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Review Article

Mouse models for pathogenic African trypanosomes: unravelling the immunology of host–parasite–vector interactions

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SUMMARY

African trypanosomiasis is a parasitic disease that affects a variety of mammals, including humans, on the sub-Saharan African continent. To understand the diverse parameters that govern the host–parasite–vector interactions, mouse models for the disease have proven to be a cornerstone. Despite the fact that most trypanosomes cannot be considered natural pathogens for rodents, experimental infections in mice have shed a tremendous amount of light on the general biology of these parasites and their interaction with and evasion of the mammalian immune system. Different aspects including inflammation, vaccine failure, antigenic variation, resistance/sensitivity to normal human serum and the influence of tsetse compounds on parasite transmission have all been addressed using mouse models. In more recent years, the introduction of various 'knock-out' mouse strains has allowed to analyse the implication of various cytokines, particularly TNF, IFNγ and IL-10, in the regulation of parasitaemia and induction of pathological conditions during infection.

Keywords *infection, mouse model, trypanosomiasis, vector biology*

INTRODUCTION

African trypanosomiasis affects humans as well as a broad range of other mammals on the sub-Sahara African continent. The causative agents of the disease are extracellular protozoan parasites of the genus *Trypanosoma*, including two *Trypanosoma* (*Trypanozoon*) *brucei* subspecies that are capable of infecting humans. Human African trypanosomiasis (HAT), or sleeping sickness, is caused by *T. b. gambiense* in 95% of the cases, while *T. b. rhodesiense* is only responsible for 5% of all human infections (1). The transmission cycle of these human pathogens nearly entirely depends on the tsetse fly (*Glossina* sp.) in which the parasite fulfils a complex life cycle that ends in the salivary glands (2). Subsequently, these parasites are transmitted by infected tsetse flies during the blood-feeding process. For *T. b. gambiense*, humans are the most important reservoir, while *T. b. rhodesiense* is a zoonotic parasite with a predominant animal reservoir. Other trypanosomes, particularly *T.* (*Nannomonas*) *congolense* and *T.* (*Duttonella*) *vivax* and to a lesser extent *T. b. brucei*, *T.* (*Trypanozoon*) *evansi* and *T.* (*Trypanozoon*) *equiperdum*, have to be considered important pathogens for the sub-Saharan livestock herd (3). Relevant for the life cycle, several trypanosome species also rely on mechanical transmission by blood-sucking insects other than tsetse flies (e.g. *Tabanus* and *Stomoxys* sp.) or on completely alternate routes, exemplified by the sexually transmitted *T. equiperdum*. To study the causes of pathogenicity of the various trypanosomes species, research of the last decades has been mainly focussed on mouse models for trypanosomiasis. Most of these studies have been conducted with *T. b. brucei*, *T. b. rhodesiense* and *T. congolense* parasites. Only a limited number of immunological studies with *T. evansi* (4,5), *T. vivax* (6,7), *T. equiperdum* (8) and *T. b. gambiense* (9) have been described in mice. In general, all models for trypanosomiasis have to be considered valuable, provided that they are interpreted in the correct context. Even comparative studies between highly virulent *T. brucei* and much less pathogenic *T. congolense* parasites can lead to

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interesting conclusions as outlined elsewhere in this article. However, it has to be reminded that *T. congolense* displays a mammalian infection cycle that is restricted to the circulation, while *T. brucei* readily invades tissues (remaining extracellular), including the brain (10,11). Hence, *T. brucei* has evolved a radically different/additional way to escape the host's immune response. It is clear that mouse models have their limitations and artefacts, such as extremely high parasitaemia levels as compared with those found in natural hosts, but, nevertheless, these models have contributed significantly to our current understanding of trypanosomiasis as a disease.

MOUSE MODELS AS A TOOL TO STUDY THE HUMORAL RESPONSES AGAINST TRYPANOSOMIASIS

To date, African trypanosomes are considered a preferred model organism to study antigenic variation. This is a mechanism of organized surface coat switching that allows these extracellular parasites to continuously escape the host adaptive immune system (12). However, recent data suggest that trypanosomes also actively undermine the adaptive response by causing rapid depletion of the B-cell compartment (13,14).

Antigenic variation was first reported using an *in vitro* antibody/complement-mediated lysis assay, showing that parasites have the capacity to alter their properties and escape elimination (15,16). Soon after, it was realized that the trypanosome genome encompassed several hundreds of genes encoding the variant surface glycoprotein (VSG) that constitutes its coat. Sequencing of the *T. brucei* genome provided evidence for around thousand VSG genes and pseudogenes, allowing the expression of a virtual unlimited number of VSG variants including mosaic proteins (17–19). In addition, the very dense surface organization of VSG was shown to shield invariant proteins or epitopes from the adaptive immune system (20).

The idea that trypanosome control relies only on the interplay between the VSG coat and the host antibody response was challenged by observations made during experimental infections in B-cell-deficient mice. Although these mice lack the capacity to generate antitrypanosome antibodies, they survived several weeks with stable levels of actively dividing *T. brucei* parasites. In addition, experimental *T. brucei* infections in IgM 'knock-out' (KO) mice only revealed a surprisingly mild phenotype with relatively little difference in parasitaemia control as compared with their wild-type littermates (21). Hence, Radwanska *et al.* (13) suggested that WT mice would be rendered similar to B-cell KO or IgM KO mice if parasites were able to successfully suppress B-cell responses. Testing this hypothesis in detail confirmed the capacity of trypanosomes to deplete the IgM^+ B-cell compartment (13). Support for a limited role of the B-cells in the control trypanosomiasis has recently come from two other independent investigations. First, Dagenais *et al.* (22) have shown that MHC-II VSG peptide presentation is hampered during progressing infections, undermining conventional adaptive immunity against later arising VSGs. These findings confirm an earlier report showing that MHC-II function is generally impaired in experimental trypanosomiasis (23). Second, Wei *et al.* (24) have shown that B-cells have no impact on parasitaemia onset or infection progression when trypanosomes are inoculated intradermally. Finally, when considering the adaptive immune reactions against the trypanosome, the role of T-cells remains a matter of debate. It is clear that the evolutionary pressure of the parasite–host interaction has lead to the accumulation of specific T-cell epitopes in the hypervariable subregion of VSG (25). Nevertheless, the role of T-cells in the absence of effective MHC-II peptide presentation is mainly limited to providing a particular cytokine environment [reviewed in (26,27)].

While antigenic variation, without any doubt, contributes to the failure of antitrypanosome vaccination, we found that the reasons extend far beyond this. Indeed, we have shown that living parasites can actively destroy B-cell memory in mice, even when raised by established and nontrypanosome-related vaccines such as the commercially available DTPa vaccine Boostrix® (13). This suggests that antitrypanosomiasis immunization as well as other vaccination campaigns might be prone to failure because of the immune disorders intrinsically caused by this parasite (28). Important to mention is that to date none of the current literature on promising vaccine candidates for trypanosomiasis has been translated into field applications. For example, actin and tubulin, as well as microtubule-associated protein (MAP), were proposed as promising vaccine candidates (29–33). However, careful reading of these reports revealed that these might document acute vaccine-induced responses rather than protection based on immunological memory. Indeed, mice were infected with trypanosomes only days after challenge with an antigen/adjuvant boost. As will be explained hereafter, trypanosomes are sensitive to killing by acute inflammatory responses (34–36). Hence, a plausible explanation for the obtained 'positive' results is the impact of innate inflammatory responses rather than vaccine-induced memory in these rapid boost/challenge experiments. To provide proof for the existence of vaccine-induced protective memory, these studies should be repeated including challenge experiments after months, and not days, following the last vaccine boost.

MOUSE MODELS AS A TOOL TO STUDY THE INNATE RESPONSES AGAINST TRYPANOSOMIASIS

As trypanosomes are extracellular parasites, most of the initial studies on host–parasite interactions have been focussed on humoral adaptive immunity. However, it rapidly became clear that T-cell suppression was one of the early hallmarks of trypanosomiasis in mice. This called for the identification of the involved soluble mediators and the cells responsible for their production (37,38). In particular, the role of TNF, IFNγ and iNOS attracted major attention from the start, not at least as TNF had initially been discovered as 'cachectin' in *T. b. brucei*-infected rabbits (39,40). The detrimental role of TNF was uncovered during the first TNF 'knock-out' mouse experimental infections. Interestingly, this cytokine was also found to be crucial for parasite control (35). The latter was explained for certain *T. b. brucei* and *T. b. gambiense* parasite stocks by a direct trypanolytic effect (34,41). In other *T. b. rhodesiense* and *T. congolense* parasite strains, this cytokine appeared to work in conjunction with NO and antibodies to exert its full trypanotoxic potential (36,42). Finally, TNF was also shown to play a role in the induction of T-cell suppression. Indeed, neutralizing anti-TNF antibodies could block the suppressive activity exerted by infection-derived macrophages in an assay that required cell–cell contact between target and effector cells (43). It was also in this type of coculture assays that the role of IFNγ was discovered. Especially in the spleen, the excessive production of this cytokine hampered both parasite antigen-driven and mitogen-driven T-cell proliferation (43). However, IFNγ KO mice failed to control excessive *T. brucei* proliferation resulting in early mortality (44). Hence, both TNF and IFNγ not only appear to be double-edge sword effectors that are needed for parasitaemia control but also contribute to infection-associated inflammatory complications. A comparative study of infections with *T. b. brucei* (as high virulent model) and *T. congolense* (as low virulent model) was very illustrative for the role of IFNγ and its expression regulation. Indeed, it was observed that an early inflammatory response initiated to control parasite development had to be counteracted by an up-regulated IL-10 production in order for a long-term low-pathogenic infection to be established (45). Differences in the pathogenicity of genetically distinct *T. b. brucei* strains have also, in part, been attributed to IL-10 signalling (46). In addition, when comparing susceptibility of different mouse strains with trypanosomiasis-associated pathology, the balance between IFNγ, TNF, NO and IL-10 turns out to be a major regulatory mechanism (47). Interestingly, IL-10 KO mice have so far been the most trypanosusceptible mice ever described (48). These findings indicate that the proper induction of IL-10 is an absolute requirement for the survival of mice during trypanosome infection.

With regard to the cells responsible for the production of the three crucial cytokines mentioned earlier (i.e. TNF, IFNγ and IL-10), various possibilities have been put forward. *In vitro*, TNF is readily produced by peritoneal macrophages when exposed to trypanosome lysate or purified VSG (49,50). *Ex vivo* staining of various cell populations for TNF has, moreover, allowed to identify a population of inflammatory TiP-DCs (TNF-iNOS-producing dendritic cells) in the liver and spleen of infected mice (51). On the other hand, IFNγ production has been mainly attributed to various T-cell subpopulations, even at infection stages where T-cell proliferative capacity is severely suppressed (52,53). An interesting hypothesis is that the early IFNγ production could be initiated by $CD8^+$ T-cells or NKT-cells, the chronic stage IFNγ would be secreted by an expanded $CD4^+$ T-cell population that has emerged from continuous antigenic stimulation. Finally, with respect to the production of IL-10, the results we obtained appear to be strongly dependent on the specific trypanosome–host interaction. While regulatory T-cells were shown to participate in IL-10 production during chronic *T. congolense* infections (45), such cells have not been identified in *T. brucei*-infected mice at time points where IL-10 was shown to be a crucial survival factor. Also alternatively activated macrophages have been shown to participate in IL-10 production (54), but once again, the data we obtained are not sufficient to explain an early-stage involvement. Hence, other cells such as regulatory or natural B-cells or specific NKT-subpopulations should be considered putative partners in the regulation of the cytokine balance in mice.

Of importance is that the results outlined previously have a particular value in understanding natural trypanosomiasis. While TNF was suggested to be a virulence factor involved in the induction of *T. congolense* and *T. vivax*-associated anaemia (55), IFNγ appears to play a major role in late-stage human sleeping sickness (56). Also, the role of IL-10 as an anti-inflammatory agent has been more recently confirmed in cattle, primate and human infections (57–59).

DISCOVERY OF THE RESISTANCE MECHANISM OF *T. B. RHODESIENSE* TO NORMAL HUMAN SERUM LYSIS

Most mouse models for trypanosomiasis have been designed to study host–parasite interactions in a nonhuman context. However, also the resistance mechanism of human-infective *T. b. rhodesiense* parasites against lysis by normal human serum (NHS) was discovered by experimental

mouse infections. Indeed, to mimic the conditions found at the human host–parasite interface, researchers at the Tropical Institute Antwerp designed an experimental set-up more than 3 decades ago in which trypanosomes were inoculated with or without NHS in mice. These infections resulted in the isolation of serum-resistant and serum-sensitive *T. b. rhodesiense* clones (60), demonstrating that resistance to NHS was not an intrinsic parasite characteristic but could be lost or regained, depending on the host environment. Transcriptional comparison of these 'mouse-derived' NHS-resistant and NHS-sensitive *T. b. rhodesiense* parasites of the Edinburgh Trypanozoon antigen type (ETat 1) gave rise to the identification of the serum resistance antigen (*SRA*) gene, expressed in both ETat1.10R(resistant) and ETat1.2R, but not in ETat1.2S(sensitive), clones (61). Subsequent transfection of *SRA* into *T. b. brucei* (AnTat 1) showed that the mere presence of this gene rendered nonhuman-infective parasites resistant against NHS-mediated lysis in the mouse model outlined earlier (62). Early on, the trypanolytic activity in NHS was discovered to be present in two different high-density lipoprotein (HDL) serum fractions, trypanosome lytic factor TLF1 (a 500-kDa lipoprotein complex) and a larger IgM containing TLF2 (>1.000 kDa) (63). So far, only the TLF1 fraction has been characterized in detail, with various authors agreeing that both ApoL1 and Hpr contribute to the antitrypanosomal activity [reviewed in (64)]. With regard to the contribution of mouse models to the full elucidation of TLF1 functioning, a recent breakthrough was made by hydrodynamic gene delivery of baboon ApoL1 to mice, conveying them with resistance to *T. b. brucei* infection (65). By comparing delivery of full-length ApoL1 with a truncated ApoL1 mutant, this molecule was shown to require its C-terminal end for activity (65).

Unfortunately, despite the discovery of SRA in *T. b. rhodesiense* 30 years ago, the proposed NHS resistance mechanism used by *T. b. gambiense* (66) remains to be further unravelled. Given that *T. b. gambiense* accounts for more than 95% of all HAT cases, a breakthrough in this area could boost the development of new control strategies.

DISSECTING THE ROLE OF THE TSETSE VECTOR IN TRYPANOSOMIASIS TRANSMISSION

Nearly all studies on the interactions between the trypanosome and the host innate and adaptive immune systems rely on experimental infections that exclude the tsetse fly vector. However, natural infections occur with infective metacyclic trypanosomes that are introduced into the host with tsetse saliva that contains proteins of which several are glycosylated and some are even shown or anticipated to possess enzymatic activities (67–70). Given that saliva was documented to enhance the onset of a trypanosome infection in a mouse model (71), these compounds could be considered vector-derived virulence factors. This observation is in line with available documentation on several species of sand flies (72,73), mosquitoes (74,75) and ticks (76,77) where salivary components are involved in the transmission dynamics of protozoan, viral and bacterial infections.

To date, very little is known about the influence of tsetse salivary compounds on the components of the innate immune system. Transcriptional analyses of the mouse dermis have illustrated that the saliva reduces the transcription of several early inflammatory genes that are responsive to a trypanosome trigger, e.g. *IL-12p35* and *IL-6* (71). These data might suggest that saliva modulates the activation of antigen-presenting cells such as dendritic cells and macrophages that are resident at or attracted to the infective bite site. Modulation of these cell types has been previously documented to occur with other disease-transmitting arthropods, including sand flies (78,79), mosquitoes (75,80) and ticks (81,82). Information from the latter indicates that the mere glycosylation pattern on insect proteins can contribute to the observed features of tsetse saliva. Indeed, binding of a tick protein (Salp15) with a mannose/galactose-containing glycosylation to the C-type lectin DC-SIGN could reduce the expression of inflammatory genes by interference at the transcriptional and post-transcriptional level (81). The underlying mechanism involves the induction of cellular conditions in which the mRNA of several inflammatory genes displays a reduced half-life and affects nucleosome remodelling events that are required for promoter accessibility (81). Other immune modulatory mechanisms could rely on interference with innate triggers that result from the tissue damage (83,84) and the inoculation of vector- and parasite-derived pathogen-associated molecular patterns (81,82). These triggers orchestrate the immune activation and include chemokines, cytokines, reactive oxygen and nitrogen species, histamine, complement components, nucleotides and nucleosides that are involved in purinergic signalling and others. Blood-feeding arthropods have developed two main strategies to overcome these: enzymatic modification/degradation (83–85) and neutralization through binding (86–90). However, functional analogies between tsetse flies and other hematophagous insects in terms of modulation of these innate immune responses remain to be uncovered.

Information on the influence of tsetse fly saliva on the adaptive immune system has also been obtained mainly from mouse infection models. Similar as for many other medically important arthropods, tsetse saliva induces a Th2-biased immune response. This was evidenced by high

IL-4 and IL-10 production by restimulated lymphocytes as well as by an IgG1/IgE-biased isotype profile in tsetse-exposed mice (91). The Th2 biasing potential of sand fly saliva was reported to exacerbate *Leishmania major* infections (72,92). For tsetse fly saliva, the effect on trypanosome infection in mice is only mild and limited to the first wave of parasitaemia (71). Another difference is that strong DTH responses against phlebotomine sand fly saliva can abrogate *Leishmania major* transmission (93,94), while mice that are immunized against saliva by repeated exposure to tsetse bites are even more prone to parasitaemia onset upon natural *T. b. brucei* infection (71). The latter might result from the presence of some highly immunogenic and allergenic proteins in tsetse saliva (including, respectively, the Tsal and TAg5 protein families) (91,95–97), where the trypanosome as extracellular parasite might benefit from local hypersensitivity reactions for invasion of the host. Comparison of the immunogenicity profile of saliva in humans and mice already indicates that the mouse model can provide useful information (91,95,96,98). However, the observed effects of tsetse saliva and antisaliva immunity on trypanosome infection onset in mice remain to be confirmed in natural hosts. The effects in a natural setting might be even more pronounced and have an actual influence on the minimal infective dose and parasite transmission dynamics.

CONCLUSION

Mouse models for trypanosomiasis have provided fundamental insights into the mechanisms of antigenic variation, the interaction of trypanosomes with the adaptive B-cell compartment, the impact of innate immunity on infection-associated disease complications and the influence of tsetse fly saliva on the onset of infection. To date, most of these data obtained from experimental infections remain to be validated in a more natural disease setting. Nevertheless, the cytokine patterns that govern the host–parasite interaction have already been proven relevant. For example, the link between VSG and TNF induction and the subsequent role of TNF in the onset of inflammation-associated anaemia are valid for cattle. On the other hand, the involvement of the IFNγ/IL-10 balance in controlling both parasite loads and pathogenicity has also been reported in human African trypanosomiasis (HAT). In contrast, experimental observations related to B-cell dysfunction and vaccine failure could be extremely relevant for natural infections but remain to be explored in field conditions.

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