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Que celui qui n'a pas

traversé la rivière

ne se moque pas

de celui qui s'est noyé

(proverbe africain)

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Abréviations

Abbreviations

Abreviaturas

µg	Microgramme
µL	Microlitre
Abs	Antibodies
ADN	Acide désoxyribonucléique
APC	Allophycocyanin
BBB	Blood-brain barrier
BCA-1	B cell attarcting chemokine 1
BHE	Barrière hémo-encéphalique
BHM	Barrière hémo-méningée
BLC	B lymphocyte chemoattractant
bp	Paires de base
CATT	Card agglutination trypanosomiasis test
CCL	Chemokine Cysteine-Cysteine motif ligand
CD	Cluster of differentiation
cDNA	Complementary desoxyribonucleic acid
CHME-5	Human microglial cell line 5
CIRMF	Centre international de recherche médicale de Franceville
CMH	Complexe majeur d'histocompatibilité
CMH-5	Cellules microgliales humaine de type 5
CNS	Central nervous system
CO_2	Dioxyde de carbone
CSF	Cerebrospinal fluid
ct	Cycle threshold
CXCL	Chemokine C-X-C motif ligand
CXCR	Chemokine C-X-C motif receptor
DALYs	Disability-adjusted life years
DC	Dendritic cell
DEAE	Diéthylaminoéthyl
DFMO	Difluoromethyl-ornithine
DMEM	Dulbecco modified Eagle medium
DNase	Desoxyribonuclease
dNTP	Desoxy-nucleic triphosphate
dpi	Day post-infection
DTT	Dithiotheritol
EDTA	Acide éthylène-diamine-tétraacétique
ELISA	enzyme-linked immunosorbent assay
FACS	Fluorescent-activated cell sorter
FBS	Fetal bovine serum
FC	Flow cytometry

Fig	Figure
FIND	Foundation for innovative new diagnostics
FITC	Fluorescein-isothiocyanate
GAPDH	D-glyceraldehyde-3-phosphate dehydrogenase
gi	Gene of interest
HAT	Human African trypanosomiasis
HAT-PCR-OC	HAT-PCR-oligochromatography
HBMEC	Human bone marrow endothelial cells
HCl	Hydrogen chlorure
hg	Housekeeping gene
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukine
IM	Intra-muscular
i.p	intraperitoneal
IU	International units
IV	Intra-venous
KCl	Potassium chlorure
LAMP	Loop-mediated isothermal amplification
LCR	Liquide céphalo-rachidien
LNB	Lyme neuroborreliosis
LPS	Lipopolysaccharide
M/F	Male/female
MCP	Monocyte chemotactic protein
MEM	Minimum essential medium
mg	Milligramme
$MgCl_2$	Magnesium chlorure
min	Minutes
MIP	Macrophage inflammatory protein
mL	millilitre
MLN	Mesenteric lymph node
mM	Millimolaire
MMLV	Moloney murine leukemia virus
mRNA	Messenger ribonucleic acid
MW	Molecular weight marker
ng	Nanogramme
NK	Natural killer

nm	Nanometer
NO	Monoxyde d'azote
OMS	Organisation mondiale de la santé
PBS	Phosphate buffer saline
PC5	Phycocyanin 5
PCR	Polymerase chain reaction
PE	Phycoerythrin
PerCP	Peridinin chlorophyll protein
PFA	Polyformaldéhyde
pg	Picogramme
PSF	Parasite soluble factor
RANTES	Regulated upon activation T cell expressed and secreted
RNA	Ribonucleic acid
RNase	Ribonuclease
RT-PCR	Reverse transcription polymerase chain reaction
s/sec	Secondes
S-1	Stage 1
S-2	Stage 2
sd	Standard deviation
SEM	Standard error of the mean
S-int	Intermediate stage
SNC	Système nerveux central
SOREM	Sleep onset rapid eye movements
T. b.	*Trypanosoma brucei*
TDR	Tropical diseases researches
Tregs/nTregs	Regulatory T cells
TGF	Transforming growth factor
Th cells	Helper T cells
THA	Trypanosomose humaine africaine
TLR	Toll-like receptor
TLTF	Trypanosome lymphocyte triggering factor
TNF	Tumor necrosis factor
UV	Ultra-violet
vs	*versus*
VSG	Variable surface glycoprotein
WHO	World health organization

Introduction

Introdução

Human African trypanosomiasis (HAT), sleeping sickness, still claims thousands of victims. In relation to mortality, of all parasitic diseases in Sub-Saharan Africa, trypanosomiasis ranks only behind malaria. As concerns disability-adjusted life years (DALYs), the health burden is similar to that of schistosomiasis (Cattand et al., 2006).

Improved clinical management, treatment and diagnostic tools are urgently needed.

During the first quarter of the 20th century, HAT spread throughout intertropical Africa. By the 1950s, the epidemic was under control because of the use of systematic population screening. However, shortly after independences, the scourge re-emerged from the so-called historic foci due to the loosening of systematic control measures, political and social unrest, and economic difficulties. Since almost 30 years, the situation keeps deteriorating, with alarming rates in areas where HAT control had vanished. Among the 400 million people living in 36 endemic countries, the WHO estimated that 60 million are exposed to the risk of contracting the disease (WHO, 1998). Nowadays, the situation is reported as being epidemic in South Sudan, Northern Uganda, Democratic Congo Republic and Angola, representing more than 90 % of reported cases (Moore and Richer, 2001; Louis et al., 2004).

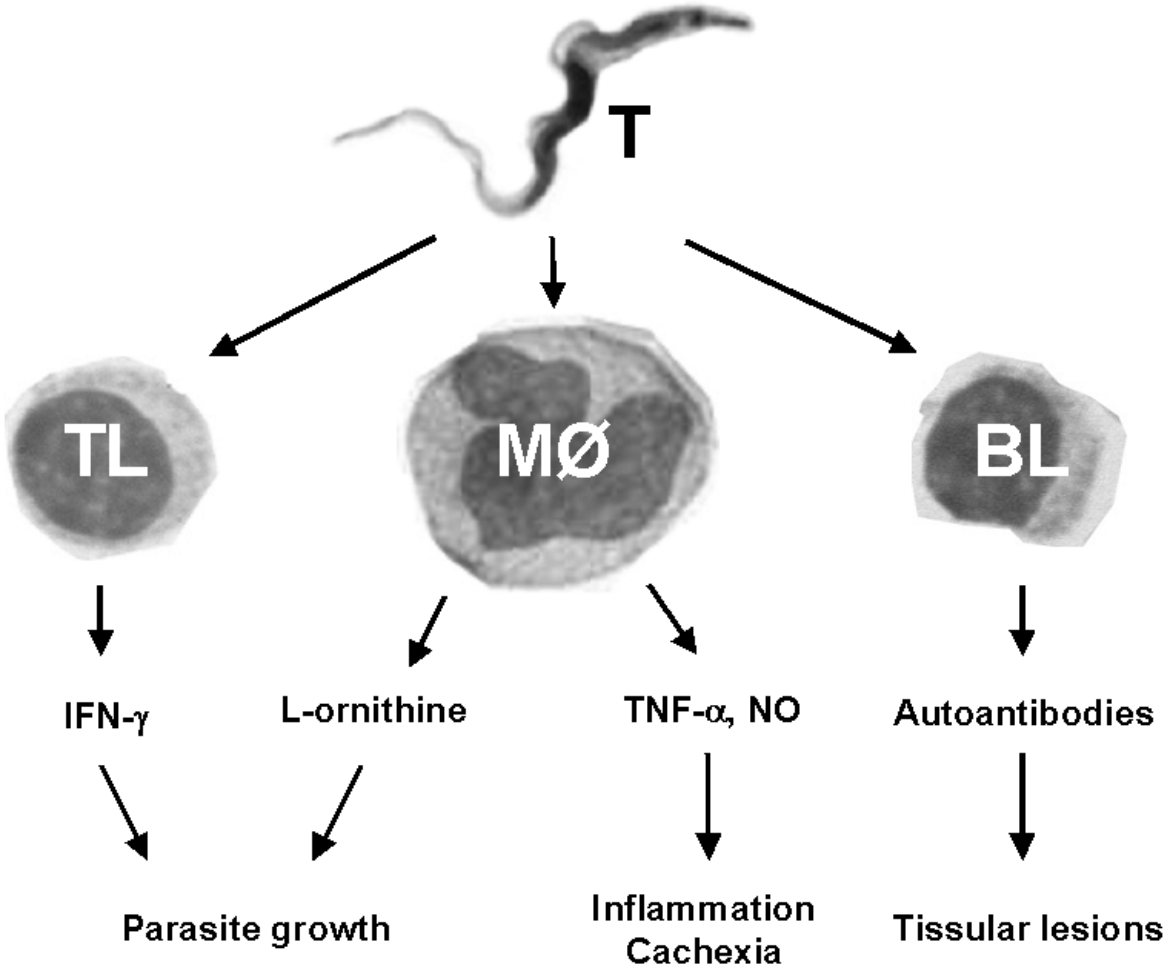
The exclusive distribution of HAT to intertropical Africa depends on that of its vector, the tsetse fly, which belongs to the *Glossina* genus. Several tsetse fly species transmit *Trypanosoma brucei* (*T. b.*) *gambiense* in Western and Central Africa. They thrive in hot humid forests along lake and river banks or even in coffee and cocoa tree plantations.

Conventionally, man represents the almost unique parasite reservoir, with anecdotic interventions of domestic animals (pigs, sheep and goats). Other species transmit *T. b.*

rhodesiense in East Africa and live in the savannah, at the forest edge or in riverine and marshland areas. Here the reservoir is almost exclusively constituted by wild games and domestic animals, human infection being sporadic on the occasion of hunting, harvesting and tourist safari activities. Several epidemic outbreaks with a heavy toll on the populations have been observed.

Trypanosomes fascinate scientists because of their unique potency of evading the host immune response through potentially unlimited antigenic variation, which accounts for vaccine development difficulties. The variable surface glycoprotein (VSG) elicits an efficient antibody response that down-regulates the number of parasites. Nevertheless, some parasites escape destruction through periodical variation of surface epitopes. Although the parasite genome has been sequenced, mechanisms of gene switching remain unclear (Pays, 2005). The whole VSG coat can be changed within only 12 minutes. The highly immunogenic VSG molecules also stimulate polyclonal B cell proliferation, particularly IgM class clones (Diffley, 1985; Vincendeau and Bouteille, 2006). This overwhelming production of immunoglobulins, only partially directed against trypanosome antigens, added to the release of VSG fragments in the blood, contributes to the disease immunopathogenicity (Figures 1 and 2).

During the first stage of the disease, HAT may cause adenopathy or splenomegaly. Later on, a number of neurological signs appear. Among those, psychiatric disorders are particularly spectacular. In Europe, patients have been reported to wander from one psychiatric institution to another without any improvement until appropriate antitrypanosomal treatment was undertaken. Such imported cases to the North are rare as tourists rarely visit the remote foci of rural Africa (Stich et al., 2002).



(T: trypanosome; Mø: macrophage; TL: T lymphocyte; BL: B lymphocyte)

Figure 1: Trypanosomes induce secretion of various components from immune cells. Besides their trypanocidal effects, these molecules are also involved in deleterious mechanisms for host tissues and/or favour parasite growth (from Vincendeau and Bouteille, 2006).

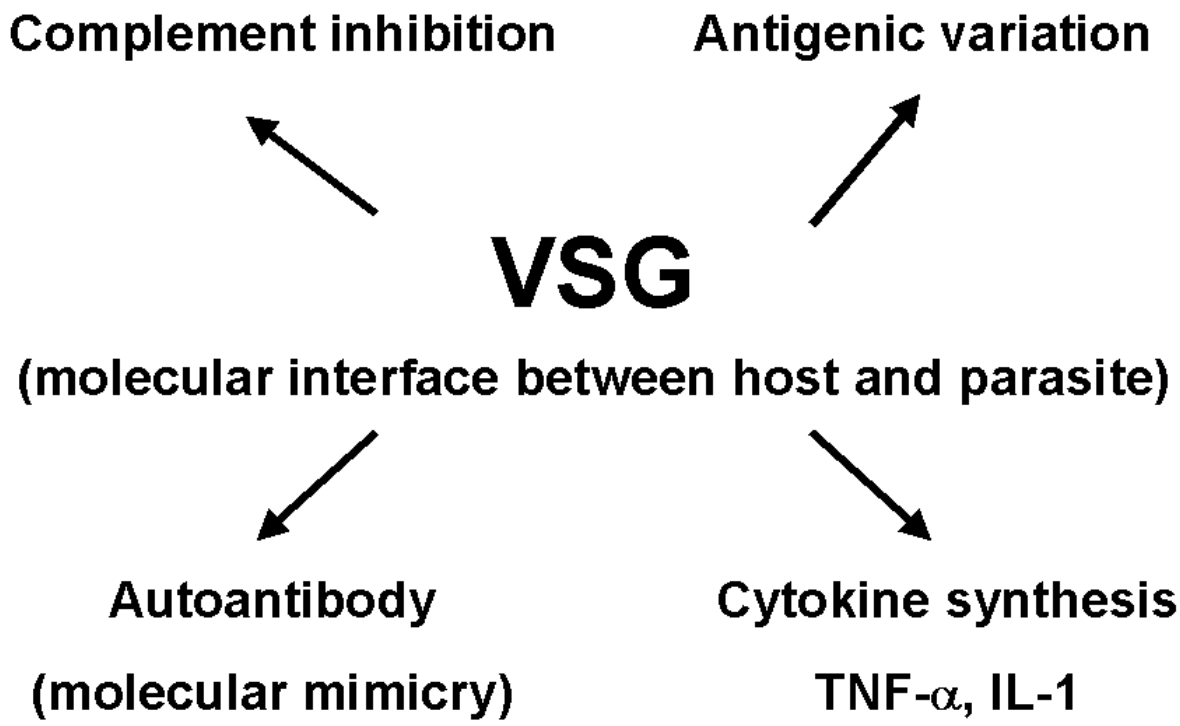


Figure 2: Variable surface glycoprotein (VSG), the major surface component of trypanosomes, is also released in host fluids. VSG induce resistance to complement lysis, escape to specific immune response, persistent cytokine production, autoantibody synthesis by molecular mimicry with host tissues (from Vincendeau and Bouteille, 2006).

The evolution of the disease also depends on the infectious agent. The *rhodesiense* form of the disease is acute and, if untreated, death occurs in several weeks to months. This will take several months or years in the *gambiense* form, giving time to develop the whole panel of neurological signs. The disease is classically divided into two successive phases (Dumas and Bisser, 1999). The hemolymphatic stage 1 follows the initial inoculation chancre and represents the parasitic invasion phase of the lymphatic organs. It is followed by the intrusion of parasites into the central nervous system (CNS), leading to the development of the stage 2 meningoencephalitis towards pre-terminal demyelization encephalitis. Conventionally, the two successive stages occur soon after the initial chancre that develops at the site of the tsetse fly bite. The chancre diameter varies from few millimeters to several centimeters. It is an erythematic pseudo-furuncle that never suppurates, but desquamates peripherally. It disappears within two or three weeks. The chancre is more easily found in Europeans than in Africans. It is also more frequently observed in East Africa than in West African endemic areas. From the chancre, trypanosomes invade the blood and lymphatic organs. This phase corresponds to the onset of hemolymphatic stage 1. General signs then appear, such as fever, fatigue, lymph node swelling, splenomegaly, hepatomegaly, skin lesions or trypanids, pruritus, and headache. Rubber consistent swollen lymph nodes, generally localised at the neck base (Winterbottom sign), are mobile and painless, and become firmer with time. They constitute a key sign of the infection in endemic foci, although its specificity is low. Cardiac abnormalities ranging from tachycardia to pericarditis or myocarditis can occur in *gambiense* forms but are more suggestive symptoms in *rhodesiense* infection and may cause patient death (Poltera et al., 1976). Although waning, general infection persists at stage 2 meningoencephalitis at the time of appearance of CNS alterations (Kennedy, 2006; Blum et al., 2006). Spectacular psychiatric disturbances may occur early or later, and may be the sole clinical sign throughout the disease. Most patients show disinterest,

indifference to external circumstances and apathy, sometimes interrupted by agitation with affective disturbances, antisocial and aggressive behavior, sudden anger and episodes of confusion or delirium. Early neurological symptoms correlate with the widespread meningeal inflammation. Intense and long lasting frontal headaches, sleep disorders, gait disturbances, hyperpathia (Kerandel's key sign), hyperesthesia, impotence, amenorrhea, and infertility correspond to trypanosome invasion of the CNS least protected zones (choroid plexus, thalamus, area postrema, median eminence, pineal, hypophyseal regions, suprachiasmatic nuclei and cerebellum). The re-appearance of primitive reflexes (palmomental reflex, sucking reflex) and the presence of a Babinski sign reveal irritation of the pyramidal tract and a lack of cortical control over the brain stem. Extrapyramidal Parkinson-like symptoms include apathy, loss of muscle tone especially in the neck muscles, drooping of the eyelids, dyskinesia, choreoathetosis, motor instability, grimaces, and clumsiness. At the terminal phase of the disease, CNS demyelization and atrophy are accompanied with disturbances in consciousness and the development of dementia with incoherence, incontinence and epileptic fits. Clinical signs remain reversible by treatment for a long time, attesting the predominance of reversible inflammatory lesions over irreversible demyelization lesions. Without treatment, the patient dies in a state of dementia and terminal cachexia.

Treatment of HAT is deceiving. Although the hemolymphatic stage 1 is treated with well-tolerated medications, the use of toxic molecules to treat the meningoencephalitic stage 2 may be highly deleterious. It is therefore crucial to diagnose precisely the stages of HAT. However, conventional stage determination is based on lumbar puncture and cerebrospinal fluid (CSF) cell counts or observation of trypanosomes. These techniques lack precision. Therapeutic efforts to treat HAT have been deceiving, especially at stage 2. Furthermore, the situation has not changed in the last 30 to 50 years (Bouteille et al., 2003). Pentamidine is the

drug of choice for *T. b. gambiense* stage 1 HAT, and suramin for stage 1 *T. b. rhodesiense* HAT (Apted, 1980; Wellde et al, 1989). Melarsoprol remains the principal medication for the treatment of stage 2 HAT in both *gambiense* and *rhodesiense* infections (Friedheim, 1949; Schmid et al., 2005). Melarsoprol is primarily neurotoxic, with a risk of fatal encephalopathy within 8-10 days of treatment (Blum et al., 2001). Overall fatality ranges from 0.95 % to 9.8 % for *T. b. gambiense*-infected patients and from 3.4 % to 12 % in *rhodesiense* infections. There is no clear course of action for reducing the incidence and severity of these events. Overall, resistance to melarsoprol is found in 10 to 30 % of patients, especially in Uganda and Angola (Legros et al., 1999; Stanghellini and Josenando, 2001). Treatment options for relapses are scarce (eflornithine or nifurtimox) and protocols are not well established. Eflornithine (difluoromethyl-ornithine, DFMO) is used against stage 2 HAT since 1992. Firstly designed as an anti-cancer drug, it acts on both stages of *T. b. gambiense* infection (Milord et al., 1992; Chappuis et al., 2005b), but yields disappointing results in *rhodesiense* forms (Iten et al., 1995). Because of its short half-life, slow infusions are administered every 6 hours during 14 consecutive days (Pépin and Milord, 1994). Adverse effects are similar to other anti-cancer drugs and white blood cell counts have to be checked weekly (Milord et al., 1992). Nifurtimox was introduced to treat Chagas' disease and has not yet been approved for HAT. It has been successfully used on both stages of *T. b. gambiense* infection, but its efficacy against *T. b. rhodesiense* remains unexplored (Bouteille et al., 2003).

As stage determination is crucial for proper treatment with existing molecules, actual diagnostic tools must be improved and new diagnostic techniques, if possible non invasive (avoiding lumbar puncture), are deeply needed to ameliorate stage determination (Chappuis et al., 2005a).

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I. Titre. Marqueurs du stade neurologique de la trypanosomose humaine africaine. Actualités et perspectives.

I. Title. Criteria for diagnosis of the CNS stage of human African trypanosomiasis. Update and perspectives.

I. Título. Marcadores de estágio do CNS da Tripanossomíase Humana Africana. Actualização e perspectivas.

Travail ayant fait l'objet d'une publication sous la référence suivante :

Courtioux B., Pervieux L., Bisser S., Bouteille B. Marqueurs du stade neurologique de la trypanosomose humaine africaine : actualités et perspectives. Médecine Tropicale 2008 ; 68 : 17-23

Résumé.

La maladie du sommeil ou trypanosomose humaine africaine (T.H.A.) a pour origine une infection parasitaire due à un protozoaire flagellé sanguicole du genre *Trypanosoma brucei*. La maladie évolue classiquement en deux stades : le stade hémo-lymphatique et le stade neurologique. Actuellement une des difficultés de cette pathologie est le diagnostic du stade neurologique dont dépend le traitement. Ce diagnostic, sur le terrain, repose uniquement sur la cytorachie et la recherche du parasite dans le liquide céphalo-rachiden des patients. Cette recherche reste très aléatoire et invasive. De nombreux travaux sont en cours pour adapter le diagnostic du stade aux conditions de terrain et développer des tests fiables, peu coûteux et non invasifs. Nous proposons ici une revue des mécanismes impliqués dans l'atteinte neurologique au cours de la THA, ainsi que les travaux en cours sur les différentes techniques qui à l'avenir pourraient simplifier et améliorer le diagnostic du stade.

Mots-clés: Trypanosomose humaine africaine*; Trypanosoma brucei;* marqueurs diagnostiques; détermination du stade.

Abstract.

Sleeping sickness or human African trypanosomiasis (HAT) is due to parasite infection by a sanguicolous flagellate protozoan of the *Trypanosoma brucei* genus. The disease is classically divided into two stages, i.e., the hemolymphatic stage and the CNS stage. Disease staging is currently a major challenge for therapeutic decision-making. In the field, diagnosis is based solely on white blood cell count and detection of the parasite in the patient's cerebrospinal fluid. This technique is unreliable and invasive. Numerous studies are now under way to adapt staging to field conditions and to develop a reliable, low-cost, non-invasive test. This article describes themechanisms underlying CNS involvement during HAT and reviews the different techniques now being studied to simplify and improve diagnosis of the CNS stage.

Key Words: Human African trypanosomiasis; *Trypanosoma brucei;* Diagnostic markers; Staging.

Resumo.

A doença do Sono ou Tripanossomíase Humana Africana (THA) é causada através da infecção pelo protozoário sangüíneo flagelado do gênero *Trypanosoma brucei*. A doença é originalmente divida em dois estágios, isto é, o estágio hemolítico e o estágio SNC onde o sistema nervoso central está comprometido. Atualmente o maior desafio é a determinação do estágio da doença para a escolha terapêutica. No campo, o diagnóstico é baseado unicamente em contagem de células brancas do sangue e pela detecção de parasitos no fluído cérebro-espinhal do paciente. Esta técnica é incerta e invasiva. Diversos estudos estão em andamento para adaptar a determinação do estágio às condições do campo e para o desenvolvimento de um teste confiável, de baixo custo e não-invasivo. Este artigo descreve os mecanismos envolvidos no estágio SNC durante a infecção causada pela THA e revisa as diferentes técnicas utilizadas atualmente no intuito de simplificar e otimizar o diagnóstico do estágio SNC.

Palavras-chave: Tripanossomíase Humana Africana; *Trypanosoma brucei;* Marcadores de diagnóstico; determinação do estágio.

II. Objectifs.

II. Objectives.

II. Objectivos

Comme la détermination du stade est essentielle pour un traitement correct de la THA avec les molécules déjà existantes, les outils de diagnostic actuel doivent être améliorés et des techniques diagnostiques doivent être recherchées et développées en rapport avec de nouvelles approches physiopathologiques. C'est la raison pour laquelle nous avons mené les objectifs suivants pour notre travail de thèse.

Objectif principal

- Rechercher de nouvelles approches diagnostiques pour déterminer le stade de la THA

Objectifs spécifiques

- Etudier la dynamique des cellules T régulatrices chez la souris infectées par *Trypanosoma brucei*

- Etudier les profils lymphocytaires immunophénotypiques dans le sang et le liquide céphalorachidien de patients souffrant de THA

- Etudier l'action de diverses espèces de trypanosomes sur l'expression de chémokines dans des cellules microgliales et endothéliales humaines

- Etudier le lien entre les taux de chémokines dans le sang et le LCR et la gravité de la maladie chez les patients souffrant de THA

As stage determination is crucial for proper treatment of HAT with existing molecules, actual diagnostic tools must be improved and diagnostic techniques must be searched for and developed from new physiopathological approaches. This is the reason why we have set the following objectives for our thesis work.

Main objective

- To search for new diagnostic approaches for staging HAT

Specific objectives

- To study the dynamics of the regulatory T cells in *Trypanosoma brucei*-infected mice

- To study immunophenotypic lymphocyte profiles in blood and in cerebrospinal fluid of patients suffering from HAT

- To study action of various species of trypanosomes on chemokines expression on human microglial and endothelial cells

- To study the link between blood and CSF chemokines levels and disease severity in patients suffering from HAT

Porque a determinação do estágio é crucial para o tratamento apropriado da THA com moléculas existentes, as ferramentas diagnósticas reais devem ser melhoradas e as técnicas diagnósticas devem ser procuradas e desenvolvidas das aproximações fisiopatológicas novas. Esta é a razão pela qual nós ajustamos os seguintes objetivos para nosso trabalho da tese.

Objetivos principal

- Procurar aproximações diagnósticas novas para a determinação do estágio da THA

Objetivos específicos

- Estudar a dinâmica das celulas T reguladoras em *Trypanosoma brucei*-contaminados ratos

- Estudar perfis immunofenotypicos dos linfócitos no sangue e no líquido cefalorraquidiano dos pacientes que sofrem da THA

- Estudar a ação das espécies diferentes dos trypanosomos na expressão das quimiocinas em celulas humanas microglial and endothelial

- Estudar a relação entre níveis das quimiocinas no sangue e no líquido cefalorraquidiano e a severidade da doença nos pacientes que sofrem da THA

III. Titre. Dynamique des cellules T régulatrices dans les ganglions lymphatiques et le thymus de souris infectées par *Trypanosoma brucei brucei.*

III. Title. Dynamics of regulatory T cells in mesenteric lymph nodes and thymus of mice infected with *Trypanosoma brucei brucei*.

III. Título. Dinâmica das celulas T reguladoras em nos de linfa mesentericos e do thymus dos ratos contaminados com *Trypanosoma brucei brucei.*

Travail à soumettre avec les références suivantes :

Pervieux L., Bouteille B., Rambert J., Semballa S., Courtois P., Vincendeau P. Dynamics of regulatory T cells in mesenteric lymph nodes and thymus of mice infected with Trypanosoma brucei brucei

Résumé.

Récemment le rôle des cellules régulatrices T CD4+CD25+ est devenu le point d'intérêt de nombreuses études à cause de l'importance de ces cellules dans la modulation de la réponse immune, mais leur rôle dans la trypanosomose humaine africaine n'a pas encore été élucidé. Nous avons étudié la dynamique de ces cellules dans les ganglions mésentériques et le thymus de souris infectées par *Trypanosoma brucei brucei*, à différentes étapes de l'infection, en comparaison avec des souris non infectées. Ces variations ont été analysées par cytométrie en flux, en utilisant deux marqueurs spécifiques des Tregs: l'anti-CD25 et l'anti-Foxp3, un membre de la famille «forkhead/winged helix» qui agit en tant que répresseur transcriptionnel. Nous avons aussi analysé, par cytométrie en flux, les effets d'un facteur immunomodulateur sécrété par les trypanosomes, le facteur soluble parasitaire (PSF), sur les Tregs : *in vitro* sur des cultures de thymocytes et *in vivo* chez la souris. Nos études ont démontré que, chez les souris infectées par *T. b. brucei*, le nombre de nTregs parmi les cellules T varie avec le temps de l'infection. Au début de la maladie, ce nombre est similaire à celui des contrôles. Soixante jours après l'infection, le nombre de nTregs diminue significativement dans les ganglions mésentériques ; cette diminution se poursuit jusqu'à 120 et 240 jours d'infection. Inversement, les nTregs augmentent dans le thymus pendant la même période d'infection. Notre travail est la première étude sur la dynamique des Tregs chez les souris infectées par *T. b. brucei* en fonction du temps d'infection. *In vitro*, une différence significative a été notée entre les thymocytes contrôles et les thymocytes en culture avec des PSF jusqu'à 120 jours d'infection ; cette différence n'était pas retrouvée après 240 jours d'infection. *In vivo*, après inoculation des souris par le PSF, seule une légère diminution non significative dans le nombre de Tregs a été trouvée. La diminution des Tregs durant l'infection semble être due à un facteur immunomodulateur sécrété par les parasites.

Mots-clés: *Trypanosoma brucei brucei*; cellules T régulatrices CD4+CD25+; Foxp3; stades d'infection; facteur soluble parasitaire.

Abstract

Recently the role of CD4+CD25+ regulatory T cells has become the focus of interest of many studies because of the importance of these cells in modulating immune response, but their role in African Trypanosomiasis has not yet been elucidated.

In our study, we investigated the dynamics of these cells in mesenteric lymph nodes and in thymus of *Trypanosoma brucei (T. b.) brucei*-infected mice, at different stages of infection, in comparison with non-infected mice. These variations were analysed by flow cytometry, using particularly two specific markers of Tregs: the anti-CD25 and the anti-Foxp3, which encodes a member of the forkhead/winged helix family and acts as a transcriptional repressor. We also analysed, by flow cytometry the effects of an immunomodulatory factor secreted by trypanosomes, the parasite soluble factor (PSF) on Tregs after thymocyte cultures *in vitro* and *in vivo* mice injection. Our studies demonstrated that in mice infected with *T. b. brucei*, the number of nTregs in T cells varied regularly. At the beginning of disease, this number remained the same one as for controls, but 60 days after infection nTregs number decreased at a significant degree and continued to decrease after 120 and 240 days of infection in mesenteric lymph nodes, and made the reverse in the thymus. Indeed, we could note that the percentage of nTregs, in thymus and mesenteric lymph nodes, had varied in an opposite way. This is the first study about dynamics of Tregs in mice infected with *T. b. brucei* according to the infection time. *In vitro*, a significant difference was noted between thymocytes controls and thymocytes cultured with PSF at the beginning of the infection, but not after 240 days of infection. *In vivo*, a weak reduction in Treg number was also found. Treg diminution during the infection seems to be due to an immunomodulatory factor secreted by parasites.

Key words: *Trypanosoma brucei brucei*; CD4+CD25+ regulatory T cells; Foxp3; infection stages; parasite soluble factor.

Resumo.

Recentemente a acção das celulas T reguladoras CD4+CD25+ tornou-se no foco de interesse de muitos estudos por causa da importância destas celulas em modular a resposta imune, mas a acção das celulas na Tripanossomíase Humana Africana não foi explicado ainda.

Em nosso estudo, nós investigamos a dinâmica destas celulas em nós de linfa mesenterica e no thymus dos ratos contaminados para *T. b. brucei*, em estágios diferentes da infecção, em comparação com ratos não contaminados. Estas variações foram analisadas pelo "flow cytometry" usando em particular dois marcadores específicos dos nTregs: o anti-CD25 e o anti-Foxp3, qual codifica um membro da família da hélice e actua como um repressor transcriptonal. Nós igualmente analisamos os efeitos de um fator immunomodulator segregado por trypanosomes, o fator solúvel do parasita dos nTregs (PSF) após as culturas dos tymócitos e a injecção *in vivo* dos PSF nos ratos. Nosso estudo demonstrou que nos ratos contaminados com *T. b. brucei*, o número dos nTregs nas celulas T varia regularmente. No início da doença, este número permaneceu mesmo que para controles, mas 60 dias depois da infecção o número dos nTregs diminuiu em um grau significativo e continuou a diminuir após 120 e 240 dias da infecção em nós da linfa mesenterica, e fêz o reverso no thymus. Certamente, nós poderíamos anotar que a porcentagem dos nTregs, no thymus e nós da linfa mesenterica, tinha variado em uma maneira oposta. Este é o primeiro estudo sobre a dinâmica dos nTregs nos ratos contaminados com o *T. b. brucei* de acordo com o tempo da infecção. *In vitro*, as diferenças significativas foram anotadas entre os tymócitos controles e os tymócitos cultivados com os PSF no início da infecção, mas não após 240 dias da infecção. *In vivo*, uma redução fraca no número dos nTregs foi encontrada igualmente. A diminuição dos Tregs

durante a infecção parece ser devido a um fator immunomodulator segregado para o trypanosomes.

Palavras-chave: *Trypanosoma brucei brucei*; celulas T reguladoras CD4+CD25+; Foxp3; estágios da infecção; fator solúvel do parasite.

IV. Titre. Profils immunophénotypiques lymphocytaires au cours de la trypanosomose humaine africaine.

IV. Title. Immunophenotypic lymphocyte profiles in human African trypanosomiasis.

IV. Título. Perfis immunophenotypíco dos linfócitos na tripanossomíase humana africana.

Travail à soumettre avec les références suivantes :

Boda C., Courtioux B., Roques P., Pervieux L., Vatunga G., Josenando T., Ayenengoye C.R., Bouteille B., Jauberteau M.O., Bisser S. Immunophenotypic lymphocyte profiles in human African trypanosomiasis.

Résumé.

La trypanosomose humaine africaine (THA) est une maladie mortelle d'Afrique tropicale. Pour mieux comprendre les spécificités cliniques de cette maladie, une analyse phénotypique des lymphocytes présents dans le sang et le LCR de patients atteints de THA a été menée. Pour cette étude, 27 contrôles et 33 patients infectés par *T. b. gambiense* et parasitologiquement confirmés (24 patients en stade 1 hémolymphatique, et 9 patients en stade 2 meningo-encéphalitique) ont été identifiés en Angola et au Gabon. Les sous groupes de lymphocytes B et T ont été étudiés, ainsi que leurs marqueurs d'activation. Dans le sang, une augmentation significative des lymphocytes B CD19+ et des lymphocytes B activés (CD19+CD69+) ou (CD19+CD95+) et une diminution des cellules T (CD4+CD25+) ont été observées, sans activation des cellules T (CD4+HLA-DR+, CD8+HLA-DR+, CD4+CD69+, CD8+CD25+). Une mise en évidence de l'altération de la fonction des cellules T a aussi été trouvée puisque les cellules T naïves et mémoires (CD4+CD45RA-) mais pas les cellules T naïves et mémoires (CD8+CD45RA-.) étaient augmentés chez les patients ayant également un déficit en cellules T CD8+ effectrices. Cependant aucune relation entre l'expression de ces différents phénotypes dans le sang et la gravité de la maladie (stade 1 *vs* 2) n'a été trouvée dans le LCR. Cependant, les cellules B exprimant le CD19 étaient augmentées en relation avec la gravité de la maladie suggérant que ce phénotype pourrait être, pour le stade 2, un marqueur de terrain plus fiable que la numération des lymphocytes totaux. Nos résultats montrent que l'activation des cellules B et T et les perturbations de leurs fonctions mènent à l'immuno-suppression chez les patients THA.

Mots-clés: *Trypanosoma brucei gambiense*; trypanosomose humaine africaine; cellules B et T activées; fonctions des cellules T; Angola; Gabon; détermination du stade; immunosuppression.

Abstract.

Human African trypanosomiasis (HAT) is a deadly disease of tropical Africa. To better understand the clinical specificities of this disease, an analysis of the lymphocytic phenotypes present in blood and CSF of HAT patients was conducted. For this study, 27 controls and 33 parasitologically confirmed *T. b. gambiense*-infected patients (24 hemolymphatic stage 1 patients, 9 meningoencephalitic stage 2 patients) were identified during active and passive screenings in Angola and Gabon. B and T lymphocytes were evaluated for cell subset, activation markers and function. In blood, evidence for increased $CD19^+$ B lymphocytes and activated B lymphocytes expressing ($CD19^+CD69^+$) or ($CD19^+CD95^+$), and decreased ($CD4^+CD25^+$) T cells were found. There was no T cell activation ($CD4^+$HLA-DR^+, $CD8^+$HLA-DR^+, $CD4^+CD69^+$, $CD8^+CD25^+$). Evidence for impaired T cell function was also found as naive memory T cells ($CD4^+CD45RA^-$) but not naïve memory T cells ($CD8^+CD45RA^-$) were increased in patients together with a lack of effector $CD8^+$ T cells. While a relationship between the expression of these different phenotypes in blood and disease severity (stage 1 *vs* 2) was not found, in cerebrospinal fluid. However, CD19-expressing B cells did increase with disease severity suggesting that this phenotype could be a more reliable field marker of stage 2-HAT than total lymphocyte count. Our results provide the cellular basis of T and B cell activation and functions impairment which leads to immunosuppression in HAT patients.

Key words: *Trypanosoma brucei gambiense*; human African trypanosomiasis; activated T and B cells; T cell functions; Angola; Gabon; stage determination; immunosuppression.

Resumo.

A Tripanossomíase Humana Africana (THA) é uma doença letal da África tropical. Para melhor compreender as especificidades clínicas desta doença foi realizada uma análise dos fenótipos de linfócitos presentes no sangue e no fluído cérebro espinhal (CSF) de pacientes acometidos de HAT. Para este estudo, 27 pacientes não-infectados (controle) e 33 pacientes parasitologicamente confirmados com *T. b. gambiense* (24 pacientes hemolíticos no estágio 1 e 9 pacientes meningoencefálicos no estágio 2) foram identificados durante a seleção ativa e passiva em Angola e no Gabão. Os linfócitos B e T foram avaliados em relação ao subconjunto celular, marcadores de ativação e função. Foram encontradas evidências no sangue do aumento de linfócitos B $CD19^+$ e linfócitos B ativados expressando ($CD19^+CD69^+$) ou ($CD19^+CD95^+$) e da diminuição de células T ($CD4^+CD25^+$). Não houve ativação de células T ($CD4^+$HLA-DR^+, $CD8^+$HLA-DR^+, $CD4^+CD69^+$, $CD8^+CD25^+$). Foram encontradas evidências de um bloqueio da função das células T uma vez que as células T de memória naïve ($CD4^+CD45RA^-$), mas não as células T de memória ($CD8^+CD45RA^-$), estavam aumentadas em pacientes juntamente com a ausência de células T efetoras $CD8^+$. Embora a relação entre a expressão destes diferentes fenótipos no sangue e a severidade da doença (estágio 1 *vs* 2) não tenha sido encontrada, as células B expressnado CD19 do fluído cérebro espinhal não aumentaram com a severidade da doença, sugerindo que este fenótipo pode ser um marcador mais confiável do estágio 2 da doença no campo do que a contagem de linfócitos totais. Nossos resultados fornecem as bases celulares da ativação e do bloqueio da função de células B e T que resultam na imunosupressão de pacientes acometidos de THA.

Palavras-chave: *Trypanosoma brucei gambiense;* Tripanossomíase Humana Africana; células T e B ativadas; funções de células T; Angola; Gabão; determinação do estágio; imunosupressão.

V. Titre. Relation entre taux de chémokines et gravité de la maladie dans la tryposomose humaine africaine.

V. Title. A link between chemokine levels and disease severity in human African trypanosomiasis.

V. Título. Uma relação entre níveis do chemokine e severidade da doença na tripanossomíase humana africana.

Travail ayant fait l'objet d'une publication sous la référence suivante :

Courtioux B., Boda C., Vatunga G., Pervieux L., Josenando T., Mengue M'Eyi P., Bouteille B., Jauberteau-Marchan M.O., Bisser S. A link between chemokine levels and disease severity in human African trypanosomiasis. International Journal for Parasitology, 2006, 36, 1057-1065

Résumé.

L'infection par *Trypanosoma brucei gambiense* est un grave problème de santé publique en Afrique sub-Saharienne. Cette maladie parasitaire est difficile à diagnostiquer à cause des signes cliniques insidieux et d'une parasitémie fluctuante. L'évolution clinique est marquée par deux stades durant lesquels la gravité de la maladie s'intensifie : un stade précoce systémique suivit du développement d'un stade meningo-encéphalitique progressif. Durant ce dernier stade, un grand nombre de signes neurologiques peuvent apparaître, menant finalement à une démyélination et à un stade fatal en l'absence de traitement. Le traitement de la phase nerveuse est toxique et difficile à administrer. C'est pourquoi des méthodes précises de diagnostic sont nécessaires pour la détermination du stade. En effet, les critères classiquement utilisés ne sont pas suffisamment spécifiques. Puisque les cytokines et les chémokines sont impliquées dans le recrutement précoce des leucocytes dans le système nerveux central (SNC), notre étude est basée sur leur valeur potentielle à définir le début de l'altération du SNC. Les taux de différentes protéines (monocyte chemoattractant protein-1/CCL-2, macrophage inflammatory protein-1a/CCL-3, IL-8/CXCL-8, regulated upon activation T cell expressed and secreted (RANTES)/CCL-5 et IL-1b) ont été mesurés à la fois dans le sérum et le liquide céphalo-rachidien (LCR) de 57 patients et 4 contrôles. Les patients ont été classés en trois groupes (stade 1, intermédiaire et stade 2) en accord avec les critères classiques de terrain (nombre de cellules dans le LCR, présence de trypanosomes dans le LCR et signes neurologiques). Dans le sérum, les taux de cytokines/chémokines ont été peu associés au stade de la maladie. Seul CXCL-8 était augmenté chez les patients en stade 1. CCL-5 était plus élevé chez les contrôles que chez les patients. A contrario, dans le LCR l'expression des cytokines sélectionnées, à l'exception de CCL-5, était associée avec la présence de signes neurologiques, démontrant leur valeur diagnostique. Dans le LCR, nous

avons observé une relation entre la présence de trypanosomes et les taux de IL-1β, CXCL-8, CCL-2 et CCL-3. La production de ces cytokines et chémokines peut être déclenchée par le parasite ; par conséquent, elles peuvent être des marqueurs potentiels de l'invasion du SNC.

Mots-Clés: *Trypanosoma brucei gambiense*; chémmokines; cytokines; stade neurologique; détermination du stade.

Abstract.

Trypanosoma brucei gambiense infection is an important public health challenge in sub-Saharan Africa. This parasitic disease is difficult to diagnose due to insidious clinical signs and transient parasitaemias. The clinical course is marked by 2 stages of increasing disease severity. An early systemic parasitic invasion is followed by the development of a progressive meningo-encephalitis. During this latter stage, a broad spectrum of neurological signs appears which finally lead to a demyelinating and fatal stage if untreated. Treatment is toxic and difficult to administer when the CNS is invaded. Therefore, accurate diagnostic methods for stage determination are needed. The classically used criteria are not sufficiently specific and mechanisms of parasite invasion through the blood-brain barrier remains poorly understood. As cytokines/chemokines are involved in the early recruitment of leukocytes into the CNS, this study has focused on their potential value to define the onset of CNS involvement. Levels of monocyte chemoattractant protein (MCP)-1/CCL-2, macrophage inflammatory protein (MIP)-1α/CCL-3, interleukin (IL)-8/CXCL-8, RANTES/CCL-5 and IL-1β were measured in paired sera and cerebrospinal fluid (CSF) from 57 patients and four controls. Patients were classified into three groups (stage 1, intermediate and stage 2) according to current field criteria for stage determination (CSF cell count, presence of trypanosomes in CSF and neurological signs). In sera, cytokine/chemokine levels were poorly related to disease stage. Only CXCL-8 was higher in stage 1 patients when compared to stage 2 and CCL-5 was higher in controls when compared to patients. In contrast, in CSF, the expression of the selected cytokines, except for CCL-5, was associated with the presence of neurological signs demonstrating their diagnostic value. We observed a relationship between the presence of trypanosomes or trypanosome related-compounds in CSF and levels of IL-

1β, CXCL-8, CCL-2 and CCL-3. These cytokines and chemokines must be triggered by the parasite and hence are potential markers of CNS invasion.

Keywords: *Trypanosoma brucei gambiense;* chemokines; cytokines; neurological stage; stage determination.

Resumo.

A infecção pelo *Trypanosoma brucei gambiense* é um importante problema de saúde pública na África Subsaariana. Esta doença parasitária é difícil de diagnosticar devido aos sinais clínicos insidiosos e parasitemia transitória. A evolução clínica é marcada por dois estágios de gravidade crescente. Uma invasão parasítica sistêmica é seguida pelo desenvolvimento de uma meningoencefalite progressiva. Durante este último estágio, um amplo espectro de sinais neurológicos parece conduzir a um estágio desmielinizante e fatal se não tratado. Quando o sistema nervoso central (SNC) já se encontra invadido, o tratamento é tóxico e de difícil administração. Por isso, métodos de diagnóstico precisos para a determinação de estágios são necessários. Os critérios utilizados classicamente não são suficientemente específicos e os mecanismos de invasão pelo parasito através da barreira hematoencefálica continua mal compreendida. Como as citocinas/quimiocinas estão envolvidas no recrutamento inicial de leucócitos para o SNC, este estudo centrou-se sobre o seu valor potencial para definir o início do envolvimento do SNC. Os níveis de proteína quimioatrente de monócitos (MCP)-1/CCL-2, Proteína Inflamatória de Macrófago (MIP)-1 α / CCL-3, interleucina (IL) -8/CXCL-8, RANTES/CCL-5 e IL-1 β foram medidos em paralelo, no soro e no líquido cefalorraquidiano (LCR) de 57 pacientes e quatro controles. Os doentes foram classificados em três grupos (estágio 1, estágio intermediário e estágio 2) de acordo com os critérios atuais previstos para a determinação de estágios (número de células no LCR, presença de tripanosomas no LCR e sinais neurológicos). Nos soros analisados, os níveis de citocinas / quimiocinas não puderam ser relacionados com o estágio da doença. Somente CXCL-8 foi maior em pacientes no estágio 1, quando comparados ao estágio 2 e CCL-5 foi superior nos controles quando comparado aos pacientes. Em contraste, no LCR, a expressão de citocinas selecionadas, exceto para a CCL-5, foi associada com a presença de sinais

neurológicos demonstrando seu valor diagnóstico. Observamos uma relação entre a presença de tripanosomas ou compostos relacionados às células de tripanosomas no LCR e os níveis de IL-1β, CXCL-8, IAC-2 e CCL-3. A presença destas citocinas e quimiocinas pode ser desencadeada pelo parasita e, portanto, são potenciais marcadores de invasão do SNC.

Palavras-chave: *Trypanosoma brucei gambiense*; quimiocinas; citocinas; estágio neurológica; determinação de estágio.

VI. Titre. *Trypanosoma brucei gambiense* mais non *Trypanosoma brucei brucei* induit l'expression du gène CXCL-13 par les lignées cellulaires humaines microgliales et endothéliales.

VI. Title. *Trypanosoma brucei gambiense* but not *Trypanosoma brucei brucei* triggers CXCL-13 gene expression in human microglial and endothelial cell lines.

VI. Título *Trypanosoma brucei gambiense* mas não *Trypanosoma brucei brucei* provoca a expressão do gene CXCL-13 em linha celular microglial e endothelial humanas.

Travail soumis à Acta Tropica avec les références suivantes :

Pervieux L., Courtioux B., Jauberteau-Marchan M.O., Lacroix A., Bernard-Jaoul A., Vincendeau P., Bouteille B., Bisser S. Trypanosoma brucei gambiense *but not* Trypanosoma brucei brucei *triggers CXCL-13 gene expression in human microglial and endothelial cell lines.*

Résumé.

La trypanosomose humaine africaine (THA) est répandue en Afrique sub-saharienne ; elle est due aux parasites du groupe *Trypanosoma brucei*. La maladie évolue en deux stades. Le parasite se multiplie d'abord dans le sang et le système lymphatique puis envahit le système nerveux central (SNC) où l'infection peut entraîner des troubles irréversibles. Le traitement de la THA au stade méningo-encéphalitique est basé sur des produits toxiques qui ne sont pas toujours efficaces dans les formes très avancées. C'est la raison pour laquelle des marqueurs pour le diagnostic précoce de l'invasion du SNC et de la gravité de la maladie sont nécessaires. La phase méningo-encéphalitique est caractérisée par une hyperlymphoctyose et la présence d'IgM dans le liquide céphalo-rachidien (LCR). CXCL-13 est une chémokine impliquée dans le recrutement précoce des lymphocytes sécrétant des IgM. Pour étudier l'expression du gène de CXCL-13 par les cellules microgliales et endothéliales, nous avons utilisé *in vitro* deux modèles de lignées cellulaires (CHME-5, HBMEC) mis en co-culture avec d'une part *T. b. gambiense,* parasite infectieux pour l'homme et d'autre part *T. b. brucei*, non infectieux. Seul *T. b. gambiense* a montré l'expression du gène de CXCL-13 par les cellules CHME-5 et HBMEC après co-culture. Nous avons montré ici *i)* l'expression du gène de CXCL-13 dans des lignées cellulaires qui ont des propriétés proches de celles des cellules cérébrales humaines et *ii)* leur implication potentielle dans la THA. L'utilisation de CXCL-13 comme marqueur diagnostique ou de gravité de la maladie devra être étudié chez les patients atteints de THA.

Mots-clés: *Trypanosoma brucei gambiense*; *Trypanosoma brucei brucei;* expression du gène CXCL-13; CMH-5; HBMEC; trypanosomose humaine africaine.

Abstract.

Human African trypanosomiasis (HAT) is widespread in sub-Saharan Africa and linked to parasites of the *Trypanosoma brucei* group. The disease evolves in two stages; the parasite multiplies in blood and lymphatic system and further invades the central nervous system (CNS) where the infection can lead to irreversible damage. Treatment of HAT meningo-encephalitis relies on toxic drugs that are not always effective in late stages. Thus, markers for early diagnosis of CNS invasion and brain disease severity are required for improved treatment procedures. HAT meningo-encephalitis is characterized by cerebrospinal fluid hyperlymphocytosis and IgM synthesis. CXCL-13 is a chemokine implicated in the early recruitment of IgM-secreting lymphocytes. We used *in vitro* models of two human cell lines (CHME-5, HBMEC) co-cultured in the presence of human infective or non-infective trypanosomes (*T. b. gambiense, T. b. brucei*, respectively) to study cells CXCL-13 gene expression. *T. b. gambiense* only triggers CXCL-13 gene expression by CHME-5 and HBMEC after co-culture. Indeed, we show here CXCL-13 gene expression in cell lines whose properties are close to those of human cerebral cells and their potential implication in HAT pathology. The further use of CXCL-13 chemokine as a diagnostic or a disease severity marker should be investigated in humans.

Keywords: *Trypanosoma brucei gambiense*; *Trypanosoma brucei brucei;* CXCL-13 gene expression; CHME-5; HBMEC; human African trypanosomiasis.

Resumo.

A Tripanossomíase humana africana (THA) é distribuída na África Subsaariana e está relacionada a parasitas do grupo do *Trypanosoma brucei*. A doença se desenvolvem em dois estágios, o parasita se multiplica no sistema sanguineo e linfático e, em seguida, invade o sistema nervoso central (SNC) onde a infecção pode ocasionar danos irreversíveis. O tratamento da meningo-encefalite associada à THA depende de drogas tóxicas que nem sempre são eficazes em estágios mais avançados. Portanto, os marcados para o diagnóstico precoce da invasão do SNC e da severidade da doença cerebral são essenciais para otimizar os procedimentos de tratamento. A meningo-encefalite associada à THA é caracterizada pela alta produção de linfócitos e síntese de IgM no fluído cérebro-espinhal. CXCL-13 é uma quimiocina associada no recrutamento inical de linfócitos produtores de IgM. Neste estudo foram utilizados duas linhagens de células humanas (CHME-5, HBMEC) co-cultivadas na presença de tripanossomas infectivos e não-infectivos (*T. b. gambiense, T. b. brucei*, respectivamente) para estudar a expressão do gene de CXCL-13. Apenas *T. b. gambiense* é capaz de deflagrar a expressão do gene CXCL-13 em linhagens de CHME-5 e HBMEC após co-cultivo. De fato, foi nós demonstramos neste trabalho que a expressão do gene CXCL-13 em linhagens celulares com propriedades semelhantes às de células de cérebro humano e a sua potencial implicação na patologia da THA. O uso posterior da quimiocina CXL-13 como um marcador de diagnóstico ou severidade da doença deve ser investigado em humanos.

Palavras-chave: *Trypanosoma brucei gambiense*, *Trypanosoma brucei brucei,* expressão do gene CXCL-13, CHME-5, HBMEC, tripanossomíase humana africana.

VII. Titre. CXCL-13 dans le diagnostic de la trypanosomose humaine africaine méningo-encéphalitique.

VII. Title. CXCL-13 in the diagnosis of human African trypanosomiasis meningo-encephalitis.

VII. Título. CXCL-13 no diagnóstico da Tripanossomíase humana africana meningo-encefalite.

Travail soumis à Tropical Medicine and International Health avec les références suivantes :

Courtioux B., Pervieux L., Vatunga G., Marin B., Josenando T., Jauberteau-Marchan M.O., Bouteille B., Bisser S. CXCL-13 in the diagnosis of human African trypanosomiasis meningo-encephalitis.

Résumé.

La trypanosomose humaine africaine ou maladie du sommeil est une maladie parasitaire due à *Trypanosoma brucei gambiense* en Afrique de l'ouest et centrale. Cette maladie évolue en deux stades: (*i*) le stade lymphatico-sanguin avec présence du parasite dans la lymphe et dans le sang et (*ii*) le stade nerveux avec présence du parasite dans le système nerveux central. Les stratégies de traitement sont basées sur un diagnostic précis du stade de la maladie. Actuellement, les critères de terrain sont à la fois non spécifiques et peu sensibles. Dans le liquide céphalorachidien (LCR), la détection des IgM est un bon candidat ainsi que celle des chémokines, particulièrement CXCL-13, qui peuvent initier et amplifier la circulation des cellules B et la production d'IgM. Pour déterminer le rôle, et l'efficacité de CXCL-13 comme marqueur dans le diagnostic de la THA au stade nerveux, 26 patients Angolais et 16 contrôles ont été inclus. Les taux de CXCL-13 ont été déterminés par ELISA dans le sérum et le LCR. Les résultats ont été comparés aux marqueurs standards de détermination du stade (présence de trypanosome dans le LCR ; cytorachie) et à la synthèse intrathécale d'IgM. Les taux de CXCL-13 dans le sérum des patients ont une valeur médiane de 386.6 pg/mL et les taux élevés sont associés avec la présence de trypanosomes dans le LCR mais pas avec les autres marqueurs de stade. Les taux de CXCL-13 dans le LCR des patients ont une valeur médiane de 80.9 pg/mL et les taux élevés sont associés avec tous les marqueurs standards de détermination du stade et avec la synthèse intrathécale d'IgM. La détection du CXCL-13 dans le LCR pourrait être un nouveau test diagnostic. Sa valeur est confortée par des découvertes récentes par rapport à son implication dans le développement de séquelles neurologiques. La valeur diagnostique du CXCL-13 devra être testée dans une étude de cohorte.

Mots-clés: *Trypanosoma brucei gambiense*; trypanosomose humaine africaine; CXCL-13; BCA-1; stade neurologique; diagnostic de stade.

Abstract.

Human African trypanosomiasis or sleeping sickness is a parasitic disease due to *Trypanosoma brucei gambiense* in west and central Africa. This disease evolves in two stages: (*i)* the lymphatic stage which corresponds to the presence of parasites in lymph and blood and *(ii)* the nervous stage which corresponds to the presence of parasites in the central nervous system. Treatment strategies rely on accurate disease staging. Actually, field criteria for staging are either non-specific or insensitive. Cerebrospinal fluid (CSF) IgM detection is a valuable candidate and B cell attracting chemokines, especially CXCL-13 may initiate B-cell trafficking and IgM production. To determine the role, and diagnostic accuracy of CXCL-13 as a marker of meningo-encephalitis in HAT, 26 patients from Angola and 16 controls were included. CXCL-13 levels were determined by ELISA on paired sera and CSF. Results were compared to standard stage determination markers and IgM intrathecal synthesis. CXCL-13 levels in patients' sera had median values of 386.6 pg/mL and high levels were associated with presence of trypanosomes in the CSF but not with other stage markers. CXCL-13 levels in patients' CSF had median values of 80.9 pg/mL and high levels were associated with all standard stage determination markers and IgM intrathecal synthesis. CXCL-13 detection in CSF could be a new diagnostic test. Its value is enhanced by recent findings with regards to its implications in the development of neurological sequelae. CXCL-13 diagnostic accuracy should be tested in a well defined cohort study.

Keywords: *Trypanosoma brucei gambiense*; human African trypanosomiasis; CXCL-13; BCA-1; neurological stage; diagnostic accuracy.

Resumo.

A Tripanosomíase humana africana (THA) ou doença do sono é uma doença parasitária causada pelo *Trypanosoma brucei gambiense* na África Ocidental e Central. Esta doença evolui em dois estágios: (i) Estágio linfático, que corresponde à presença de parasitos nos vasos linfáticos e no sangue e (ii) Estágio do sistema nervoso que corresponde à presença de parasitos no sistema nervoso central. As estratégias de tratamento dependem de uma determinação exata do estágio da doença. Na verdade, os critérios de determinação campo são insensíveis ou não-específicos. A detecção dos IgM no Líquido cefalorraquidiano (LCR) é um valioso candidato e quimiocinas capazes de atrair células B, especialmente CXCL-13 podem dar início ao tráfego de células B e produção de IgM. Determinar o papel, e precisão do diagnóstico utilizando CXCL-13 como um marcador de meningoencefalite-encefalite no LCR, foram utilizados vinte e seis pacientes de Angola e 16 controles. Os níveis de CXCL-13 foram determinados, em análises pareadas, por ELISA em soros e em LCR. Os resultados foram comparados ao padrão de marcadores de determinação de estágio e síntese de IgM no canal espinal. Os níveis de CXCL-13 nos soros analisados dos pacientes apresentaram valores médios de 386,6 pg/mL e estes níveis elevados foram associados a presença de tripanosomas no LCR, mas não com outros marcadores de fase. Os níveis de CXCL-13 no LCR dos pacientes apresentaram valores médios de 80,9 pg/mL e os níveis elevados foram associados com todos os marcadores de determinação de estágio e síntese de IgM no canal espinal. A detecção de CXCL-13 em LCR pode ser um novo teste de diagnóstico. O seu valor é reforçado pelos resultados mais recentes em relação às suas implicações no desenvolvimento de seqüelas neurológicas. O valor da dosagem de CXCL-13 deve ser testado em um estudo com grupos bem definidos.

Palavras-chave: *Trypanosoma brucei gambiense*; Tripanossomíase humana africana; CXCL-13; BCA-1; estágio neurológico; precisão de diagnóstico.

Conclusion-Perspectives

Conclusão-Perspectivas

The CNS may no longer be considered as excluded from the mechanisms of immune surveillance. Although immunoglobulin concentrations in the CSF are very low compared to blood levels, and in spite of the restricted traffic of lymphocytes within brain tissue, the CNS is tightly linked to the immune system by means of the preferential passage of activated lymphocytes and/or monocytes through the blood-brain barrier. This passage depends upon the activation state of inflammatory cells. Activated leucocytes and the endothelium of brain capillaries express specific adhesion molecules leading to strong attachment of leucocytes to the endothelium and their trans-endothelial migration. An upregulated expression of the chemokine/cytokine network is also required for this migration.

In HAT, host response to parasite invasion produces a vascularitis, with peri-vascularitis and trypanosome spread into the CNS via the subarachnoidal spaces and perivascular extensions. A chronic reversible immune related meningoencephalitis occurs, which subsequently evolves to an irreversible demyelinating process. The relative roles of humoral immunity (antibodies) and cellular immunity (T cells, B cells) are variable and unresolved. CSF analysis remains the best way to detect a CNS immune response and to differentiate its components. CSF analysis has diagnostic and prognostic values which are routinely applied for HAT. However, the use of toxic drugs in HAT emphasises the need for standardised criteria to determine CNS involvement in this disease.

The main objective of our thesis work was to identify new diagnostic markers during HAT. To reach our objective, we explored new physiopathological approaches.

First, we had to identify and characterise cells implicated in immune dysregulations observed during human and experimental trypanosomiasis, mechanisms and signaling ways implicated, and finally parasitic molecules which induced them. For that, we chose to study and characterise a lymphocytic population known to act in prevention of auto-immune diseases and in tolerance to self antigen. Several regulatory T cells populations have been

described and studied in humans and mice but only nTregs are really implicated in defense of the organism against attacks of foreign substances. These nTregs are secreted constitutively by the thymus (Maggi et al., 2005) and are also known as natural regulatory T cells (CD4+CD25+ T cells). This population is characterized by a particular and specific marker, the Foxp3 gene. This gene is predominantly express in thymus and in periphery in the spleen, mesenteric lymph nodes, and liver in mice and humans (Maggi et al., 2005). Our study characterized nTregs in thymus and mesenteric lymph nodes of mice infected by *T. b. brucei* after different times of infection. Our results showed a constant increase in nTregs in the thymus of infected mice. For mesenteric lymph nodes, nTregs decreased regularly with infection time. These results could be explained by a maturation blocking of nTregs in thymus of old mice or by blocking the exit of nTregs from the thymus. This second hypothesis could explain the constant nTreg decrease in the periphery, observed during our study. Our results showed that the dynamic of nTregs was age-dependent and not only due to infection, as has been demonstrated in another studies (Nishioka et al., 2006; Zhao et al., 2007). However, Nishioka et coll., in 2006, showed that the decrease, linked to age, in T cells resulted from changes in the CD4+CD25- T cell population and not from an increase in suppressive functions of CD4+CD25+ T cells. Contrary to our results, the CD4+CD25+ nTreg percentage expressing specific the transcriptional Foxp3 factor increased significantly in the periphery (blood, spleen, and mesenteric lymph nodes) in 20 month age old Balb/c mice (Zhao et al., 2007). Moreover, other studies showed that Foxp3+ nTregs increased in spleen and liver of mice infected by *T. congolense* (Guilliams et al., 2007). This difference with our results found during *T. brucei brucei* infection could be due to the higher intraspecific diversity known in *T. congolense* (Frame et al., 1991). Always with the same objective, we tested soluble parasitic factors (PSF) to study their action on dynamic nTreg cells. Thymocytes cultured with or

without PSF showed their inhibitory role on nTreg dynamics, on a central level (thymus) and in the periphery (mesenteric lymph nodes).

nTregs played an important role in protection against auto-immune diseases but were regulated via external factors during *Trypanosoma brucei* infection. Age of mice was the main factor characteristic of nTreg dynamics during infection, on a central level with thymus involution with age or in the periphery with the possible blocking of nTregs in the thymus or during their maturation.

During HAT, a marked hypergammaglobulinemia and hyperlymphocytose were observed. To confirm these results, we examined immunophenotypic lymphocyte profiles in blood and cerebrospinal fluid from patients infected with HAT. B and T lymphocytes from patients and controls included during studies in Angola and in Gabon were analysed. In blood, a significant increase in CD19+ B lymphocytes and activated B lymphocytes was accompanied by decrease in T cells. Results of our study also showed that there was no relationship between the expression of different lymphocyte phenotypes and disease severity in blood. However, in CSF, the increase in CD19+ B cells was corelated with disease severity, suggesting that this phenotype was a better indicator of the nervous stage of HAT than the total lymphocyte count and that it could be used as a stage marker in the field. These results confirmed the immunosuppression observed during HAT and the immune response characterized by polyclonal activation of B cells that proliferate and secrete high antibody levels (Anthoons et al., 1986; Kazyumba et al., 1986). Futhermore, no increase in CD5+ B cells was found in patient blood. These cells are known to produce non-specific anti-trypanosome antibodies (Buza et al., 1997), suggesting the intervention of another mechanism in antibody production. These antibodies could be induced by molecular mimicry mechanisms such as the expression of epitopes shared by trypanosomes and CNS antigens (Ayed et al.,

1997; Girard et al., 2000). This large increase in B cells during infection could also be associated with a change in homeostasis of T cell groups as has been observed in HIV patients (Ho et al., 1995; Mohri et al., 1998). Immunosuppressive T cell mechanisms have been reported in experimental models of HAT (Millar et al., 1999; Reinitz and Mansfield, 1990; Sendashonga and Black, 1986). These mechanisms described the apparition of activated macrophages (Namangala et al., 2000; Namangala et al., 2001). They were activated in the presence of IL-4 and contributed to the development of a Th2 cytokine response which decreased inflammatory responses in hosts (Gobert et al., 2000).

For this reason, we also decided to study the action and variations of cytokine/chemokine levels during HAT, in comparison to disease severity. Chemokines are produced after activation of cells such as astrocytes, microglia, macrophages, T cells and endothelial cells. They have a key role in early events of inflammation, and are putative candidates for the migration of leukocytes from blood to CNS by chemotactic gradients at the inflammation site (Luster, 1998; Sharafeldin et al., 2000). Indeed, as cytokines and chemokines are implicated in the early recruitement of leucocytes to the CNS, our study was based on their potential value to define the beginning of CNS alterations. Different proteins were studied in the blood and CSF from HAT patients. In blood, cytokine/chemokine levels were not associated with disease stage. Conversely, in CSF, cytokine levels, except CCL-5, were associated with the presence of neurological signs, demonstrating their diagnostic value. In HAT, cytokine/chemokine secretions were not correlated with the presence of parasites in blood but with their presence in CSF, and therefore correlated with disease severity. In CSF, some cytokine/chemokine levels were elevated in late stage disease, and were not present during early events of parasite invasion of the BBB. It could be supposed that the presence of parasites in the CSF directly induced release of certain cytokines/chemokines, depending on some parasitic factors or immune cell activation mediated by the presence of trypanosomes.

This result was found in our study for IL-1β, in agreement with other reports (Tachado and Schofield, 1994; Quan et al., 1998). Moreover, IL-1β has been shown to activate chemokine production, particularly CCL-2 by endothelial cells of the CNS leading to leucocyte recuitement through the BBB (Harknesse et al., 2003). Recently, it has been demonstrated that CCL-2 was implicated in BBB permeability by alterations of protein junctions in endothelial cells (Stamatovic et al., 2005). Therefore, CCL-2 could play a key role for parasite entry into the brain. Chemotactic and proinflammatory effects of CCL-3 in cell recruitement into the CSF was of major importance in HAT and confirmed stage 2 patients.

We have so shown that cytokine and chemokine production could be induced by parasites. Therefore, they could be potential markers of CNS invasion. Indeed, increased chemokine levels in patient CSF corresponded to late stage 2 patients. These chemokines could be used as additional stage markers in HAT for doubtful cases, in addition to diagnostic criteria frequently used in the field, resulting in a more accurate treatment of patients. In *T. b. gambiense* and *rhodesiense* infections, IL-10 concentrations in CSF of stage 2 patients are elevated and could be an interesting marker (MacLean et al., 2001; Lejon et al., 2002).

The CXC chemokine family was represented in our study by CXCL-8 and CXCL-13. CXCL-8 was associated with parasite detection in CSF and with the presence of neurological signs. In blood elevated CXCL-13 levels were associated with the presence of trypanosomes in the CSF but not with other stage markers (neurological signs, IgM synthesis, and cell numbers in CSF), which excluded this chemokine as a valid diagnostic marker in blood. However, in CSF, elevated levels were associated with all standard stage markers and with intrathecal IgM synthesis. The value of CXCL-13 as a HAT stage marker was confirmed by recent studies concerning its implication in the development of neurological sequelae. These results were similar to those reported for Lyme neuroborreliosis (Narayan et al., 2005; Rupprecht et al., 2006) and suggested early CXCL-13 release and the probability for CXCL-

13 to be an early marker of CSF invasion. Early CXCL-13 release could be confirmed by recent experimental findings on systemic CXCL-13 activation which attracted B lymphocytes into the CNS and induced a mechanism of transit through the BBB (Kanemitsu et al., 2005; Ransohoff et al., 2007). CXCL-13 is mainly synthetized by follicular dendritic cells (DCs) in lymphoid tissues (Cyster et al., 2000; Magliozzi et al., 2004) but circulating DCs exist in the CSF (Hatterer et al., 2006), thus making a early CXCL-13 release in the CSF possible. Results of our study always confirmed results found concerning cell counts, showing that this criterium seems to be the best actually used (Miézan et al., 1998; Jamonneau et al., 2003; Lejon et al., 2008). Experimental results suggested an association between high expression of CXCL-13 coding genes and susceptibility to disease (Kierstein et al., 2006). In addition, CXCL-13 could be used as a demyelinisation marker and could determine the severity of neurological signs and persistence of neuro-psychiatric sequelae (Corcione et al., 2004; Rupprecht et al., 2005).

Finally, we demonstrated that *T. b. gambiense* could activate CXCL-13 expression in microglial and endothelial cell lines, suggesting a direct role for trypanosomoses in regulation. To study CXCL-13 gene expression by microglial and endothelial cells, we used two *in vitro* cell line models (CHME-5, HBMEC) co-cultured with first *T. b. gambiense,* infectious parasite for human and then *T. b. brucei*, non-infectious. Only *T. b. gambiense* showed CXCL-13 gene expression by CHME-5 and HBMEC cells after co-culture. Our results confirmed the possibility of specific mechanisms linked to human infectious parasites (Nikolskaia et al., 2006 ; Masocha et al., 2007). Identifying these mechanisms could elucidate *T. b. gambiense* pathogenicity in the CSF. Recently, in experimental models of resistance/susceptibility to *T. congolense* infection, strong expression of genes coding for chemokines, and particularly CXCL-13, was associated with disease susceptibility. High IL-10 production during HAT could also explain some results. Even if IL-10 generally

suppresses inflammatory chemokine production in DCs and other cell lines, it also induces the expression of genes link to B cell differenciation and function including CXCL-13 in DCs (Perrier et al., 2004).

The perspectives of our thesis work concern new diagnostic markers we have described during this study. They must be validated on a large scale in the field. This validation is difficult because there is no real gold standard for the classification of patients depending on disease stage. HAT stage diagnosis must be determined by a combination of several diagnostic tests and the identification of new diagnostic stage markers must be promoted. It is also necessary to perform post-therapeutic follow-ups of patients over a long time period, on cohort studies. Our studies on cells and cytokines/chemokines involved show the necessity of their evaluation as stage markers by multicentric studies with standardized processes.

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Résumé. La trypanosomose humaine africaine (THA), ou maladie du sommeil est une parasitose due à des trypanosomes du groupe *Trypanosoma brucei* (*T. b.*). Ces parasites sont transmis par piqûre d'une mouche du genre *Glossina*, la mouche tsé-tsé. La THA pose un réel problème de santé publique en Afrique noire et Subsaharienne. Deux stades sont classiquement décrits dans l'évolution de la maladie, le stade lymphatico-sanguin et le stade nerveux. Le diagnostic et la détermination du stade, dont dépend le traitement, restent difficiles. En effet, les critères couramment utilisés sont peu sensibles ou peu spécifiques et nécessitent une ponction lombaire avec prélèvement de liquide céphalo-rachidien (LCR). C'est pourquoi notre travail de thèse avait pour objectif la recherche de nouvelles approches diagnostiques permettant de déterminer le stade de la THA. Pour cela, nous avons étudié diverses sous-populations de lymphocytes T et B, et les cytokines/chémokines les régulant. Nous avons d'abord caractérisé les lymphocytes T régulateurs CD4+CD25+Foxp3+ (nTregs), chez des souris infectées par *T. b. brucei*. L'augmentation des nTreg dans le thymus jusqu'à 120 jours d'infection puis leur diminution à 240 jours, peuvent être dues à un mécanisme de blocage de maturation dans le thymus ou à un blocage de leur sortie en dehors du thymus. Inversement, dans les ganglions mésentériques, nous avons trouvé une constante diminution des nTregs au cours de l'infection. Ces résultats suggèrent que la variation de leur nombre est due à l'évolution de l'infection, mais est aussi âge-dépendante. Notre travail a ensuite permis de préciser le type de cellules lymphocytaires impliquées dans le sang et dans le LCR de patients atteints de THA. Dans le sang, tous stades confondus, la proportion de lymphocytes B (CD19) augmente alors que celle des lymphocytes T diminue confirmant le caractère immunosuppressif de la maladie. Dans le LCR, l'augmentation des cellules B CD19 chez les patients en stade 2, pourrait constituer un nouveau critère du stade nerveux. D'autre part nous avons cherché à comprendre quels étaient les mécanismes d'attraction des lymphocytes dans le système nerveux central par le dosage dans le sérum et le LCR de différentes cytokines/chémokines. Dans le sérum, les taux de cytokines/chémokines étaient uniquement associés à la présence du trypanosome dans le LCR. Dans le LCR, leur expression était associée avec la présence de signes neurologiques, démontrant leur intérêt pour le diagnostic du stade nerveux. Enfin, nous avons montré que *T. b. gambiense* peut activer l'expression de CXCL-13 dans les lignées cellulaires microgliales et endothéliales, suggérant un rôle direct des trypanosomes dans le processus de régulation. Nos travaux sur les cellules lymphocytaires et les cytokines/chémokines impliquées montrent la nécessité de leur évaluation en tant que marqueurs de stade par des études multicentriques de terrain.
Mots clés. Trypanosome humaine africaine ; diagnostic de stade ; lymphocytes T régulateurs ; lymphocytes B ; profils lymphocytaires ; chémokines.

Abstract. Human African trypanosomiasis (HAT), or sleeping sickness is a parasitic disease due to trypanosomes of the *Trypanosoma brucei* (*T. b.*) group. These parasites are transmitted by the bite of a tse-tse fly. HAT represents a real public health problem in Africa and sub-Saharan Africa. Two stages are classically described in disease progression, the heamo-lymphatic stage and the nervous stage. Diagnosis and stage determination, which are crucial to determine treatment, remain difficult. Frequently used criteria are not very sensitive or very specific and require a lumbar puncture to obtain a sample of cerebrospinal fluid (CSF). The objective of our PhD was to identify new approaches diagnostic to determine disease stage. For that, we studied various subpopulations of T and B lymphocytes, and the cytokines/chemokines controlling them. We initially characterized the regulatory T cells CD4+CD25+Foxp3+ (nTregs), in *Trypanosoma brucei*-infected mice. nTregs increased in the thymus until 120 days post infection and decreased at 240 days. This may be due to a mechanism blocking nTreg maturation in the thymus, or their exit from the thymus. In contrast, in mesenteric lymph nodes, a nTregs constantly decreased was found during infection. These results suggest that the variation in their number was due to the progression of the infection, but was also age-dependent. Our study identified the lymphocytes present in the blood and CSF of HAT infected patients. In blood, regardless of the HAT stage, the proportion of B cells (CD19) increased whereas the number of T cells decreased, thus confirming the immunosuppressive character of the disease. In the CSF, the increased number of CD19 cells observed for stage 2 patients could constitute a new criterion of the nervous stage. Furthermore, we tried to understand by which mechanisms lymphocytes were attracted into the central nervous system by measurement of different cytokines/chemokines levels in serum and CSF. In serum, cytokines/chemokines levels were only associated with the presence of trypanosomes in CSF. In CSF, their expression was associated with the presence of neurological signs, suggesting a possible use for diagnosis of the nervous stage. Finally, we showed that *T. b. gambiense* activated CXCL-13 expression in microglial and endothelial cell lines, suggesting a direct role of trypanosomes in regulation. Our studies on lymphocytes and cytokines/chemokines implicated highlights the necessity of their evaluation as stage markers by multicentric studies in the field.
Key words. Human african trypanosomiasis; stage diagnosis; regulatory T lymphocytes; B lymphocytes; lymphocytic populations; chemokines.

Resumo. A tripanossomíase humana africana (THA), o doença do sono é uma doença parasítica induzida por trypanosomas do groupo *Trypanosoma brucei* (*T. b.*). Estos parasitos são transmitidos pela punctura de uma mosca tse-tse. A THA é responsavel por um grande problema de saúde pública em Africa e em Africa subsariana. Duas fases são descritas na evolução da doença, a fase linfaticosanguínea e a fase nervosa. O diagnóstico e a determinação, quais são cruciais para determinar o tratamento apropriado, permaneça difícil. Certamente, os critérios geralmente usados permanecem atualmente discutível porque não são específicos. O nosso trabalho de these teve como objetivos a busca para as novas aproximações diagnósticas que tornam possível determinar o estágio da doença. Para isso, nós estudamos os vários subpopulações dos linfócitos T e B, e as citocinas/ quimiocinas que controlando os. Nós caracterizamos inicialmente os linfócitos em particular as celulas T reguladoras CD4+CD25+Foxp3+ (nTregs), em ratos contaminados para *T. brucei*. O aumento dos nTregs no thymus até 120 dias da infecção e a diminução em 240 dias, pode ser devido a um mecanismo da obstrução da maturação dos nTregs no thymus, o a uma obstrução da saída delos fora do thymus. Inversamente, em nós de linfa mesenterica, nós encontramos que os nTregs diminuem durante a infecção. Estes resultados sugerem que a variação de seu número seja devido à evolução da infecção, mas são igualmente idade-dependentes. Nós caracterizamos as celulas linfocitarias implicadas na THA, tanto no sangue como no líquido cefalorraquidiano (LCR) de doentes. No sangue, em todas as fases, a proporção de linfócitos B aumenta enquanto que a dos linfócitos T diminui confirmando o carater immunosuppresivo da doença. No LCR, o aumento das celulas CD19 positivas nos doentes em fase 2, poderia constituir um novo critério da fase nervosa. Por outro lado procuramos compreender quais eram os mecanismos de atração dos linfócitos no sistema nervoso central pela medida no soro e no LCR das diferente citocinas/quimiocinas. No soro, os níveis das citocinas/ quimiocinas foram associados somente com a presença de trypanosomes no CSF. No LCR, sua expressão estêve associada com a presença de sinais neurológicos, mostrando seu valor diagnóstico. Finalmente, nós mostramos que *T. b. brucei* pode ativar a expressão do CXCL-13 nas linhas celulas, sugerindo uma acção direta dos trypanosomes no processo da regulação. O nosso trabalho nos linfócitos e nas citocinas/quimiocinas implicadas mostraram a necessidade de sua avaliação como marcadores do estágio por estudos no campo.
Palavras chave. Tripanossomíase humana africana; diagnóstico de fase; linfócitos T reguladores; B linfócitos; populações linfócitos; quimiocinas.

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