionated human *HPR* expressing AID$^{-/-}$ murine plasma (Superose 6). Milliliters containing IgM-HPR-HDL complexes are marked by 'immunocomplex' and milliliters containing HPR-HDL that does not co-migrate with IgM are marked by 'HPR-HDL.' The HPR western blot was transferred from a non-reduced PAG which efficiently separates HPR (45 kDa) from HP (90 kDa). The IgM and APOA-I were transferred from a reduced PAG to follow the IgM heavy chain (63 kDa) and APOA-I (28 kDa) (HP and HPR beta chains co-migrate on reducing gels). C. Western blot analysis (IgM) of a co-immunoprecipitation of pooled milliliters 66 and 66.5 from A using an antibody recognizing human HPR (Sigma H6395). D. Schematic representation of the vector used to transiently induce *APOL1* expression in AID$^{-/-}$ mice. The labeling is analogous to the labeling in Fig 1*A*. E. Western blots of size fractionated human *APOL1* expressing AID$^{-/-}$ murine plasma (Superose 6). F. Western blot of size fractionated untransfected AID$^{-/-}$ murine plasma (Superose 6). G. Far western blot using TLF2 as the bait protein to detect prey proteins in murine plasma, murine HDL, murine HDL after transfection with human *HPR*, and murine HDL after transfection with human *APOL1*. The band in lane 3 at ~45 kDa likely corresponds to human HPR as denoted by the 'huHPR' caption between the 35 and 55 kDa MW markers. H. Far western blot showing the interactions between TLF2-IgMs (bait) and prey proteins in human plasma, bovine plasma, murine plasma, murine HDL after mock transfection with saline, murine HDL after transfection with human *HPR*, and purified human TLF1. Four identical membranes were prepared and probed as indicated. Panel 'a' was probed with an anti-HP antibody that recognizes both HP (high molecular weight bands) and HPR (~45 kDa). Panel 'b' was probed with a secondary antibody that recognizes human IgM including the IgM component of TLF2. Panel 'c' was probed with human IgM, followed by the secondary antibody that recognizes human IgM. Panel 'd' was probed with purified human TLF2, followed by the secondary antibody that recognizes human IgM. Panel 'c' was probed with

pooled human IgM at a concentration equimolar to that which was used in panel 'd'. The arrowhead marks the 45 kDa human HPR protein, present in lanes 1, 5, and 6 of panels 'a' and 'd'.

Figure 3. TLF2-IgMs Purified from Human Plasma Interact with Trypanosome Proteins. A. Native dot blot using TLF2-IgMs as bait and non-denatured trypanosome lysates, human IgM, or human HP as prey. B. Far western blot using TLF2-IgMs as bait and an SDS-PAGE denatured trypanosome lysate preparation as prey. C. Native dot blot using TLF2-IgMs as bait and murine HDL after mock transfection with saline, murine HDL after transfection with human *HPR*, murine HDL after transfection with human *APOL1*, VSG2, 3, and 13 preparations, or non-denatured trypanosome lysate as prey. We estimate that by this technique, TLF2-IgMs have an approximately 5-6-fold higher affinity for VSG3 as compared to VSGs 2 or 13.

Figure 4. TLF2-IgM Production Increases Substantially Upon Trypanosome Infection in Wildtype Mice. A. Anti-APOL1 western blots of size fractionated (Superdex 200 10/300) murine plasma samples expressing human *APOL1* and *HPR*. Top panel: A/J mouse infected with and cured of *T. b. brucei*. Middle panel: Swiss Webster mouse infected with and cured of *T. b. brucei*. Bottom panel: Uninfected Swiss Webster mouse. B. Western blot showing the interaction between murine IgMs and SDS PAGE separated proteins from human plasma, bovine plasma, murine plasma after mock transfection with saline, murine HDL after mock transfection with saline, and murine HDL after transfection with human *HPR*. Samples were probed with non-specific murine IgM (a), healthy murine plasma (b), and murine plasma from a *T. b. brucei* AnTat 1.1-infected Swiss-Webster mouse (c). The amount of non-specific IgM used in 'a' was at an equimolar concentration relative to panel 'c' as determined by ELISA. The bands present in lane 1 cannot be identified by this technique. The bands present in lane 3 of each blot are likely murine IgM. The bands marked by two arrowheads below HPR (marked by one arrowhead) in lanes 4 and 5 could correspond to murine APOA-I based on the molecular weight of the protein, although this is speculation.

Figure 5. Trypanosome Infection Increases TLF2-IgM Production in Sleeping Sickness Patients. A-E. Samples were taken from patients infected with *T. b. rhodesiense* before or after treatment with suramin or melarsoprol. A. Plasma IgM levels determined by ELISA (Invitrogen) in sleeping sickness patients compared to control samples from healthy donors (error bars indicate the median and IQR). B. Determination of the ratio of TLF2-associated APOL1 to TLF1-associated APOL1 by western blot following native agarose gel electrophoresis in sleeping sickness patients compared to control samples from healthy donors (error bars indicate the median and IQR). C. Plasma IFN-gamma levels determined by ELISA in sleeping sickness patients compared to control samples from healthy donors (error bars indicate the median and IQR). The American group did not exist at the time that this analysis was performed and are thus not included (*see* Method details). D. Plasma APOL1 levels determined by western blot in sleeping sickness patients compared to control samples from healthy donors (error bars indicate the median and IQR). E. Plasma APOA-I levels determined by western blot in sleeping sickness patients compared to control samples from healthy donors (error bars indicate the median and IQR).

Table 1. Details of Patients and Controls. Staging criteria and treatment regimens were fully described by MacLean et al, 2010. By definition, early cases are in the hemolymphatic stage of infection, while late cases are in the meningoencephalitic stage.

STAR Methods

RESOURCE AVAILABILITY

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jayne Raper (raper@genectr.hunter.cuny.edu).

Materials Availability

This study did not generate any unique distributable reagents.

Data and Code Availability

This study did not generate any digitally accessible data or code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice—To generate TLFs in mice, we transiently expressed *APOL1* and *HPR* by HGD. The genes of interest were cloned into the pRG977 (Molina-Portela et al., 2008) mammalian expression vector (Fig. 1*A*, 2*A*, and 2*D*). Each gene of interest is flanked by an intron (5' with respect to the coding sequence) and a polyadenylation signal (3' with respect to the coding sequence) to stabilize the resulting RNA transcripts. The expression of each gene is driven by a mammalian ubiquitin promoter, which functions in any murine cell type. Each plasmid contains a beta-lactamase gene for antibiotic selection and an origin of replication for propagation in *E. coli*. Plasmids are isolated from bacteria such that all contaminating endotoxin is removed (Qiagen). Mice were transfected by HGD as described in (Kovacsics and Raper, 2014). Briefly, 25 μg of plasmid DNA diluted in approximately 2 to 3 milliliters of saline is then injected directly into the tail vein of an adult mouse (amount of saline is 10% of the weight of the mouse in vol/g). The majority of gene expression in mice receiving plasmids encoding TLF genes by tail vein injection is observed in hepatocytes.

To generate TLF2 in mice that had increased levels of germline IgMs, we used male and female *AID* knockout mice (8-12 weeks old). To generate TLF2 in mice that had been previously infected with trypanosomes, we used female A/J mice (6-12 weeks old) and outbred female Swiss Webster mice (6-12 weeks old). Trypanosome infections were carried out by infecting on day 0 with 5000 monomorphic *T. b. brucei* Lister-427 parasites intraperitoneally followed by treatment with 200 ul of 1.25 mg/ml diminazene aceturate on day 4 post-infection. Mice were confirmed parasite free by blood smear on days 5, 6, and 7. For the generation of (anti-trypanosome IgMs) antibodies used in Far western blots, mice were infected with 5000 pleomorphic AnTat 1.1 parasites intraperitoneally and monitored for 12 days before plasma harvest.

All animal experiments were approved by the Institutional Animal Care and Use Committee at Hunter College, which both have currently approved Animal Welfare Assurance Agreements with the National Institutes of Health Office for Protection from Research Risks. 1-4 mice were housed per cage, and the mice were maintained on a 12-hour light cycle.

Cell lines—The trypanosomes used in the *in vivo* experiments in this study were the Lister-427 and AnTat 1.1 strains of *Trypanosoma brucei brucei*. The Lister-427 line was also grown in vitro using HMI-9 media supplemented with 10% FBS, 10% Serum Plus, and penicillin/streptomycin at 37°C with 5% CO_2. The human cell lines used in this study were HepG2 cells (ATCC HB-8065) and NTera-2 cells (ATCC CRL-1973). The human cells were grown in DMEM supplemented with 10% FBS.

Human plasma—Human African trypanosomiasis patient and control plasma samples were a randomly selected subset of those previously described in (Maclean et al., 2007). Subjects or their guardians signed consent forms after receiving standard information in their local language. Protocols were approved by the Grampian Research Ethics Committee (Aberdeen) and the Ministry of Health (Uganda). Patient demographics and details of treatment are summarised in Table 1. "Before treatment" samples were taken on admission prior to commencement of treatment. "After treatment" samples were taken as part of the diagnostic confirmation of cure 6 weeks after admission. Staging criteria and treatment regimens were fully described previously in (MacLean et al., 2010).

METHOD DETAILS

Experimental design—All experiments were performed without blinding or randomization. All murine-based TLF2 generation experiments were repeated at least once, with representative experiments shown throughout the manuscript. All Far western blots were repeated extensively with reproducible results. All statistical analyses are detailed in "quantification and statistical analysis."

TLF2 detection in $AID^{-/-}$ mice after HGD—Mice were exsanguinated using 200 μl of heparin (Abraxis) per mouse 24 hours after HGD (2 mice per transgenic group; 1 mouse with saline control) and the isolated plasma was pooled (800 μl per group; 400 μl for saline group) for immediate size fractionation on a Superose 6 column (1.5 by 60 cm) via Fast Performance Liquid Chromatography

(FPLC) (V_0: 34 ml, flow rate: 0.4 ml/min, fraction size: 500 µl). Collected fractions were diluted in SDS-PAGE loading buffer and run on 10% tris-glycine gels. Where shown, IgM, APOA-I, and APOL1 were visualized on the same membrane for all groups, while HPR was visualized from a separate membrane. The elution volumes are used in the labeling of the blots, rather than arbitrary fraction numbers, for the purposes of promoting future reproducibility. Fractions containing TLF2 were concentrated to a 500 µl volume (Centricon – Fisher Scientific) and subjected to co-immunoprecipitation (Co-IP) (APOL1/HPR dual expression: fractions 67 ml and 67.5 ml, HuHPR expression: fractions 65.5 ml – 67.5 ml). Co-immunoprecipitation was performed using magnetic protein A/G beads (Thermo-Pierce). Briefly, antibodies were cross-linked to beads using disuccinimidyl suberate, which were then incubated with protein samples for either 1 hour at room temperature or overnight at 4°C. Beads were then washed 5X with tris-buffered saline (TBS) (pH 6.7 in order to increase the stringency of the wash steps and prevent non-specific interactions between the TLFs and the beads) before being boiled in SDS-PAGE gel loading buffer. Antibodies used for Co-IP: Anti-Hu HP mouse monoclonal (Sigma H6396), Mouse IgG1 (Invitrogen 02-6100. All membranes for western blots were blocked using 5% milk in TBS with 0.05% Tween-20.

TLF2 detection in infected and cured mice after HGD—Swiss Webster and A/J mice were infected on day 0 with 5000 monomorphic *T. b. brucei* Lister-427 parasites I.P. followed by treatment with diminazene aceturate on day 4 post-infection. Mice were transiently transfected with a vector encoding *APOL1* and *HPR* by HGD on day 12 post infection and were subsequently exsanguinated using 200 µl of heparin (Abraxis) per mouse on day 14 post infection. 400 µl (to maximize APOL1 yield and achieve detection on western blots) of plasma was immediately loaded onto a Superdex 200 10/300 column and fractionated via FPLC (V0: 7 ml, flow rate 1 ml/min, fraction size: 500 µl). Collected fractions were diluted in SDS-PAGE loading buffer and run on 10% tris-glycine gels.

Purification of human TLF2 and murine HDL—TLF2 was purified as described previously with modifications (Raper et al., 1999). Peripheral venous blood from healthy donors in New York (United States) was obtained. Approval was obtained from the institutional review board (IRB) at New York University School of Medicine. Briefly, the density of the isolated plasma (100 ml, HP haplotype 1-1) was adjusted to 1.25 g/ml using KBr. The plasma was then ultracentrifuged at 49,000 RPM, 10°C, 16 hours. The bottom 50% of the resulting density gradient was collected and dialyzed using three changes of TBS-2.5 mM EDTA at 4°C. The dialyzed product was concentrated (Centricon – Fisher Scientific) to roughly 40 ml and sized (5 ml per run) on a Superose 6 column (1.5 by 60 cm, V_0: 34 ml, flow rate: 1.5 ml/min, fraction size: 1.5 ml). Fractions in the TLF2 range were pooled, concentrated to 10 ml, and immunoaffinity purified using a mouse monoclonal antibody raised against human HP that recognizes human HPR (Sigma H6396). The antibody was coupled via amino groups to a HiTrap *N*-hydroxysuccinimide column as per the manufacturer's instructions (GE Healthcare Bio-Sciences). The pooled TLF2 was then loaded onto the column and allowed to bind for 10 minutes, followed by a wash with five column volumes of TBS. The bound TLF2 was then eluted with a 100 mM glycine-150 mM NaCl solution at pH 2.8. Eluted fractions were immediately neutralized with 1.5 M Tris-HCL (pH 8), pooled, and concentrated. The affinity-purified sample was reduced in SDS-PAGE loading buffer containing β-mercaptoethanol and run on 10% polyacrylamide gels for silver staining or transferred to PVDF membranes for immunoblotting. The quality of the purified TLFs is shown in supplementary figure 2.

Murine HDL was prepared using a simplified protocol. One day post-plasmid HGD, mice were sacrificed and exsanguinated. The density of the collected plasma was adjusted to 1.25 g/ml with KBr in 12 ml of TBS and the samples were ultracentrifuged as described above (49,000 RPM, 10°C, 16 hours). At this point, the top 33% of the density gradient was collected, dialyzed into TBS, and concentrated. There is no need to separate the HDL from other lipoproteins by density, as the concentration of contaminating LDL is negligible in mice and can be efficiently removed by size fractionation.

24-hour Trypanosome in vitro lysis assay—Cultured *T. b. brucei* Lister-427 parasites were diluted to 5×10^5 ml^{-1} in HMI-9 medium with 10% fetal calf serum (Laboratory Disposable Products) and 10% Serum Plus (SAFC Biosciences) and 100 µl was added to each well of an opaque 96 well plate. Parasites

were then diluted 1:1 with serially diluted concentrations of purified human TLF2. After 20 hours of incubation at 37°C, 20 µl of alamarBlue (Invitrogen) was added to each well. The assay involves the reduction of resazurin by metabolically active cells to the fluorescent resorufin, allowing quantitation of non-lysed cells at 1-4 hours post-reagent addition. Where indicated, the trypanosomes were pre-treated for 30 minutes with 10 mM ammonium chloride (which remained present throughout the assay), a weak base that inhibits APOL1-mediated lysis of trypanosomes by neutralizing the endocytic compartments of the organism.

Native agarose gel electrophoresis—0.7% SeaKem agarose (Lonza) was prepared in a running buffer composed of 90 mM Tris and 90 mM borate at pH 8.5. A pigmented Ferritin marker (Pharmacia) that migrates the same distance as the bulk of human HDL was used to determine when to stop gel electrophoresis. Protein samples were prepared via 1:1 dilution in 15% Ficoll 400 buffered with Tris-HCl. A total of 10 µl of concentrated (10 fold) murine TLF2 fractions or 1.5 µl of whole human or murine plasma was loaded into the gels and run at a constant voltage of 100V for 2 hours. Proteins were transferred to PVDF membranes for western blotting via capillary transfer overnight.

Far western blotting—Protein-protein interactions were analyzed by Far western blotting, reviewed here (Wu et al., 2007). SDS-PAGE gels were used to separate proteins by size, which were then transferred to PVDF membranes. Protein samples were reduced with 100 mM β-mercaptoethanol where indicated. Membranes were blocked using 5% BSA in TBS with 0.05% Tween-20. Where indicated, membranes were then probed using 20 µg/ml of purified human TLF2, or an equimolar amount of pooled human IgM (Sigma, Cat: I8260), in the presence of 5% BSA in TBS with 0.05% Tween-20 for 1 hour at room temperature. This was followed by three washes (ten minutes each) in TBS with 0.05% Tween-20. Membranes were visualized using an anti-Hu IgM polyclonal antibody conjugated to HRP, meaning that any detectable bands would be resolved by the secondary antibody binding to the TLF2-IgM at that particular site. The Far western blot protocol was performed analogously for the dot blot (blot overlay assay) experiments with the following exception: Proteins were not denatured in SDS loading buffer in order to further facilitate the maintenance of native protein structure. Protein samples (2 µl) were applied directly to a nitrocellulose membrane and allowed to dry for ~15 minutes prior to probing.

Quantifications of plasma proteins in humans—To quantify IgM, plasma samples were diluted between 10,000 and 100,000-fold prior to being screened in a commercially purchased sandwich ELISA (Invitrogen, biological limit of detection: 15.6 ng/ml).

Plasma interferon (IFN) gamma concentrations were measured using ELISA (BD OptEIA, BD Biosciences, Oxford, UK, biological limit of detection: 1.8 pg/ml). Note that the "before treatment" data are a subset of values previously published in (Maclean et al., 2007) but are included for clarity and for comparison with "after treatment" data. Specifically, the data is reproduced in part from Fig 2a. Lorna MacLean et al. Spatially and Genetically Distinct African Trypanosome Virulence Variants Defined by Host Interferon-γ Response. *The Journal of Infectious Diseases* (2007) 196 (11): 1620-1628. By permission of Oxford University Press on behalf of the Infectious Diseases Society of America, available at: https://academic.oup.com/jid/article/196/11/1620/1992925?searchresult=1.

To quantify APOL1, 0.375 ul of each plasma sample was electrophoresed on 10% polyacrylamide gels alongside a serially diluted sample of purified recombinant APOL1 at a known concentration. The gels were blotted onto PVDF membranes for western blotting and pixel density quantification studies via FIJI (Schindelin et al., 2012). The levels of APOA-I were measured by the same method except that only 10-20 nl of each plasma sample was electrophoresed alongside a serially diluted sample of recombinant APOA-I (Peprotech 350-11).

The ratio of TLF2:TLF1 was calculated by western blot and pixel density quantification via FIJI (Schindelin et al., 2012) after native agarose gel electrophoresis. 1.5 ul of each plasma sample was electrophoresed for 2 hours in 0.7% agarose and blotted onto PVDF membranes. The membranes were probed for the presence of APOL1 which was used to indicate how much of each TLF species was present in each sample. The ratio was determined by comparing the pixel density of the signal corresponding to TLF2-associated APOL1 to TLF1-associated APOL1.

QUANTIFICATION AND STATISTICAL ANALYSIS

Pixel density quantifications were carried out using FIJI (Schindelin et al., 2012). Graphical and statistical analyses were carried out in GraphPad Prism version 7 (GraphPad Software, La Jolla, California, USA). Statistical analyses were only applied to the data in figure 5. Each data set was initially analyzed by the Kruskal-Wallis test to determine if statistically significant differences existed. If so, we applied individual Mann-Whitney tests to compared differences between groups before performing Bonferroni corrections: ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-human APOL1 (1:10,000)	ProteinTech Group	Cat#16139-1-AP
Anti-human HP/R (1:10,000)	Sigma Aldrich	Cat#H8636
Anti-mouse APOA-I (1:10,000)	Abcam	Cat#Ab20453
Anti-human APOA-I (1:5000)	Novus Biologicals	Cat#NB400-147
Anti-mouse IgM (1:10,000)	Jackson Immuno Research Labs	Cat#115-036-075
Anti-human IgM (1:5000)	Jackson Immuno Research Labs	Cat#09-035-129
Anti-rabbit TrueBlot (1:10,000)	Rockland Antibodies	Cat#18-8813-33
Pooled human IgM	Sigma Aldrich	Cat#I8260
Pooled mouse IgM	Sigma Aldrich	Cat#PP50
Biological Samples		
Ugandan human plasma	See table 1	N/A
American human plasma	New York University Medical Center	N/A
Chemicals, Peptides, and Recombinant Proteins		
Sodium pyruvate	Thermo Fisher	Cat#MT-25-000-CI
Bathocuprione di-sulfonic acid	Thermo Fisher	Cat#AC164060010
Penicillin/Streptomycin	Thermo Fisher	Cat#15140122
L-cysteine	Sigma Aldrich	Cat#C7352
Hypoxanthine	Sigma Aldrich	Cat#H9636
Thymidine	Sigma Aldrich	Cat#T1895
2-mercaptoethanol	Invitrogen	Cat#21985-023
Fetal bovine serum	Gemini Bio-Products	Cat#S01520; Lot#A95E82G
Serum plus	Sigma Aldrich	Cat#14008C
Iscoves modified DMEM	Laboratory Disposable Products	Cat#10-016-CV
DMEM	Laboratory Disposable Products	Cat#10-017-CM
Diminazene aceturate	Sigma Aldrich	Cat#D7770
Sterile saline	Thermo Fisher	Cat#NC9054335
Experimental Models: Cell lines		
Human HepG2 cells	ATCC	Cat#HB-8065
Human NTera-2 cells	ATCC	Cat#CRL-1973
Trypanosoma brucei Lister-427	Gift from George Cross	N/A
Trypanosoma brucei AnTat 1.1	Gift from George Cross	N/A
Experimental Models: Organisms/Strains		
Mouse A/J	Jackson Laboratories	Cat#000646
Mouse Swiss Webster	Taconic Biosciences	SW Murine Pathogen Free
Mouse AID knockout	Jackson Laboratories	Cat#007770
Recombinant DNA		

pRG977-APOL1/HPR	Molina-Portela *et al*., 2008 – Vector backbone originally from Regeneron Pharmaceuticals	N/A
pRG977-HPR	Molina-Portela *et al*., 2008 – Vector backbone originally from Regeneron Pharmaceuticals	N/A
pRG977-APOL1	Molina-Portela *et al*., 2008 – Vector backbone originally from Regeneron Pharmaceuticals	N/A
Software and Algorithms		
GraphPad Prism	GraphPad software	https://www.graphpad.com/scientific-software/prism/
FIJI	Schindelin *et al.*, 2012	N/A

Table 1

	Patients (n=21)	Controls (n=19)
Male:Female	10:11	9:10
Median Age (range)	25 (6-65)	35 (8-59)
Early:Late stage	4:17	n/a

Figure 1

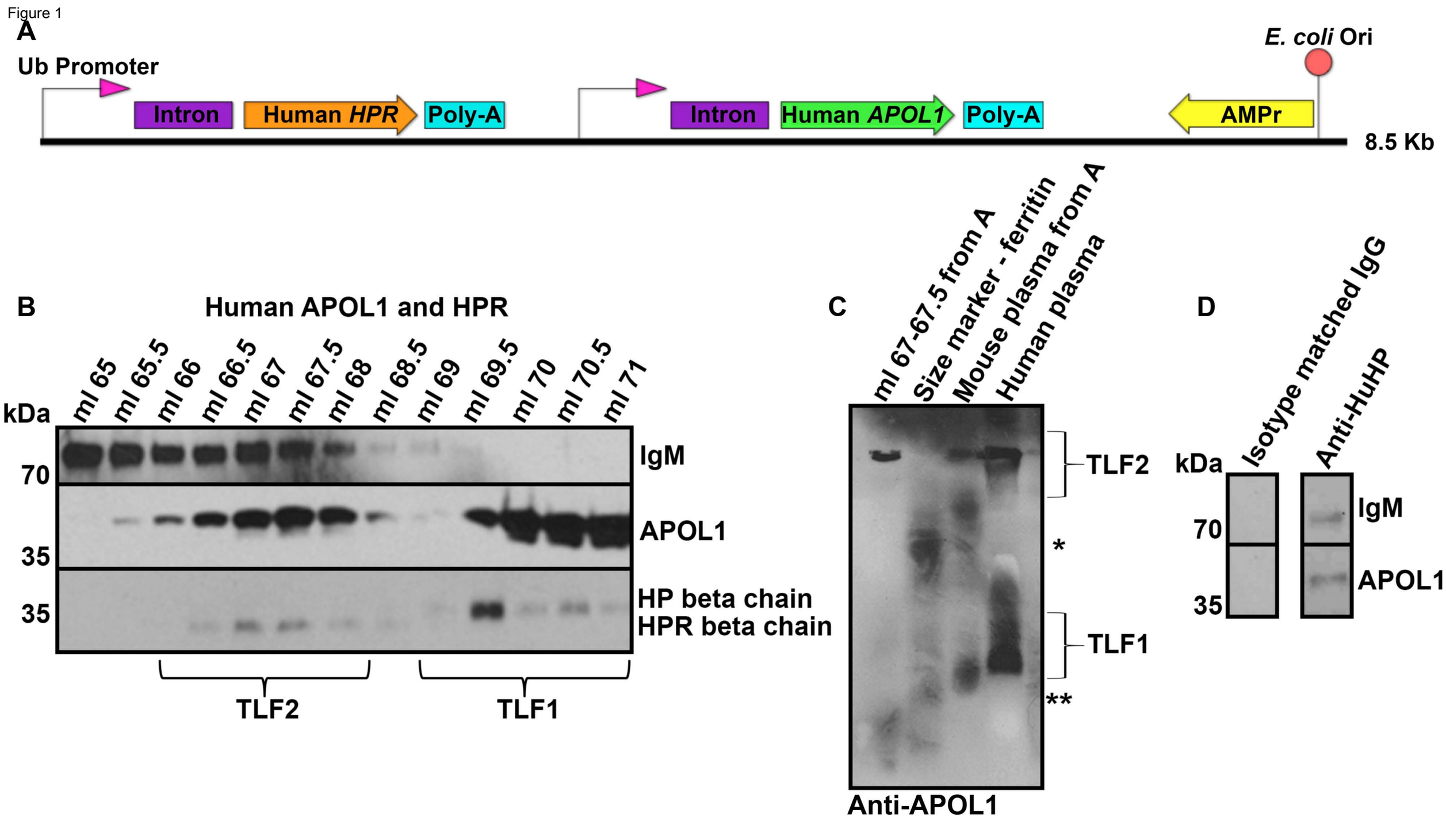

A
Ub Promoter
Intron
Human HPR
Poly-A
Intron
Human APOL1
Poly-A
AMPr
E. coli Ori
8.5 Kb
B
Human APOL1 and HPR
kDa
ml 65
ml 65.5
ml 66
ml 66.5
ml 67
ml 67.5
ml 68
ml 68.5
ml 69
ml 69.5
ml 70
ml 70.5
ml 71
70
35
35
IgM
APOL1
HP beta chain
HPR beta chain
TLF2
TLF1
C
ml 67-67.5 from A
Size marker - ferritin
Mouse plasma from A
Human plasma
TLF2
*
TLF1
**
Anti-APOL1
D
Isotype matched IgG
Anti-HuHP
kDa
70
35
IgM
APOL1

Figure 2

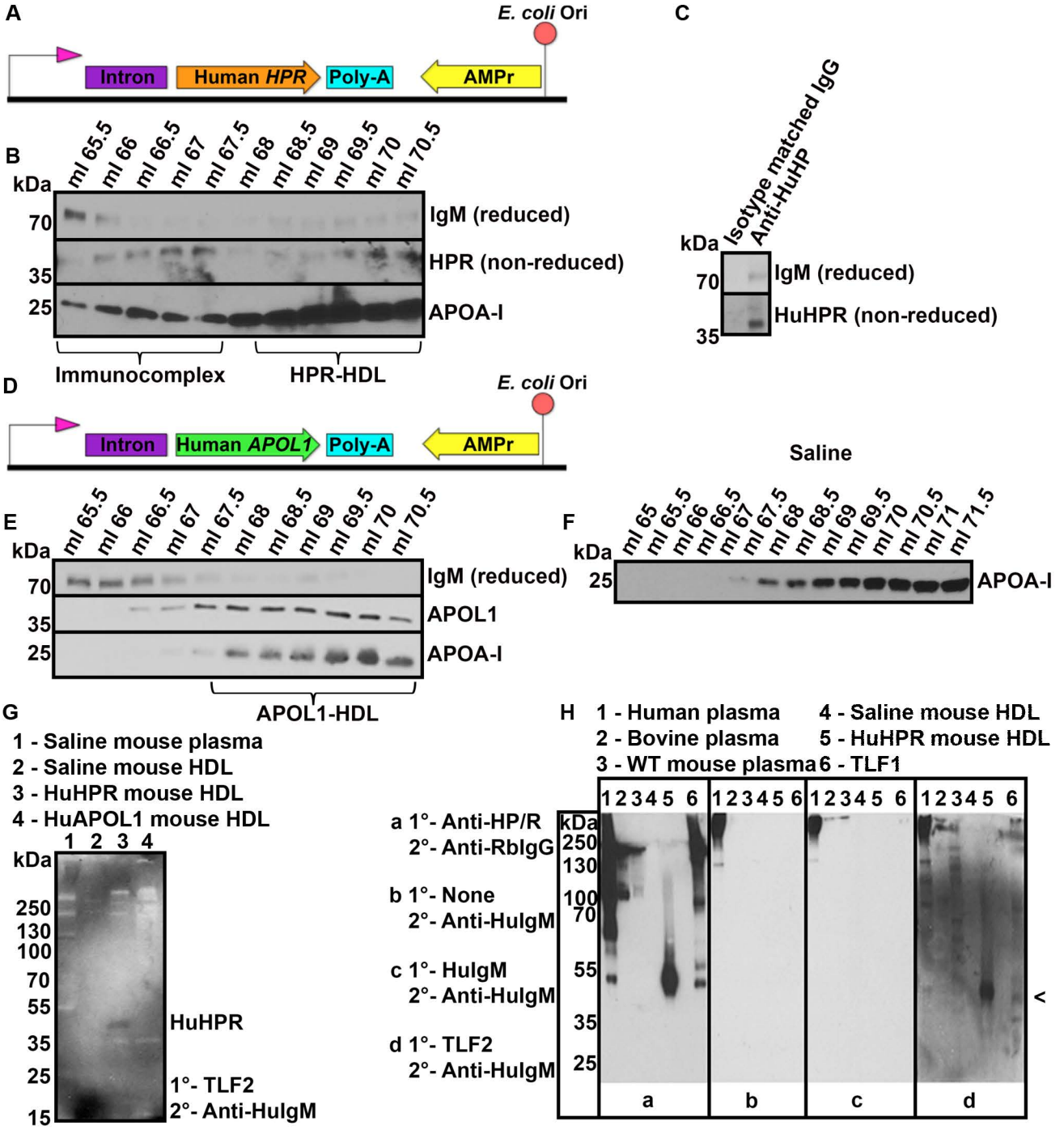

A
E. coli Ori
Intron
Human HPR
Poly-A
AMPr
B
kDa
ml 65.5
ml 66
ml 66.5
ml 67
ml 67.5
ml 68
ml 68.5
ml 69
ml 69.5
ml 70
ml 70.5
70
35
25
IgM (reduced)
HPR (non-reduced)
APOA-I
Immunocomplex
HPR-HDL
C
Isotype matched IgG
Anti-HuHP
kDa
70
35
IgM (reduced)
HuHPR (non-reduced)
D
E. coli Ori
Intron
Human APOL1
Poly-A
AMPr
E
kDa
ml 65.5
ml 66
ml 66.5
ml 67
ml 67.5
ml 68
ml 68.5
ml 69
ml 69.5
ml 70
ml 70.5
70
35
25
IgM (reduced)
APOL1
APOA-I
APOL1-HDL
Saline
F
kDa
ml 65
ml 65.5
ml 66
ml 66.5
ml 67
ml 67.5
ml 68
ml 68.5
ml 69
ml 69.5
ml 70
ml 70.5
ml 71
ml 71.5
25
APOA-I
G
1 - Saline mouse plasma
2 - Saline mouse HDL
3 - HuHPR mouse HDL
4 - HuAPOL1 mouse HDL
1 2 3 4
kDa
250
130
100
70
55
35
25
15
HuHPR
1°- TLF2
2°- Anti-HulgM
H
1 - Human plasma
2 - Bovine plasma
3 - WT mouse plasma
4 - Saline mouse HDL
5 - HuHPR mouse HDL
6 - TLF1
a 1°- Anti-HP/R
2°- Anti-RblgG
b 1°- None
2°- Anti-HulgM
c 1°- HulgM
2°- Anti-HulgM
d 1°- TLF2
2°- Anti-HulgM
kDa
250
130
100
70
55
35
25
1 2 3 4 5 6
a
b
c
d
<

Figure 3

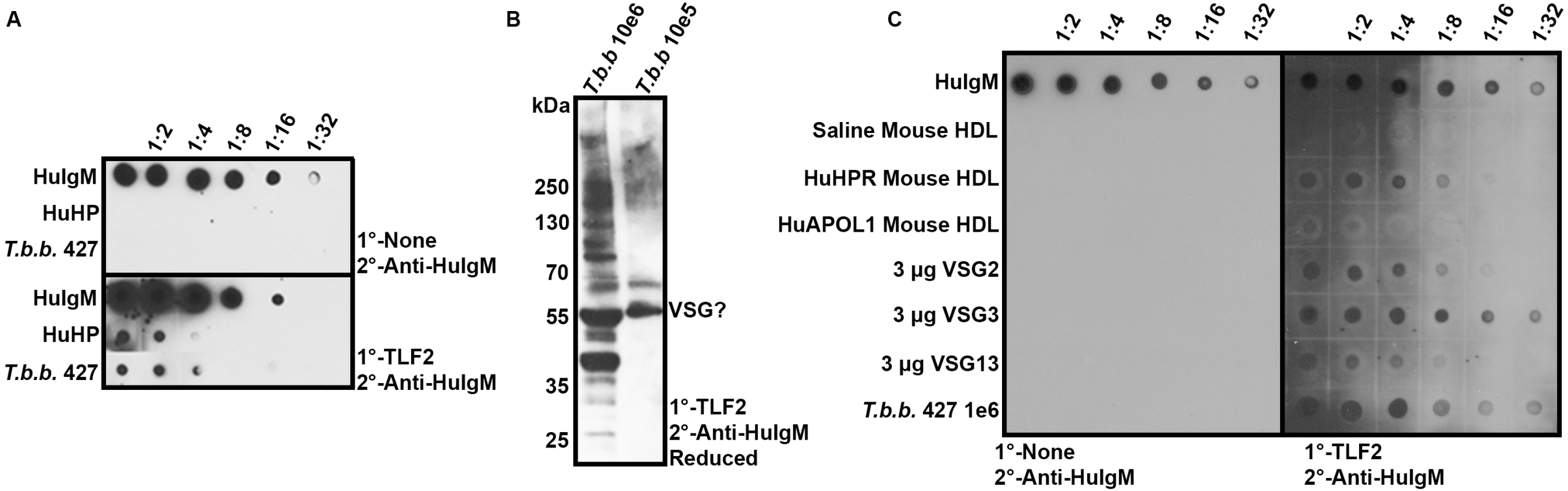

A
1:2
1:4
1:8
1:16
1:32
HuIgM
HuHP
T.b.b. 427
1°-None
2°-Anti-HuIgM
HuIgM
HuHP
T.b.b. 427
1°-TLF2
2°-Anti-HuIgM
B
T.b.b 10e6
T.b.b 10e5
kDa
250
130
70
55
35
25
VSG?
1°-TLF2
2°-Anti-HuIgM
Reduced
C
1:2
1:4
1:8
1:16
1:32
1:2
1:4
1:8
1:16
1:32
HuIgM
Saline Mouse HDL
HuHPR Mouse HDL
HuAPOL1 Mouse HDL
3 µg VSG2
3 µg VSG3
3 µg VSG13
T.b.b. 427 1e6
1°-None
2°-Anti-HuIgM
1°-TLF2
2°-Anti-HuIgM

Figure 4

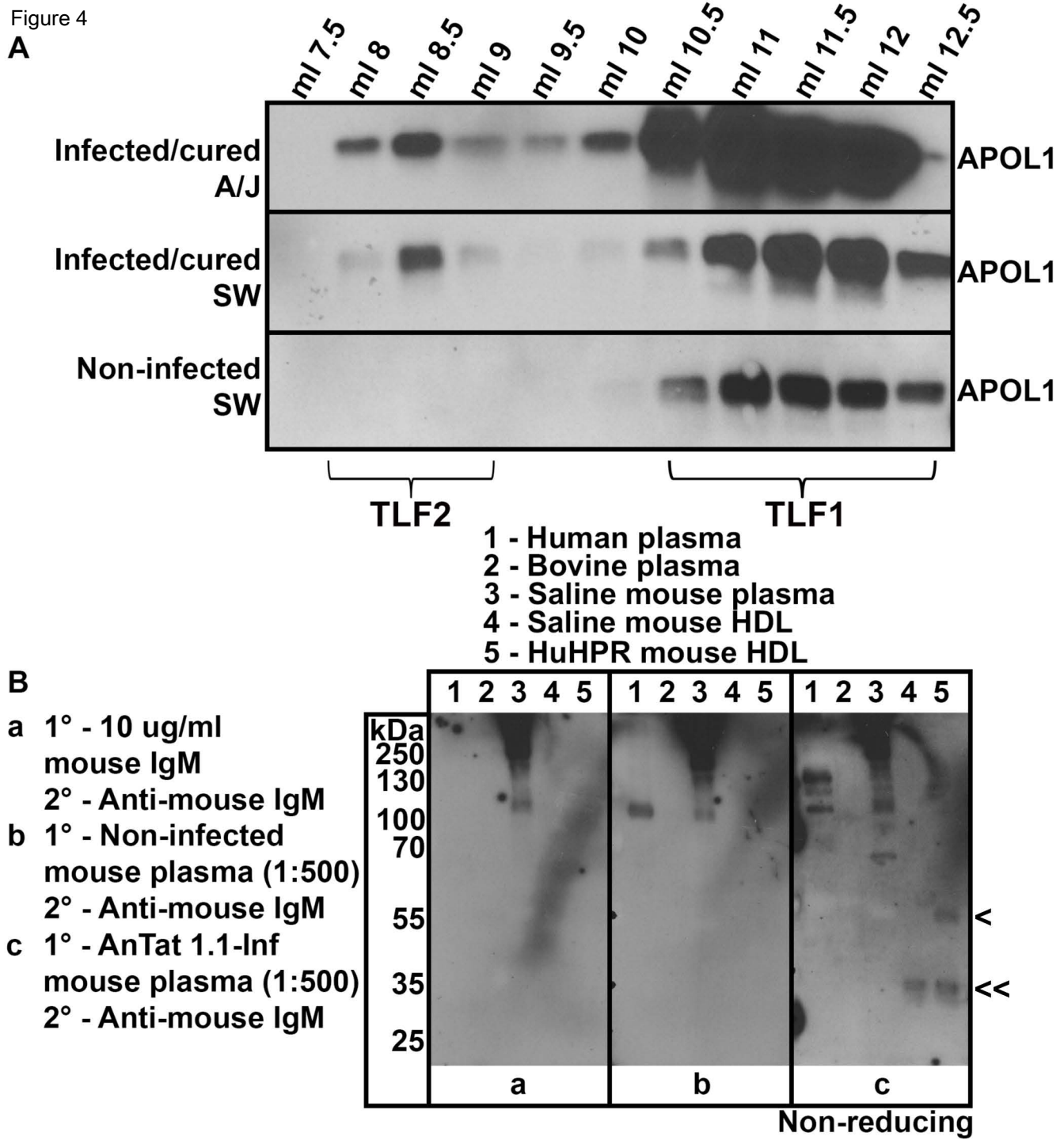
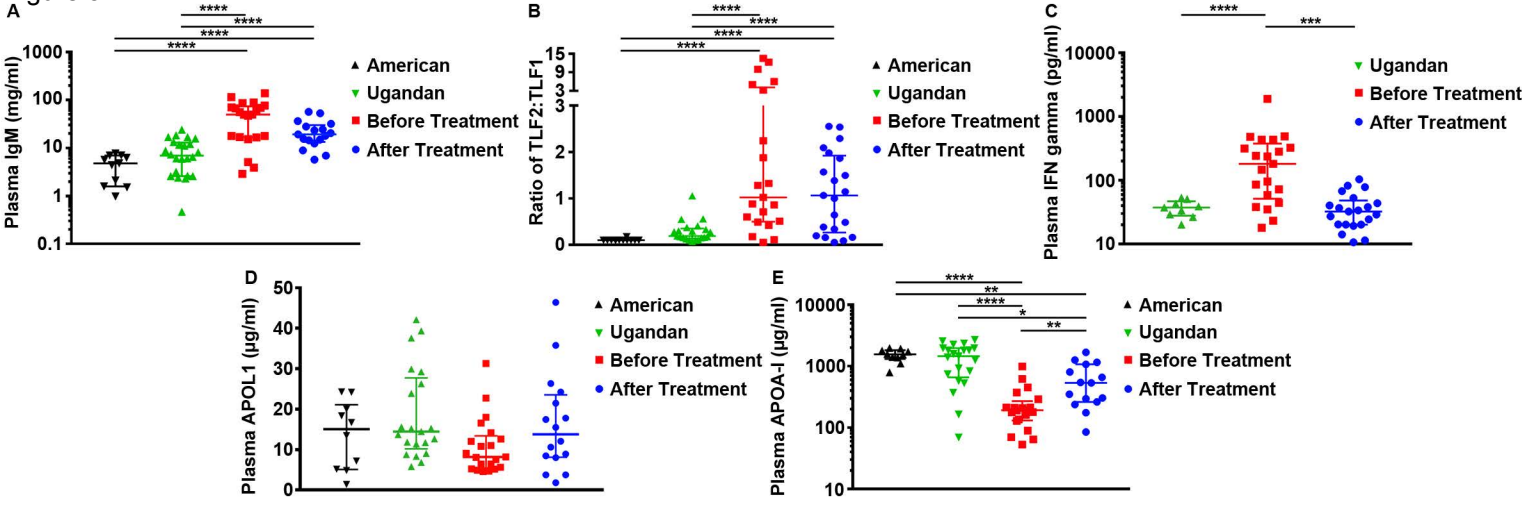

A
ml 7.5
ml 8
ml 8.5
ml 9
ml 9.5
ml 10
ml 10.5
ml 11
ml 11.5
ml 12
ml 12.5
Infected/cured A/J
Infected/cured SW
Non-infected SW
APOL1
APOL1
APOL1
TLF2
TLF1
1 - Human plasma
2 - Bovine plasma
3 - Saline mouse plasma
4 - Saline mouse HDL
5 - HuHPR mouse HDL
B
a 1° - 10 ug/ml mouse IgM
2° - Anti-mouse IgM
b 1° - Non-infected mouse plasma (1:500)
2° - Anti-mouse IgM
c 1° - AnTat 1.1-Inf mouse plasma (1:500)
2° - Anti-mouse IgM
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
kDa
250
130
100
70
55
35
25
<
<<
a
b
c
Non-reducing

Figure 5


A
Plasma IgM (mg/ml)
1000
100
10
1
0.1
****
****
****
****
American
Ugandan
Before Treatment
After Treatment
B
Ratio of TLF2:TLF1
15
9
3
2
1
0
****
****
****
****
American
Ugandan
Before Treatment
After Treatment
C
Plasma IFN gamma (pg/ml)
10000
1000
100
10
****
***
Ugandan
Before Treatment
After Treatment
D
Plasma APOL1 (µg/ml)
50
40
30
20
10
0
American
Ugandan
Before Treatment
After Treatment
E
Plasma APOA-I (µg/ml)
10000
1000
100
10
****
**
****
*
**
American
Ugandan
Before Treatment
After Treatment