Inflammation following trypanosome infection and persistence in the skin

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Highlights

- The skin is transformed from an initial barrier to a reservoir tissue.
- Skin-dwelling trypanosomes sustain transmission and complicate diagnosis.
- Trypanosomes modulate and adopt innate skin responses to enhance early infection.
- Motility enables trypanosomes to avoid immune elimination in skin and bloodstream.
- Potential of $CD4^+$ T-cell based vaccination strategies against conserved parasite and salivary epitopes.

Abstract

Human African trypanosomes rely for their transmission on tsetse flies (*Glossina* sp.) that inoculate parasites into the skin during blood feeding. The absence of a protective vaccine, limited knowledge about the infection immunology and the existence of asymptomatic carriers sustaining transmission are major outstanding challenges towards elimination. All these relate to the skin where (*i*) parasites persist and transmit to tsetse flies and (*ii*) a successful vaccination strategy should ideally be effective. Host immune processes and parasite strategies that underlie early infection and skin tropism are essential aspects to comprehend the transmission-success of trypanosomes and the failure in vaccine development. Recent insights into the early infection establishment may pave the way to novel strategies aimed at blocking transmission.

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Introduction

Human African trypanosomiasis (HAT) or sleeping sickness is caused by extracellular protozoan parasites transmitted by tsetse flies (*Glossina* sp.). Approximately 97% of the HAT cases are caused by *Trypanosoma brucei gambiense,* 3% by *T. b. rhodesiense*, whereas atypical infections with veterinary trypanosome species have also been described [1]. As a result of continued control efforts, the incidence has been reduced to 997 reported cases in 2018 (WHO website; URL: https://www.who.int/trypanosomiasis_african/en/). A sustained active transmission in remaining HAT foci involves the skin as (*i*) a stringent barrier where trypanosomes overcome host immune responses to establish infection and (*ii*) an anatomical reservoir where transmissible trypanosomes persist and remain undetected in asymptomatic individuals [2*-4]. Major constraints in developing a successful vaccine have been the well-known hallmarks of trypanosomiasis: antigenic variation and severe suppression and exhaustion of lymphocyte responses [5]. The earliest skin – trypanosome immunological interactions may offer windows of opportunity for intervention although current insights remain scarce because experimental infection models mostly bypass the skin and exclude the tsetse vector.

The skin as an immunological barrier and site of parasite persistence: to transmit or not to transmit, that is the question!

African trypanosomes use the pool feeding behaviour of tsetse flies to breach the skin barrier and enabling deposition in the dermis (BOX). Infected tsetse flies display an altered feeding behavior, enhancing the likelihood of parasite transmission and inoculation at multiple sites in the skin [6]. The introduced infective metacyclic forms (MCF) subsequently need to escape immune elimination and differentiate into proliferative slender bloodstream form (BSF) trypomastigotes (Fig. 1). Parasites remain in the bite site vicinity, distribute in the skin or move into afferent lymphatic ducts and draining lymph nodes as route of systemic colonization [3]. From the blood stream, parasites also re-access the dermis and subcutaneous tissue where they maintain stable levels [4]. Trypanosomes in extravascular spaces in the skin show a backward-forward movement [4,7] with entanglement between collagen fibres and intricate interactions with dermal adipocytes [3]. These may support the long-term persistence in the skin, compatible with uptake by the bloodfeeding vector [3,4], a feature also reported for other pathogens such as *Leishmania* [8] and *Borrelia* [9]. Essential for successful acquisition by the tsetse fly is differentiation of BSFs into insect-preadapted stumpy forms (Fig. 1, [10]) which are estimated to constitute on average approximately 20% of the skin burden [4].

Human exposure experiments have shown substantial interindividual variation in susceptibility within a geographical area, with a >5-fold difference between the lowest infective dose (<200) and the highest non-infective MCF dose (>1000) [11]. Epidemiological studies suggest that human genetic polymorphisms in some cytokine genes (IL-1, IL-1RN) and loci involved in serum trypanolysis (Apolipoprotein L1 allele variant G2, haptoglobin/haptoglobin related protein) are associated with level of resistance [12,13]. All these polymorphisms point to involvement of innate responses that may already act during early skin infection.

BOX: The complexity of the infectious inoculum provides decoys and immune modulators

Microbial diversity in the infection inoculum

Several parasite forms are present in the salivary glands, recently studied in higher resolution using a single-cell transcriptome approach [14**]. The mature metacyclic trypanosomes are considered the only infectious stage, whereas the impact of immature stages in the skin remains unclear. MCF are particularly infective when compared to BSF parasites [3], suggesting stage-specific adaptations to support dermal infection establishment such as upregulation of spermidine/trypanothione biosynthetic pathway genes, variant-specific surface glycoproteins (VSG) and other surface proteins (calflagins, Fam10) (Fig. 1).

Insect and skin microbiota can also significantly impact parasite transmission and exacerbate disease, by affecting dermal IL-1β levels and neutrophil recruitment [15*,16]. Unlike *Leishmania*-infected sand flies with an obstructed anterior midgut, tsetse flies do not show a regurgitation response and are less likely to introduce large numbers of bacteria. Related to viral determinants, epidemiological studies did so far not reveal substantial co-presence of trypanosomes and viruses (*e.g.* the salivary gland hypertrophy virus) in tsetse flies [17]. Nevertheless, an infective bite is likely to trigger a (recall) response that is broader than to trypanosomal antigens only.

The tsetse salivary potion

Tsetse saliva was shown to favor early infection onset linked to suppression of inflammatory gene (*il-1*, *il-6*) transcription in the skin [18]. The saliva composition is strongly conserved amongst tsetse species [19] and consist of factors that may disarm danger signals and inflammatory triggers (*e.g.* lectins and inhibitors of purinergic signaling, complement, elastase and cathepsin G), modulate B cell responses and trigger T_H2-skewed immunity [20]. Several of the saliva proteins associate with the parasite surface (unpublished data), ensuring their activity in the immediate parasite microenvironment. However, immunization against total saliva by repeated tsetse exposure does not confer resistance but in the contrary promotes early systemic colonization, re-emphasizing the complexity of the parasite-vector-host tripartite interaction that needs to be considered when studying infections and evaluating vaccines [21].

The skin immunological repertoire in face of invading trypanosomes

Transcriptional studies showed that the bite-inflicted skin damage is sufficient to trigger inflammatory cytokines and chemokines (*il-1*, *il-6*, *cxcl1*, *cxcl5*) with a concomitant neutrophil recruitment within hours of infection [22**]. Infective bites occasionally result in the formation of a skin ulceration or chancre, most often with the more virulent *T. b. rhodesiense* infections [1]. After primary infiltration of neutrophils, CD4+ and CD8+ lymphocytes, plasma cells and monocytes access the skin site from 24 hours onwards [22**,23]. The role of the steady-state and recruited skin immunological repertoire during trypanosomiasis and parasite evasion strategies largely remain blind spots in our knowledge, although some assumptions can be made based on experimental infections in mice (Fig. 2).

Innate humoral factors act early upon trypanosome infection

Upon introduction in the human skin during tsetse probing and the generation of a dermal blood pool, trypanosomes need to escape lysis by a range of innate humoral factors: complement and apolipoprotein L1 (ApoL1) as active constituent of two lytic high-density lipoprotein fractions (trypanosome lytic factor-1 and -2, TLF-1 and TLF-2). The parasite surface VSG coat, already expressed on MCFs (Fig. 1), confers resistance to complement lysis by preventing progression of the alternative complement activation pathway beyond the C3 convertase [24,25]. While ApoL1 confers innate resistance against animal trypanosome species by forming pores in lysosomal and mitochondrial membranes, *T. b. rhodesiense* and *T. b. gambiense* have evolved different strategies to overcome lysis (reviewed in [26,27]). This is achieved by inhibiting the pore-forming activity (serum resistance-associated protein, SRA), reducing uptake (mutation or reduced expression of the haptoglobin-hemoglobin receptor), inducing membrane stiffening (*T. b. gambiense*-specific glycoprotein, TgsGP) or increasing proteolytic turnover in digestive vacuoles (cysteine protease activity). Indications of an evolutionary arms race between the trypanosome and host humoral factors are given by the frequency in African populations of ApoL1 allelic variants that are less potently neutralized by SRA and therefore kill *T. b. rhodesiense*, but as a downside may trigger chronic kidney disease [28,29]. *Trypanosoma evansi* and *T. b. brucei* also show a remarkable phenotypic plasticity enabling resistance to prolonged TLF exposure and imposing a potential future risk of emerging atypical human infections [26].

Keratinocytes, fibroblasts and adipocytes: somatic cells with immunological functions

Somatic cells of the epidermis (keratinocytes) and dermis (fibroblasts and adipocytes) fulfill immunological functions during infections by the production of cytokines, chemokines and antimicrobial peptides (AMP) [30,31]. Following bite-induced tissue injury, keratinocytes can sense danger signals and trigger inflammation (*e.g.* by releasing IL-1β and IL-18) following recognition by TLRs and produce AMPs [30]. Especially cathelicidins may kill *T. brucei,* although BSF are more resistant than insect forms [32]. Surprisingly, trypanosomes adhere to adipocytes [3] that also represent a source of cathelicidins. As trypanosomes preferentially target adipose tissue, this suggests a favorable metabolic/immunological interaction with these cells from which fatty acids may be catabolized as an alternative energy source [33]. Early *in vitro* culture work showed that adipocytes and fibroblasts trigger parasite expansion which may be important during early infection and differentiation [34].

Mast cells and neutrophils: innate responses may assist early trypanosome infection onset

Skin-resident mast cells (MC) are immune sentinels with pattern recognition receptors and IgG/IgE responsive Fc receptors enabling rapid and selective responses to danger signals, trypanosomes and a major allergen in tsetse saliva (*i.e.* tsetse antigen 5, TAg5). MCs can selectively release prestored and *de novo* synthetized pro-inflammatory and vasoactive mediators, cytokines and chemokines. MCs thereby stimulate cellular recruitment and the activation of a plethora of somatic cells and immune cells at the local infection site as well as cells distally in draining lymph nodes (reviewed in [35]). For instance, release of prestored TNF can stimulate the recruitment of neutrophils by activation of vascular endothelium, trigger DC maturation and induce lymph node hypertrophy due to lymphocyte retention. MCs are also increasingly believed to play a role as nonconventional antigen-presenting cells [36]. Although their action against trypanosomes in unknown, MCs may exert direct antiparasitic activity through release of microbicidal factors including AMPs, production of reactive oxygen species,

pathogen internalization and formation of MC extracellular traps [37]. Despite their role as sentinels and catalysts/modulators of innate and adaptive immune responses, MCs remain underexplored as target cell during parasitic diseases [35,38].

During early infection, two recruitment waves of neutrophils were observed resulting in multifocal infiltrates centered around blood vessels: one associated with the skin damage and one triggered by the expanding parasites [4,22**]. Neutrophils can potentially contribute to parasite control through (*i*) engulfment and destruction in phagolysosomes, (*ii*) secretion of microbicidal factors, (*iii*) inducing hostile inflammatory conditions and (*iv*) the release of neutrophil extracellular traps (NETs) (reviewed in [39]). Despite their presumed role as barrier to infection, neutrophils fail to engulf and eliminate viable trypanosomes in which parasite motility seems to play a role as escape mechanism [22**]. The local inflammatory environment with the degranulation of MCs/neutrophils in the skin may be preferred by stumpy forms that are more resistant to external proteolytic/acidic stress [40]. Surprisingly, neutropenic mouse models revealed that parasites benefit from neutrophil functions during infection onset, without major changes in systemic cytokine levels (IFN-γ, IL1β, TNF-α, IL10, IL-12) [22**]. This identified neutrophils as important regulators of the early infection, as is the case during *Leishmania* infections [41]. Unravelling the trypanosome-neutrophil interaction and the exploration of single gene targets in parasite motility amenable for drug discovery [42**] could offer novel therapeutic options.

Macrophages and dendritic cells: role in trypanosome control, antigen presentation and Th-skewing

Classically activated (M1) macrophages are major effectors against trypanosomes relying on reactive oxygen and nitrogen species, trypanolytic activity of soluble TNF and engulfment of (opsonized) parasites. Macrophages respond to VSG moieties (scavenger receptor type A) and CpG motifs in trypanosomal genomic DNA (TLR9) and produce a plethora of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-12, especially following priming with IFN-γ [43]. Given their abundance in the skin and additional monocyte-recruitment within the first days of infection [22**], these cells could be major contributors to the skin barrier function. The importance of iNOS/NO and TNF in parasite control during intradermal infections was demonstrated for *T. congolense* [44]. Nevertheless, other trypanosome species and strains may exhibit different levels of susceptibility to these M1 mediators. Moreover, trypanosomes can manipulate the antitrypanosomal activity of macrophages from outside and from within the cell: (*i*) upon parasite engulfment, transmembrane receptor-like adenylate cyclases can reduce TNF-α synthesis [45], (*ii*) secreted factors such as Kinesin Heavy Chain 1 (TbKHC1) can suppress iNOS activity levels by inducing IL-10 [46] and (*iii*) metabolites (*i.e.* ketoacids) inhibit IL-1β induction [47*]. Many more parasite factors are likely involved in intercellular communication and virulence and may be enriched in high fusogenic extracellular vesicles (EVs) [48]. Some of the constituents may act as protein mimetics or exert so-called “moonlighting” activities, *i.e.* targeting unexpected host components [49].

Dermal dendritic cells and Langerhans cells are the major antigen-presenting cells of the skin, differentiating upon uptake of antigens and migrating to T cell-rich areas of the draining lymph nodes [30,31]. The waves of neutrophil influx observed in infected skin [22**] could participate in the recruitment and regulation of activation of monocyte-derived dendritic cells. The crosstalk of moderate numbers of neutrophils with DCs could trigger both DC activation and inactivation (reviewed in [50]). Following engulfment of apoptotic/necrotic neutrophils that may accumulate early upon

intradermal infection, DCs can induce tolerogenic conditions as a result of a reduced expression of costimulatory molecules and inflammatory cytokines [50]. Experiments with murine DCs indicate that both TNF and VSG trigger T_H2 instructing maturation profiles favouring the induction of CD4+ T cells without antitrypanosomal activity [51].

ILC, NK, NKT and T cells: sources of IFN-γ and paradigm shift to T cells as important anti-parasitic effectors

Early IFN-γ production contributes to macrophage M1-priming and parasite control. Dermal γδ T cells and dendritic epidermal T cells (DETCs) are non-MHC restricted T cells responding to skin injury and represent putative sources of IFN-γ [30]. During viral skin infections, γδ T cells can form immune synapses with proximal MCs resulting in γδ T cell proliferation and production of IFN-γ [36]. ILC1 innate lymphoid cells and recruited NK and NKT cells can be additional IFN-γ sources. Infections in perforin-deficient ($Prf1^{-/-}$) mice suggest little effect of NK cytolytic activity on early *T. brucei* control [52], whereas NK cells do play a critical perforin-dependent role during *T. congolense* infection [53]. In liver and spleen, NK and NKT cells are reported to be the earliest systemic IFN-γ sources, CD8+ and to a lesser extent CD4+ T cells predominate a few days later [54]. The relatively limited contribution of CD4+ T cells may arise from T_H2 skewing and exclusive responsiveness against peptides of the hypervariable N-terminal domain of VSG, which would reduce recall and limit antigen-specific IFN-γ production [55]. In contrast, IFN-γ production by CD8+ T cells is triggered non-specifically by the trypanosome-derived lymphocyte triggering factor TLTF [56]. Depletion experiments showed that CD4+ T lymphocytes play a key role in chancre development, but without significantly impacting infection and time of parasite appearance in blood [57]. Major reasons may be recognition of hypervariable T cell epitopes and profound immunosuppression that occurs rapidly upon experimental infection. Various mechanisms contribute hereto (reviewed in [5,58]): the membrane protein Trypanosome Suppression Immunomodulating Factor (TSIF), NO and prostaglandins from myeloid derived suppressor cells, reduced MHC class II-presentation and lower interleukin-2 (IL-2) and IL-2 receptor (IL-2R) expression by T cells.

B cells: facing the extensive evasion strategies of the trypanosome

Experimental infections of B cell deficient ($\mu MT^{-/-}$) mice and T/B cell deficient ($RAG\text{-}2^{-/-}$) mice with BSF *T. brucei* indicate that the intrinsic skin barrier function is independent of B and T lymphocytes [44]. Trypanosomes are in fact masters in escape from B cell responses, mainly based on (*i*) antigenic variation of the dense VSG coat (including changes in glycosylation [59*]) and of antibody-exposed regions of other surface proteins (*e.g.* transferrin receptor [60*]), (*ii*) rapid VSG recycling to remove surface-bound IgGs [61], (*iii*) induction of polyclonal B cell responses [62] giving rise to poly-specific antibodies that may saturate opsonization mechanisms and (*iv*) (partial) impairment of B cell lymphopoiesis and memory which is particularly the case in mice [63,64]. Understanding the mechanistic basis of B cell dysfunction and the implication of NK cytolytic activity [52] and IFN-γ related inflammatory mechanisms [58] will be essential in further guiding vaccine development. Currently, substantial research is also conducted on the mechanisms of antigenic variation which depends on gene control to ensure single (monoallelic) VSG expression and a machinery for antigen switching. Upon inoculation in the host, MCF already express a protective (M-)VSG coat and, following

differentiation into BSF, maintenance of monoallelic B-VSG expression is essential to prevent rapid immune clearance [65**]. Understanding the underlying mechanisms [66] could offer future therapeutic opportunities.

Trypanosome infection of the skin: from an initial barrier to a reservoir tissue

Although the skin represents a stringent barrier for infection, it transforms into a reservoir tissue where parasites dwell without triggering major inflammatory cell infiltration [4]. Although intriguing, the underlying host and parasite factors are currently unknown. Trypanosomes can be very resilient and subvert the antimicrobial functions of the skin immunological repertoire (see "The skin immunological repertoire in face of invading trypanosomes"). How the characteristics of the dermal parasite population differ from those in the blood stream and adipose tissue may shed some light on specific interactions with this tissue. While IFN-γ production and macrophage activation is important for parasite control, they can be countered by IL-10, of which transcripts are detected in the skin within 4.5 hours post-infection [22**]. IL-10 is crucial to limit tissue damage due to the uncontrolled type 1 inflammation during trypanosome infections [67]. Several parasite factors are capable of subverting inflammatory responses (*vide supra*, [45-47*]), including TbKHC1 that induces IL-10 and arginase-1 in myeloid cells [46]. The accumulation of myeloid-derived suppressor cells (MDSCs) in infected tissues can also suppress T cell proliferation and IFN-γ production [68]. Regulatory T cells (Tregs) are another known source of IL-10 during trypanosomiasis, limiting IFN-γ production and reducing macrophage TNF-α and NO production [67,69]. Tregs thereby also suppress the antiparasitic responses mediated by activation of macrophages [69,70] and their expansion in draining lymph nodes and spleen enhances susceptibility to skin infection [71]. Such contribution of Tregs in the skin may balance inflammatory responses and enable chronic infection without pathogenic damage.

The skin-trypanosome interface: opportunities for vaccination?

Vaccination approaches targeting the MCFs at initial dermal infection may act before parasite and/or host factors undermine adaptive immunity. A recent immunization study demonstrated only a moderate impact of antibodies against a metacyclic surface antigen [14**], suggesting that combination or alternative vaccination approaches are required to confer superior resistance [72]. Targeting of virulence factors to interfere with infection establishment in the skin could be an attractive strategy. Reports from *Leishmania* indicate that some glycolytic enzymes, which are enriched in EVs, deserve exploration as vaccine candidates [73]. Strategies to induce T_H1-memory to conserved epitopes (membrane-proximal/C-terminal VSG domains or invariant surface glycoproteins) prior to infection [72], especially in the form of skin-resident memory T cells (T_{RM}), may also be attractive to trigger rapid protective responses. This concept is being explored in leishmaniasis [74*] where IFN-γ producing CD4+ T_{RM} can contribute to parasite control at the dermal site of infection. Such approach would ensure a rapid local IFN-γ release, additional recruitment of IFN-γ producing (short-lived) T effector (T_{EFF}) cells, and activation of antiparasitic activities in macrophages and recruited inflammatory monocytes at the bite site. Vaccination strategies to efficiently raise T_{RM} and maintain T_{EFF} cells or obtain a similar type of response would be highly beneficial [74*]. Given the implication of

excessive IFN-γ in pathogenicity, a localized T_H1 recall/delayed type hypersensitivity response against salivary antigens could serve as an attractive adjunct to parasite antigens [21].

Conclusions and perspectives

Limited studies have so far addressed the early interactions between tsetse transmitted trypanosomes and the skin immunological repertoire, although this may be essential to trigger novel intervention strategies. This is especially relevant because traditional vaccination approaches against African trypanosomiasis have not been rewarding. In addition, the remarkable phenotypic plasticity of veterinary trypanosome species conferring increased human serum resistance may represent an emerging threat to human health.

Intriguingly, the skin switches from an initial barrier to a reservoir where parasites can persist. Understanding how parasites not only avoid/modulate host immunity (*i.e.* serum lysis, macrophage activation, antigen-presentation, T and B cell responses) but also benefit from early innate immune responses in the dermis (*e.g.* neutrophil functions) are major outstanding challenges. Skin-dwelling trypanosomes can also escape parasitological diagnosis, sustaining transmission and hampering elimination campaigns. Given this major hurdle, novel point-of-care detection methods and treatment schemes to efficiently eliminate dermal parasite burdens need to be established. Parasite motility, maintenance of monoallelic expression, parasite differentiation and metabolic processes all have immunological implications and may represent important future targets for drug discovery.

Since the finding that T cells not only stimulate B cell responses but also constitute important effectors during trypanosomiasis, new vaccination approaches may be triggered aimed at stimulating macrophage antiparasitic activity in the skin by inducing T_H1 (T_{RM} and T_{EFF}) responses, similar to what is explored for leishmaniasis. Identification of conserved metacyclic surface epitopes and T_H1-stimulating tsetse salivary antigens will be valuable in this process. Preclinical evaluation also will require the highly needed inclusion of natural transmission models to obtain a strong proof-of-concept.

References

1. Buscher P, Cecchi G, Jamonneau V, Priotto G: **Human African trypanosomiasis**. *Lancet* 2017, **390**:2397-2409.
2. *Capewell P, Atkins K, Weir W, Jamonneau V, Camara M, Clucas C, Swar NK, Ngoyi DM, Rotureau B, Garside P, et al.: **Resolving the apparent transmission paradox of African sleeping sickness**. *PLoS Biol* 2019, **17**:e3000105.

Following identification of the skin as an anatomical reservoir for *T. brucei*, this study emphasizes the role of asymptomatic carriers with dermal trypanosome burdens in sustaining transmission.

3. Caljon G, Van Reet N, De Trez C, Vermeersch M, Pérez-Morga D, Van Den Abbeele J: **The dermis as a delivery site of *Trypanosoma brucei* for tsetse flies**. *Plos Pathogens* 2016, **12**:e1005744.
4. Capewell P, Cren-Travaille C, Marchesi F, Johnston P, Clucas C, Benson RA, Gorman TA, Calvo-Alvarez E, Crouzols A, Jouvion G, et al.: **The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes**. *Elife* 2016, **5**.
5. Onyilagha C, Uzonna JE: **Host Immune Responses and Immune Evasion Strategies in African Trypanosomiasis**. *Front Immunol* 2019, **10**:2738.
6. Van Den Abbeele J, Caljon G, De Ridder K, De Baetselier P, Coosemans M: ***Trypanosoma brucei* modifies the tsetse salivary composition, altering the fly feeding behavior that favors parasite transmission**. *PLoS Pathog* 2010, **6**:e1000926.
7. Alfituri OA, Ajibola O, Brewer JM, Garside P, Benson RA, Peel T, Morrison LJ, Mabbott NA: **Effects of host-derived chemokines on the motility and viability of *Trypanosoma brucei***. *Parasite Immunol* 2019, **41**:e12609.
8. Aslan H, Oliveira F, Meneses C, Castrovinci P, Gomes R, Teixeira C, Derenge CA, Orandle M, Gradoni L, Oliva G, et al.: **New Insights Into the Transmissibility of *Leishmania infantum* From Dogs to Sand Flies: Experimental Vector-Transmission Reveals Persistent Parasite Depots at Bite Sites**. *J Infect Dis* 2016, **213**:1752-1761.
9. Bernard Q, Grillon A, Lenormand C, Ehret-Sabatier L, Boulanger N: **Skin Interface, a Key Player for *Borrelia* Multiplication and Persistence in Lyme Borreliosis**. *Trends Parasitol* 2020, **36**:304-314.
10. Szoor B, Silvester E, Matthews KR: **A Leap Into the Unknown - Early Events in African Trypanosome Transmission**. *Trends Parasitol* 2020, **36**:266-278.
11. Fairbairn H, Burtt E: **The infectivity to man of a strain of *Trypanosoma rhodesiense* transmitted cyclically by *Glossina morsitans* through sheep and antelope; evidence that man requires a minimum infective dose of metacyclic trypanosomes**. *Ann Trop Med Parasitol* 1946, **40**:270-313.
12. Kamoto K, Noyes H, Nambala P, Senga E, Musaya J, Kumwenda B, Bucheton B, Macleod A, Cooper A, Clucas C, et al.: **Association of APOL1 renal disease risk alleles with *Trypanosoma brucei rhodesiense* infection outcomes in the northern part of Malawi**. *PLoS Negl Trop Dis* 2019, **13**:e0007603.
13. Ofon E, Noyes H, Mulindwa J, Ilboudo H, Simuunza M, Ebo'o V, Njiokou F, Koffi M, Bucheton B, Fogue P, et al.: **A polymorphism in the haptoglobin, haptoglobin related protein locus is associated with risk of human sleeping sickness within Cameroonian populations**. *PLoS Negl Trop Dis* 2017, **11**:e0005979.
14. **Vigneron A, O'Neill MB, Weiss BL, Savage AF, Campbell OC, Kamhawi S, Valenzuela JG, Aksoy S: **Single-cell RNA sequencing of *Trypanosoma brucei* from tsetse salivary glands unveils metacyclogenesis and identifies potential transmission blocking antigens**. *Proc Natl Acad Sci U S A* 2020, **117**:2613-2621.

First single cell transcriptional profiling of *T. brucei* in tsetse salivary glands, including the mature MCF. Immunization with a conserved metacyclic surface protein shows a limited, antibody-mediated impact on natural challenge.

15. *Dey R, Joshi AB, Oliveira F, Pereira L, Guimaraes-Costa AB, Serafim TD, de Castro W, Coutinho-Abreu IV, Bhattacharya P, Townsend S, et al.: **Gut Microbes Egested during Bites of Infected Sand Flies Augment Severity of Leishmaniasis via Inflammasome-Derived IL-1beta**. *Cell Host Microbe* 2018, **23**:134-143 e136.

Demonstration of the impact of egested insect microbiota on *Leishmania* infection onset, inflammation and disease exacerbation. These data illustrate the complexity of the parasite-vector-host interplay.

16. Gimblet C, Meisel JS, Loesche MA, Cole SD, Horwinski J, Novais FO, Misic AM, Bradley CW, Beiting DP, Rankin SC, et al.: **Cutaneous Leishmaniasis Induces a Transmissible Dysbiotic Skin Microbiota that Promotes Skin Inflammation**. *Cell Host Microbe* 2017, **22**:13-24 e14.
17. Ouedraogo GMS, Demirbas-Uzel G, Rayaisse JB, Gimonneau G, Traore AC, Avgoustinos A, Parker AG, Sidibe I, Ouedraogo AG, Traore A, et al.: **Prevalence of trypanosomes, salivary gland hypertrophy virus and Wolbachia in wild populations of tsetse flies from West Africa**. *BMC Microbiol* 2018, **18**:153.
18. Caljon G, Van Den Abbeele J, Stijlemans B, Coosemans M, De Baetselier P, Magez S: **Tsetse fly saliva accelerates the onset of *Trypanosoma brucei* infection in a mouse model associated with a reduced host inflammatory response**. *Infect Immun* 2006, **74**:6324-6330.
19. Attardo GM, Abd-Alla AMM, Acosta-Serrano A, Allen JE, Bateta R, Benoit JB, Bourtzis K, Caers J, Caljon G, Christensen MB, et al.: **Comparative genomic analysis of six *Glossina* genomes, vectors of African trypanosomes**. *Genome Biol* 2019, **20**:187.
20. Caljon G, Van Den Abbeele J, Sternberg JM, Coosemans M, De Baetselier P, Magez S: **Tsetse fly saliva biases the immune response to Th2 and induces anti-vector antibodies that are a useful tool for exposure assessment**. *Int J Parasitol* 2006, **36**:1025-1035.
21. Reed SG, Coler RN, Mondal D, Kamhawi S, Valenzuela JG: ***Leishmania* vaccine development: exploiting the host-vector-parasite interface**. *Expert Rev Vaccines* 2016, **15**:81-90.
22. **Caljon G, Mabille D, Stijlemans B, De Trez C, Mazzone M, Tacchini-Cottier F, Malissen M, Van Ginderachter JA, Magez S, De Baetselier P, et al.: **Neutrophils enhance early *Trypanosoma brucei* infection onset**. *Sci Rep* 2018, **8**:11203.

First demonstration that tsetse-transmitted trypanosomes benefit from neutrophil recruitment for enhanced infection onset. Parasites escape eengulfment which appear to be linked to motility.

23. Mwangi DM, Hopkins J, Luckins AG: **Cellular phenotypes in *Trypanosoma congolense* infected sheep: the local skin reaction**. *Parasite Immunol* 1990, **12**:647-658.
24. Ferrante A, Allison AC: **Alternative pathway activation of complement by African trypanosomes lacking a glycoprotein coat**. *Parasite Immunol* 1983, **5**:491-498.
25. Devine DV, Falk RJ, Balber AE: **Restriction of the alternative pathway of human complement by intact *Trypanosoma brucei* subsp. *gambiense***. *Infect Immun* 1986, **52**:223-229.
26. Radwanska M, Vereecke N, Deleeuw V, Pinto J, Magez S: **Salivarian Trypanosomosis: A Review of Parasites Involved, Their Global Distribution and Their Interaction With the Innate and Adaptive Mammalian Host Immune System**. *Front Immunol* 2018, **9**:2253.
27. Pays E, Vanhollebeke B, Uzureau P, Lecordier L, Perez-Morga D: **The molecular arms race between African trypanosomes and humans**. *Nat Rev Microbiol* 2014, **12**:575-584.
28. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, et al.: **Association of trypanolytic ApoL1 variants with kidney disease in African Americans**. *Science* 2010, **329**:841-845.
29. Uzureau S, Lecordier L, Uzureau P, Hennig D, Graversen JH, Homble F, Mfutu PE, Oliveira Arcolino F, Ramos AR, La Rovere RM, et al.: **APOL1 C-Terminal Variants May Trigger Kidney Disease through Interference with APOL3 Control of Actomyosin**. *Cell Rep* 2020, **30**:3821-3836 e3813.
30. Di Meglio P, Perera GK, Nestle FO: **The multitasking organ: recent insights into skin immune function**. *Immunity* 2011, **35**:857-869.
31. Pasparakis M, Haase I, Nestle FO: **Mechanisms regulating skin immunity and inflammation**. *Nat Rev Immunol* 2014, **14**:289-301.
32. McGwire BS, Olson CL, Tack BF, Engman DM: **Killing of African trypanosomes by antimicrobial peptides**. *J Infect Dis* 2003, **188**:146-152.

33. Trindade S, Rijo-Ferreira F, Carvalho T, Pinto-Neves D, Guegan F, Aresta-Branco F, Bento F, Young SA, Pinto A, Van Den Abbeele J, et al.: ***Trypanosoma brucei*** **Parasites Occupy and Functionally Adapt to the Adipose Tissue in Mice**. *Cell Host Microbe* 2016, **19**:837-848.
34. Balber AE: **Primary murine bone marrow cultures support continuous growth of infectious human trypanosomes**. *Science* 1983, **220**:421-423.
35. Abraham SN, St John AL: **Mast cell-orchestrated immunity to pathogens**. *Nat Rev Immunol* 2010, **10**:440-452.
36. Mantri CK, St John AL: **Immune synapses between mast cells and gammadelta T cells limit viral infection**. *J Clin Invest* 2019, **129**:1094-1108.
37. Naqvi N, Ahuja K, Selvapandiyan A, Dey R, Nakhasi H, Puri N: **Role of Mast Cells in clearance of *Leishmania* through extracellular trap formation**. *Sci Rep* 2017, **7**:13240.
38. Lu F, Huang S: **The Roles of Mast Cells in Parasitic Protozoan Infections**. *Front Immunol* 2017, **8**:363.
39. Kolaczkowska E, Kubes P: **Neutrophil recruitment and function in health and inflammation**. *Nat Rev Immunol* 2013, **13**:159-175.
40. Nolan DP, Rolin S, Rodriguez JR, Van Den Abbeele J, Pays E: **Slender and stumpy bloodstream forms of *Trypanosoma brucei* display a differential response to extracellular acidic and proteolytic stress**. *Eur J Biochem* 2000, **267**:18-27.
41. Hurrell BP, Regli IB, Tacchini-Cottier F: **Different *Leishmania* Species Drive Distinct Neutrophil Functions**. *Trends Parasitol* 2016, **32**:392-401.
42. **Shimogawa MM, Ray SS, Kisalu N, Zhang Y, Geng Q, Ozcan A, Hill KL: **Parasite motility is critical for virulence of African trypanosomes**. *Sci Rep* 2018, **8**:9122.
Illustration that propulsive motility is essential for infection and escape from antibody-mediated claerence in the bloodstream. The LC1 dynein subunit may serve as a drug target as mutations in this single gene are sufficient to cause the infection defect.
43. Paulnock DM, Freeman BE, Mansfield JM: **Modulation of innate immunity by African trypanosomes**. *Parasitology* 2010, **137**:2051-2063.
44. Wei G, Bull H, Zhou X, Tabel H: **Intradermal infections of mice by low numbers of african trypanosomes are controlled by innate resistance but enhance susceptibility to reinfection**. *J Infect Dis* 2011, **203**:418-429.
45. Salmon D, Vanwalleghem G, Morias Y, Denoeud J, Krumbholz C, Lhomme F, Bachmaier S, Kador M, Gossmann J, Dias FB, et al.: **Adenylate cyclases of *Trypanosoma brucei* inhibit the innate immune response of the host**. *Science* 2012, **337**:463-466.
46. De Muylder G, Daulouede S, Lecordier L, Uzureau P, Morias Y, Van Den Abbeele J, Caljon G, Herin M, Holzmuller P, Semballa S, et al.: **A *Trypanosoma brucei* kinesin heavy chain promotes parasite growth by triggering host arginase activity**. *PLoS Pathog* 2013, **9**:e1003731.
47. *Campbell NK, Williams DG, Fitzgerald HK, Barry PJ, Cunningham CC, Nolan DP, Dunne A: ***Trypanosoma brucei*** **Secreted Aromatic Ketoacids Activate the Nrf2/HO-1 Pathway and Suppress Pro-inflammatory Responses in Primary Murine Glia and Macrophages**. *Front Immunol* 2019, **10**:2137.
Identification of secreted metabolites of *T. brucei*, *i.e.* ketoacids, as macrophage immunomodulators.
48. Szempruch AJ, Sykes SE, Kieft R, Dennison L, Becker AC, Gartrell A, Martin WJ, Nakayasu ES, Almeida IC, Hajduk SL, et al.: **Extracellular Vesicles from *Trypanosoma brucei* Mediate Virulence Factor Transfer and Cause Host Anemia**. *Cell* 2016, **164**:246-257.
49. Gomez-Arreaza A, Acosta H, Quinones W, Concepcion JL, Michels PA, Avilan L: **Extracellular functions of glycolytic enzymes of parasites: unpredicted use of ancient proteins**. *Mol Biochem Parasitol* 2014, **193**:75-81.
50. Schuster S, Hurrell B, Tacchini-Cottier F: **Crosstalk between neutrophils and dendritic cells: a context-dependent process**. *J Leukoc Biol* 2013, **94**:671-675.
51. Pletinckx K, Stijlemans B, Pavlovic V, Laube R, Brandl C, Kneitz S, Beschin A, De Baetselier P, Lutz MB: **Similar inflammatory DC maturation signatures induced by TNF or *Trypanosoma brucei* antigens instruct default Th2-cell responses**. *Eur J Immunol* 2011, **41**:3479-3494.

52. Frenkel D, Zhang F, Guirnalda P, Haynes C, Bockstal V, Radwanska M, Magez S, Black SJ: ***Trypanosoma brucei*** **Co-opts NK Cells to Kill Splenic B2 B Cells**. *PLoS Pathog* 2016, **12**:e1005733.
53. Onyilagha C, Kuriakose S, Ikeogu N, Kung SKP, Uzonna JE: **NK Cells Are Critical for Optimal Immunity to Experimental *Trypanosoma congolense* Infection**. *J Immunol* 2019, **203**:964-971.
54. Cnops J, De Trez C, Stijlemans B, Keirsse J, Kauffmann F, Barkhuizen M, Keeton R, Boon L, Brombacher F, Magez S: **NK-, NKT- and CD8-Derived IFNgamma Drives Myeloid Cell Activation and Erythrophagocytosis, Resulting in Trypanosomosis-Associated Acute Anemia**. *PLoS Pathog* 2015, **11**:e1004964.
55. Dagenais TR, Demick KP, Bangs JD, Forest KT, Paulnock DM, Mansfield JM: **T-cell responses to the trypanosome variant surface glycoprotein are not limited to hypervariable subregions**. *Infect Immun* 2009, **77**:141-151.
56. Olsson T, Bakhiet M, Hojeberg B, Ljungdahl A, Edlund C, Andersson G, Ekre HP, Fung-Leung WP, Mak T, Wigzell H, et al.: **CD8 is critically involved in lymphocyte activation by a T. brucei brucei-released molecule**. *Cell* 1993, **72**:715-727.
57. Naessens J, Mwangi DM, Buza J, Moloo SK: **Local skin reaction (chancre) induced following inoculation of metacyclic trypanosomes in cattle by tsetse flies is dependent on CD4 T lymphocytes**. *Parasite Immunol* 2003, **25**:413-419.
58. Stijlemans B, Radwanska M, De Trez C, Magez S: **African Trypanosomes Undermine Humoral Responses and Vaccine Development: Link with Inflammatory Responses?** *Front Immunol* 2017, **8**:582.
59. *Pinger J, Nesic D, Ali L, Aresta-Branco F, Lilic M, Chowdhury S, Kim HS, Verdi J, Raper J, Ferguson MAJ, et al.: **African trypanosomes evade immune clearance by O-glycosylation of the VSG surface coat**. *Nat Microbiol* 2018, **3**:932-938.

First demonstration that antigenic variation of *T. brucei* not only includes VSG amino-acid sequence variations but also surface post-translational modifications.

60. *Trevor CE, Gonzalez-Munoz AL, Macleod OJS, Woodcock PG, Rust S, Vaughan TJ, Garman EF, Minter R, Carrington M, Higgins MK: **Structure of the trypanosome transferrin receptor reveals mechanisms of ligand recognition and immune evasion**. *Nat Microbiol* 2019, **4**:2074-2081.

Structural studies into the transferrin receptor indicate that polymorphic sites and N-linked glycans are important for immune escape rather than for binding of transferrin from different hosts. This broadens the scope of antigenic variation to other surface glycoproteins than VSG.

61. Engstler M, Pfohl T, Herminghaus S, Boshart M, Wiegertjes G, Heddergott N, Overath P: **Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes**. *Cell* 2007, **131**:505-515.
62. Drennan MB, Stijlemans B, Van den Abbeele J, Quesniaux VJ, Barkhuizen M, Brombacher F, De Baetselier P, Ryffel B, Magez S: **The induction of a type 1 immune response following a *Trypanosoma brucei* infection is MyD88 dependent**. *J Immunol* 2005, **175**:2501-2509.
63. Radwanska M, Guirnalda P, De Trez C, Ryffel B, Black S, Magez S: **Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses**. *PLoS Pathog* 2008, **4**:e1000078.
64. Lejon V, Mumba Ngoyi D, Kestens L, Boel L, Barbe B, Kande Betu V, van Griensven J, Bottieau E, Muyembe Tamfum JJ, Jacobs J, et al.: **Gambiense human african trypanosomiasis and immunological memory: effect on phenotypic lymphocyte profiles and humoral immunity**. *PLoS Pathog* 2014, **10**:e1003947.
65. **Aresta-Branco F, Sanches-Vaz M, Bento F, Rodrigues JA, Figueiredo LM: **African trypanosomes expressing multiple VSGs are rapidly eliminated by the host immune system**. *Proc Natl Acad Sci U S A* 2019, **116**:20725-20735.

Disruption of monoallelic VSG expression results in a host antibody response against a broader VSG repertoire and parasite control by the adaptive immune system. Therapeutic opportunities may arise from disrupting mechanisms that control monoallelic VSG expression.

66. Faria J, Glover L, Hutchinson S, Boehm C, Field MC, Horn D: **Monoallelic expression and epigenetic inheritance sustained by a *Trypanosoma brucei* variant surface glycoprotein exclusion complex**. *Nat Commun* 2019, **10**:3023.
67. Guilliams M, Oldenhove G, Noel W, Herin M, Brys L, Loi P, Flamand V, Moser M, De Baetselier P, Beschin A: **African trypanosomiasis: naturally occurring regulatory T cells favor trypanotolerance by limiting pathology associated with sustained type 1 inflammation**. *J Immunol* 2007, **179**:2748-2757.
68. Onyilagha C, Kuriakose S, Ikeogu N, Jia P, Uzonna J: **Myeloid-Derived Suppressor Cells Contribute to Susceptibility to *Trypanosoma congolense* Infection by Suppressing CD4(+) T Cell Proliferation and IFN-gamma Production**. *J Immunol* 2018, **201**:507-515.
69. Wei G, Tabel H: **Regulatory T cells prevent control of experimental African trypanosomiasis**. *J Immunol* 2008, **180**:2514-2521.
70. Okwor I, Onyilagha C, Kuriakose S, Mou Z, Jia P, Uzonna JE: **Regulatory T cells enhance susceptibility to experimental *Trypanosoma congolense* infection independent of mouse genetic background**. *PLoS Negl Trop Dis* 2012, **6**:e1761.
71. Onyilagha C, Okwor I, Kuriakose S, Singh R, Uzonna J: **Low-dose intradermal infection with *Trypanosoma congolense* leads to expansion of regulatory T cells and enhanced susceptibility to reinfection**. *Infect Immun* 2014, **82**:1074-1083.
72. Black SJ, Mansfield JM: **Prospects for vaccination against pathogenic African trypanosomes**. *Parasite Immunol* 2016, **38**:735-743.
73. Keerti, Yadav NK, Joshi S, Ratnapriya S, Sahasrabuddhe AA, Dube A: **Immunotherapeutic potential of *Leishmania* (*Leishmania*) *donovani* Th1 stimulatory proteins against experimental visceral leishmaniasis**. *Vaccine* 2018, **36**:2293-2299.
74. *Hohman LS, Peters NC: **CD4(+) T Cell-Mediated Immunity against the Phagosomal Pathogen *Leishmania*: Implications for Vaccination**. *Trends Parasitol* 2019, **35**:423-435.
Review raising various important considerations for vaccine development for Leishmaniasis, with the requirements for protective CD4+ mediated immunity under conditions associated with physiological vector transmission.
75. Telleria EL, Benoit JB, Zhao X, Savage AF, Regmi S, Alves e Silva TL, O'Neill M, Aksoy S: **Insights into the trypanosome-host interactions revealed through transcriptomic analysis of parasitized tsetse fly salivary glands**. *PLoS Negl Trop Dis* 2014, **8**:e2649.

Figures:

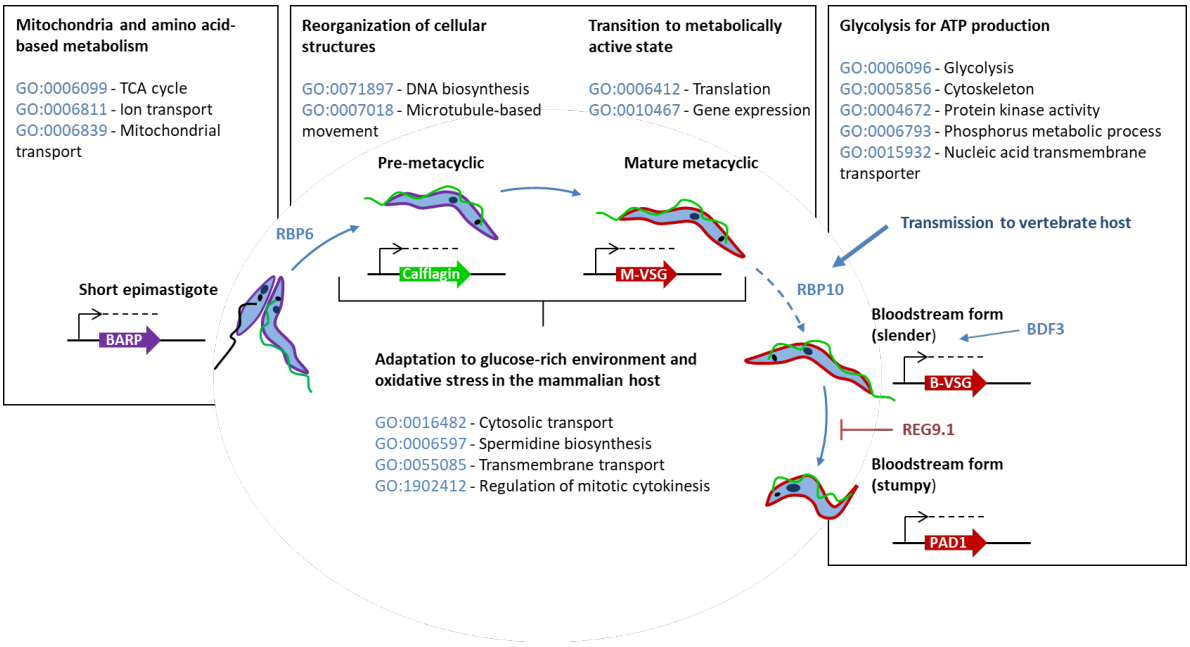

Figure 1. *T. brucei* changes upon transition from the tsetse salivary gland to the mammalian host. Selection of gene ontology terms (indicated in blue, [10,14**,75]), changes in surface properties and specific genes associated with the different life cycle stages of *T. brucei* (epimastigote, pre-metacyclic, mature MCF and BSF) in the insect vector and the mammalian host. Indicated in red are the genes involved in parasite transition from one life cycle stage to another. TCA: tricarboxylic acid, M-VSG and B-VSG: metacyclic and bloodstream variant surface glycoprotein, BARP: *brucei* alanine rich protein, PAD1: protein associated with differentiation, RBP: RNA-binding protein, REG9.1: regulator of ESAG 9, BDF3: bromodomain factor.

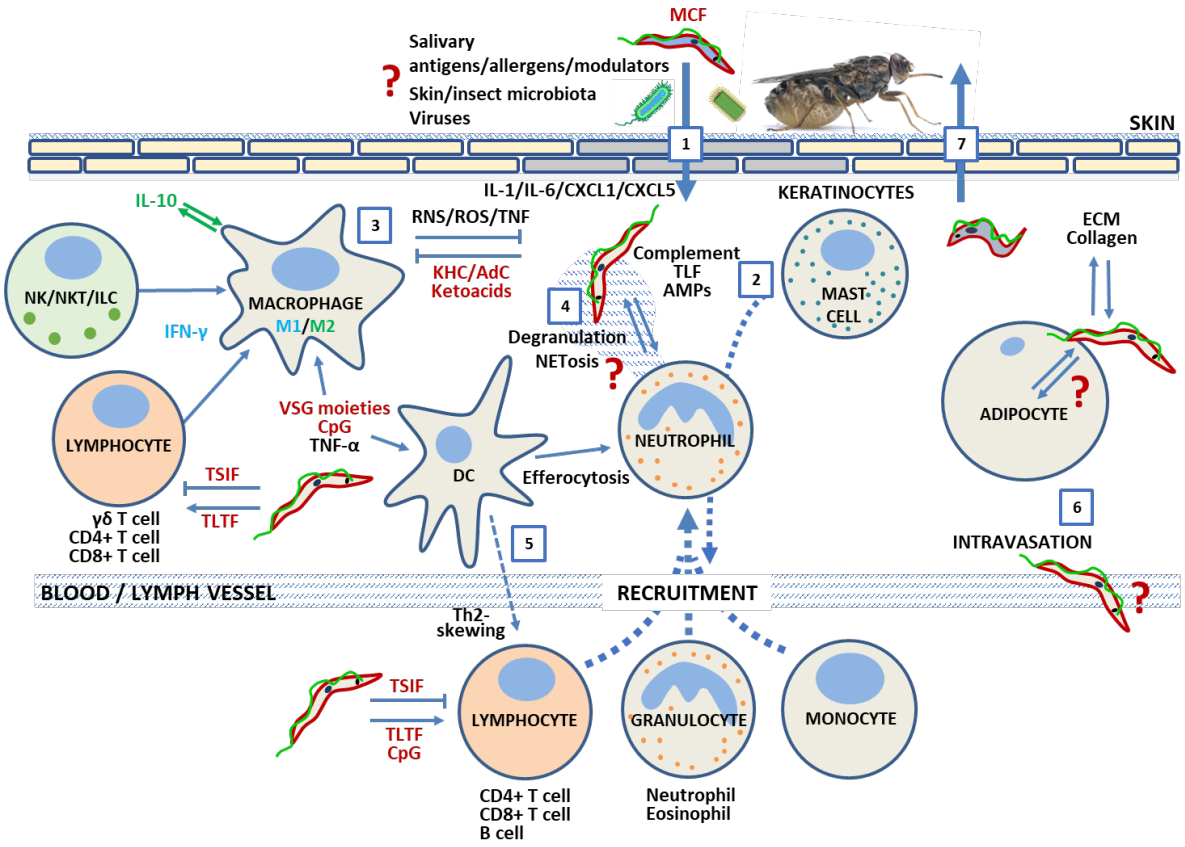

Figure 2. Immune interactions of the skin with tsetse fly transmitted trypanosomes. The early immunological processes in the skin following a natural infection are yet poorly understood. (**1**) Infected tsetse flies inoculate a complex mixture of MCF with microbial and salivary factors (BOX) that cause inflammation and modulate various responses at the infection site. MCF need to overcome innate killing by complement, TLF and AMPs and differentiate into BSF trypanosomes. (**2**) Somatic and skin-resident immune cells, *e.g.* keratinocytes and mast cells, are responding to the tissue injury and pathogen exposure and orchestrate the recruitment and activation of immune cells. (**3**) Macrophages in a classical/M1 activation state are the major contributors to parasite control, responding to trypanosomal pathogen-associated molecular patterns especially in the presence of IFN-γ that may come from various cellular sources. Trypanosomes and host IL-10 can suppress macrophage M1 activation. (**4**) Recruited neutrophils are unable to eliminate trypanosomes by phagocytosis and enhance early systemic infection. The role of degranulation and NETosis remain to be understood as well as the parasite mechanisms to overcome effective killing by neutrophils. (**5**) DCs engulf apoptotic neutrophils and are triggered by TNF and VSG to acquire a T_H2-skewing phenotype. T and B cell responses are modulated by various parasite virulence factors. (**6**) From the dermis, parasites gain access to the afferent lymph through a yet unknown mechanism as a route to systemic infection. Trypanosomes persisting in the skin interact with the extracellular matrix and dermal adipocytes. (**7**) Stumpy BSF in the skin are transmissible to tsetse flies. Parasite immunomodulators are indicated in red. ECM: extracellular matrix; TSIF: trypanosome suppression immunomodulating factor; TLTF: T lymphocyte-triggering factor; KHC: kinesin heavy chain; AdC: adenylate cyclase.