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**Role of chemokines and cytokines in the neuropathogenesis of African trypanosomiasis**

Masocha W. Chemokines, cytokines in African trypanosomiasis

Willias Masocha

**Willias Masocha,** Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Kuwait University, Safat 13110, Kuwait

**Author contributions**: Masocha W solely wrote the manuscript.

**Correspondence to: Willias Masocha, B Pharm (Hons), PhD, Associate Professor,** Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Kuwait University, PO Box 24923, Safat 13110, Kuwait. masocha@hsc.edu.kw

**Telephone:** +965-24636078   **Fax:** +965-24636841

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**Abstract**

Trypanosoma brucei spp cause human African trypanosomiasis (HAT) or sleeping sickness in humans and nagana in animals. The early stages of the disease have none specific symptoms, however, the late stage of the disease involves neurological signs of the disease including disturbance of sleep patterns from which the disease derives the name sleeping sickness. During the late stage of African trypanosomiasis parasites, increased numbers of white blood cells, increased levels of cytokines and/or chemokines are found in the brain parenchyma and/or cerebrospinal fluid of animal models and HAT patients. In this minireview contemporary findings on how chemokines and cytokines are thought to play an important role in the central nervous system invasion by the parasites, inflammation and the neuropathology of the disease are discussed. The levels of various cytokines and chemokines, such as interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), C-C motif chemokine 2 (CCL2), CCL3, C-X-C motif chemokine 8 (CXCL8, IL-8) and CXCL10 in the cerebrospinal fluid (CSF) of HAT patients correlate with the severity or stage of the disease. Thus, these molecules are possible candidates for differentiating between early and late stage HAT. The role of cytokines and chemokines in parasite invasion of the central nervous system (CNS) is also being elucidated. IFN-γ, TNF-α, and CXCL-10 are some of the cytokines and chemokines now known to facilitate parasite penetration of the brain parenchyma. Interestingly, they also constitute some of the candidate molecules with potential to differentiate between stage 1 and stage 2 of HAT. The increased levels of cytokines such as IL-1β, IL-6, IFN-γ and TNF-α, as well as prostaglandins, during African trypanosomiasis might contribute to the neurological dysfunctions that occur during the HAT.

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**Key words:** African trypanosomiasis; Chemokine; Cytokine; Central nervous system; Brain parenchyma; Cerebrospinal fluid; Neuroinvasion; Neuroinflammation; Neurological disturbances

**Core tip:** Human African trypanosomiasis (HAT) or sleeping sickness, caused by Trypanosoma brucei spp, is staged into an early hemolymphatic stage and a late meningoencephalitic stage. During the late stage parasites, increased numbers/levels of white blood cells, cytokines and/or chemokines are found in the cerebrospinal fluid of patients. In this minireview contemporary findings on how chemokines and cytokines such as interferon-gamma (IFN-γ), TNF-α, C-X-C motif chemokine 8 (CXCL8) and CXCL10, are thought to play an important role in the central nervous system invasion by the parasites, inflammation and the neuropathology of the disease and might be candidates to differentiate between early and late stage HAT are discussed.

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**INTRODUCTION**

Three morphologically identical subspecies ofthe haemoflagellate protozoan parasiteTrypanosoma brucei(T. b.), T. b. brucei, T. b. gambiense and T. b. rhodesiense, cause African trypanosomiasis, the latter two species are human infective. The disease is endemic to sub-Saharan Africa and is transmitted through a bite of a tsetse fly (Glossina sp.) during a blood-meal. T. b. gambiense which is found in foci in large areas of West and Central Africa causes a chronic form of human African trypanosomiasis (HAT) that lasts several months to years. On the other hand T. b. rhodesiense with a much more limited distribution is found in East and Southeast Africa and causes an acute form of the disease that lasts several weeks to months[[1](#_ENREF_1),[2](#_ENREF_2)].

Clinically HAT is divided in to two stages: an early hemolymphatic stage (Stage 1) and a late encephalitic stage (Stage 2)[[1](#_ENREF_1),[3](#_ENREF_3),[4](#_ENREF_4)]. However, the demarcations between these two stages of the disease are not clear, more so for disease caused by T. b. rhodesiense where there is rapid transition from stage 1 to stage 2[[3](#_ENREF_3)]. In the early stage of HAT a chancre might develop at the site of inoculation followed by involvement of blood and lymphatic systems, which presents with general signs and symptoms of infection, chronic intermittent fever, headache, lymphadenopathy, splenomegaly and pruritus. In the late stage of the disease there are signs of nervous system involvement, which present as sleep disorders, *i.e.*, dysregulation of the circadian rhythm of the sleep–wake cycle and a fragmentation of the sleeping pattern, neurological symptoms including confusion, tremor, fasciculations, general motor weakness, hemiparesis, akinesia or dyskinesia, sensory disturbances with diffuse hyperpathia, abnormal movements and speech disorders, and psychiatric symptoms. If untreated, the disease will lead to coma and death in most of the cases. The patients die in a state of cachexia and also because of opportunistic infections[[4](#_ENREF_4)]. Clinical symptoms of HAT are of non-specific nature, thus, its diagnosis is confirmed by finding trypanosomes in the blood and lymph nodes or in the cerebrospinal fluid (CSF) using microscopy, the latter during the late stage of HAT. The serological test card agglutination trypanosomiasis test is used to screen for T. b. gambiense infections. The world health organization criteria for diagnosing Stage 2 HAT is the finding of trypanosomes and/or a white blood cell (WBC) count of > 5/μL in the CSF[[1](#_ENREF_1),[4](#_ENREF_4)].

Differentiating between the two stages of the disease is imperative before treatment can be begun[[1](#_ENREF_1)] because of the differences between the drugs used to treat early and late stages of HAT in terms of ability to cross the blood-brain barrier (BBB) and toxicity. The drugs which are used to treat the late stage of the disease, melarsoprol, eflornithine and the nifurtimox-eflornithine combination treatment, permeate the BBB better but are more toxic than the drugs used to treat the early stage of the disease, suramin and pentamidine[[3](#_ENREF_3),[4](#_ENREF_4)].

In this minireview the role of chemokines and cytokines in the invasion of the central nervous system (CNS) by the parasite and the ensuing inflammation and neuropathology which makes the disease intractable and fatal in most of the cases, will be discussed. Cytokines are a large group of immunoregulatory molecules. They play an important role in the control and pathogenesis of infectious diseases. Chemokines are involved in recruitment and retention of immune cells during inflammation and infection.

**Chemokine and cytokine expression in the CNS**

Trypanosome infection results in activation of the immune system and induction of expression of various cytokines and chemokines in both HAT patients and animal models of the disease[[5-11](#_ENREF_5)]. However, eventually the infection results in immunosuppression[[12-14](#_ENREF_12)]. The cytokines and chemokines that are induced both in the periphery and the CNS play an important role in the control of the parasites but they also contribute to the inflammation and immunosuppression which occurs during the disease[[6-11](#_ENREF_6),[15-17](#_ENREF_15)].

Increased expression of chemokines in the CNS has been observed during African trypanosomiasis. The expression of the chemokines C-X-C motif chemokine (CXCL) 1, CXCL2 (MIP-2), CXCL5, CXCL9, CXCL10, CXCL12, CXCL13, CXCL14, CXCL16, C-C motif chemokine (CCL) 2 (MCP-1), CCL3 (MIP-1alpha), CCL4, CCL5 (RANTES), CCL7, CCL9, CCL12 and CCL28 was found to be up-regulated in the brains of rodents infected with T. b. brucei[[5](#_ENREF_5),[9](#_ENREF_9),[18](#_ENREF_18),[19](#_ENREF_19)]. Some of these chemokines are expressed at higher levels during late than early stage African trypanosomiasis (Table 1). CXCL9 and CXCL10 were the most highly up-regulated cytokines in the brain at later stages, when parasites had invaded the CNS, compared to early stages of the disease before CNS invasion. The increased expression of both chemokines was found to be dependent on interferon (IFN)-γ[[5](#_ENREF_5)]. CXCL10 was found to be predominantly up-regulated in parenchymal astrocytes of hypothalamic regions, optic chiasm, and optic tracts at later stages of the disease[[5](#_ENREF_5)]. Of these chemokines CCL2, CCL3, CXCL8 (IL-8), CXCL10 and, CXCL13 have been found to be increased in the CSF of patients with late stage HAT infected with either T. b. gambiense or T. b. rhodesiense more than non-infected control patients or patients with early stage HAT (Table 1)[[5](#_ENREF_5),[7](#_ENREF_7),[8](#_ENREF_8),[20-25](#_ENREF_20)].

Several studies have reported the increased expression of cytokines in the CNS during trypanosome infection. The cytokines IFN-α/β, IFN-γ, interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-10, IL-13, transforming growth factor (TGF)-β and tumor necrosis factor (TNF)-α, were found increased in the brains of rodents infected with T. b. brucei[[10](#_ENREF_10),[18](#_ENREF_18),[19](#_ENREF_19),[26-30](#_ENREF_26)]. Some of these cytokines are also expressed at higher levels during late than early stage African trypanosomiasis (Table 1). It has been suggested that astrocytes might be the source of some of these cytokines since the levels of these cytokines were found to correlate with astrocyte activation[[19](#_ENREF_19)]. Lymphocytes are the major source of IFN-γ in the brains of T. b. brucei infected mice[[10](#_ENREF_10)]. T. b. brucei CpG-DNA stimulates macrophages to increase the production of Il-12 and TNF-α[[31](#_ENREF_31)], thus, macrophages and possibly microglia might be the some of the major producers of these cytokines in the brain during T. b. brucei infections. Of these cytokines IFN-γ, IL-1β, IL-6, IL-10, TNF-α have been found to be increased in the CSF of patients with late stage HAT infected with either T. b. gambiense or T. b. rhodesiense more than non-infected controls or patients with early stage HAT (Table 1)[[5](#_ENREF_5),[7](#_ENREF_7),[8](#_ENREF_8),[20](#_ENREF_20),[22](#_ENREF_22),[32-34](#_ENREF_32)]. On the other hand, the levels of whereas TGF-β was decreased in the CSF of patients with late stage HAT infected with T. b. rhodesiense compared to patients with early stage HAT but was higher than non-infected control, where it was not detected in the latter[[34](#_ENREF_34)].

**Cytokines, chemokines and trypanosome brain invasion**

Taking into consideration that the expression of various chemokines and cytokines in the CNS correlate with presence of trypanosomes in the brains of animal models of the disease and CSF of HAT patients there is a possibility these molecules play a role in the recruitment, mobility and retention, and also in the control of the levels, of the parasites in the CNS. The role which some of these molecules play in trypanosome invasion of the brain have been studied using transgenic animal models (Table 2)[[35](#_ENREF_35)].

Of these molecules the role of IFN-γ in trypanosome invasion of the brain was the first to be studied using transgenic mice[[10](#_ENREF_10)]. Mice deficient of IFN-γ or its receptor had higher parasites in the blood but had less parasites, and lymphocytes as well, in the brain parenchyma compared to wild type (WT) mice. In these transgenic mice the parasites accumulated in the perivascular space between the endothelial basement membrane and parenchymal of the post-capillary venules[[10](#_ENREF_10)], suggesting that IFN-γ or factors induced by it are important for parasite crossing of the parenchymal basement membrane. The source of the IFN-γ was most likely lymphocytes since the levels of IFN-γ did not increase in RAG deficient mice (lacking mature B and T lymphocytes) and parasite penetration into the brain parenchyma was reduced in these mice. Mice deficient of IL-12 have reduced IFN-γ levels[[36](#_ENREF_36)] and also have less parasites penetrating the brain parenchyma[[10](#_ENREF_10)].

IFN-γ induces the production of the chemokine CXCL10, also known as IFN-γ-induced protein 10 (IP-10). Mice deficient of IFN-γ have reduced expression of CXCL10 compared to WT mice during trypanosome infection[[5](#_ENREF_5)]. Transgenic mice lacking CXCL10 or its receptor CXCR3 also showed reduced parasites penetrating the brain parenchyma although they had similar parasites in the blood compared to WT mice[[5](#_ENREF_5)]. CXCL10 deficient mice did not have accumulation of parasites in the perivascular space suggesting that other IFN-γ-induced molecules instead of CXCL10 play a role in IFN-γ dependent passage of parasites across the parenchymal basement membrane.

The role of TNF-α in trypanosome invasion of the brain was also studied using transgenic mice[[15](#_ENREF_15)]. Mice deficient of TNF-α receptor 1 had higher parasites in the blood but had less numbers of both parasites and T lymphocytes in the brain parenchyma compared to WT mice[[15](#_ENREF_15)]. T. b. brucei infected mice deficient of TNFR1 had less adhesion molecules, vascular cell adhesion protein 1 (VCAM-1) and intercellular Adhesion Molecule 1 (ICAM-1), compared to WT mice, suggesting that the induction of adhesion molecules through TNF-α signalling might play a role in the TNF-α facilitated parasite and T cell invasion of the brain parenchyma. Mice deficient of IFN-α/βR had reduced numbers of T lymphocytes and parasites in the brain parenchyma compared to WT mice, but the magnitude was not as pronounced as mice deficient of IFN-γ, TNF-α or CXCL-10 signalling[[15](#_ENREF_15)]. Mice lacking the receptors of two other cytokines IL-1R and IL-18R had similar parasites in the blood as well as parasites and T lymphocytes in the brain parenchyma as WT mice[[15](#_ENREF_15)], suggesting that these cytokines do not play a significant role in parasite penetration in to the CNS.

**Cytokines, chemokines and neuropathology during African trypanosomiasis**

 Chemokines such as CXCL10 play a role in the attraction, mobiltiy and/or retention of inflammatory cells into the CNS during African trypanosomiasis[[5](#_ENREF_5)], thus contribute to the neuroinflammation and morbidity seen in the late stage of the disease. High levels of CCL2, CCL3, CXCL8 and CXCL10 in the CSF were found to be associated with the severity of the disease and neurological signs which are characteristic of late stage HAT[[5](#_ENREF_5),[7](#_ENREF_7)].

High levels of cytokines such as IL1β, IL-6, IFN-γ and TNF-α in the plasma or CSF have also been found to be associated with the severity of the disease and neurological signs which are characteristic of late stage HAT[[7](#_ENREF_7),[8](#_ENREF_8),[22](#_ENREF_22),36,[37](#_ENREF_37)]. However, beside neuroinflammation, the role of cytokines in causing brain dysfunctions which result in neuroendocrine dysfunctions, neurological symptoms and/or sleep disorders (Table 3) have also been studied. In HAT patients high plasma concentrations of IL-6 and TNF-α have been found to correlate with hypopituitarism and endocrine dysfunctions[[38](#_ENREF_38)]. Endocrine dysfunctions result in some of the signs and symptoms of HAT such as impotence, amenorrhea, infertility and lethargy. Chronically elevated concentrations of IL-6 and/or TNF-α during HAT might have a direct inhibitory effects on the hypothalamus-pituitary-thyroid or adrenal axis resulting in reduced thyroid hormone and cortisol secretion[[38](#_ENREF_38)]. TNF-α inhibitors have been shown to restore the hypothalamic-pituitary-adrenal axis in other chronic inflammatory diseases such rheumatoid arthritis[[39](#_ENREF_39)].

IL-6, IFN-γ and TNF-α can alter synaptic functions and are implicated in causing sleep pattern disruptions[[40-45](#_ENREF_40)]. IFN-γ alters clock gene expression and circadian rhythms in the suprachiasmatic nucleus (SCN)[[41](#_ENREF_41),[46](#_ENREF_46)]. The SCN is essential for the generation and maintenance of daily rhythms in physiology and behavior[[47-49](#_ENREF_47)] . IL1β, TNF-α and IFN-γ also affects hypothalamic and brainstem neurons which are involved in sleep-wakefulness regulation[[41](#_ENREF_41),[50](#_ENREF_50),[51](#_ENREF_51)]. Apart from HAT, IL-6 and/or TNF-α are elevated in other disorders associated with excessive daytime sleepiness, such as sleep apnea, narcolepsy, and idiopathic hypersomnia[[43](#_ENREF_43),[44](#_ENREF_44),[52](#_ENREF_52)].

Cytokines and chemokines can sensitize and stimulate nociceptors in the periphery and/or synaptic targets in the CNS, which can result in neuropathic pain[[53](#_ENREF_53)]. Administration or up-regulation of IL1β, IL-6, IFN-γ and TNF-α can induce neuropathic pain in rodents[[40](#_ENREF_40),[54-59](#_ENREF_54)]. It has been suggested that IL-1 and IFN-γ might be implicated in the thermal hyperalgesia observed in T. b. brucei infected rats[[60](#_ENREF_60),[61](#_ENREF_61)]. Hyperaesthesia is one of the clinical features reported in HAT patients[[3](#_ENREF_3),[4](#_ENREF_4)]. Thus, these cytokines, together with other inflammatory molecules, most likely contribute to the hyperalgesia/hyperaesthesia and pain observed in HAT.

In rats infected with T. b. brucei apoptosis of some cells and degeneration of some nerve fibres, though modest, in the brain have been found to be spatially associated with mRNA expression of the cytokines IL1β, and TNF-α[[29](#_ENREF_29)]. Intraventricular infusion of an antagonist of TNF-α, but not IL-1, was found to reduce trypanosome-induced neurodegeneration[[28](#_ENREF_28)]. Infusion of antagonists of both cytokines further reduced the trypanosome-induced neurodegeneration, thus, implying that TNF-α is a principle mediator of trypanosome-induced neurodegeneration and its effects are augmented by IL-1[[28](#_ENREF_28)].

CONCLUSIONThe expression of cytokines and chemokines in the brain and/or CSF is increased in animal models of African trypanosomiasis and HAT patients and the levels of these molecules correlate with the severity or stage of the disease. The high levels of chemokines and cytokines in the brain and CSF during late compared to early stage African trypanosomiasis are most likely due to the invasion of the CNS by trypanosomes and/or WBCs in the late stage resulting in neuroinflammation. Thus, these molecules are possible candidates for differentiating between early and late stage HAT. In the future clinicians could utilise this knowledge to treat patients with high levels of these molecules in the CSF as late stage patients, thus, possibly reducing the occurrence of relapses in late stage HAT patients who might have been wrongfully diagnosed as early stage and treated as such using the current staging criteria. Recently, extensive research has been undertaken to evaluate the suitability of these molecules as stage biomarker and also as markers for treatment outcome in HAT patients [[5](#_ENREF_5),[7](#_ENREF_7),[8](#_ENREF_8),[20-25](#_ENREF_20),[32-34](#_ENREF_32)]. The role of cytokines and chemokines in parasite invasion of the CNS is also being elucidated. IFN-γ, TNF-α, and CXCL-10 are some of the cytokines and chemokines now known to have a facilitative role in parasite penetration of the brain parenchyma. Interestingly, they also constitute some of the molecules with potential to differentiate between stage 1 and stage 2 of HAT[[5](#_ENREF_5),[20](#_ENREF_20),[22](#_ENREF_22)]. Moreover, neopterin which is a stable product produced by IFN-γ activated immune cells has been suggested to have potential to differentiate between these two stages of HAT[[24](#_ENREF_24)]. The increased levels of cytokines such as IL-1β, IL-6, IFN-γ and TNF-α during African trypanosomiasis contribute to the neurological dysfunctions that occur during HAT. Thus, studying cytokines and chemokines during African trypanosomiasis not only aids in understanding the neurobiology of the disease but also provides candidate diagnostic markers and possible therapeutic targets to reduce the neurological sequelae in surviving patients.

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**REFERENCES**

1 Control and surveillance of African trypanosomiasis. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 1998; **881**: I-VI, 1-114 [PMID: 10070249]

2 **Simarro PP,** Diarra A, Ruiz Postigo JA, Franco JR, Jannin JG. The human African trypanosomiasis control and surveillance programme of the World Health Organization 2000-2009: the way forward. *PLoS Negl Trop Dis* 2011; **5**: e1007 [PMID: 21364972 DOI: 10.1371/journal.pntd.0001007]

3 **Kennedy PG.** Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). *Lancet Neurol* 2013; **12**: 186-194 [PMID: 23260189 DOI: 10.1016/S1474-4422(12)70296-X]

4 **Malvy D,** Chappuis F. Sleeping sickness. *Clin Microbiol Infect* 2011; **17**: 986-995 [PMID: 21722252 DOI: 10.1111/j.1469-0691.2011.03536.x]

5 **Amin DN,** Rottenberg ME, Thomsen AR, Mumba D, Fenger C, Kristensson K, Buscher P, Finsen B, Masocha W. Expression and role of CXCL10 during the encephalitic stage of experimental and clinical African trypanosomiasis. *J Infect Dis* 2009; **200**: 1556-1565 [PMID: 19827943]

6 **Bancroft GJ,** Sutton CJ, Morris AG, Askonas BA. Production of interferons during experimental African trypanosomiasis. *Clin Exp Immunol* 1983; **52**: 135-143 [PMID: 6190591]

7 **Courtioux B,** Boda C, Vatunga G, Pervieux L, Josenando T, M'Eyi PM, Bouteille B, Jauberteau-Marchan MO, Bisser S. A link between chemokine levels and disease severity in human African trypanosomiasis. *Int J Parasitol* 2006; **36**: 1057-1065 [PMID: 16765963 DOI: 10.1016/j.ijpara.2006.04.011]

8 **Lejon V,** Lardon J, Kenis G, Pinoges L, Legros D, Bisser S, N'Siesi X, Bosmans E, Buscher P. Interleukin (IL)-6, IL-8 and IL-10 in serum and CSF of Trypanosoma brucei gambiense sleeping sickness patients before and after treatment. *Trans R Soc Trop Med Hyg* 2002; **96**: 329-333 [PMID: 12174791]

9 **Liu Y,** Li Z, Bakhiet M. Upregulation of the chemokines Rantes, MCP-1, MIP-1a and MIP-2 in early infection with Trypanosoma brucei brucei and inhibition by sympathetic denervation of the spleen. *Trop Med Int Health* 1999; **4**: 85-92 [PMID: 10206261]

10 **Masocha W,** Robertson B, Rottenberg ME, Mhlanga J, Sorokin L, Kristensson K. Cerebral vessel laminins and IFN-gamma define Trypanosoma brucei brucei penetration of the blood-brain barrier. *J Clin Invest* 2004; **114**: 689-694 [PMID: 15343387]

11 **Okomo-Assoumou MC,** Daulouede S, Lemesre JL, N'Zila-Mouanda A, Vincendeau P. Correlation of high serum levels of tumor necrosis factor-alpha with disease severity in human African trypanosomiasis. *Am J Trop Med Hyg* 1995; **53**: 539-543 [PMID: 7485714]

12 **Askonas BA.** Macrophages as mediators of immunosuppression in murine African trypanosomiasis. *Curr Top Microbiol Immunol* 1985; **117**: 119-127 [PMID: 2411475]

13 **Goodwin LG,** Green DG, Guy MW, Voller A. Immunosuppression during trypanosomiasis. *Br J Exp Pathol* 1972; **53**: 40-43 [PMID: 5014242]

14 **Hudson KM,** Byner C, Freeman J, Terry RJ. Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. *Nature* 1976; **264**: 256-258 [PMID: 1087372]

15 **Amin DN,** Vodnala SK, Masocha W, Sun B, Kristensson K, Rottenberg ME. Distinct Toll-like receptor signals regulate cerebral parasite load and interferon alpha/beta and tumor necrosis factor alpha-dependent T-cell infiltration in the brains of Trypanosoma brucei-infected mice. *J Infect Dis* 2012; **205**: 320-332 [PMID: 22116836 DOI: 10.1093/infdis/jir734]

16 **de Gee AL,** Sonnenfeld G, Mansfield JM. Genetics of resistance to the African trypanosomes. V. Qualitative and quantitative differences in interferon production among susceptible and resistant mouse strains. *J Immunol* 1985; **134**: 2723-2726 [PMID: 2579155]

17 **Lucas R,** Magez S, Songa B, Darji A, Hamers R, de Baetselier P. A role for TNF during African trypanosomiasis: involvement in parasite control, immunosuppression and pathology. *Res Immunol* 1993; **144**: 370-376[PMID: 8278660]

18 Hunter CA, Gow JW, Kennedy PG, Jennings FW, Murray M. Immunopathology of experimental African sleeping sickness: detection of cytokine mRNA in the brains of Trypanosoma brucei brucei-infected mice. *Infect Immun* 1991; **59**: 4636-4640 [PMID: 1718878]

19 **Hunter CA,** Jennings FW, Kennedy PG, Murray M. Astrocyte activation correlates with cytokine production in central nervous system of Trypanosoma brucei brucei-infected mice. *Lab Invest* 1992; **67**: 635-642 [PMID: 1434541]

20 **Amin DN,** Ngoyi DM, Nhkwachi GM, Palomba M, Rottenberg M, Buscher P, Kristensson K, Masocha W. Identification of stage biomarkers for human African trypanosomiasis. *Am J Trop Med Hyg* 2010; **82**: 983-990 [PMID: 20519589]

21 **Courtioux B,** Pervieux L, Vatunga G, Marin B, Josenando T, Jauberteau-Marchan MO, Bouteille B, Bisser S. Increased CXCL-13 levels in human African trypanosomiasis meningo-encephalitis. *Trop Med Int Health* 2009; **14**: 529-534 [PMID: 19298637 DOI: 10.1111/j.1365-3156.2009.02263.x]

22 **Hainard A,** Tiberti N, Robin X, Lejon V, Ngoyi DM, Matovu E, Enyaru JC, Fouda C, Ndung'u JM, Lisacek F, Muller M, Turck N, Sanchez JC. A combined CXCL10, CXCL8 and H-FABP panel for the staging of human African trypanosomiasis patients. *PLoS Negl Trop Dis* 2009; **3**: e459 [PMID: 19554086]

23 **Hainard A,** Tiberti N, Robin X, Ngoyi DM, Matovu E, Enyaru JC, Muller M, Turck N, Ndung'u JM, Lejon V, Sanchez JC. Matrix metalloproteinase-9 and intercellular adhesion molecule 1 are powerful staging markers for human African trypanosomiasis. *Trop Med Int Health* 2011; **16**: 119-126 [PMID: 20958893]

24 **Tiberti N,** Hainard A, Lejon V, Courtioux B, Matovu E, Enyaru JC, Robin X, Turck N, Kristensson K, Ngoyi DM, Vatunga GM, Krishna S, Buscher P, Bisser S, Ndung'u JM, Sanchez JC. Cerebrospinal fluid neopterin as marker of the meningo-encephalitic stage of Trypanosoma brucei gambiense sleeping sickness. *PLoS One* 2012; **7**: e40909 [PMID: 22815865 DOI: 10.1371/journal.pone.0040909]

25 **Tiberti N,** Matovu E, Hainard A, Enyaru JC, Lejon V, Robin X, Turck N, Ngoyi DM, Krishna S, Bisser S, Courtioux B, Buscher P, Kristensson K, Ndung'u JM, Sanchez JC. New biomarkers for stage determination in Trypanosoma brucei rhodesiense sleeping sickness patients. *Clin Transl Med* 2013; **2**: 1 [PMID: 23369533 DOI: 10.1186/2001-1326-2-1]

26 **Masocha W,** Amin DN, Kristensson K, Rottenberg ME. Differential invasion of Trypanosoma brucei brucei and lymphocytes into the brain of C57BL/6 and 129Sv/Ev mice. *Scand J Immunol* 2008; **68**: 484-491 [PMID: 18822108]

27 **Masocha W,** Rottenberg ME, Kristensson K. Minocycline impedes African trypanosome invasion of the brain in a murine model. *Antimicrob Agents Chemother* 2006; **50**: 1798-1804 [PMID: 16641452]

28 **Quan N,** He L, Lai W. Intraventricular infusion of antagonists of IL-1 and TNF alpha attenuates neurodegeneration induced by the infection of Trypanosoma brucei. *J Neuroimmunol* 2003; **138**: 92-98 [PMID: 12742658 DOI: 10.1016/S0165-5728(03)00122-X]

29 **Quan N,** Mhlanga JD, Whiteside MB, McCoy AN, Kristensson K, Herkenham M. Chronic overexpression of proinflammatory cytokines and histopathology in the brains of rats infected with Trypanosoma brucei. *J Comp Neurol* 1999; **414**: 114-130 [PMID: 10494082]

30 **Sternberg JM,** Rodgers J, Bradley B, Maclean L, Murray M, Kennedy PG. Meningoencephalitic African trypanosomiasis: Brain IL-10 and IL-6 are associated with protection from neuro-inflammatory pathology. *J Neuroimmunol* 2005; **167**: 81-89 [PMID: 16054238 DOI: 10.1016/j.jneuroim.2005.06.017]

31 **Shoda LK,** Kegerreis KA, Suarez CE, Roditi I, Corral RS, Bertot GM, Norimine J, Brown WC. DNA from protozoan parasites Babesia bovis, Trypanosoma cruzi, and T. brucei is mitogenic for B lymphocytes and stimulates macrophage expression of interleukin-12, tumor necrosis factor alpha, and nitric oxide. *Infect Immun* 2001; **69**: 2162-2171 [PMID: 11254571 DOI: 10.1128/IAI.69.4.2162-2171.2001]

32 **MacLean L,** Odiit M, Sternberg JM. Nitric oxide and cytokine synthesis in human African trypanosomiasis. *J Infect Dis* 2001; **184**: 1086-1090 [PMID: 11574928 DOI: 10.1086/323479]

33 **Maclean L,** Odiit M, Sternberg JM. Intrathecal cytokine responses in Trypanosoma brucei rhodesiense sleeping sickness patients. *Trans R Soc Trop Med Hyg* 2006; **100**: 270-275 [PMID: 16343570 DOI: 10.1016/j.trstmh.2005.03.013]

34 **MacLean L,** Reiber H, Kennedy PG, Sternberg JM. Stage progression and neurological symptoms in Trypanosoma brucei rhodesiense sleeping sickness: role of the CNS inflammatory response. *PLoS Negl Trop Dis* 2012; **6**: e1857 [PMID: 23145191 DOI: 10.1371/journal.pntd.0001857]

35 **Masocha W,** Kristensson K, Rottenberg ME. Neurobiology of African trypanosomiasis. In: Bentivoglio M, Cavalheiro EA, Kristensson K, Patel N, editors. Neglected tropical diseases and conditions of the nervous system: Springer, In press

36 Barkhuizen M, Magez S, Atkinson RA, Brombacher F. Interleukin-12p70-dependent interferon- gamma production is crucial for resistance in African trypanosomiasis. *J Infect Dis* 2007; **196**: 1253-1260 [PMID: 17955445 DOI: 10.1086/521681]

37 **Maclean L,** Odiit M, Macleod A, Morrison L, Sweeney L, Cooper A, Kennedy PG, Sternberg JM. Spatially and genetically distinct African Trypanosome virulence variants defined by host interferon-gamma response. *J Infect Dis* 2007; **196**: 1620-1628 [PMID: 18008245 DOI: 10.1086/522011]

38 **Reincke M,** Arlt W, Heppner C, Petzke F, Chrousos GP, Allolio B. Neuroendocrine dysfunction in African trypanosomiasis. The role of cytokines. *Ann N Y Acad Sci* 1998; **840**: 809-821 [PMID: 9629307]

39 **Atzeni F,** Straub RH, Cutolo M, Sarzi-Puttini P. Anti-TNF therapy restores the hypothalamic-pituitary-adrenal axis. *Ann N Y Acad Sci* 2010; **1193**: 179-181 [PMID: 20398027 DOI: 10.1111/j.1749-6632.2009.05366.x]

40 **Gruber-Schoffnegger D,** Drdla-Schutting R, Honigsperger C, Wunderbaldinger G, Gassner M, Sandkuhler J. Induction of thermal hyperalgesia and synaptic long-term potentiation in the spinal cord lamina I by TNF-alpha and IL-1beta is mediated by glial cells. *J Neurosci* 2013; **33**: 6540-6551 [PMID: 23575851 DOI: 10.1523/JNEUROSCI.5087-12.2013]

41 **Kristensson K,** Nygard M, Bertini G, Bentivoglio M. African trypanosome infections of the nervous system: parasite entry and effects on sleep and synaptic functions. *Prog Neurobiol* 2010; **91**: 152-171 [PMID: 19995590]

42 **Shin HC,** Oh S, Jung SC, Park J, Won CK. Differential modulation of short and long latency sensory responses in the SI cortex by IL-6. *Neuroreport* 1997; **8**: 2841-2844 [PMID: 9376515]

43 **Vgontzas AN,** Chrousos GP. Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders. *Endocrinol Metab Clin North Am* 2002; **31**: 15-36 [PMID: 12055986]

44 **Vgontzas AN,** Zoumakis M, Papanicolaou DA, Bixler EO, Prolo P, Lin HM, Vela-Bueno A, Kales A, Chrousos GP. Chronic insomnia is associated with a shift of interleukin-6 and tumor necrosis factor secretion from nighttime to daytime. *Metabolism* 2002; **51**: 887-892 [PMID: 12077736 DOI: 10.1053/meta.2002.33357]

45 **Vikman KS,** Owe-Larsson B, Brask J, Kristensson KS, Hill RH. Interferon-gamma-induced changes in synaptic activity and AMPA receptor clustering in hippocampal cultures. *Brain Res* 2001; **896**: 18-29 [PMID: 11277968 DOI: 10.1016/S0006-8993(00)03238-8]

46 **Kwak Y,** Lundkvist GB, Brask J, Davidson A, Menaker M, Kristensson K, Block GD. Interferon-gamma alters electrical activity and clock gene expression in suprachiasmatic nucleus neurons. *J Biol Rhythms* 2008; **23**: 150-159 [PMID: 18375864 DOI: 10.1177/0748730407313355]

47 **Green CB,** Menaker M. Circadian rhythms. Clocks on the brain. *Science* 2003; **301**: 319-320 [PMID: 12843400 DOI: 10.1126/science.1087824]

48 **Saper CB.** The central circadian timing system. *Curr Opin Neurobiol* 2013 [PMID: 23706187 DOI: 10.1016/j.conb.2013.04.004]

49 **Stephan FK,** Nunez AA. Elimination of circadian rhythms in drinking, activity, sleep, and temperature by isolation of the suprachiasmatic nuclei. *Behav Biol* 1977; **20**: 1-61 [PMID: 194576]

50 **Kubota T,** Li N, Guan Z, Brown RA, Krueger JM. Intrapreoptic microinjection of TNF-alpha enhances non-REM sleep in rats. *Brain Res* 2002; **932**: 37-44 [PMID: 11911859 DOI: 10.1016/S0006-8993(02)02262-X]

51 **Yi PL,** Tsai CH, Lu MK, Liu HJ, Chen YC, Chang FC. Interleukin-1beta mediates sleep alteration in rats with rotenone-induced parkinsonism. *Sleep* 2007; **30**: 413-425 [PMID: 17520785]

52 **Tam CS,** Wong M, McBain R, Bailey S, Waters KA. Inflammatory measures in children with obstructive sleep apnoea. *J Paediatr Child Health* 2006; **42**: 277-282 [PMID: 16712558 DOI: 10.1111/j.1440-1754.2006.00854.x]

53 **Ellis A,** Bennett DL. Neuroinflammation and the generation of neuropathic pain. *Br J Anaesth* 2013; **111**: 26-37 [PMID: 23794642 DOI: 10.1093/bja/aet128]

54 **DeLeo JA,** Colburn RW, Nichols M, Malhotra A. Interleukin-6-mediated hyperalgesia/allodynia and increased spinal IL-6 expression in a rat mononeuropathy model. *J Interferon Cytokine Res* 1996; **16**: 695-700 [PMID: 8887053]

55 **Robertson B,** Xu XJ, Hao JX, Wiesenfeld-Hallin Z, Mhlanga J, Grant G, Kristensson K. Interferon-gamma receptors in nociceptive pathways: role in neuropathic pain-related behaviour. *Neuroreport* 1997; **8**: 1311-1316 [PMID: 9175135]

56 **Vikman KS,** Duggan AW, Siddall PJ. Interferon-gamma induced disruption of GABAergic inhibition in the spinal dorsal horn in vivo. *Pain* 2007; **133**: 18-28 [PMID: 17407800 DOI: 10.1016/j.pain.2007.02.010]

57 **Vikman KS,** Hill RH, Backstrom E, Robertson B, Kristensson K. Interferon-gamma induces characteristics of central sensitization in spinal dorsal horn neurons in vitro. *Pain* 2003; **106**: 241-251 [PMID: 14659507 DOI: 10.1016/S0304-3959(03)00262-8]

58 **Wei XH,** Na XD, Liao GJ, Chen QY, Cui Y, Chen FY, Li YY, Zang Y, Liu XG. The up-regulation of IL-6 in DRG and spinal dorsal horn contributes to neuropathic pain following L5 ventral root transection. *Exp Neurol* 2013; **241**: 159-168 [PMID: 23261764 DOI: 10.1016/j.expneurol.2012.12.007]

59 **Zimmermann M.** Pathobiology of neuropathic pain. *Eur J Pharmacol* 2001; **429**: 23-37 [PMID: 11698024 DOI: 10.1016/S0014-2999(01)01303-6]

60 **Kristensson K,** Eneroth A, Olsson T, Wiesenfeld-Hallin Z. A new approach for the pathogenesis of human African trypanosomiasis. *Bull Soc Pathol Exot* 1994; **87**: 319-322 [PMID: 7496193]

61 **Wiesenfeld-Hallin Z,** Kristensson K, Samuelsson EB, Schultzberg M. Studies of hyperalgesia induced by Trypanosoma brucei brucei infection in rats. *Acta Trop* 1991; **48**: 215-222 [PMID: 1671623]

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**Table 1 Cytokines and chemokines with increased expression in the brain parenchyma of rodents and cerebrospinal fluid of human patients more during late than early stage African trypanosomiasis**

|  |  |  |
| --- | --- | --- |
| **Site**  | **Cytokine/chemokine** | **Ref.** |
| **Chemokines** |
| **Rodent brain parenchyma** | CCL21, CCL4, CCL5, CCL7, CCL9, CCL12, CCL19, CCL28 CXCL1, CXCL5, CXCL9, CXCL101, CXCL12, CXCL131, CXCL14, CXCL16,  | [[5](#_ENREF_5),[19](#_ENREF_19)] |
|  **HAT patient CSF** | CCL21, CCL3, CXCL8 (IL-8), CXCL101, CXCL131 | [[5](#_ENREF_5),[7](#_ENREF_7),[8](#_ENREF_8),[20-25](#_ENREF_20)] |
| **Cytokines** |
| **Rodent brain parenchyma** | IFN-γ1, IL-1α, IL-1β1, IL-6, IL-101, TGF-β, TNF-α1 | [[10](#_ENREF_10),[19](#_ENREF_19),[26-28](#_ENREF_26),[30](#_ENREF_30)] |
| **HAT patient CSF** | IFN-γ1, IL-1β1, IL-61, IL-101, TNF-α1 | [[5](#_ENREF_5),[7](#_ENREF_7),[8](#_ENREF_8),[20](#_ENREF_20),[22](#_ENREF_22),[32-34](#_ENREF_32)] |

1Expressed in both late stage rodent brains and HAT patients CSF.

CCL: C-C motif chemokine; CSF: Cerebrospinal fluid; CXCL: C-X-C motif chemokine; HAT: Human African trypanosomiasis; IFN: Interferon; IL: Interleukin; TGF: Transforming growth factor; TNF: Tumor necrosis factor.

**Table 2 Cytokines and chemokines involved in Trypanosoma brucei spp. neuroinvasion**

|  |  |  |
| --- | --- | --- |
| **Cytokine/Chemokine** | **Trypanosome levels in the brain parenchyma of transgenic mice compared to WT mice** | **Ref.** |
| **Chemokines** |
| CXCL10 | Cxcl10-/- and Cxcr3-/- mice had less trypanosomes in the brain parenchyma compared with WT mice. |  [[5](#_ENREF_5)] |
| **Cytokines** |
| IFNα/β  | Ifn-α/βr -/- mice had slightly less trypanosomes in the brain parenchyma compared with WT mice. | [[15](#_ENREF_15)] |
| IFN-γ  | Ifn-γ -/- and Ifn-γr -/- had less trypanosomes in the brain parenchyma compared with WT mice. Trypanosomes accumulated in the perivascular compartment, confined between the endothelial and the parenchymal basement membranes, in certain areas of the brains of both transgenic mice | [[10](#_ENREF_10)] |
| IL-12 | Il-12p40-/- mice had less trypanosomes in the brain parenchyma compared with WT mice. | [[10](#_ENREF_10)] |
| TNF-α | Tnfr1*−/−* mice had less trypanosomes in the brain parenchyma compared with WT mice. | [[15](#_ENREF_15)] |

CXCL: C-X-C motif chemokine; IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor; WT: Wild-type.

**Table 3 Selected cytokines associated neurological and neuroendocrine features of African trypanosomiasis**

|  |  |  |
| --- | --- | --- |
| **Cytokine** | **Possible neurological and neuroendocrine features associated with** | **Ref.** |
| IFN-γ  | Sleep pattern disruptions, hyperalgesia/hyperaesthesia and pain | [[41](#_ENREF_41),[60](#_ENREF_60)] |
| IL1β  | Hyperalgesia/ hyperaesthesia and pain, neurodegeneration | [[28](#_ENREF_28),[29](#_ENREF_29),[61](#_ENREF_61)] |
| IL-6 | Hypopituitarism and endocrine dysfunctions, sleep pattern disruptions, hyperalgesia/ hyperaesthesia and pain | [[38](#_ENREF_38)] |
| TNF-α | Hypopituitarism and endocrine dysfunctions, sleep pattern disruptions, hyperalgesia/hyperaesthesia and pain, neurodegeneration | [[28](#_ENREF_28),[29](#_ENREF_29),[38](#_ENREF_38),[41](#_ENREF_41)] |

IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor.