Review

CellPress

African trypanosome control in the insect vector and mammalian host

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The life cycle of African trypanosomes involves adaptations to the defense mechanisms of two completely different hosts, the insect vector *Glossina* and the mammalian host. This interplay ultimately determines host resistance and/or tolerance to parasite infection. In the tsetse fly, the immune deficiency (IMD)-regulated pathway, the scavenger receptor peptidoglycan-recognition protein LB (PGRP-LB), and the reactive oxygen species (ROS)-mediated response modulate the insect's capacity to transmit the parasite. In experimental mice, control of parasite burden and tissue pathogenicity relies on timely regulated interactions between myeloid cells exhibiting distinct activation states (M1 versus M2 type). Tsetse fly saliva and various trypanosome components including adenylate cyclases, DNA, a kinesin heavy chain, and variant surface glycoprotein (VSG) interfere with resistance and tolerance to infection.

African trypanosomosis outcome: when immune response matters

African trypanosomosis is a parasitic disease of medical and veterinary importance affecting mainly sub-Saharan Africa. The causative agents, the African trypanosomes, are hemoflagellate, blood-borne unicellular protozoans that are transmitted through the bite of tsetse fly species (*Glossina* spp.) and cause often-fatal diseases in various mammals. Only two species, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, are infectious to humans, causing human African trypanosomosis (HAT) (commonly called sleeping sickness). More than 98% of cases are caused by *T. b. gambiense* in West and Central Africa. The disease can last for months to years without major symptoms before the development of encephalitis. *T. b. rhodesiense* infection is found in East Africa and causes an acute disease with neurological complications appearing within weeks. These human-infecting trypanosomes have evolved mechanisms to resist killing by blood immune factors (Box 1). Although the number of new cases has dropped to <8000 in 2012 (with 20 000 estimated unreported/undiagnosed cases), HAT remains a neglected disease that requires further research on prevention, diagnosis, and treatment procedures to prevent its re-emergence [1,2]. In addition to human infections, animal

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Keywords: inflammation; liver; monocytes; macrophages; myeloid cells; tsetse fly.

1471-4922/

 http://dx.doi.org/10.1016/j.pt.2014.08.006

Glossary

Adenylate cyclase: enzyme converting ATP into cAMP. In African trypanosomes, several dozens of genes encode isoforms that share the structure of transmembrane receptor-like proteins exposed at the plasma membrane surface with a large, relatively variable extracellular domain and a unique conserved intracellular catalytic site.

Antimicrobial peptide (AMP): produced as part of the innate immune response to kill pathogens.

C–C chemokine receptor 2 (CCR2): receptor mainly expressed by myeloid cells with the main chemokine ligand CCL2. It causes emigration of Ly6C^{high} monocytes from the bone marrow, thus exerting a central role in the trafficking of these monocytes to inflammation sites.

Immune deficiency (IMD) pathway: a major signaling pathway of the insect immune response mainly activated by Gram-negative bacteria.

Monocytes: circulating cells generated from bone marrow-derived macrophage and DC precursors (MDPs), divided into subsets by their phenotype, function, and gene signature. In mice, the major Ly6C^{high}CX3CR1^{Int}CCR2^{high} monocyte subset is rapidly recruited to inflammation sites. The minor Ly6C^{low}CX3CR1^{high}CCR2^{-} monocyte subset patrols along the blood vessel endothelial surface, coordinating its repair. Ly6C^{low} monocytes may not represent *bona fide* monocytes but blood-resident macrophages [83]. The equivalents of mouse Ly6C^{high} and Ly6C^{low} monocytes in humans (and other vertebrates) are respectively the CD14^{+} monocytes (including a CD16^{+} and a CD16^{-} subset) and the CD14^{low}CD16^{+} monocytes.

Myeloid cells: leukocytes from the mononucleated phagocyte system (granulocytes and various monocyte, DC, and macrophage subsets) characterized by the common expression of the αM integrin CD11b and differing ontogeny [83]. M1-type/M2-type myeloid cells refer to a pathogenic insult-dependent continuum of activation states ranging from inflammation induction to tissue-healing function.

NADPH-oxidase (Nox) and the related dual oxidase (Duox): play various biological and pathogenic roles via regulated generation of ROS.

Peptidoglycan-recognition proteins (PGRPs): enable the host immune system to recognize bacteria by their unique cell-wall component peptidoglycan.

Resistance: ability of a host to limit the establishment of or eliminate parasites.

Tolerance: ability of a host to limit lesions resulting from the immune response mounted to limit parasite burden, thus remaining asymptomatic.

Variant surface glycoprotein (VSG): the main antigen of the African trypanosome cell surface. This elongated glycoprotein of about 60 kDa, organized in a tight network of 5 million homodimers all around the parasite, is bound perpendicular to the plasma membrane through GPI anchoring. On live trypanosomes, only surface peptidic loops can be recognized by the host. Antigenic variation results from continuous sequence changes in these loops following either gene conversion or transcriptional switching between VSG genes.

Box 1. Innate resistance mechanisms to HAT

Humans (and some other primates) have developed innate immune molecules, the trypanolytic factor (TLF) 1 and 2 complexes, that kill most trypanosome species. TLF-1 and TLF-2 contain haptoglobin-related protein (HPR) and apolipoprotein L1 (APOL1), which respectively allow TLF-1 to enter via the haptoglobin–hemoglobin receptor of the trypanosome (TbHpHbR) [93] and kill the parasite [94–98]. Under the acidic environment of the endocytic compartment, APOL1 is released from TLFs and inserts into endosomal membranes, where this protein forms ionic pores that initiate a cascade leading to parasite death [95,99,100]. Both human infective trypanosomes possess the capacity to escape the TLF lytic mechanism. *Trypanosoma brucei rhodesiense* expresses serum resistance-associated (SRA) protein, which neutralizes the lytic activity of APOL1 [95,101]. *Trypanosoma brucei gambiense* resists APOL1 toxicity by expressing *Trypanosoma gambiense*-specific glycoprotein (TgsGP), which stiffens endo/lysosomal membranes, presumably blocking the insertion of APOL1 into these membranes [102]. In addition, mutations in *T. b. gambiense* TbHpHbR and altered lysosomal pH lower the uptake of TLF-1 and accelerate the degradation of APOL1, respectively [102,103].

African trypanosomosis, caused by tsetse fly-transmitted *Trypanosoma congolense*, *Trypanosoma vivax* (also mechanically transmitted by tabanid and *Stomoxys* flies), and *T. b. brucei*, represents a major obstacle in the agricultural economics of sub-Saharan areas (the 'tsetse belt') with devastating effects on nutrition and public health. Equine and camel trypanosomosis, caused by sexually transmitted *Trypanosoma equiperdum* and mechanically transmitted *Trypanosoma evansi*, respectively, also contribute to the disease burden. Cattle are an important reservoir for the human-infective parasite *T. b. rhodesiense*. Due to their other means of transmission, *T. vivax*, *T. equiperdum*, and *T. evansi* also represent constraints on livestock outside the tsetse belt, in Africa, in Asia, the Middle East, and Central and South America, and in Australia and even Europe, and their geographical distribution is still evolving [3].

As pure extracellular parasites, African trypanosomes are continuously confronted with components of the insect and mammalian host innate and/or adaptive immune system. The actions of the hosts to reduce parasite burden define their relative resistance to infection (see Glossary) [4,5]. However, mounting resistance to infection comes at a cost of severe tissue damage caused by disproportionate immune responses. Lesions to the spleen, lymph nodes, myocardium, brain, kidneys, and liver are common in natural African trypanosomosis [6]. The ability of the host to limit the tissue pathogenic consequences of the disease is defined as tolerance to infection [4,5]. Therefore, to develop new treatment strategies, it is important to identify the components involved in host–parasite interactions while balancing the development of tissue pathogenicity.

Here we summarize knowledge of the immune mechanisms of resistance and tolerance to African trypanosome infection developed by natural insects (focusing on the tsetse fly) and experimental (murine) hosts. We also describe the immune-evasion molecules developed by African trypanosomes to survive without killing their hosts, thereby ensuring their effective transmission.

Resistance and tolerance of the insect host to African trypanosome infection

The adult tsetse fly shows high resistance to African trypanosomes (especially *Trypanosoma brucei* s.l.), which is reflected by low infection rates in experimental infections (<15%) and natural populations (<1%) [7,8]. Moreover, most flies are susceptible to trypanosome infection only shortly after their emergence, at their first blood meal [9]. Successful development of *T. congolense* and *T. brucei* sp. in this vector is crucial, because it is their sole mean of transmission to mammalian hosts. When ingested by the tsetse fly, trypanosomes have to pass through several developmental barriers localized in specific microenvironments of the alimentary tract and salivary glands [10–13]. During this journey, trypanosomes are challenged by the fly innate defense system, which pre-exists at a basic level in the adult fly and is induced systemically (in the fat body) as well as locally (epithelial response in the alimentary tract) by the ingested parasites (Figure 1). As indicated by genome sequencing, the tsetse fly possesses a well-developed innate immune system similar to that described for *Drosophila* [14,15]. The development of a fully functioning innate immune system in the mature adult tsetse fly depends on the establishment of a bacterial microbiome population in the preceding larval stage; in the freshly emerged adult fly, the immaturity of the immune system is responsible for the high susceptibility to trypanosome infection [16,17]. In particular, in the tsetse midgut, the peritrophic matrix regulates the timing of the immune induction following parasite challenge [18]. Starved young and adult tsetse flies show lower resistance to trypanosome infection coinciding with reduced immune responsiveness in these flies [19].

The IMD pathway and PGRP-LB control trypanosome infection

A key experiment demonstrating the modulating role of the tsetse fly innate immune system in trypanosome infection is that bacterial stimulation of the fly IMD pathway before a trypanosome-containing blood meal increases the fly's resistance to the establishment of a parasite midgut infection. Differential regulation of antimicrobial peptide (AMP) genes (*defensin*, *attacin*, *cecropin*) occurs after stimulation by bacteria or by different trypanosome stages, indicating that the fly immune system can discriminate between different pathogen molecular signals [20]. Similarly, following trypanosome challenge in adult parasite-resistant tsetse flies, higher expression levels of genes associated with IMD pathway-related immunity (*attacin*, *pgrp-lb*) are observed [18]. RNAi-mediated suppression of the *attacin* gene or the IMD transcriptional activator gene *relish*, which regulates in tsetse flies the expression of *attacin* and *cecropin*, increases the establishment of trypanosome infection in the tsetse midgut, suggesting that IMD-regulated AMPs contribute to trypanosome resistance in the tsetse fly midgut [21]. Nothing is known about the parasite-associated components or tsetse fly recognition proteins involved in triggering this IMD pathway. Microbe detection and the subsequent response of the insect innate immune system is a multistep process that requires direct contact between insect pattern-recognition