OPINION

The trypanolytic factor of human serum

Etienne Pays, Benoit Vanhollebeke, Luc Vanhamme, Françoise Paturiaux-Hanocq, Derek P. Nolan and David Pérez-Morga

Abstract | African trypanosomes (the prototype of which is *Trypanosoma brucei brucei*) are protozoan parasites that infect a wide range of mammals. Human blood, unlike the blood of other mammals, has efficient trypanolytic activity, and this needs to be counteracted by these parasites. Resistance to this activity has arisen in two subspecies of *Trypanosoma brucei* — *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* — allowing these parasites to infect humans, and this results in sleeping sickness in East Africa and West Africa, respectively. Study of the mechanism by which *T. b. rhodesiense* escapes lysis by human serum led to the identification of an ionic-pore-forming apolipoprotein — known as apolipoprotein L1 — that is associated with high-density-lipoprotein particles in human blood. In this Opinion article, we argue that apolipoprotein L1 is the factor that is responsible for the trypanolytic activity of human serum.

Similar to other species of African trypanosome (such as *Trypanosoma congolense* and *Trypanosoma vivax*), *Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* are protozoan parasites that are transmitted to various mammals by tsetse flies (*Glossina palpalis* and *Glossina morsitans*) (FIG. 1). During a blood meal by the tsetse fly, infective trypanosome forms, known as metacyclic forms, that are present in the salivary glands of the fly are inoculated into mammalian hosts, such as humans, cattle, antelopes, buffaloes and lions. This allows the transformation of the parasites into long, slender bloodstream forms, which actively divide and colonize the blood until a quorum-sensing signal triggers their differentiation into short, stumpy forms. These short, stumpy forms do not divide and are competent for transformation into procyclic forms as soon as they are ingested by a tsetse fly. The procyclic forms then proliferate in the mid-gut of the fly and eventually differentiate into metacyclic forms after a complex journey in the insect vector. The metacyclic forms are quiescent and are ready to be transferred back to mammals.

Because they reside extracellularly in their mammalian hosts, the bloodstream forms of trypanosomes need to resist all components of host defence. The main protective feature of these parasites is a thick and dense surface coat, which covers the entire plasma membrane. This coat consists of a monolayer of ~10^7 molecules of a single glycoprotein known as variant surface glycoprotein (VSG). The VSG molecules are attached to the plasma membrane by glycosylphosphatidylinositol (GPI) anchors, and they form homodimers[1]. Close interactions between adjacent VSG homodimers prevent antibodies from reaching the inner antigenic determinants of the coat, and the elongated shape of the VSGs keeps toxins away from the plasma membrane.

In addition to these protective functions, VSGs undergo continuous variation, which leads to the periodic change of the VSG loops that are exposed at the surface of the parasite. These loops contain the only antigenic determinants of living intact trypanosomes that are recognized by the immune system. Therefore, such variation is known as antigenic variation, and it allows the parasite to evade antibody-mediated clearance[2–5]. Only one VSG variant is produced at any one time, because only one VSG allele at a time can be transcribed from the repertoire of VSG genes, which consists of more than 1,000 sequences. This transcription occurs at one of several VSG gene expression sites (ESs), and antigenic variation can result from two distinct mechanisms: transcriptional switching between VSG ESs, and homologous recombination between the active VSG gene and another VSG gene from the repertoire.

In addition to the defences that are usually encountered in mammals, African trypanosomes need to defy a novel innate immune mechanism that evolved in humans and in some non-human primates — an efficient trypanolytic factor that is present in serum. Here, we summarize current knowledge of the trypanolytic activity of human serum and of how trypanosomes that infect humans resist this activity. Two serum proteins — haptoglobin-related protein (HPR) and apolipoprotein L1 (APOL1) — have been proposed as candidates for providing the trypanolytic activity. In this Opinion article, we argue that the characterization of the mechanism of resistance of *T. b. rhodesiense*, as well as the study of the phenotype of lysis induced by APOL1, indicates that APOL1 is the sole factor that is responsible for trypanolysis.

Studies of trypanolytic activity

Between 1902 and 1912, Laveran and Mesnil[6] reported that sera from humans and other primates — such as various *Papio* (baboon), *Cercocebus* (mangabey) and

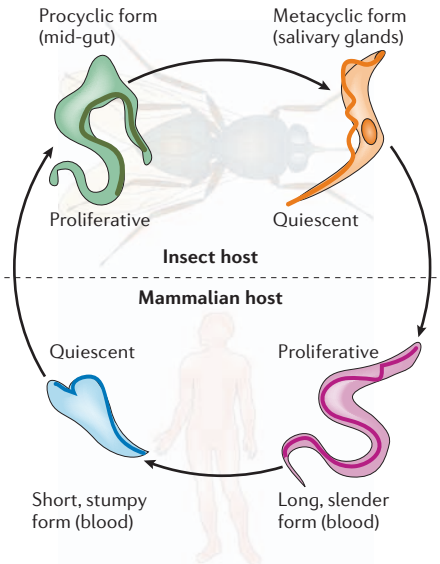

Figure 1 | **Life cycle of *Trypanosoma brucei*.** The life cycle of *T. brucei* alternates between the insect host (the tsetse fly) and a mammalian host (such as humans, cattle, antelopes, buffaloes and lions). In both the insect vector and the mammalian host, colonization occurs through the proliferation of rapidly dividing trypanosome forms. These forms eventually transform into resting (quiescent) parasite cells that are preprogrammed for cellular differentiation after changes in the environment (that is, transfer from one host to the other). The long, slender bloodstream forms also adapt to their environment while present in the mammalian host, and this adaptation involves continuous variation of the main surface antigen, which is known as variant surface glycoprotein.

Mandrillus (forest baboon) species — kill trypanosomes, although they did note differences in activity between these sera. Human serum did not affect the *T. brucei* subspecies that infect humans: that is, *T. b. rhodesiense* and *T. b. gambiense*. By contrast, sera from various *Papio* species lysed *T. b. rhodesiense* and showed 10–25-fold higher activity than human serum against human-serum-sensitive trypanosomes[6]. Sera from *Cercocebus* and *Mandrillus* species were much less active against human-serum-sensitive trypanosomes, and serum from *Pan troglodytes* (chimpanzees) was devoid of activity, despite the close evolutionary link between chimpanzees and humans[6]. These findings were confirmed in subsequent studies, which also showed that serum from *Gorilla gorilla* (gorillas) has trypanolytic activity[7–9].

Trypanolysis occurs through high-density-lipoprotein-particle-mediated endocytosis. Mainly as a result of the pioneering work of Mary Rifkin[10,11], the trypanolytic activity was shown to be associated with high-density lipoprotein (HDL) particles (BOX 1), in particular with the densest HDL subfraction, HDL3 (REFS 12–15). Further studies indicated that receptor-mediated endocytosis of these HDL particles by the trypanosome was involved in lysis[16–22]. In this case, internalization of the lytic particles would involve their delivery to increasingly acidic compartments of the endocytic pathway. That these processes occur is supported by the findings that either neutralization of the pH of endosomes (with a membrane-permeable weak base, such as chloroquine)[16,17] or inhibition of endocytosis itself (by RNA-interference-mediated knockdown of actin mRNA)[22] results in inhibition of lysis. The identity of the trypanosome cell-surface receptor for HDL particles remains elusive, but it is probably a lipoprotein scavenger receptor, because HDL particles and low-density lipoprotein (LDL) particles have both been shown to compete with binding of the trypanolytic factor to the trypanosome surface[23]. However, the lipoprotein scavenger receptor of trypanosomes must considerably differ from that of other eukaryotes, because sequence analysis did not uncover any candidate genes in the trypanosome genome.

HPR as the trypanolytic factor. Purification of the trypanolytic HDL particles in the HDL3 subfraction yielded two types of complex, which differed in their lipid content but contained several of the same proteins, including APOA1 and the serine-protease-like protein HPR[24–27]. The involvement of APOA1 in trypanolysis was quickly discounted because of its wide distribution among different types of HDL complex and because of its failure to show trypanolytic activity[28–30]. Several lines of evidence, however, suggested that HPR has a role in trypanolysis. First, haptoglobin-specific antibodies, which also recognize HPR, inhibited trypanolysis when added to lytic HDL particles[24]. Second, haptoglobin, which is similar to HPR, also prevented trypanolysis when added to these assays of trypanolysis, possibly as a result of competition[30,31] (although a differential effect was observed for the two types of trypanolytic complex that had been purified[32–34]). Third, HPR is not expressed in chimpanzees, the serum of which lacks trypanolytic activity (as indicated earlier)[6–9,35]. Last, HPR was also thought to be the specific component of the trypanolytic HDL particles that is recognized by the parasite cell-surface receptor[36].

The trypanolytic effect of the HPR-containing HDL particles was thought to be a consequence of damage to the lysosomal membrane. According to Hajduk and colleagues[16,24,37], HDL particles caused lipid peroxidation of the lysosomal membrane of the parasite, leading to disruption of this membrane, release of proteolytic enzymes into the cytoplasm and auto-digestion of the parasite cell. The peroxidase activity of HDL particles was initially ascribed to a putative HPR–haemoglobin dimer[24] and then to the Fenton reaction between hydrogen peroxide and iron (an iron-salt-dependent decomposition of hydrogen peroxide, which generates highly reactive hydroxyl radicals)[37]. Findings supporting these views included the inhibition of lysis by antioxidants and protease inhibitors (factors that were proposed to block lipid peroxidation and cellular auto-digestion, respectively)[16,24,37], the detection of end-products of lipid peroxidation during trypanolysis[37] and the observation (using electron microscopy) of discontinuities in the lysosomal membrane of cells undergoing lysis[16]. By contrast, Raper, Tomlinson and colleagues[27,38] could not detect binding of haemoglobin to HPR, found no evidence of lipid peroxidation, observed no effect of antioxidants and protease inhibitors on trypanolysis, and found no reactive oxygen intermediates associated with trypanolysis. As detailed later, one explanation for these conflicting findings could be that both the proposed identity and the proposed mode of action of the trypanolytic factor were incorrect.

Box 1 | **High-density lipoprotein (HDL) particles**

In the blood, most lipids are contained in soluble complexes known as lipoproteins. HDL particles are spherical particles that comprise a hydrophobic lipid core (which mainly consists of triglycerides and cholesteryl esters) surrounded by a hydrophilic layer (which consists of phospholipids, unesterified cholesterol and several proteins that are collectively known as apolipoproteins). In terms of protein content, HDL particles mainly contain apolipoprotein A1 (APOA1), which specifically captures and solubilizes free cholesterol, thereby enabling HDL particles to function as cholesterol scavengers. Several HDL-particle subfractions can be separated on the basis of density. The subfraction known as HDL3, which contains both APOL1 and haptoglobin-related protein (HPR), is the densest. The high protein content of HDL particles renders them denser than other lipoprotein particles, including low-density lipoprotein (LDL) particles.

Studies of resistance to trypanolysis

In addition to studies of the trypanolytic activity, research on the resistance of certain trypanosome subspecies to lysis provided another angle from which to approach identification of the trypanolytic factor. In contrast to *T. b. brucei*, the subspecies *T. b. gambiense* and *T. b. rhodesiense* escape the trypanolytic activity of human serum and cause sleeping sickness, a lethal disease in humans. *T. b. gambiense* is permanently resistant to human serum. By contrast, *T. b. rhodesiense* loses resistance after being isolated from humans and transferred to other animals: for example, after 30–70 passages in mice[6,39]. Following the injection of these mice with human serum, *T. b. rhodesiense* regains resistance to lysis, and this acquisition of resistance was shown to be associated with antigenic variation[39].

Specifically, for a given isolate of *T. b. rhodesiense*, such resistance arising on selection in human serum involved a change in expression of the VSG to a particular VSG variant known as ETat 1.10. This VSG, however, was not itself responsible for resistance to lysis, because other human-serum-resistant *T. b. rhodesiense* clones were shown to express different VSGs, including VSGs that are expressed by clones that are sensitive to lysis[39]. This paradox was resolved by the discovery that, in these trypanosomes, selection in human serum involved transcriptional switching to a particular VSG ES, which was named R-ES[40]. Although

R-ES initially contained the gene encoding the VSG variant ETat 1.10, replacement of this gene by gene conversion resulted in conservation of the resistance phenotype, indicating that a component of R-ES, but not the VSG gene itself, conferred resistance. All VSG ESs have polycistronic transcription units that contain several genes (known as expression-site-associated genes, ESAGs) in addition to the VSG gene[41], and these are under monoallelic control of expression, similar to the VSG genes. R-ES was found to contain a gene known as serum-resistance associated (*SRA*) (FIG. 2), which had previously been identified as being transcribed only in resistant clones[42]. The presence of this gene in a VSG ES that is systematically selected for transcription in human serum provided a straightforward explanation for its exclusive expression in human-serum-resistant clones. Transfection of *SRA* alone into *T. b. brucei* (which is sensitive to lysis by human serum) conferred full resistance, thereby identifying *SRA* as necessary and sufficient for resistance of *T. b. rhodesiense*[40]. The finding that *SRA* is an ESAG present in a particular VSG ES explained the link between resistance to lysis by human serum and antigenic variation.

SRA encodes a truncated VSG that is devoid of surface-exposed loops, owing to an in-frame deletion[43–45]. Together with the localization of this gene in a VSG ES, this finding indicates that *SRA* was generated by antigenic-variation-associated homologous recombination of VSG genes. Interestingly, another component that is involved in adaptation of the parasite to the host, the parasite surface receptor for host transferrin, was also found to originate from a VSG gene, and the genes that encode this heterodimeric receptor are also located in VSG ESs[41]. So, the VSG ESs are loci that result in the generation of various VSG-derived proteins that help the parasite to adapt to the host.

SRA and R-ES seem to be conserved in most isolates of *T. b. rhodesiense*[46–50]. So, these genetic elements are clearly important for the propagation of this trypanosome subspecies, for which they are also excellent specific markers[51]. Given the high probability that *SRA* evolved only once, *T. b. rhodesiense* is likely to have arisen as a clone of *T. b. brucei* that differs mainly or solely by its ability to express SRA on selection in human serum. The genetic variability that is observed between *T. b. rhodesiense* isolates is probably a result of spreading of R-ES into various backgrounds, through genetic exchange[48]. Using reverse-transcription PCR, it has also been shown that R-ES

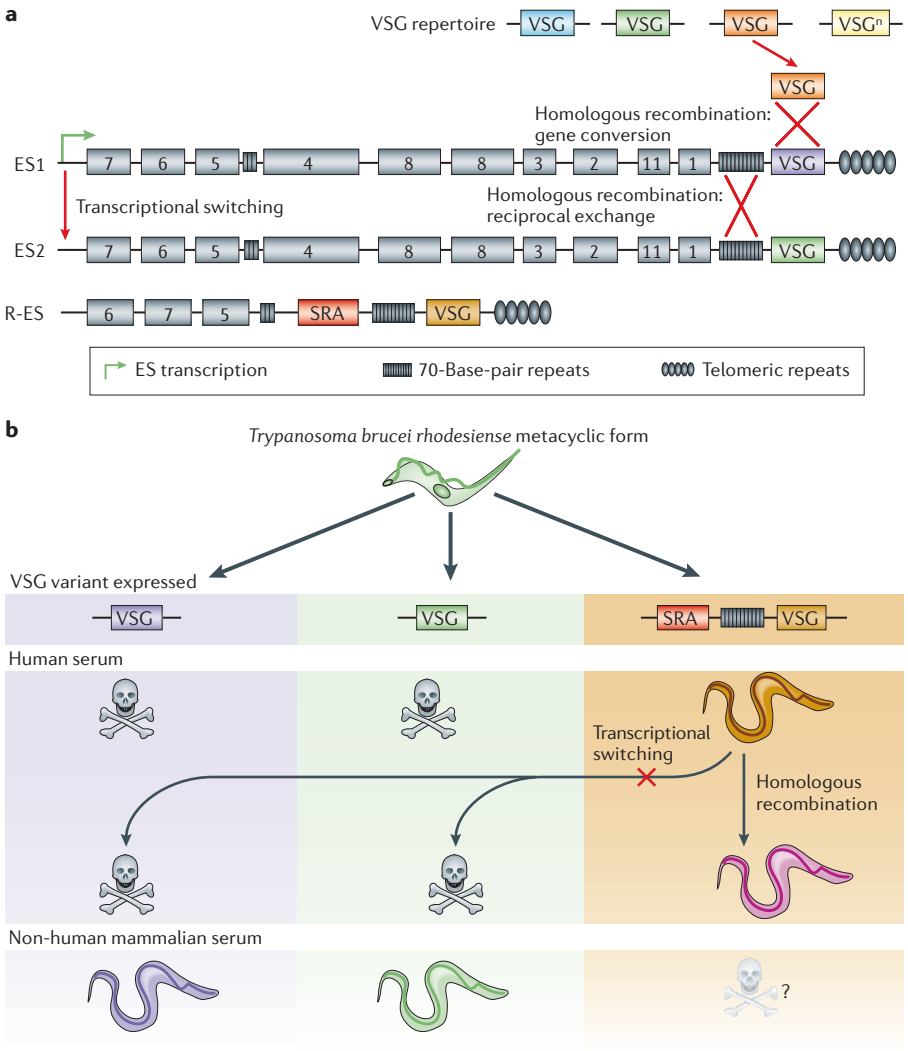

Figure 2 | **Antigenic variation and resistance to human serum of *Trypanosoma brucei rhodesiense*.** **a** | Genetic mechanisms of antigenic variation. The *Trypanosoma brucei* genome contains more than 1,000 variant surface glycoprotein (VSG) genes (shown as coloured boxes). Only one of these VSG variants is expressed at any one time. Gene expression occurs in a telomeric expression site (ES), where the VSG gene is co-transcribed with expression-site-associated genes (ESAGs; shown as numbered boxes).The *T. brucei* genome contains a set of 15–20 similar (but not identical) ESs, 3 examples of which are depicted. In *T. b. rhodesiense*, one particular ES, known as R-ES, contains the serum-resistance associated (*SRA*) gene, which confers resistance to lysis by human serum[40]. Antigenic variation (that is, the expression of a different VSG variant) can result from two mechanisms (indicated in red): transcriptional switching and homologous recombination. Transcriptional switching occurs by a process known as *in situ* activation, in which expression of the active ES is turned off, and expression of a previously silent ES is turned on. This process does not involve DNA rearrangement. By contrast, homologous recombination involves replacement of the active VSG gene. This can occur by one of two mechanisms: gene conversion, which involves replacement with a copy of a VSG gene from the repertoire; or reciprocal exchange, which involves replacement with a VSG gene from another ES (and thereby exchange of a VSG gene between two ESs). These replacements occur through recombination between homologous regions, such as 70-base-pair repeats. **b** | Gain and loss of resistance to human serum. Tsetse flies inject the mammalian host with *T. b. rhodesiense* in the metacyclic form, and these trypanosomes then transform into long, slender bloodstream forms (FIG. 1), in which different VSG ESs can be activated. Only trypanosomes in which the R-ES is active can resist lysis by human serum. Under these conditions, *in situ* activation (transcriptional switching) of other VSG ESs is counter-selected (indicated by a small red cross), owing to the requirement for *SRA* expression to resist lysis. Therefore, antigenic variation only occurs through homologous recombination targeted to the active VSG gene. In non-human hosts, expression of the R-ES seems to be counter-selected, although *in situ* inactivation of the R-ES does require many passages in mice[6,39]. This counter-selection might result from the absence of most ESAGs from the R-ES, because these ESAGs might not be completely dispensable.

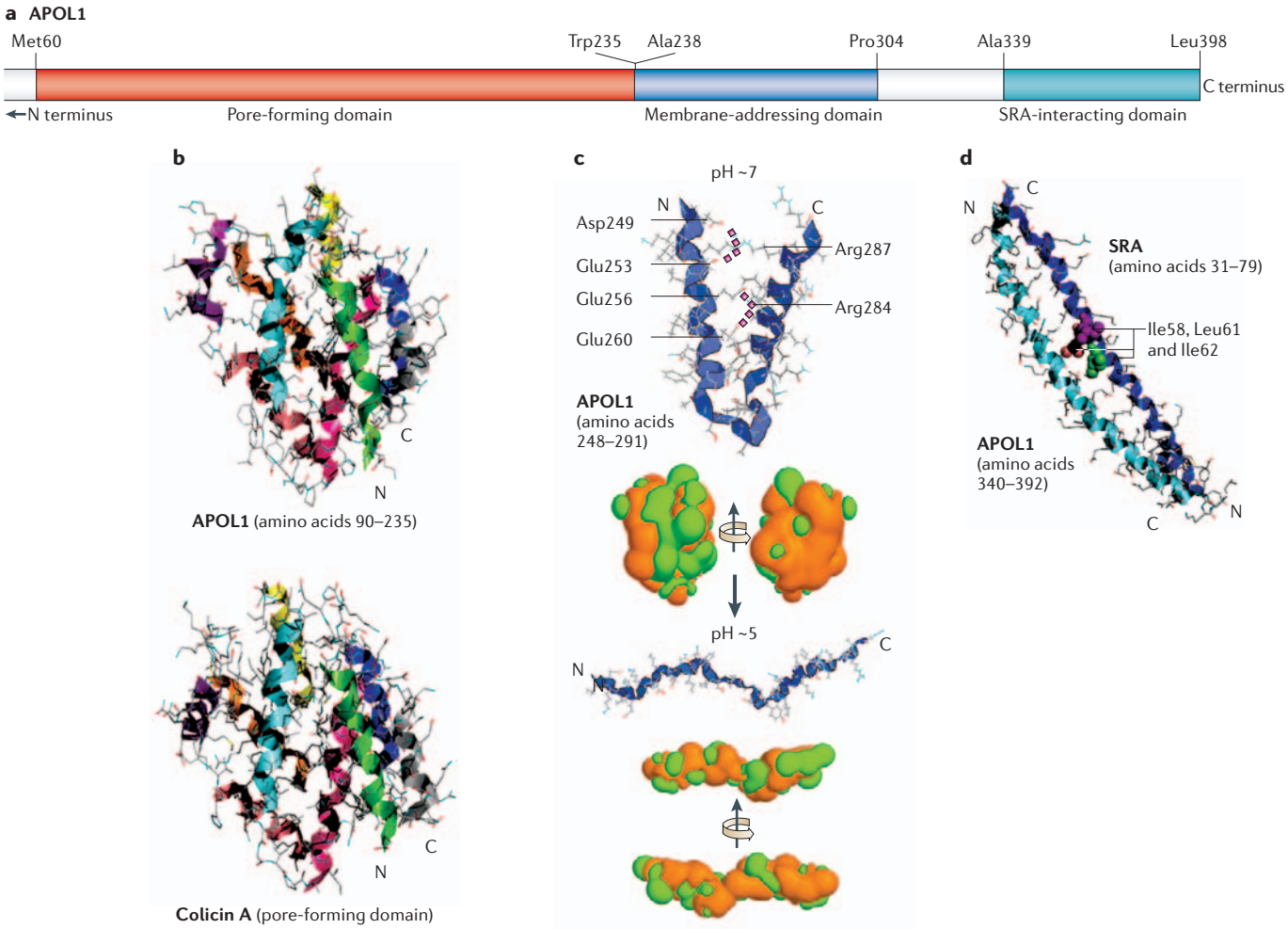

Figure 3 | **Models of the three domains of apolipoprotein L1 (APOL1).** A diagrammatic representation of APOL1 and the location of each of its domains is shown in **a**. The structure of each domain is also shown in a ribbon-diagram format in **b**,**c**,**d**, where the protein backbone is shown as a coloured ribbon and the amino-acid side chains are shown in black (uncharged atoms), blue (positively charged atoms) and red (negatively charged atoms). **a** | APOL1 contains three domains: a pore-forming domain, a membrane-addressing domain and an SRA (serum-resistance associated)-interacting domain. **b** | The region of APOL1 that spans the methionine residue at position 60 and the tryptophan residue at position 235 contains a domain that is structurally and functionally similar to the ionic-pore-forming domain of bacterial colicins such as colicin A. Analogous helices of the two proteins are shown in the same colour. Colicin A diagram modified with permission from REF. 64 © (2005) American Association for the Advancement of Science. **c** | The region of APOL1 that spans the alanine residue at position 238 and the proline residue at position 304 contains a membrane-addressing domain. Computer modelling of this region predicts a pH-dependent structure. At neutral pH, such as in the blood, the two α-helices of the membrane-addressing domain interact through two salt bridges (indicated by pink squares), which are formed by the side chains of the amino acids indicated. This hairpin structure shows a segregation between hydrophobic (orange) and hydrophilic (green) surfaces, as indicated by space-filling models. At acidic pH, such as in the lysosome (pH 5.3), the salt bridges are predicted to dissociate as a result of neutralization of the negatively charged residues, and this would lead to loss of the large hydrophobic surface. Experimental evidence indicates that this surface is required for the association of APOL1 with high-density lipoprotein (HDL) particles and that treatment with acid results in dissociation of APOL1 from HDL particles (B.V. and E.P., unpublished observations). Figure modified with permission from REF. 64 © (2005) American Association for the Advancement of Science. **d** | The region of APOL1 that spans the alanine residue at position 339 and the leucine residue at position 398 forms a long α-helix that strongly and specifically interacts with the N-terminal α-helix of the *Trypanosoma brucei rhodesiense* protein SRA. The residues of SRA that are important for this interaction, as determined by studies involving point mutations, are indicated. Figure modified with permission from *Nature* REF. 54 © (2003) Macmillan Publishers Ltd.

is not active when the parasite is present in non-human sera[52]. This finding indicates that R-ES, which lacks several ESAGs that are usually found in other VSG ESs[40] (FIG. 2a), is counter-selected when the parasite is not exposed to human serum (FIG. 2b). The data also confirm and explain the early observations of Laveran and Mesnil[6], as well as more recent observations in the field[47], that *T. b. rhodesiense* is sensitive to human serum when grown in non-primate animals. Therefore, when living in non-human hosts, *T. b. rhodesiense* probably does not differ from *T. b. brucei*, but the difference arises when *T. b. rhodesiense* is exposed to human serum, which triggers selection of R-ES and transcription of *SRA*. Interestingly, *T. b. gambiense* does not carry *SRA*, despite its constitutive resistance to human serum[53]. Therefore, the mechanism ensuring resistance of this subspecies to lysis must differ from that of *T. b. rhodesiense*, and this mechanism is under investigation at present.

APOL1

Although SRA has a signal peptide, it is not targeted to the cell surface of the parasite (unlike the VSGs from which it is derived) but accumulates in the lysosome[54]. This could result from the absence of a GPI anchor (REFS 55,56, and D.P.N., F.P.H. and E.P., unpublished observations). Similar to VSGs, SRA is characterized by a long N-terminal hairpin that contains two amphipathic α-helices[1,44]. The analysis of various mutated forms of SRA expressed in *T. b. brucei* (which itself does not express SRA) showed that only the N-terminal amphipathic α-helix helix A is required to provide resistance[54]. In VSGs, this helix is usually involved in coil-coiling interactions with the other amphipathic α-helix, helix B, and with helices of adjacent VSGs, to form a dimer[1]. So, by analogy, it was envisaged that SRA interacts with the trypanolytic factor of human serum by coil-coiling. Chromatographic separation of human serum on SRA affinity columns showed that the protein APOL1 bound strongly and specifically to SRA. This binding was found to result from hydrophobic coil-coiling interactions between helix A of SRA and the C-terminal α-helix of APOL1 (REF. 54) (FIG. 3a,d). In addition, point mutations that disrupt helix A of SRA were shown to result in loss of binding to APOL1 (REF. 54).

APOL1 is associated with the HDL3 subfraction of human serum[57]. Trypanosomes internalize APOL1-containing particles, and APOL1 is then routed to the lysosome, where it colocalizes with SRA[54]. Under conditions that dissociate serum lipoprotein complexes (that is, in the presence of detergent), separation of human serum by affinity chromatography on either APOL1-specific antibodies or SRA selectively removed APOL1, and the only other detectable components that were subsequently eluted with APOL1 were serum albumin and antibodies[54]. This chromatographic step, followed by dialysis to remove the detergent, resulted in total loss of the trypanolytic activity of the serum[54]. Applying the same protocol but using affinity chromatography on an SRA molecule with a disrupted helix A did not result in the removal of APOL1 or in the loss of trypanolytic activity, showing that the depletion of APOL1 (and not the treatment with detergent) was responsible for the loss of trypanolytic activity[54]. Furthermore, addition of purified native or recombinant APOL1 to the depleted serum or to fetal calf serum (which naturally lacks APOL1) restored or conferred, respectively, the ability to lyse human-serum-sensitive trypanosomes but not human-serum-resistant trypanosomes[54]. The resistance of trypanosomes to lysis was associated with the C-terminal helix of APOL1 (which interacts with helix A of SRA), because removal of this helix from APOL1 conferred the ability to lyse both human-serum-sensitive and human-serum-resistant trypanosomes[54]. Finally, the natural phenotype of this system can be entirely reconstituted with recombinant proteins: recombinant-SRA-expressing *T. b. brucei* were found to be resistant to lysis following exposure to recombinant APOL1, whereas wild-type *T. b. brucei* were readily lysed by APOL1 (REF. 54). This finding indicates that APOL1 mimics the natural killing activity of human serum, and it also proves that SRA blocks the activity of APOL1 on trypanosomes.

From these studies, it was concluded that APOL1 is the trypanolytic factor of human serum and that, in *T. b. rhodesiense*, SRA neutralizes APOL1 through coil-coiling interactions with the C-terminal helix of APOL1. Accordingly, APOL1 was found to be absent from chimpanzee serum, which is non-trypanolytic (as discussed earlier)[6,8,9], and sequencing of the chimpanzee genome provided a straightforward explanation for this finding: the *APOL1* gene is absent from these animals[58].

Resistance to APOL1 during the* T. b. rhodesiense *life cycle. In long, slender bloodstream forms of *T. b. rhodesiense* (FIG. 1), the expression of SRA allows neutralization of APOL1 in the lysosome[54]. This mechanism of resistance considerably differs from the previously proposed mechanism, the selective inhibition of endocytosis of the trypanolytic factor[19]. Recent data obtained using *T. b. brucei* transfected with an epitope-tagged version of SRA indicate that neutralization of the trypanolytic factor by SRA can occur in endosomes, before the lytic factor reaches the lysosome[59]. These findings are consistent with the observation that the interaction between SRA and APOL1 can occur under both neutral and acidic conditions (that is, between pH 7.5 and 5.0)[54]. In accordance with the apparent lability of SRA[40,46,59], the distribution of SRA between the endosomes and the lysosome might depend on the relative level of endocytic-protease activity, as indicated by studies in which the endo-lysosomal protease trypanopain was inhibited (L.V. and E.P., unpublished observations). Therefore, it can be speculated that SRA is routed to the lysosome through the endocytic pathway and that the interaction of SRA with APOL1 can occur at different steps of this pathway and is followed by the proteolytic cleavage of this complex. Degradation of APOL1, however, is not required for the trypanosome to be resistant to lysis, because the presence of both APOL1 and SRA in the lysosome (in contrast to APOL1 alone) has been shown to prevent lysis from occurring[54]. That is, interaction with SRA seems to be sufficient for inactivation of APOL1.

In procyclic forms of *T. b. rhodesiense* (FIG. 1), which are present in the tsetse fly, R-ES is inactive, so SRA is not expressed. However, procyclic forms can resist lysis by human serum *in vitro*[60], and this is probably a consequence of the low level of endocytosis by trypanosomes at this stage[22].

Metacyclic forms of *T. b. rhodesiense* (FIG. 1), which are transferred from the tsetse fly to the mammalian host, are the first to be confronted with mammalian blood. Because R-ES is probably inactive in these forms of the parasite[3], it is not clear how they resist APOL1 before their transformation into bloodstream forms. *In vitro*, metacyclic forms of *T. b. rhodesiense* were found to be mostly sensitive to lysis by human serum[61]. In the same assay, metacyclic forms of *T. b. brucei* and *T. b. gambiense* were always sensitive and resistant, respectively, to lysis by human serum. Therefore, metacyclic forms of *T. b. rhodesiense* are not constitutively resistant to human serum. After inoculation by the tsetse fly, trypanosomes can remain for several days in tissue spaces or lymphatic vessels, so it is possible that some metacyclic forms escape exposure to APOL1 before they begin to express SRA.

Proposed mechanism of APOL1-mediated trypanolysis. The physiological function of APOL1 in humans remains elusive, although there is indirect evidence that it is involved in lipid metabolism[62], as well as evidence of a link between overexpression in the brain and schizophrenia[63]. For this reason, the way in which APOL1 kills trypanosomes was not apparent. A weak similarity was, however, found between the N-terminal region of APOL1 and the pore-forming domain of colicins, which are bacterial toxins that kill competing bacteria by forming ionic pores in the inner cell membrane. This finding led to experiments that uncovered the capacity of APOL1 to generate ionic pores in biological membranes, both *in vivo* and *in vitro*[64]. Briefly, the region of APOL1 that spans amino acids 60 to 235, which was predicted by computer modelling to form a structure

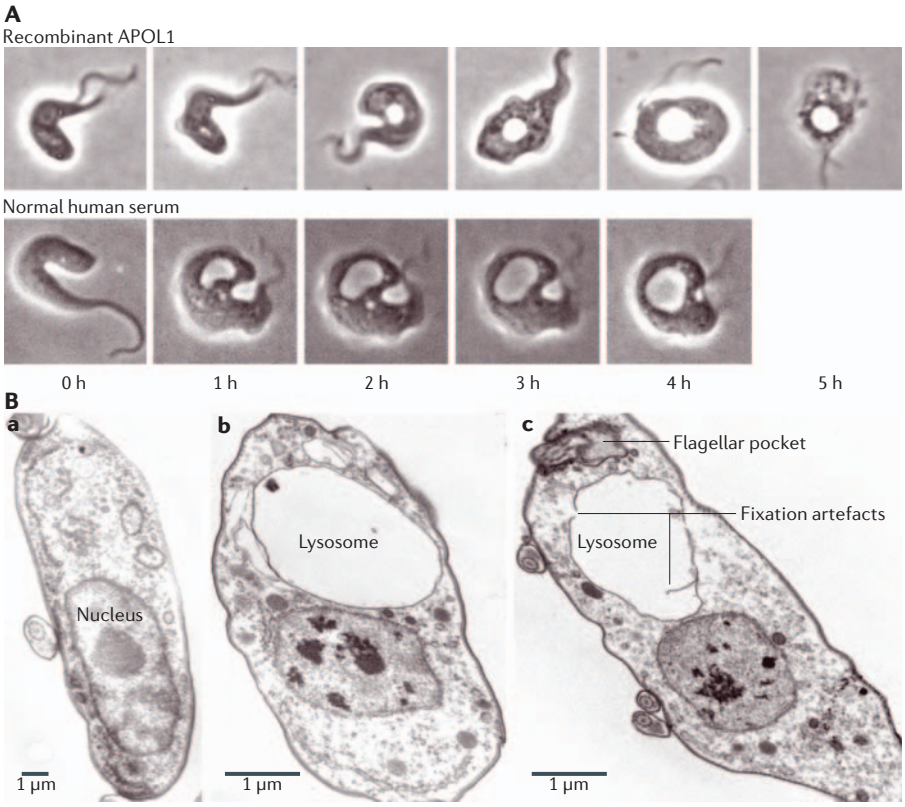

Figure 4 | **Phenotype of trypanosome lysis by human serum or by recombinant apolipoprotein L1 (APOL1). A** | Micrographs show the expansion of the lysosome of *Trypanosoma brucei brucei* over time in the presence of 1 μg per ml recombinant APOL1 or 10% normal human serum. Lower panels are reproduced with permission from REF. 64 © (2005) American Association for the Advancement of Science. **B** | Transmission electron micrographs show fixed *T. b. brucei* that either were not exposed to human serum (**a**) or were exposed to human serum for 1.5 hours (**b**,**c**). In control cells (**a**), the lysosome is small and dispersed, and cannot be seen clearly. In treated cells (**b**,**c**), the swollen lysosome is indicated. Fixation artefacts, which have led to the suggestion that the lysosomal membrane is disrupted during lysis[16], are also shown (**c**). Quantitative measurements of lysosomal surface area and cytoplasmic surface area over time are shown in Supplementary information S1 (figure)). Both the swelling of the lysosome and the absence of general cellular swelling can be observed in Supplementary information S2 (movie).

that resembles the pore-forming domain of colicins (FIG. 3a,b), showed bactericidal activity with a marked similarity to that of colicins and generated anionic pores in synthetic lipid-bilayer membranes. This pore-forming domain of APOL1 was found to be adjacent to a pH-sensitive membrane-addressing domain, which was predicted to bind HDL particles at neutral pH only (that is, in the blood but not in the late endosomes or the lysosome)[64] (FIG. 3a,c). Both domains were required for the toxic activity of APOL1 *in vivo*, against bacteria and against trypanosomes. By contrast, the C-terminal α-helix of APOL1 (which interacts with SRA and is required for resistance to lysis) was dispensable for toxic activity in both cases.

In trypanosomes, recombinant APOL1 was found to mimic completely the lytic activity of normal human serum, causing depolarization of the lysosomal membrane (that is, loss of the differential charge between the two faces of the membrane), followed by continuous swelling of the lysosome until the parasite cell lysed[64] (FIG. 4). In addition, APOL1 was detected at the periphery of the lysosome but not in the lumen[64]. Given the predicted structure of its membrane-addressing domain (FIG. 3c), APOL1 is thought to undergo a conformational change that allows its dissociation from HDL particles at low pH (such as in the late endosomes and the lysosome) and its subsequent insertion into the lysosomal membrane, where it forms a pore. On exposure to recombinant APOL1, similar to normal human serum, lysosomal swelling was found to be the first detectable morphological change, and as this process continued, there were no marked alterations in other intracellular structures (FIG. 4; see Supplementary information S1,S2 (figure and movie)), in particular no general vacuolization and cellular swelling (although this is in contrast to findings reported in REF. 65; discussed later).

Lysosomal swelling was found to be associated with the influx and intracellular accumulation of chloride ions (Cl^-). Both of these processes were blocked by either depletion of extracellular Cl^- or addition of the anion-channel blocker DIDS (4,4′-diisothiocyano-2,2′-disulphonate stilbene)[64]. Although DIDS completely inhibited the influx of Cl^- into trypanosomes and the subsequent lysis, it had no direct effect on the pore-forming activity of APOL1 *in vitro*, indicating that additional Cl^- channels are involved in trypanolysis. So, it was concluded that APOL1 triggers the lysosomal influx of Cl^- from the cytoplasm, where the concentration of this anion is high (106 mM)[66]. This movement would then reduce the cytoplasmic Cl^- concentration, activating the compensatory entry of extracellular Cl^- through DIDS-sensitive channels in the plasma membrane[64]. This model of the trypanolytic process is shown in FIG. 5. The cascade of Cl^- movement would be accompanied by the influx of water into the lysosome and osmotic swelling of this compartment. This accounts for the observed inhibition of trypanolysis by osmotically active molecules such as sucrose (the presence of which in the cytosol reduces the influx of water into the lysosome)[67]. The internal pressure resulting from the continuous enlargement of the lysosome would be responsible for the lysis of the parasite, because this pressure is expected to irreversibly damage the plasma membrane of the parasite.

This model also explains the observed fraying of the parasite surface coat and the leakage of ions that occur before lysis[67]. The finding that trypanosomes with considerably swollen lysosomes can survive for a period of time clearly conflicts with the idea that lysis involves disruption of the lysosomal membrane following lipid peroxidation, a mechanism that is still proposed by some researchers[16,20,24]. Indeed, the extreme swelling of the lysosome seems to be incompatible with a damaged lysosomal membrane. The main argument for lysosomal-membrane disruption is that discontinuities can be detected in the membrane, using electron microscopy[16]; however, these are probably the result of common fixation artefacts[68] (FIG. 4Bc).

A recent study proposed an alternative mechanism for trypanolysis: that lytic HDL particles generate cation-selective pores that are active at the plasma membrane after they have been recycled from the lysosome[69].

Given the cation concentration gradients at the plasma membrane (that is, extracellular to cytoplasmic concentration ratios of 140 mM/14 mM and 6 mM/116 mM for sodium ions (Na^+) and potassium ions (K^+), respectively[66]), these pores would allow an influx of Na^+ into the cytoplasm. However, in this study[69], the electrophysiological measurements were carried out on a crude population of lipoproteins that contained various pore-forming proteins and peptides, and this population generated complex ion currents, possibly owing to the formation of irrelevant (non-physiological) pores or the influx of ions through nonspecific channels.

In addition, although pore-forming activity was monitored under conditions that mimic the interface between the lysosomal and cytoplasmic compartments, its involvement in trypanolysis was entirely attributed to ionic fluxes produced at the plasma membrane, and we think that this conclusion is questionable for two main reasons. First, it is unlikely that plasma-membrane fluxes are relevant to the trypanolytic process that is induced by normal human serum, because we have found that this process is characterized by swelling of the lysosome but not of the cytoplasm[64] (FIG. 4; see Supplementary information S1, S2 (figure and movie)). This phenotype is in contrast to that observed using purified lytic HDL particles[65], indicating that the lysis that occurs in the presence of purified lytic HDL particles is likely to involve nonspecific toxicity. Second, the specificity of the pores for particular cations was not measured in the study reported in REF. 69. However, if the pores conduct Na^+ to a similar extent to which they seem to conduct K^+, then (because the Na^+ and K^+ gradients across the plasma membrane are approximately equal in magnitude but opposite in direction[66]) the most likely initial outcome would be the electroneutral exchange of the two cations, which would have no osmotic consequences. Therefore, in our opinion, the hypothesis that the trypanolytic factor generates cationic pores is not proven by the data in REF. 69, and pore formation at the plasma membrane does not account for the phenotype of trypanolysis by normal human serum.

Identification of the trypanolytic factor of human serum: APOL1 or HPR? Initially, HPR was considered to be the trypanolytic factor of human serum, and recent papers present evidence for why this protein, in addition to APOL1, is a trypanolytic factor[9,69,70]. This view is based, in part, on the observation that serum from baboons is trypanolytic and contains HPR but not APOL1 (REFS 8,9). However, serum from these primates kills both human-serum-sensitive and human-serum-resistant *T. b. rhodesiense* clones, and it seems to have considerably more lytic activity than does human serum[6,8,9]. In our opinion, these observations indicate that the trypanolytic activity of baboon serum differs from the trypanolytic activity of human serum and that HPR cannot be the common trypanolytic factor in both cases, because HPR does not lyse human-serum-resistant *T. b. rhodesiense*. It is also argued that HPR is a trypanolytic factor because HPR-specific antibodies inhibit the activity of lytic HDL particles[9,24,70]. These experiments, however, involved lipoprotein complexes that were not dissociated with detergent. In addition, it has been reported that antibodies specific for APOA1 (a protein that does not have trypanolytic activity[28–30]) inhibit lysis[70]. So, one possible explanation for the inhibitory activity of HPR-specific antibodies is that antibodies specific for any exposed constituent of HDL particles can inhibit lysis through a nonspecific mechanism, perhaps by aggregation of HDL particles or by interference in the endocytic process.

In their recent study, Hajduk and colleagues[70] propose that both HPR and APOL1 are trypanolytic but that these proteins need to be associated in the same HDL particle for maximal activity. This proposal is based on trypanolytic assays carried out using purified HPR and APOL1 or using human HDL particles fractionated into particles that contain both HPR and APOL1, either protein alone or neither protein. In the first case (using purified proteins), toxicity was

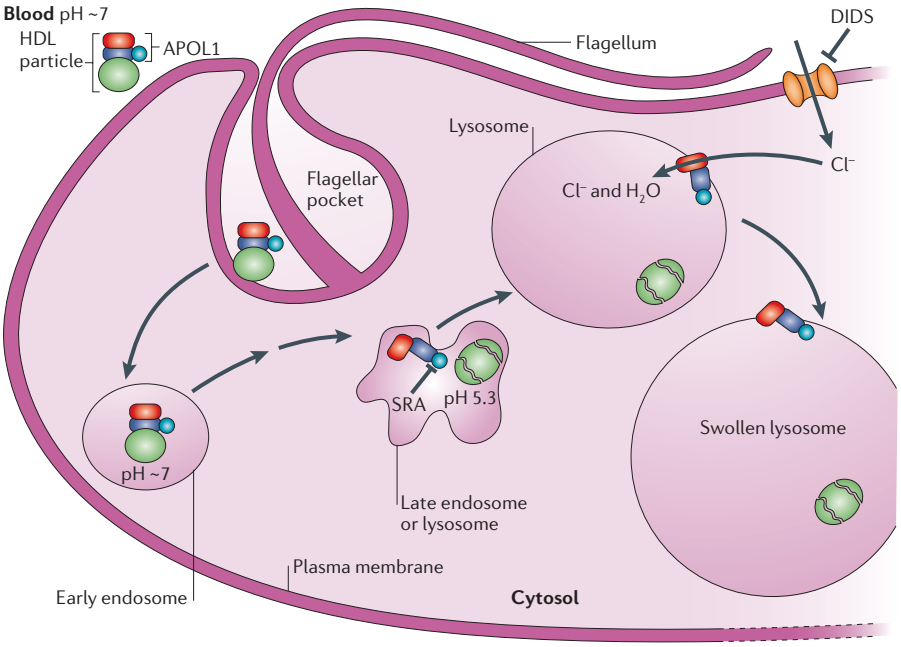

Figure 5 | **Model of the mechanism of trypanolysis by apolipoprotein L1 (APOL1).** APOL1 contains a pore-forming domain (red) and a membrane-addressing domain (blue). It is associated with high-density lipoprotein (HDL) particles (green) that are present in the blood. These particles are internalized by bloodstream-form trypanosomes through HDL-receptor-mediated endocytosis that is initiated in the flagellar pocket. Following internalization, HDL particles progress through the endocytic pathway, from early endosomes (which have neutral pH) to late endosomes (which are acidic) and then to the lysosome (which is also acidic). We think that most data indicate that trypanolysis occurs by the following process. The lysosomal pH induces a conformational change in the membrane-addressing domain of APOL1, leading to dissociation from the HDL particle and binding to the lysosomal membrane. The pore-forming domain of APOL1 enables it to form a pore in the membrane, and this leads to the flux of chloride ions (Cl^-) from the cytoplasm to the lumen of the lysosome. Cytoplasmic Cl^- deprivation is compensated by the activity of a DIDS (4,4′-diisothiocyano-2,2′-disulphonate stilbene)-sensitive Cl^- transporter in the plasma membrane. This ionic flux is presumably accompanied by secondary cationic movements[64,67,69], and it triggers the movement of water (H_2O) into the lysosome and osmotic swelling of this compartment. The resultant uncontrolled swelling of the lysosome increases intracellular pressure, probably leading to damage to the plasma membrane (dashed line), which is ultimately the cause of cell death. The *Trypanosoma brucei rhodesiense* protein serum-resistance associated (SRA) interacts with APOL1 in late endosomes and the lysosome, and this prevents APOL1 from forming pores, possibly through sequestration of APOL1 followed by proteolytic degradation.

Box 2 | **The function of apolipoproteins (APOLs)**

In evolutionary terms, *APOL1* appeared recently, as it has been identified only in humans and gorillas[8,75]. It belongs to a multigene family that has six members[76,77], and homologous sequences are present in other eukaryotes (particularly in other mammals). Among APOL1-like proteins, the sequence of the pore-forming domain is similar, whereas that of the membrane-addressing domain is more variable; the C-terminal helix is the most conserved region (see Supplementary information S3 (figure)). Unlike other APOL1-family members, APOL1 is secreted (probably because of the presence of a signal peptide), but sequestration in high-density lipoprotein (HDL) particles presumably neutralizes its activity in serum. Other APOL-family members are most probably intracellular proteins. Given the pore-forming activity of APOL1 (REF. 64), intracellular APOLs could be ion channels in organelles, such as the lysosome, and might thereby control the volume of these organelles. The potential of the C-terminal helix of APOL1 to interact with the *Trypanosoma brucei rhodesiense* protein serum -resistance associated (SRA) and thereby neutralize APOL1 activity, together with the evidence that this helix is dispensable for trypanolytic activity despite its high level of sequence conservation[54,64], raises the interesting possibility that this region controls the ionic-pore-forming activity of APOLs through interaction with proteins that have SRA-like helices.

It is tempting to propose that the generation of a secreted version of these pore-forming proteins in the great apes of Africa was linked to the presence of trypanosomes in this region and that it was selected because of its trypanolytic activity. However, the conservation of *APOL1* in humans (despite the wide geographical dispersion of humans) does not support this idea but indicates that APOL1 fulfils additional functions. In this regard, it is interesting that the expression of APOL1, APOL2 and APOL3 is strongly induced by the pro-inflammatory cytokine tumour-necrosis factor[75,78] and that overexpression of APOL6 triggers apoptosis, presumably through a BH3 domain (B-cell lymphoma 2 (BCL-2)-homology domain 3)[79]. So, APOLs might be involved in various cellular responses to danger signals.

Pore-forming proteins and cell death

The ability of pore-forming proteins to trigger cell death can be illustrated by several well-known examples — bacterial colicins, diphtheria toxin and apoptotic proteins from the BCL-2 family — although different mechanisms are involved in the cell death that is mediated by these three types of protein (reviewed in REF. 80). Despite a low level of sequence homology, these proteins all have pore-forming domains with a similar structure: that is, several α-helices organized around a conserved hydrophobic hairpin. Computer modelling and experimental data indicate that the pore-forming domain of APOL1 also belongs to this category[64] (FIG. 3). As is the case for BCL-2-family members, the pore-forming domain of APOL1 might have been inherited from bacteria.

HDL particles and innate immunity

The association of APOL1 with HDL particles is likely to occur through hydrophobic interactions that are mediated by the membrane-addressing domain at neutral pH[64] (FIG. 3). The presence of pore-forming microbicidal components in HDL particles is not an unprecedented finding. Various APOLs that are associated with HDL particles can show bactericidal activity[81]. In addition, defensins and cathelicidins, which are pore-forming antibacterial peptides, also seem to be associated with lipoproteins in serum[82]. The anchoring of microbicidal proteins in lipoprotein particles would ensure the sequestration of these proteins when their activity is not required, thereby preventing cytotoxic effects while maintaining high serum concentrations. Therefore, lipoproteins could have a role as carriers for components of the innate immune system[21,70].

detected following direct incubation of trypanosomes with high concentrations of either protein. However, we argue that these data cannot be fully interpreted without information relating to the phenotype of lysis. For example, when the antimicrobial peptides cathelicidins are purified from lipoprotein complexes, they can kill trypanosomes *in vitro* through disruption of the plasma membrane[71]; however, there is no evidence that these cathelicidins are active against trypanosomes *in vivo*. In the second case (using fractionated HDL particles), almost 100% of the trypanolytic activity was recovered in the HDL-particle fraction that contained both HPR and APOL1, whereas less than 0.4% of the trypanolytic activity was detected in fractions that contained either APOL1 or HPR. We think that the small trypanolytic effect of these HDL particles that contain undefined amounts of either HPR or APOL1 could be a result of non-specific toxicity conferred by the experimental treatment. Indeed, fractionation does seem to confer non-physiological toxicity, because the phenotype of trypanolysis by fractionated HDLs differed from that of human serum and involved generalized vacuolization and cellular swelling[65]. Furthermore, fractionation resulted in a higher trypanolytic activity than that naturally present in serum[31,33], and it rendered chimpanzee HDL particles trypanolytic[9]. One possibility is that HDL-particle fractionation activates microbicidal peptides, including cathelicidins[71]. A definitive assessment of this idea could be made by determining the effect of fractionated HDL particles on trypanosomes that are naturally resistant to human serum.

In summary, we outline the following five arguments in support of our contention that APOL1 is the only trypanolytic factor of human serum. First, the addition of recombinant APOL1 to fetal calf serum, which lacks HPR, rendered the serum trypanolytic, and the phenotype of lysis was identical to that observed for human serum[54,64]. It should be noted that free APOL1 (that is, APOL1 not associated with HDL particles), whether native or recombinant protein, seems to have less trypanolytic activity than human serum[64,70], and this finding could be interpreted in two ways. The reduced activity of free APOL1 might result from a lack of synergy with HPR[69,70]: that is, both APOL1 and HPR are required for maximal trypanolytic activity. Alternatively, it is our contention that the reduced activity of free APOL1 reflects a requirement for APOL1 to be associated with HDL particles for efficient binding and uptake by the HDL receptor of the parasite[11,23]. Indeed, kinetic studies indicated that free recombinant APOL1 seemed to be as effective as human serum at lysis of trypanosomes, except that lysis was delayed[64]. Accordingly, trypanolysis by recombinant APOL1 was strongly accelerated on reconstitution of APOL1 in lipoproteins[54,64]. Therefore, we think that the activity of APOL1 does not require synergy with HPR. *APOL1*-transgenic mice would be useful for evaluating the *in vivo* efficiency of trypanosome killing by APOL1 alone, but such mice are not available at present. Injection of recombinant APOL1 into wild-type mice, however, was shown to be necessary and sufficient for complete inhibition of infection with *T. b. brucei*[72]. It remains to be determined whether similar experiments with *HPR*-transgenic mice would show increased parasite killing by APOL1.

Second, the presence of HPR in APOL1-free serum did not trigger trypanolysis and did not affect trypanosome growth. This was observed *in vivo* for transgenic mice that expressed HPR at a level similar to that of humans[73], as well as for human serum that was specifically depleted of APOL1 (through separation of the proteins present in detergent-dissociated HDL particles by affinity chromatography on SRA)[54,64].

Third, depletion of APOL1 from human serum without removal of HPR (as above) led to a complete loss of trypanolytic activity[54,64]. The converse experiment — selective depletion of HPR without removal of APOL1— has not yet been carried out.

Fourth, trypanolysis did not occur when recombinant APOL1 that was mutated in either the pore-forming domain or the membrane-addressing domain was added to serum specifically depleted of APOL1, even if HPR was present[64]. Conversely, recombinant APOL1 that lacked the C-terminal (SRA-interacting) helix was necessary and sufficient to kill *T. b. rhodesiense* independent of HPR[54,72].

Fifth, expression of SRA alone conferred complete resistance to either human serum or recombinant APOL1. This finding can be explained by the interaction between SRA and APOL1 (REF. 54), and there is no evidence that SRA could interfere with HPR. For example, no trace of HPR has been found in the APOL1-containing serum fraction that binds SRA affinity columns in the presence of detergent (discussed earlier)[54].

We propose, therefore, that APOL1 is responsible for most, if not all, of the trypanolytic activity that is associated with human serum. In our opinion, the idea that APOL1 operates synergistically with HPR for maximal trypanolytic activity in the context of a subclass of HDL particles that contains APOL1, HPR and APOA1 is not supported by the available evidence.

Conclusions and future directions

Despite considerable progress, many questions that relate to the trypanolytic activity of human serum remain to be answered. In addition, the physiological function of APOL1 and other APOLs remains unclear (BOX 2; see Supplementary information S3 (figure)). At present, the most important points that require further investigation are the identity of the trypanosome receptor for the trypanolytic HDL particles, the basis of resistance of *T. b. gambiense* to APOL1 and the putative involvement of HPR in the mechanism of trypanosome killing by APOL1. To resolve the last issue, a crucial experiment will be to compare the trypanosome-infection characteristics of mice that are transgenic for *HPR*, *APOL1* or both genes. So far, however, attempts to generate *APOL1*-transgenic mice have not been successful.

Recent advances in this field of research include a breakthrough in the diagnosis of sleeping sickness, for which the presence of *SRA* has proved to be a reliable marker of infection with *T. b. rhodesiense*[47–51], and the use of APOL1 as a treatment that might cure the disease. As discussed in this article, when APOL1 is not bound to HDL particles, it kills trypanosomes but with delayed kinetics compared with human serum[64]. So, to convert APOL1 into a drug that is effective against trypanosomes that infect humans, two modifications have been carried out. First, the C-terminal region, which is recognized by SRA, has been removed. Second, this truncated APOL1 molecule has been fused to an antibody module (known as a nanobody) that is derived from single-chain camel antibodies, thereby targeting the toxin (APOL1) to invariant determinants (high-mannose side chains) of the trypanosome surface[74]. The efficacy of trypanolysis by this protein construct was shown in mice: injection of the modified APOL1 resulted in inhibition of parasitaemia caused by either *T. b. brucei* or *T. b. rhodesiense*[72].

APOL1 might also be useful for solving the problem of nagana, a lethal disease that is caused by infection of cattle with *T. b. brucei*. Transgenic cattle expressing APOL1 derivatives such as the truncated-APOL1–nanobody conjugate would be expected to resist infection by *T. b. brucei* and *T. b. rhodesiense*, because this truncated trypanolytic factor cannot be neutralized by the *T. b. rhodesiense* 'antidote' SRA. As well as allowing healthy animal production in areas in which *T. b. rhodesiense* is endemic, the use of such 'trypanolytic cattle' should lead to a marked reduction in the main reservoir of *T. b. rhodesiense*, thereby contributing to the prevention of epidemics of sleeping sickness.

Etienne Pays, Benoit Vanhollebeke, Luc Vanhamme, Françoise Paturiaux-Hanocq and David Pérez-Morga are at the Laboratory of Molecular Parasitology, Institute of Molecular Biology and Medicine (IBMM), Université Libre de Bruxelles, 12 rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium.

Derek P. Nolan is at the Department of Biochemistry, Trinity College, Dublin 2, Ireland.

Correspondence to E.P.
e-mail: epays@ulb.ac.be

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1. Blum, M. L. *et al*. A structural motif in the variant surface glycoproteins of *Trypanosoma brucei*. *Nature* **362**, 603–609 (1993).
2. Cross, G. A. M. Antigenic variation in trypanosomes. *Proc. R. Soc. Lond. B* **202**, 55–72 (1978).
3. Barry, J. D. The relative significance of mechanisms of antigenic variation in African trypanosomes. *Parasitol. Today* **13**, 212–218 (1997).
4. Borst, P. Antigenic variation and allelic exclusion. *Cell* **109**, 5–8 (2002).
5. Pays, E., Vanhamme, L. & Pérez-Morga, D. Antigenic variation in *Trypanosoma brucei*: facts, challenges and mysteries. *Curr. Opin. Microbiol.* **7**, 369–374 (2004).
6. Laveran, A. & Mesnil, F. in *Trypanosomes et Trypanosomiases* 126–183 (Libraires de l'Académie de Médecine, Paris, 1912) (in French).
7. Seed, J. R., Sechelski, J. B. & Loomis, M. R. A survey for a trypanocidal factor in primate sera. *J. Protozool.* **37**, 393–400 (1990).
8. Poelvoorde, P., Vanhamme, L., Van Den Abbeele, J., Switzer, W. M. & Pays, E. Distribution of apolipoprotein L-I and trypanosome lytic activity among primate sera. *Mol. Biochem. Parasitol.* **134**, 155–157 (2004).
9. Lugli, E. B., Pouliot, M., Portela, M. P., Loomis, M. R. & Raper, J. Characterization of primate trypanosome lytic factors. *Mol. Biochem. Parasitol.* **138**, 9–20 (2004).
10. Rifkin, M. R. Identification of the trypanocidal factor in normal human serum: high density lipoprotein. *Proc. Natl Acad. Sci. USA* **75**, 3450–3454 (1978).
11. Rifkin, M. R. Role of phospholipids in the cytotoxic action of high density lipoprotein on trypanosomes. *J. Lipid Res.* **32**, 639–647 (1991).
12. Hajduk, S. L. *et al*. Lysis of *Trypanosoma brucei* by a toxic subspecies of human high-density lipoprotein. *J. Biol. Chem.* **264**, 5210–5217 (1989).
13. Gillett, M. P. & Owen, J. S. *Trypanosoma brucei brucei*: differences in the trypanocidal activity of human plasma and its relationship to the level of high density lipoproteins. *Trans. R. Soc. Trop. Med. Hyg.* **85**, 612–616 (1991).
14. Gillett, M. P. & Owen, J. S. Comparison of the cytolytic effects *in vitro* on *Trypanosoma brucei brucei* of plasma, high density lipoproteins, and apolipoprotein A-I from hosts both susceptible (cattle and sheep) and resistant (human and baboon) to infection. *J. Lipid Res.* **33**, 513–523 (1992).
15. Lorenz, P., Owen, J. S. & Hassall, D. G. Human serum resistant *Trypanosoma brucei rhodesiense* accumulates similar amounts of fluorescently-labelled trypanolytic human HDL3 particles as human serum sensitive *T. b. brucei*. *Mol. Biochem. Parasitol.* **74**, 113–118 (1995).
16. Hager, K. M. *et al*. Endocytosis of a cytotoxic human high density lipoprotein results in disruption of acidic intracellular vesicles and subsequent killing of African trypanosomes. *J. Cell Biol.* **126**, 155–167 (1994).
17. Lorenz, P., Barth, P. E., Rudin, W. & Betschart, B. Importance of acidic intracellular compartments in the lysis of *Trypanosoma brucei brucei* by normal human serum. *Trans. R. Soc. Trop. Med. Hyg.* **88**, 487–488 (1994).
18. Ortiz-Ordonez, J. C., Sechelski, J. B. & Seed, J. R. Mechanism of lysis of *Trypanosoma brucei gambiense* by human serum. *J. Parasitol.* **80**, 924–930 (1994).
19. Hager, K. M. & Hajduk, S. L. Mechanism of resistance of African trypanosomes to cytotoxic human HDL. *Nature* **385**, 823–826 (1997).
20. Shimamura, M., Hager, K. M. & Hajduk, S. L. The lysosomal targeting and intracellular metabolism of trypanosome lytic factor by *Trypanosoma brucei brucei*. *Mol. Biochem. Parasitol.* **115**, 227–237 (2001).
21. Vanhamme, L. & Pays, E. The trypanosome lytic factor of human serum and the molecular basis of sleeping sickness. *Int. J. Parasitol.* **34**, 887–898 (2004).
22. Garcia-Salcedo, J. A. *et al*. A differential role for actin during the life cycle of *Trypanosoma brucei*. *EMBO J.* **23**, 780–789 (2004).
23. Green, H. P., del Pilar Molina Portela, M., St Jean, E. N., Lugli, E. B. & Raper, J. Evidence for a *Trypanosoma brucei* lipoprotein scavenger receptor. *J. Biol. Chem.* **278**, 422–427 (2003).
24. Smith, A. B, Esko, J. D. & Hajduk, S. L. Killing of trypanosomes by the human haptoglobin-related protein. *Science* **268**, 284–286 (1995).
25. Raper, J., Nussenzweig, V. & Tomlinson, S. The main lytic factor of *Trypanosoma brucei brucei* in normal human serum is not high density lipoprotein. *J. Exp. Med.* **183**, 1023–1029 (1996).
26. Tomlinson, S., Muranjan, M., Nussenzweig, V. & Raper, J. Haptoglobin-related protein and apolipoprotein AI are components of the two trypanolytic factors in human serum. *Mol. Biochem. Parasitol.* **86**, 117–120 (1997).
27. Muranjan, M., Nussenzweig, V. & Tomlinson, S. Characterization of the human serum trypanosome toxin, haptoglobin-related protein. *J. Biol. Chem.* **273**, 3884–3887 (1998).
28. Rifkin, M. R. *Trypanosoma brucei*: cytotoxicity of host high-density lipoprotein is not mediated by apolipoprotein A-I. *Exp. Parasitol.* **72**, 216–218 (1991).
29. Owen, J. S., Gillett, M. P. & Hughes, T. E. Transgenic mice expressing human apolipoprotein A-I have sera with modest trypanolytic activity *in vitro* but remain susceptible to infection by *Trypanosoma brucei brucei*. *J. Lipid Res.* **33**, 1639–1646 (1992).
30. Tomlinson, S. *et al*. High-density-lipoprotein-independent killing of *Trypanosoma brucei* by human serum. *Mol. Biochem. Parasitol.* **70**, 131–138 (1995).

31. Smith, A. B. & Hajduk, S. L. Identification of haptoglobin as a natural inhibitor of trypanocidal activity in human serum. *Proc. Natl Acad. Sci. USA* **92**, 10262–10266 (1995).
32. Raper, J., Nussenzweig, V. & Tomlinson, S. Lack of correlation between haptoglobin concentration and trypanolytic activity of normal human serum. *Mol. Biochem. Parasitol.* **76**, 337–338 (1996).
33. Raper, J., Fung, R., Ghiso, J., Nussenzweig, V. & Tomlinson, S. Characterization of a novel trypanosome lytic factor from human serum. *Infect. Immun.* **67**, 1910–1916 (1999).
34. Barker, C., Barbour, K. W., Berger, F. G. & Hajduk, S. L. Activity of human trypanosome lytic factor in mice. *Mol. Biochem. Parasitol.* **117**, 129–136 (2001).
35. McEvoy, S. M. & Maeda, N. Complex events in the evolution of the haptoglobin gene cluster in primates. *J. Biol. Chem.* **263**, 15740–15747 (1988).
36. Drain, J., Bishop, J. R. & Hajduk, S. L. Haptoglobin-related protein mediates trypanosome lytic factor binding to trypanosomes. *J. Biol. Chem.* **276**, 30254–30260 (2001).
37. Bishop, J. R., Shimamura, M. & Hajduk, S. L. Insight into the mechanism of trypanosome lytic factor-1 killing of *Trypanosoma brucei brucei*. *Mol. Biochem. Parasitol.* **118**, 33–40 (2001).
38. Molina Portela, M. P., Raper, J. & Tomlinson, S. An investigation into the mechanism of trypanosome lysis by human serum factors. *Mol. Biochem. Parasitol.* **110**, 273–282 (2000).
39. Van Meirvenne, N., Magnus, E. & Janssens, P. G. The effect of normal human serum on trypanosomes of distinct antigenic type (ETat 1 to 12) isolated from a strain of *Trypanosoma brucei rhodesiense*. *Ann. Soc. Belg. Med. Trop.* **56**, 55–63 (1976).
40. Xong, H. V. *et al*. A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* **95**, 839–846 (1998).
41. Pays, E., Lips, S., Nolan, D., Vanhamme, L. & Pérez-Morga, D. The VSG expression sites of *Trypanosoma brucei*: multipurpose tools for the adaptation of the parasite to mammalian hosts. *Mol. Biochem. Parasitol.* **114**, 1–16 (2001).
42. De Greef, C., Imberechts, H., Matthyssens, G., Van Meirvenne, N. & Hamers, R. A gene expressed only in serum-resistant variants of *Trypanosoma brucei rhodesiense*. *Mol. Biochem. Parasitol.* **36**, 169–176 (1989).
43. De Greef, C. & Hamers, R. The serum resistance-associated (*SRA*) gene of *Trypanosoma brucei rhodesiense* encodes a variant surface glycoprotein-like protein. *Mol. Biochem. Parasitol.* **68**, 277–284 (1994).
44. Campillo, N. & Carrington, M. The origin of the serum resistance associated (*SRA*) gene and a model of the structure of the SRA polypeptide from *Trypanosoma brucei rhodesiense*. *Mol. Biochem. Parasitol.* **127**, 79–84 (2003).
45. Vanhamme, L. *et al*. The *Trypanosoma brucei* reference strain TREU927/4 contains *T. b. rhodesiense*-specific *SRA* sequences, but displays a distinct phenotype of relative resistance to human serum. *Mol. Biochem. Parasitol.* **135**, 39–47 (2004).
46. Milner, J. D. & Hajduk, S. L. Expression and localization of serum resistance associated protein in *Trypanosoma brucei rhodesiense*. *Mol. Biochem. Parasitol.* **104**, 271–283 (1999).
47. Welburn, S. C. *et al*. Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (*SRA*) gene. *Lancet* **358**, 2017–2019 (2001).
48. Gibson, W., Backhouse, T. & Griffiths, A. The human serum resistance associated gene is ubiquitous and conserved in *Trypanosoma brucei rhodesiense* throughout East Africa. *Infect. Genet. Evol.* **1**, 207–214 (2002).
49. Radwanska, M. *et al*. The serum resistance-associated gene as a diagnostic tool for the detection of *Trypanosoma brucei rhodesiense*. *Am. J. Trop. Med. Hyg.* **67**, 684–690 (2002).
50. Gibson, W. & Ferris, V. Conservation of the genomic location of the human serum resistance associated gene in *Trypanosoma brucei rhodesiense*. *Mol. Biochem. Parasitol.* **130**, 159–162 (2003).
51. Gibson, W. C. The *SRA* gene: the key to understanding the nature of *Trypanosoma brucei rhodesiense*. *Parasitology* **131**, 143–150 (2005).
52. Vanhamme, L. *et al*. Differential RNA elongation controls the variant surface glycoprotein gene expression sites of *Trypanosoma brucei*. *Mol. Microbiol.* **36**, 328–340 (2000).
53. De Greef, C., Chimfwembe, E., Kihang'a Wabacha, J., Bajyana Songa, E. & Hamers, R. Only the serum-resistant bloodstream forms of *Trypanosoma brucei rhodesiense* express the serum resistance associated (SRA) protein. *Ann. Soc. Belg. Med. Trop.* **72** (Suppl. 1), 13–21 (1992).
54. Vanhamme, L. *et al*. Apolipoprotein L-I is the trypanosome lytic factor of human serum. *Nature* **422**, 83–87 (2003).
55. Triggs, V. P. & Bangs, J. D. Glycosylphosphatidylinositol-dependent protein trafficking in bloodstream stage *Trypanosoma brucei*. *Eukaryot. Cell* **2**, 76–83 (2003).
56. Wang, J., Böhme, U. & Cross, G. A. M. Structural features affecting variant surface glycoprotein expression in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* **128**, 135–145 (2003).
57. Duchateau, P. N. *et al*. Apolipoprotein L, a new human high density lipoprotein apolipoprotein expressed by the pancreas. Identification, cloning, characterization, and plasma distribution of apolipoprotein L. *J. Biol. Chem.* **272**, 25576–25582 (1997).
58. Mikkelsen, T. S. *et al*. Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* **437**, 69–87 (2005).
59. Oli, M. W., Cotlin, L. F., Shiflett, A. M. & Hajduk, S. L. Serum resistance-associated protein blocks lysosomal targeting of trypanosome lytic factor in *Trypanosoma brucei*. *Eukaryot. Cell* **5**, 132–139 (2006).
60. Moore, D. R. *et al*. Developmentally regulated sensitivity of *Trypanosoma brucei brucei* to the cytotoxic effects of human high-density lipoprotein. *Exp. Parasitol.* **81**, 216–226 (1995).
61. Brun, R. & Jenni, L. Human serum resistance of metacyclic forms of *Trypanosoma brucei brucei*, *T. brucei rhodesiense* and *T. brucei gambiense*. *Parasitol. Res.* **73**, 218–223 (1987).
62. Duchateau, P. N. *et al*. Plasma apolipoprotein L concentrations correlate with plasma triglycerides and cholesterol levels in normolipidemic, hyperlipidemic, and diabetic subjects. *J. Lipid Res.* **41**, 1231–1236 (2000).
63. Mimmack, M. L. *et al*. Gene expression analysis in schizophrenia: reproducible up-regulation of several members of the apolipoprotein L family located in a high-susceptibility locus for schizophrenia on chromosome 22. *Proc. Natl Acad. Sci. USA* **99**, 4680–4685 (2002).
64. Pérez-Morga, D. *et al*. Apolipoprotein L-I promotes trypanosome lysis by forming pores in lysosomal membranes. *Science* **309**, 469–472 (2005).
65. Raper, J., Molina Portela, M. P., Lugli, E., Frevert, U. & Tomlinson, S. Trypanosome lytic factors: novel mediators of innate immunity. *Curr. Opin. Microbiol.* **4**, 402–408 (2001).
66. Nolan, D. P. & Voorheis, H. P. Factors that determine the plasma-membrane potential in bloodstream forms of *Trypanosoma brucei*. *Eur. J. Biochem.* **267**, 4615–4623 (2000).
67. Rifkin, M. R. *Trypanosoma brucei*: biochemical and morphological studies of cytotoxicity caused by normal human serum. *Exp. Parasitol.* **58**, 81–93 (1984).
68. Cardoso de Almeida, M. L., Geuskens, M. & Pays, E. Cell lysis induces redistribution of the GPI-anchored variant surface glycoprotein on both faces of the plasma membrane of *Trypanosoma brucei*. *J. Cell Sci.* **112**, 4461–4473 (1999).
69. del Pilar Molina-Portela, M., Lugli, E. B., Recio-Pinto, E. & Raper, J. Trypanosome lytic factor, a subclass of high-density lipoprotein, forms cation-selective pores in membranes. *Mol. Biochem. Parasitol.* **144**, 218–226 (2005).
70. Shiflett, A. M., Bishop, J. R., Pahwa, A. K. & Hajduk, S. L. Human HDLs are platforms for the assembly of multi-component innate immune complexes. *J. Biol. Chem.* **280**, 32578–32585 (2005).
71. McGwire, B. S., Olson, C. L., Tack, B. F. & Engman, D. M. Killing of African trypanosomes by antimicrobial peptides. *J. Infect. Dis.* **188**, 146–152 (2003).
72. Baral, T. N. *et al*. Experimental therapy of African trypanosomiasis with a nanobody-conjugated human trypanolytic factor. *Nature Med.* **12**, 580–584 (2006).
73. Hatada, S. *et al*. No trypanosome lytic activity in the sera of mice producing human haptoglobin-related protein. *Mol. Biochem. Parasitol.* **119**, 291–294 (2002).
74. Stijlemans, B. *et al*. Efficient targeting of conserved cryptic epitopes of infectious agents by single domain antibodies. African trypanosomes as paradigm. *J. Biol. Chem.* **279**, 1256–1261 (2004).
75. Monajemi, H., Fontijn, R. D., Pannekoek, H. & Horrevoets, A. J. The apolipoprotein L gene cluster has emerged recently in evolution and is expressed in human vascular tissue. *Genomics* **79**, 539–546 (2002).
76. Page, N. M., Butlin, D. J., Lomthaisong, K. & Lowry, P. J. The human apolipoprotein L gene cluster: identification, classification, and sites of distribution. *Genomics* **74**, 71–78 (2001).
77. Duchateau, P. N., Pullinger, C. R., Cho, M. H., Eng, C. & Kane, J. P. Apolipoprotein L gene family: tissue-specific expression, splicing, promoter regions; discovery of a new gene. *J. Lipid Res.* **42**, 620–630 (2001).
78. Horrevoets, A. J. *et al*. Vascular endothelial genes that are responsive to tumor necrosis factor-α *in vitro* are expressed in atherosclerotic lesions, including inhibitor of apoptosis protein-1, stannin, and two novel genes. *Blood* **93**, 3418–3431 (1999).
79. Liu, Z., Lu, H., Jiang, Z., Pastuszyn, A. & Hu, C. A. Apolipoprotein L6, a novel proapoptotic Bcl-2 homology 3-only protein, induces mitochondria-mediated apoptosis in cancer cells. *Mol. Cancer Res.* **3**, 21–31 (2005).
80. Lazebnik, Y. Why do regulators of apoptosis look like bacterial toxins? *Curr. Biol.* **11**, R767–R768 (2001).
81. Concha, M. I. *et al*. Apolipoproteins A-I and A-II are potentially important effectors of innate immunity in the teleost fish *Cyprinus carpio*. *Eur. J. Biochem.* **271**, 2984–2990 (2004).
82. Sorensen, O., Bratt, T., Johnsen, A. H., Madsen, M. T. & Borregaard, N. The human antibacterial cathelicidin, hCAP-18, is bound to lipoproteins in plasma. *J. Biol. Chem.* **274**, 22445–22451 (1999).

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Competing interests statement

The authors declare no competing financial interests.

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