Original article

Mutual self-defence: the trypanolytic factor story

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Abstract

Around 1900 Laveran and Mesnil discovered that African trypanosomes (prototype: \textit{Trypanosoma brucei brucei}) do not survive in the blood of some primates and humans. The nature of the trypanolytic factor present in these sera has been the focus of a long-standing debate between different groups, but recent developments have allowed the proposal of a coherent model incorporating most seemingly divergent views and providing an interesting example of the complex interplay that continuously occurs between hosts and parasites. Possibly as an adaptation to their natural environment, great African apes and humans have acquired a new member of the apolipoprotein-L family, termed apoL1. This protein is the only one of the family to be secreted in the blood, where it binds to a subset of HDL particles that also contain another human-specific protein, haptoglobin-related protein or Hpr. \textit{T. b. brucei} possesses a specific surface receptor for the haptoglobin—hemoglobin (Hp-Hb) complex, as a way to capture heme into hemoproteins that contribute to cell growth and resistance to the oxidative stress of the host. As this receptor does not discriminate between Hp and Hpr, Hpr-containing HDL particles of human serum are efficiently taken up by the parasite, leading to the simultaneous internalization of apoL1, Hpr and Hb-derived heme. Once in the lysosome, apoL1 is targeted to the lysosomal membrane, where its colicin-like anionic pore-forming activity triggers an influx of chloride ions from the cytoplasm. Osmotic effect linked to this ionic flux leads to uncontrolled swelling of the lysosome, ultimately causing the death of the parasite. Two \textit{T. brucei} clones, termed \textit{Trypanosoma brucei rhodesiense} and \textit{Trypanosoma brucei gambiense}, have managed to resist this lysis mechanism and, therefore, cause sleeping sickness in humans. While the mechanism of this resistance is still not known in the case of \textit{T. b. gambiense}, the dominant factor responsible for resistance of \textit{T. b. rhodesiense} has been identified. This protein, named SRA for Serum Resistance-Associated, is a truncated version of the major and variable surface antigen of the parasite, the Variant Surface Glycoprotein or VSG. Presumably due to its defective nature, SRA is not targeted to the plasma membrane as do regular VSGs, but ends up in the late endosomal compartment. In this location SRA is thought to neutralize apoL1 through coiled–coil interactions between \(\alpha\)-helices. We discuss the potential of these discoveries in terms of fight against the disease.

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1. Trypanolytic activity of human blood

African trypanosomes can infect a wide variety of mammals, and only some mammalian sera are capable of trypanolysis. This is the case in some primates like baboons (\textit{Papio sp.}), \textit{Gorilla} and humans, clearly indicating that this innate defence was acquired recently during evolution, possibly as an adaptation of the host to local parasites [1]. The main carrier of trypanolytic activity, termed trypanosome lytic factor 1 (TLF-1), was early recognized as being discrete HDL particles of the densest subfraction (HDL3) [2]. These particles were found to be actively taken up by the parasite through their binding to a specific surface receptor, followed by intracellular trafficking to the lysosome via the endocytic pathway [3]. The second lytic fraction (TLF-2) is not linked to HDLs, but is characterized by the presence of IgMs [4]. Apart from this difference, TLF-1 and TLF-2 contain the same limited subset of proteins, and their trypanolytic activity appears to be phenotypically identical.

2. Parasite response: resistance in two clones

The \textit{Trypanosoma brucei} parasites responsible for human sleeping sickness are \textit{Trypanosoma brucei gambiense} and
Trypanosoma brucei rhodesiense, respectively, present in Western and Eastern Africa. These parasites are likely to be individual clones derived from T. brucei, and are both able to fully resist TLF. The mechanism of this resistance is clearly different between the two clones. Whereas resistance is constitutive in T. b. gambiense, it is conditional in T. b. rhodesiense. Specifically, in the latter case it is linked to variation of the main parasite antigen, the Variant Surface Glycoprotein or VSG. The nature of this linkage could be explained when it was discovered that T. b. rhodesiense contains a specific gene necessary and sufficient to encode resistance to TLF, and that this gene, termed SRA for Serum Resistance-Associated, is present in one of the multiple polycistronic and telomeric transcription units where expression of the VSG gene can occur [5]. Only a single one of these genomic sites is transcribed at a time, but switching between sites can achieve antigenic variation. In T. b. rhodesiense resistance to TLF was found to require transcriptional activation of the SRA-containing VSG expression site, which also triggers transcription of the VSG gene contained in this site, hence variation of the VSG. SRA is a truncated VSG devoid of most of the surface-exposed loops of the antigen. Instead of being targeted to the cell surface, SRA is routed to the endocytic pathway for degradation in the lysosome, possibly due to improper folding. It is in this subcellular compartment that neutralization of TLF occurs. In T. b. gambiense SRA is not present, and the mechanism of resistance to TLF is unknown.

3. Identification of the main components of the lytic factor

In vitro analysis of purified TLF-1 allowed the conclusion that Hpr, an HDL-bound and human-specific protein evolved from Hp, was the lytic factor [6,7]. As both Hp and Hpr bind Hb [8], the mechanism of trypanolysis was considered to be disruption of the lysosomal membrane through lipid peroxidation triggered by Fenton reaction due to heme contained in Hpr-bound Hb [9]. However, this model was not supported by further biochemical analysis [10], and several reports failed to detect physiologically significant trypanolytic activity associated with Hpr, whether native, recombinant or in transgenic models [11–14]. In addition, another distinct function of Hpr in trypanolysis was proposed: this protein was shown to act as a ligand to mediate one of the binding processes of TLF to the trypanosome surface [13,14]. Nevertheless, the Hpr-mediated Fenton hypothesis is still currently proposed as involved in at least part of the lytic process [15].

Another approach for identifying the lytic factor was the search for the human serum component neutralized by SRA. This led to the unexpected discovery that another HDL-bound and human-specific protein termed apoL1 was responsible for trypanosome lysis [13,16–18]. ApoL1 contains an anion-selective membrane pore, similar to that of bacterial colicins [17]. When inserted in the lysosomal membrane of the parasite following apoL1 uptake by endocytosis, this pore allows the influx of chloride ions into the lysosome, which triggers the simultaneous entry of water and uncontrolled swelling of the vacuole until the parasite dies [17–19]. When present, SRA strongly interacts with the C-terminal α-helix of apoL1, thereby neutralizing the activity of this protein [17].

4. The apoL family

In humans apoL1 belongs to a family of six members, but in other mammalian orders this family has undergone spectacular expansions. The apoL1 gene results from a recent duplication that conferred an N-terminal signal peptide to the protein. As far as known, the other protein members are intracellular. Therefore, it is likely that the original function of apoLs is not linked to lipid transport or metabolism. A detailed analysis of available information, as well as some experimental data, led to the conclusion that these proteins share structural and functional similarities with the pro- and anti-apoptotic proteins of the Bcl-2 family [20]. However, apoLs seem to be involved in programmed cell necrosis rather than apoptosis [20]. The presence of an apoL member on HDL particles provided humans with innate immunity against African trypanosomes, in keeping with the known role of HDLs in antimicrobial activity [18,21].

5. Respective roles of Hpr and apoL1

It is currently recognised by the different researchers in the field that optimal trypanolytic activity results from the simultaneous presence of apoL1 and Hpr on the same subset of HDL particles [13,21]. It is also consensual that physiological levels of apoL1 alone, either recombinant or native, are sufficient to cause complete trypanolysis, although less rapidly than when Hpr is present [13,16–19,21]. In particular, natural human mutants lacking Hpr but possessing physiological levels of apoL1 are not susceptible to trypanosome infection, but the rate of trypanosome killing by the serum from these individuals is lower than normal [13]. The accelerating effect of Hpr could be entirely explained following the identification of the parasite receptor for TLF-1 [13,22]. This receptor, termed TbHpHbR for T. brucei Hb-Hp receptor, is a GPI-anchored glycoprotein that binds either Hpr–Hb or Hpr–Hb with high affinity. This affinity is similar to that exhibited by the human receptor for the Hpr–Hb complex, termed CD163. However, in contrast to CD163, TbHpHbR recognizes Hpr–Hb equally well as Hpr–Hb. While in human serum TbHpHbR was required for binding and uptake of TLF-1, hence for trypanolysis by this serum fraction, in non-human serum like that of mice TbHpHbR appeared to confer better virulence to the parasite, notably by increasing its resistance to the innate defence of the host [22]. Therefore, while apoL1 is necessary and sufficient for trypanolysis, Hpr is involved in optimal trypanolytic activity by allowing faster uptake of this toxin by the parasite through its involvement in the binding of the carrier particles to a specific surface receptor.

The question that remains is the eventual additional contribution of Hpr to the process of lysis [15,21]. In our view the function of the parasite Hpr–Hb receptor is impossible to reconcile with a Fenton-based trypanolytic activity of Hpr. Indeed, heme can now be considered as a growth factor for
trypanosomes. Heme enters the trypanosome indistinguishably associated with either Hpr or Hp, and in non-trypanolytic sera such as that of mice, the evidence for active uptake of Hp-associated heme obviously contradicts any toxic activity of this molecule. This conclusion was also independently reached by detailed dissection of the contribution of Hpr to either trafficking or activity of the lytic factor within the trypanosome, which ruled out the involvement of this protein in toxic activity [13]. Finally, the fact that continuous swelling of the lysosome typically characterizes the process of trypanolysis [19] further argues against peroxidation-driven disruption of the lysosomal membrane. Therefore, in our view the only involvement of Hpr in trypanolysis is to allow specific binding of the trypanolytic particles to the parasite.

6. Model

Following a reverse order of steps with respect to the timing of the successive discoveries, an integrated model of the trypanosome-host interplay can now be proposed (Fig. 1). The free-living style of African trypanosomes, not only *T. brucei* but also *Trypanosoma congolense* and *Trypanosoma vivax*, imposes these parasites the ability to permanently resist the efficient response of the host, particularly the toxic effect of reactive oxygen and nitrogen species produced by macrophages. Presumably due to this requirement, these different trypanosomes all contain a specific receptor for the Hp–Hb complex. The internalized heme is incorporated into hemoproteins such as P450 and b5 cytochromes that help the parasite to grow and resist the innate defence of the host, possibly through modifications of membrane lipids [22]. *T. brucei* cannot synthesize heme [23], which explains the necessity for a surface receptor. Moreover, in order to compete with the efficient Hp–Hb scavenging by the functionally analogous receptor of macrophages (CD163), the trypanosome receptor had to exhibit an affinity comparable to that of CD163. However, in contrast to CD163, TbHpHbR also recognizes the Hpr–Hb complex. While beneficial for the parasite in non-human serum, the presence of TbHpHbR became detrimental in human serum because of the presence of Hpr on the apoL1-containing HDL particles of this serum. Indeed, the Hpr–Hb complex bound to these particles is efficiently recognized by TbHpHbR, which allows the uptake of the trypanolytic particles into the parasite. Following dissociation from HDLs in acidic endosomes, apoL1 is targeted to the lysosomal membrane where its colicin-like pore-forming activity triggers a flux of chloride anions towards the lysosome lumen. Osmotic swelling resulting from this flux is presumably responsible for trypanolysis. Some *T. brucei* clones have managed to adapt to human serum, and are able to infect humans causing the sleeping sickness disease. This is the case of *T. b. rhodesiense*, which synthesizes...
a truncated form of VSG termed SRA, able to strongly interact with apoL1. The intracellular routing of this defective VSG into the endocytic system allows its co-localization with apoL1, hence the neutralization of TLF. The precise mechanism by which SRA blocks the activity of apoL1 is not known, but it is remarkable that SRA interacts with an amphipatic helix of apoL1 that appears to be dispensable, being not required for either the pore-forming activity or the membrane-addressing capacity of this protein [17]. As this helix is the most conserved sequence between members of the apoL family [20], it is likely that it is involved in controlling the protein activity, either by blocking the pore-forming domain or by sequestering the protein away from target membranes, presumably through coil—coiling interactions in either case. Therefore, the fact that this sequence was targeted by the parasite is presumably significant.

7. Perspectives

ApoL1 being a potent trypanosome toxin, its conversion into a drug was an obvious theoretical development. When not present together with Hpr on HDL particles, this protein is inefficiently taken up by the parasite [13,21]. Therefore, in most conserved sequence between members of the apoL family [20], it is likely that it is involved in controlling the protein activity, either by blocking the pore-forming domain or by sequestering the protein away from target membranes, presumably through coil—coiling interactions in either case. Therefore, the fact that this sequence was targeted by the parasite is presumably significant.

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