Role of cytokines in *Trypanosoma brucei*-induced anaemia: A review of the literature

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Abstract

Background

Anaemia is an important complication of trypanosomiasis. The mechanisms through which trypanosomal infection leads to anaemia are poorly defined. A number of studies have implicated inflammatory cytokines, but these data are limited and inconsistent. In this article, we reviewed the published literature on cytokines associated with *Trypanosoma brucei* infections and their role in the immunopathology leading to anaemia.

Methodology

Articles were searched in PubMed through screening of titles and abstracts with no limitation on date of publishing and study design. Articles in English were searched using keywords "African trypanosomiasis", "sleeping sickness", "*Trypanosoma brucei*", in all possible combinations with "anaemia" and/or "cytokines".

Results

Twelve articles examining cytokines and their role in trypanosome-induced anaemia were identified out of 1095 originally retrieved from PubMed. None of the articles identified were from human-based studies. A total of eight cytokines were implicated, with four cytokines (IFN-γ, IL-10, TNF-α, IL-12) showing an association with anaemia. These articles reported that mice lacking TNF-α were able to control anaemia, and that IFN-γ was linked to severe anaemia given its capacity to suppress erythropoiesis, while IL-10 was shown to regulate IFN-γ and TNF-α, providing a balance that was associated with severity of anaemia. IFN-γ and TNF-α have also been reported to work in concert with other factors such as nitric oxide and iron in order to induce anaemia.

Conclusion

IFN-γ, IL-10, and TNF-α were the three major cytokines identified to be heavily involved in anaemia caused by *Trypanosoma brucei* infection. The anti-inflammatory cytokine, IL-10, was shown to counter the effects of proinflammatory cytokines in order to balance the severity of anaemia. The mechanism of anaemia is multifactorial and therefore requires further, more elaborate research. Data from human subjects would also shed more light.

Introduction

The protozoan parasites that cause trypanosomiasis, also known as sleeping sickness in humans and nagana in animals in Africa, are extracellular haemoflagellates of the species *Trypanosoma brucei* that are transmitted by a tsetse fly (*Glossina* spp.) bite. Other important species causing nagana that circulate alongside *Trypanosoma brucei* include *Trypanosoma vivax* and *Trypanosoma congolense*. Infection from the subspecies *Trypanosoma brucei gambiense*, typically found in western and central Africa, can progress (over a few months to over several years) from the haemolymphatic stage, during which the parasites are in the peripheral blood, to the meningoencephalitic stage, after the trypanosomes cross the blood-brain barrier. The subspecies *Trypanosoma brucei rhodesiense* in eastern and southern Africa generally causes a more acute form of human African trypanosomiasis (HAT). Presently 98% of all reported HAT cases are caused by *Trypanosome brucei gambiense*[1]. *Trypanosoma brucei brucei* is sensitive to trypanolytic factors present in human serum and therefore fails to establish infection in humans. *T. brucei brucei* can, however, cause disease in some livestock[2]. The disease runs a complex course leading to death if left untreated in both animals and humans.

Though HAT is a well-known parasitic infection with neurological involvement, the most common complication associated with it is anaemia. Anaemia occurs in humans, livestock[3,4] and experimental mouse models[5–7]. In humans, anaemia is the common characteristic during the haemolymphatic stage and in animals, it is the main diagnostic tool, followed by parasite detection in the blood[8]. The pathogenic pathways are not fully understood but may involve several mechanisms. For example, there is the mechanism of cellular injury by which active biological substances such as phospholipase, pyruvate, and proteases produced by live and dead trypanosomes are thought to cause cellular injury[9]. Immunological mechanisms have also been advanced, related to the removal of erythrocytes, mostly owing to the presence of autologous immunoglobulin antibodies on the red cell surfaces and also to cytokines, which are thought to mediate the loss of erythrocytes[9–12]. Anaemia occurs during all stages of infection, from the initial stage, which is characterised by high levels of parasitaemia,[13,14] to the chronic aparasitaemic phase, where parasites are less detectable in peripheral blood but tend to establish themselves in extravascular tissue. The recovery phase is characterised by a low presence or absence of parasitaemia and the erythrocyte value returns to preinfection levels[12].

The persistence of anaemia beyond the initial wave of parasitaemia (after which low numbers of parasites are in circulation) indicates that anaemia may be directly induced by the invasion of red cells by parasites, and has led to a postulation that it might be mediated by cytokines[15]. Cytokines are important signalling molecules that, through interaction with other molecular messengers (such as cytokine inhibitors and soluble cytokine receptors), affect the behaviour of cells and regulate the host immune response[16]. These effects include regulation of inflammation, as well as control of cell growth[17].

A number of studies on HAT have implicated inflammatory cytokines in the pathophysiology of anaemia[15,18,19], but the precise roles of each individual cytokine as well as any variations in response between host and parasite species remain unclear. The aim of this review was to assess the known cytokines that are implicated in trypanosome-associated anaemia in order to guide possible biomarker development for the detection and management of anaemia in trypanosomiasis. It is envisioned that the review may direct further research on other cytokines that have not yet been linked to trypanosome-associated anaemia but have been observed in trypanosomiasis. In this regard, a review was done on published articles, found using the PubMed electronic database, related to cytokines that have been

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shown to be involved in trypanosome-associated anaemia.

Materials and Methods

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines[20] were followed in this review as described in the following subsections.

Context

The focus of this review was to identify cytokines that are upregulated or downregulated in trypanosomiasis and have been specifically shown to be associated with the anaemia that is observed in the course of the disease. Trypanosomiasis can be caused by different trypanosome species and the host-parasite immune response may differ from species to species. For the purpose of this review, we restricted the focus to publications that dealt with the *Trypanosoma brucei* subspecies that cause human and animal African trypanosomiasis (HAT/AAT).

Search strategy and selection criteria

We conducted a literature search on the PubMed database to identify relevant original articles using the keywords "African trypanosomiasis", "sleeping sickness", "*Trypanosoma brucei*", in all possible combinations with " anaemia" and "cytokines". The search was restricted to English but was neither limited by date of publication nor by study design. Both human and animal studies were included in the search.

Initially, titles and abstracts were screened. At this stage, we excluded review articles and articles describing anaemia caused by trypanosome species other than *Trypanosoma brucei*. Articles identified as possibly relevant were retrieved as full papers and were further screened. The reference lists of included articles were assessed for further relevant publications meeting our inclusion criteria. The results of the review are presented below.

Results

This section describes how anaemia was assessed on the reviewed articles and describes the role individual cytokines played in relation to anaemia in *Trypanosoma brucei* infection.

Diagnosis of anaemia in African trypanosomiasis

Most studies assessed the development of anaemia by looking at the red blood cell (RBC) counts at different time points in experimentally infected mice, and comparing these counts to preinfection levels or those of controls (uninfected mice)[11,21,22]. This assessment assumes that preinfection levels were normal, with anaemia being defined as a drop in RBC count by a specified magnitude. Other studies assessed the occurrence of anaemia by comparing preinfection packed cell volume (PCV) of red blood cells in mice with their PCV after being infected by *T. brucei*[15,23]. This assessment assumes that, in control mice, the percentage volume occupied by the red blood cells forms the normal range and therefore any measurements below this range are indicative of anaemia. All retrieved articles reported anaemia in experimental murine models and no articles on human studies were retrieved.

Anaemia and cytokines

The search in the PubMed database identified 1095 potentially relevant articles with the selected keywords. From these search results, 279 articles were selected based on the titles, and further abstract assessment identified 23 articles suitable for full article review. Eleven of the 23 articles were excluded, as two were reviews, six studied species of trypanosome parasites other than *Trypanosoma brucei*, and three compared cytokines using parameters other than anaemia (Figure 1).

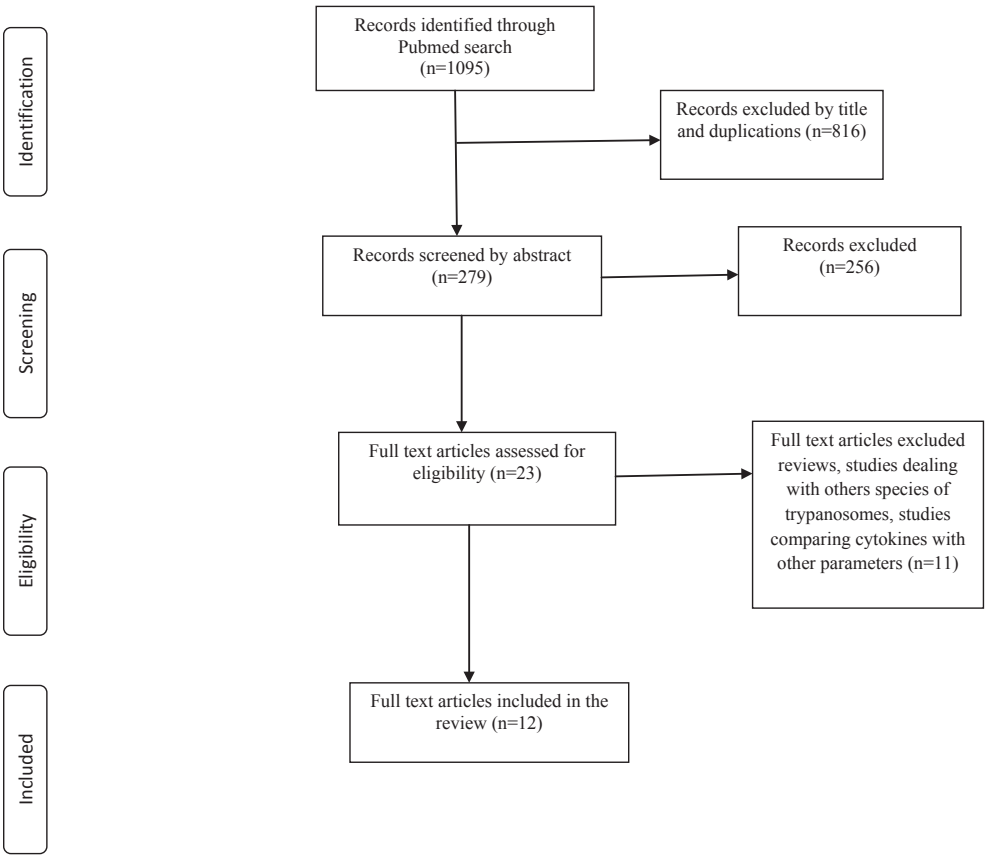

Figure 1. Flow diagram describing different steps taken to select appropriate articles for review

Table 1. Publications on the cytokines studied in trypanosomiasis-associated anaemia ordered by year

Publication	Infecting trypanosome	Clinical sample	Cytokines	Other factors associated with anaemia
Magez et al. 1999	T.b.b AnTat1.1E	Blood from TNF-α $^{-/-}$	TNF-α	-
Namangala et al. 2001	T.b.b AnTat1.1E T.b.b PLC$^{-/-}$	Plasma Culture supernatant	IL-10,IL-4*, IFN-γ, TNF	NO
Magez et al. 2002	T.b.b AnTat1.1E	Serum	IFN-γ, LT-α*, TNF	-
Magez et al. 2004	T.b.b AnTat1.1E	Serum	IL-10, IFN-γ TNF, IL-6	-
Naessen et al. 2005	T.b.r T. congolense	Blood from TNF-α $^{-/-}$	TNF-α	EPO
Guilliams et al. 2008	T.b.b AnTat1.1E	Culture supernatant	IL-10, IFN-γ	-
Stijlemans et al. 2008	T.b.b AnTat1.1E	Serum	IL-10,IL-6, IFN-γ	-
Guilliams et al. 2009	T.b.b AnTat1.1E	Culture supernatant	IL-10, TNF	-
Nishimura et al. 2009	T.b.b	Plasma Culture supernatant	TNF-α, IL-10, IFN-γ, IL-12	NO
Morrison et al. 2010	T. brucei TREU927 T. brucei STIB247	Plasma	IL-10, IL-12, IFN-γ, TNF, TGF-β	NO EPO
Stijlemans et al. 2010	T.b.b AnTat1.1E	Serum	IL-10, IL-6, IFN-γ, TNF	-
Vankrunkelsven et al. 2010	T.b.b AnTat1.1E	Serum	IL-10, IFN-γ TNF	Galectin-3

NO = Nitric Oxide; EPO = Erythropoietin; T.b.b = *Trypanosoma brucei brucei*; T.b.r = *Trypanosoma brucei rhodesiense*; IL-10 = Interleukin 10; IL-12 – Interleukin 12; IFN-γ = Interferon gamma; TNF/TNF-α = Tumour necrosis factor alpha; PLC$^{-/-}$ = Phospholipase C null mutant.

Twelve articles were finally selected for the literature review. A total of eight cytokines were suspected as being involved in trypanosomiasis-associated anaemia. These were interferon gamma (IFN-γ), interleukins (IL-10, IL-6, IL-12 and IL-4), tumour necrosis factor-alpha (TNF/TNF-α), lymphotoxin-alpha (LT-α), and transforming growth factor-beta (TGF-β), but only four were shown to be actually involved in trypanosomiasis-associated anaemia (IFN-γ, IL-10, TNF-α, IL-12). Table 1 describes the published articles, reflecting the cytokines studied and their involvement in anaemia. Each of the cytokines identified will now be described in the context of their roles in *Trypanosoma brucei*-induced anaemia.

Interferon gamma (IFN-γ)

IFN-γ is released by (among other immune cells) T lymphocytes and natural killer cells upon activation by specific antigens. It has potent immunoregulatory properties (notably macrophage activation) and antiproliferative effects[24]. It is known to inhibit bone marrow proliferation and has been implicated in the pathogenesis of aplastic anaemia. Overexpression of the IFN-γ gene in mice results in chronic anaemia. It is indicated that trypanosomiasis is associated with increased expression of IFN-γ, which may suppress erythropoiesis and result in anaemia[22,25]. It is also suggested that suppressed erythropoiesis is a result of apoptosis of haematopoetic progenitor cells[11], leading to impaired red blood cell maturation. The increase in IFN-γ expression following *Trypanosoma brucei brucei* infection is associated with a rise in nitric oxide (NO)[15,26], and it has been suggested that IFN-γ may exert its effect via NO[26]. Indeed, disruption of the IFN-γ receptor prevents the increase in NO synthesis and anaemia following infection[15].

IFN-γ might also contribute to anaemia through perturbation of iron metabolism by upregulating expression of the iron transporter DMT-1, which increases iron influx and sequestration into cells[11,27].

Tumour necrosis factor alpha (TNF-α)

TNF-α is secreted predominantly by macrophages and is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. In trypanosomiasis, studies suggest that it may play a crucial role in the pathogenesis of anaemia, through modulation of cell growth and the phagocytosis of erythrocytes by macrophages[28]. Mice with TNF-α knockout have less severe anaemia and overall morbidity following trypanosomal infection[29]. The effect of TNF-α appears to be receptor-

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specific, with TNF receptor 1 (TNF-R1) associated with severe anaemia, but not TNF-R2[30]. It is important to note that, in mice, TNF-α deficiency was only protective of anaemia following *Trypanosoma brucei brucei* infection but not with *Trypanosoma congolense* infection[15], suggesting that the TNF-α host-parasite combination is critical in determining the effects of TNF-α on anaemia.

Interleukin 10 (IL-10)

Several articles indicated that IL-10 works in concert with other cytokines in trypanosomiasis-associated anaemia[11,21–23]. IL-10 has been shown to downregulate the production of IFN-γ and TNF-α by classically activated (M1) macrophages[21,31]. Thus, the balance between IL-10 and IFN-γ or IL-10 and TNF-α appears to be a critical determinant of the severity of anaemia associated with trypanosomiasis[11,22,23]. These articles also presented data suggesting that IL-10 might be protective against anaemia, as IL-10 knockout (IL-10-/-) mice exhibit severe anaemia, in addition to other pathological features such as neurologic symptoms and systemic inflammation[26].

There is also evidence that IL-10 may have an influence on iron uptake. For example, one study showed that the changes in mRNA levels of transferrin (Tf)—the universal transporter of iron (Fe^{3+})—during the different phases of trypanosomal infection closely paralleled those of IL-10 expression[25].

Interleukin 12 (IL-12)

There was only one article that reported a relationship between IL-12 and anaemia in trypanosomiasis. In this study, BALB/c mice were infected with either strain 927 or strain 247 of *Trypanosoma brucei*. Infected mice were associated with increased levels of IL-12 compared to uninfected mice at all time points for both parasite strains, with levels in IL-12 in strain 247-infected mice being greater than those in strain 927-infected mice[22].

Discussion

Cytokines play a number of roles in biological processes and have been implicated in the pathogenesis of several disorders, notably immunoinflammatory conditions. In HAT, trypanosomes trigger a strong cytokine response with a type I immune response during the early stage, followed by a type II cytokine response in the late, chronic phase[11,32]. This type I/type II switch is considered important in controlling the severity of the disease. It is detrimental for the host to be locked in one type of cytokine environment throughout the infection. Here, we reviewed published data on the role cytokines play in immunopathology leading to anaemia in trypanosomiasis.

The literature search yielded 12 articles, which reported eight different cytokines that were thought to be involved in anaemia associated with *Trypanosoma brucei* infection. Of the eight cytokines, four were shown to play a key role, either by increasing the severity of anaemia or attenuating it. Those associated with more severe disease were TNF-α, IFN-γ and IL-12, all of which classically have proinflammatory properties. In contrast, IL-10, which is an anti-inflammatory cytokine, was shown to reduce the severity of anaemia.

Most studies identified TNF-α and IFN-γ as key cytokines that play greater roles in trypanosomiasis-associated anaemia, with each cytokine being reported in 10 of the 12 articles that qualified for the review. These data showed that the early stage of infection has been characterised by significant upregulation of TNF-α and IFN-γ. Prolonged overexpression of TNF-α correlated with disease severity (including anaemia and cachexia)[33], and extended survival of the host, dependent on the changes of cytokine profile towards anti-inflammatory ones, such as IL-10. IL-10 was also commonly cited; it was reported in eight of the articles that were reviewed. High levels of IL-10 were shown to down-regulate TNF-α and to attenuate the severity of the anaemia.

The molecular details of how cytokines mediate their effects to influence the severity of anaemia are largely unknown. However, a number of studies in mice have implicated NO as a key mediator of the cytokine response. NO is a pivotal effector molecule and possesses both cytostatic and cytolytic properties, as observed in *Trypanosoma congolense* infection[34]. Sternberg and Mabbot demonstrated that anaemia in the murine model was mediated by suppressor macrophages releasing nitric oxide. In in vitro studies, they recorded NO-dependent suppression of T cell proliferation when splenocyte cultures were exposed to IFN-γ and trypanosomes, similar to the characteristic suppression seen in the spleen of trypanosome-infected mice, therefore suggesting the role of NO in IFN-γ-associated anaemia[35]. Similar studies by the same authors have demonstrated the relationship between anaemia and bone marrow NO production, with bone marrow cell populations from *Trypanosoma brucei*-infected mice exhibiting increased levels of NO synthase activity, while suppression of bone marrow T cell proliferation coincided with NO production. It was therefore postulated that increased NO production in the bone marrow of *Trypanosoma brucei*-infected mice was probably playing a significant role in the anaemia resulting from *Trypanosoma brucei* infection[10]. In these studies, the authors also reported a correlation between serum nitrate concentrations and rising parasitaemia in *Trypanosoma brucei*-infected mice, in whom a 50% reduction in erythrocyte count and lowered haemoglobin concentrations were also noted. However, these conditions were reversed when infected mice were treated with NG-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, prompting a postulation that NO may be acting on the proliferation of immature erythrocytes or haematopoetic stem cells, resulting in anaemia[10].

Another potential mechanism explaining the effects of cytokines on anaemia might involve regulation of iron metabolism. Stijlemans et al. showed that bone marrow iron levels decline during the course of *Trypanosoma brucei* infection, and that this affects erythropoesis[27]; the total amount of erythrocytes isolated using a marker based on cell-surface erythroid-specific Ter 119 antigen and transferrin receptor (CD71) showed a significant drop in infected animals as compared to uninfected animals at the bone marrow level[27]. Additionally, trypanosomiasis may induce anaemia through the direct destruction of red cells infected by the parasites. For example, goats experimentally infected with *T. congolense* and *T. brucei* exhibit increased destruction of erythrocytes in the blood by the infecting trypanosomes, leading to reduced haemoglobin levels, despite increased erythrogenesis in response to increased erythrocyte loss[36].

These data and interactions indicate anaemia associated with trypanosomiasis results from a cascade of responses. It is evident that there is a need for a holistic approach to gain a better understanding of the aetiological factors.

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Moreover, the role of any particular cytokine or factor in the pathogenesis of anaemia during trypanosomiasis appears to be dependent on the host as well as the parasite itself.

No human data were retrieved in this review therefore the generalisation of the findings to human disease should be made with great caution, even if there is a 95% genomic level of identity between mice and humans[37]. Future understanding on the pathogenesis of anaemia in human African trypanosomiasis would be better informed by human-based studies.

Conclusion

The objective of this review was to investigate the published data regarding the role certain cytokines play in the immunopathology leading to anaemia in trypanosomiasis disease. The PubMed database was searched and articles were included using the PRISMA method. Though the search yielded many articles on trypanosomiasis, few reported on cytokines associated with anaemia in trypanosomiasis. This could be as a result of the limitation of this review, as it only focused on cytokines involved in anaemia caused by *Trypanosoma brucei*, the only species known to cause human and animal African trypanosomiasis. This restriction was based on the fact that trypanosomiasis is caused by different trypanosome species and the host-parasite immune responses may differ from species to species.

The immune responses associated with anaemia in *Trypanosoma brucei* infection include upregulation or downregulation of cytokines. Three cytokines (IL-10, TNF-α and IFN-γ) have been shown to play an important role: the IL-10 acting to counter the effects of the two proinflammatory cytokines, TNF-α and IFN-γ, to reduce the severity of the disease. The cytokines appear to work in concert with other regulatory pathways, notably nitric oxide and regulators of iron metabolism, to mediate their effects on bone marrow and circulating erythrocytes. Further research is recommended to explore other cytokines that might be involved in this cascade and to gain a better understanding of the processes involved.

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