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Comparison of cytokine plasma levels in human African trypanosomiasis

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Summary

BACKGROUND Immunological studies suggest that human African trypanosomiasis (HAT) is associated with inflammatory responses. A better understanding of the complex cytokine interactions regulating HAT infections is essential to elucidate the mechanisms of generalized immunosuppression.
METHOD We determined levels of interleukin (IL)-2, IL-4, IL-6, IL-10, tumour necrosis factor (TNF)-α and interferon (IFN)-γ protein levels in plasma samples from three groups of individuals from the Democratic Republic of Congo: (i) HAT cases; (ii) seropositive individuals for whom parasite detection was negative and (ii) controls.
RESULTS Plasma levels of six cytokines were significantly higher in HAT cases than in both controls ($P < 0.003$) and seropositive individuals ($P < 0.016$). IL-2 and IL-10 concentrations were significantly lower ($P < 0.02$) in the seropositive group than in the control one.
CONCLUSION Human African trypanosomiasis leads to the development of strong cytokine responses, indicating the potential involvement of IL-2 and IL-10 in the phenomenon of seropositivity without parasitological confirmation. This strongly suggests the involvement of immunity in this particular aspect of HAT epidemiology.

keywords gamma interferon, human African trypanosomiasis, interleukin, *Trypanosoma brucei gambiense*, tumour necrosis factor-alpha

Introduction

Human African trypanosomiasis (HAT) in West Africa is caused by *Trypanosoma brucei gambiense* (*Tbg*). *Tbg* HAT is classically described as a chronic form: after infection, trypanosomes proliferate in blood and lymph during the early stage, which is followed by a meningo-encephalitic or late stage characterized by cerebrospinal fluid invasion and central nervous system disorders (Buguet *et al.* 2001). The duration of the early stage can be up to several years, and the appearance of neurological disorders during the late stage often progresses rapidly.

As for numerous infectious diseases, clinical presentations and evolutions can strongly differ from the classical ones. Concerning *Tbg* HAT, acute (Truc *et al.* 1997) and asymptomatic (Jamonneau *et al.* 2000, 2004)forms have been described. Both the infectious agent and host immunity could play a role in this variability (Garcia *et al.* 2000; Jamonneau *et al.* 2004; Courtin *et al.* 2006). A particular attention has been paid for people positive to card agglutination test for trypanosomiasis (CATT) (Magnus *et al.* 1978), for which no parasite can be detected (Kanmogne *et al.* 1996; Simo *et al.* 1999). In a longitudinal follow-up, Garcia *et al.* (2000) confirmed that some of these individuals have probably been infected by *Tbg*, and the complete absence of disease after 32 months could be due to a strongly suited immune response.

The purpose of the present preliminary study was to compare interleukin (IL)-2, IL-4, IL-6, IL-10, tumour necrosis factor (TNF)-α and interferon (IFN)-γ protein levels in plasma samples between HAT cases, seropositive apparently aparasitemic subjects, and controls in order to explore the possible role of human immunity in this complex phenomenon.

Materials and methods

Field sampling

Blood was sampled in the field from 107 CATT positive on whole blood and 56 CATT negative on whole blood subjects in the Bandundu province (Democratic Republic of Congo) by the HAT National Control Program (HAT-NCP). Consent forms were signed by all subjects included in this study and the protocol was approved by ethic committee of health ministry.

Definition of groups

As we were interested in a specific seropositive profile such as defined by Garcia *et al.* (2000), the serological status of these 107 CATT positive and 56 CATT negative on whole blood were blindly tested by means of trypanolysis tests (TL) (Van Meirvenne *et al.* 1995). In the field, parasitological investigation on the 107 CATT-positive subjects was based on direct examination of thick blood smear as proposed by the HATNCP. Because of the very poor sensitivity of this method (Chappuis *et al.* 2005), we decided to base the HAT diagnosis on a *T. brucei s.l.* polymerase chain reaction (PCR) test (Tbsl PCR) (Penchenier *et al.* 1996) using specific primers (Moser *et al.* 1989) performed blindly, which is described as more sensitive (Jamonneau *et al.* 2003; Chappuis *et al.* 2005). Malaria and loiasis status of each individual were assessed by means of *Plasmodium falciparum* and/or *Loa loa* PCR (Pf PCR and Ll PCR) using specific primers (Toure *et al.* 1997; Singh *et al.* 1999).

Of 107 subjects testing CATT positive on whole blood, the following two groups were defined, based on TL and Tbsl PCR results. (i) Group 1, HAT cases: positive CATT, positive TL and positive Tbsl PCR ($n = 21$) and (ii) group 2, seropositive individuals: positive CATT, positive TL and negative Tbsl PCR ($n = 9$). The remaining 77 subjects CATT positive on whole blood who were negative to TL were excluded (Figure 1). Co-infections by *P. falciparum* and/or *Loa loa* have been taken into account during multivariate linear regression analysis (see below).

From the 56 subjects CATT negative on whole blood, a control group, based on the result of TL and PCR, was defined as follows: negative CATT, negative TL and negative Tbsl PCR. To avoid cytokine plasma level variations due to malaria or loiasis infections, positive *P. falciparum* and/or *Loa loa* PCR subjects were excluded. Thus, 34 individuals were included in the control group (Figure 1).

Biological methods

Cytokines levels were determined for the individuals of groups 1, 2 and 3. The human Th1/Th2 Cytokine Cytometry Bead Array (CBA) kit II and the BD CBA software (BD Biosciences) were used to quantitatively measure IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ protein levels. After the acquisition of sample data using a flow cytometer, the sample results are generated in graphical and tabular format using the BD CBA analysis software.

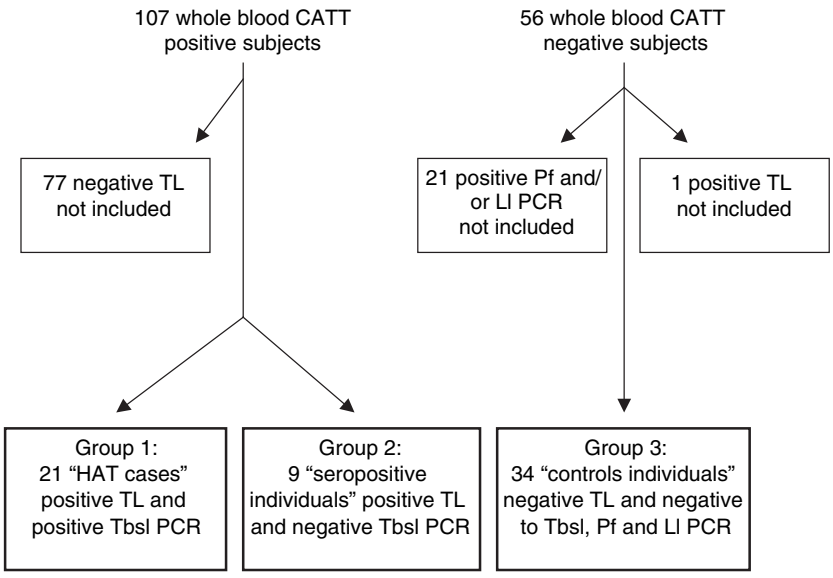

Figure 1 Constitution of study groups.

Statistical analysis

Multivariate linear regression was applied to test for the effect of HAT on cytokine circulating plasma levels, adjusted on co-infection status. As the distributions of cytokine circulating plasma levels were not normally distributed, we performed a log-transformation in order to set the skewness of each variable to 0. Interactions between co-infection and HAT status were also tested. All statistical analyses were performed using STATA software (StataCorp., College Station, Texas, USA, 1999, Release 8.0).

Results

All subjects belonged to the Yansi ethnic group. Age did not differ between the three groups. There was no association between cytokine plasma levels and age or sex. Seven individuals in the HAT case group and four in the seropositive group were infected with *P. falciparum*. Only one individual from the HAT case group was positive to loiasis PCR and was kept in the analyses without taking into account his loiasis status.

Figure 2 displays plasma concentration of the six cytokines for each individual before the log-transformation in HAT cases, seropositive and control individuals. For all cytokines, the circulating plasma levels for HAT cases were significantly higher than for controls (Table 1). HAT cases positive for *P. falciparum* presented a significantly lower IL-10 plasma level ($P = 0.03$) than controls. No interaction between *P. falciparum* status and HAT status has been significant, meaning that the effect of HAT on IL-10 plasma level was similar whatever *P. falciparum* status was.

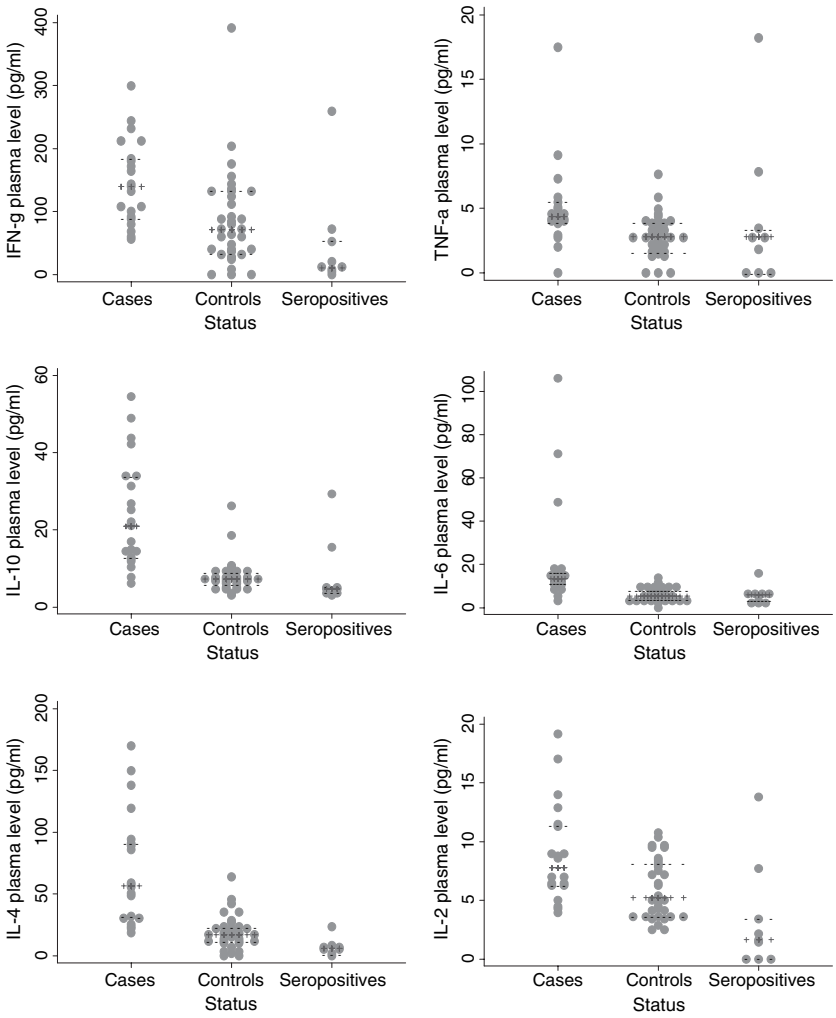

Figure 2 Plasma cytokine concentration in human African trypanosomiasis cases, non-infected controls and seropositive persons. The dot plot graphs indicate the level cytokine value for each individual and display median and 25th and 75th percentiles for each group.

Table 1 Multivariate linear regression comparing the cytokines plasma levels of HAT cases and seropositives with controls

Cytokines	Status	β*	95% CI	*P*-value
IFN-γ	Control	–		
	HAT case	0.40†	[0.14, 0.67]	0.003
	Seropositive	−0.34‡	[−0.69, 0.01]	0.06
	Plasmodium falciparum PCR positive	0.01§	[−0.32, 0.34]	0.95
TNF-α	Control	–		
	HAT case	0.86	[0.33, 1.39]	0.002
	Seropositive	−0.01	[−0.72, 0.70]	0.98
	P. falciparum PCR positive	0.47	[−0.19, 1.13]	0.16
IL-10	Control	–		
	HAT case	1.13	[0.67, 1.58]	$<10^{-4}$
	Seropositive	−0.79	[−1.40, −0.17]	0.01
	P. falciparum PCR positive	−0.65	[−1.22, −0.07]	0.03¶
IL-6	Control	–		
	HAT case	1.05	[0.62, 1.48]	$<10^{-4}$
	Seropositive	−0.07	[−0.65, 0.50]	0.80
	P. falciparum PCR positive	−0.06	[−0.59, 0.47]	0.83
IL-4	Control	–		
	HAT case	1.38	[0.88, 1.88]	$<10^{-4}$
	Seropositive	−0.25	[−0.93, 0.43]	0.47
	P. falciparum PCR positive	0.26	[−0.37, 0.88]	0.41
IL-2	Control	–		
	HAT case	0.46	[0.17, 0.74]	0.002
	Seropositive	−0.55	[−0.93, −0.16]	0.006
	P. falciparum PCR positive	0.23	[−0.12, 0.59]	0.19

CI, confidence interval; HAT, human African trypanosomiasis; INF, interferon; TNF, tumour necrosis factor; IL, interleukin; PCR, polymerase chain reaction.
*Coefficient of regression; a positive (negative) value indicates a higher (lower) level of cytokines.
†Effect of HAT cases (adjusted on *P. falciparum* status) on the level of cytokines compared with the control group.
‡Effect of seropositives (adjusted on *P. falciparum* status) on the level of cytokines compared with the control group.
§Effect of *P. falciparum* on the level of cytokines compared with the control group.
¶No interaction.

In seropositive individuals, the levels of both IL-10 and IL-2 were significantly lower than in controls ($P = 0.01$ and $P = 0.006$ respectively) (Table 1). All plasma cytokine levels were significantly lower in seropositive individuals than in HAT cases (not shown in the table). The same pattern of results was obtained when the individual with positive *Loa loa* PCR was excluded.

Discussion

Circulating levels of the six cytokines exhibited higher levels in patients than in controls as previously described (Okomo-Assoumou *et al.* 1995; Rhind *et al.* 1997; MacLean *et al.* 2001; MacLean *et al.* 2004). The specific result of this preliminary report concerns seropositive subjects. We show that they differ from controls for IL-2 and IL-10 suggesting that these cytokines could be involved in this phenomenon. The importance of IL-10 in HAT is known, but its role remains unclear (Lejon *et al.* 2002). An important finding is that the normal physiological equilibrium between TNF-α and IL-10 is disrupted by a chronic exposure and that the influence of IL-10 can be either beneficial or detrimental (for a review see Dumas *et al.* 1999). These results have been recently emphasized by Courtin *et al.* (2006) who showed that a polymorphism of the gene encoding IL-10 is associated with a lower risk to develop the disease. There is poor information in HAT concerning the involvement of IL-2 although its role seems important in animal trypanosomiasis (Sileghem & Flynn 1992). Nevertheless it has been demonstrated that both IL-10 and IL-2 could be involved in a complex process leading to the impairment of monocytes to provide co-stimulatory signal for resting T-cell proliferation (Moore *et al.* 1993).

In the field, three categories of individuals are encountered: (i) healthy individuals; (ii) HAT cases and

(iii) subjects with positive serology, but negative parasitology. This last category can arise from (i) false seropositivity, mainly due to cross-reactivity with animal trypanosomes or other infectious diseases (Noireau *et al.* 1986, 1988); (ii) weak parasitaemia not detectable due to lack of sensibility of tests and (iii) a potential phenomenon of control of infection in subjects with suited immune system (Garcia *et al.* 2000). The study of seropositivity with negative parasitology progressed along two complementary approaches, the first being whether these individuals have to be treated or not. In these studies, seropositivity was defined as CATT positivity during a cross-sectional survey and this group of subjects involved obvious HAT cases (Kanmogne *et al.* 1996; Simo *et al.* 1999). We adopted a second approach, questioning about the physiological significance of this phenomenon. In a previous study (Garcia *et al.* 2000), with a more specific definition of seropositivity, we have shown that some of these individuals have probably been infected by *Tbg*. The complete absence of the disease after 32 months of follow-up could be due to particular immune response allowing infection control. The results obtained in the present preliminary study seem to support this view. Even though, the presence of cryptic infections within seropositive group cannot be excluded, the observed cytokine profile of seropositive subjects seems to be different from both cases and controls. However, the low number of subjects and the absence of follow-up need further investigation. These preliminary results are consistent with the development of complex cytokines responses in HAT that could be involved in the variability of responses to infection.

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Comparación de los niveles de citocinas en plasma en la tripanosomiasis humana africana

ANTECEDENTES Estudios inmunológicos sugieren que la tripanosomiasis humana africana (THA) está asociada con respuestas inflamatorias. Un mejor entendimiento de las complejas interacciones entre las citocinas que regulan la infección por THA es esencial para elucidar los mecanismos de inmunosupresión generalizada.

MÉTODO Hemos determinado los niveles de interleuquina (IL)-2, IL-4, IL-6, IL-10, el factor de necrosis tumoral (TNF)-α e interferon (IFN)-γ, en muestras de plasma provenientes de 3 grupos de individuos de la República Democrática del Congo: (1) casos THA; (2) individuos seropositivos con resultado negativo en la detección del parásito (3) controles.

RESULTADOS Los niveles en plasma de las 6 citocinas fueron significativamente mayores en los casos que en los controles ($P < 0.003$) y en los individuos seropositivos ($P < 0.016$). Las concentraciones de IL-2 e IL-10 fueron significativamente menores ($P < 0.02$) en el grupo de seropositivos que en el grupo control.

CONCLUSIÓN La THA conlleva al desarrollo de respuestas mediadas por citocinas, indicando una implicación potencial de la IL-2 y la IL-10 en el fenómeno de la seropositividad sin confirmación parasitológica. Esto parece sugerir la implicación de la inmunidad en este aspecto particular de la epidemiología del THA.

palabras clave tripanosomiasis humana Africana, factor de necrosis tumoral-alfha, interleuquina, gama interferón, *Trypanosoma brucei gambiense*

Comparaison des taux de cytokines plasmatiques dans la trypanosomiase humaine africaine

DONNÉES DE BASE Les études immunologiques suggèrent que la trypanosomiase humaine africaine (THA) est associée à des réponses inflammatoires. Une meilleure compréhension des interactions complexes des cytokines régulant les infections par la THA est essentielle pour élucider le mécanisme d'immunosuppression généralisée.

MÉTHODE Nous avons déterminé les taux d'interleukines (IL)-2, -4,-IL-6, IL-10, de TNF-α et de protéine d'interféron gamma (INF-γ) dans des échantillons de plasma provenant de 3 groupes d'individus de la République Démocratique du Congo: 1) des cas de THA, 2) des individus séropositives mais chez qui le parasite n'était pas dètecté et 3) des cas contrôles.

RÉSULTATS Les taux plasmatiques des 6 cytokines étaient significativement plus élevés dans les cas de THA que chez les contrôles ($P < 0{,}003$) et les individus séropositives ($P < 0{,}016$). Les concentrations de IL-2 et IL-10 étaient significativement plus basses ($P < 0{,}02$) dans le groupe des séropositifs que dans le groupe contrôle.

CONCLUSION La THA donne lieu au développement de fortes réponses de cytokines, indiquant l'implication potentielle des IL-2 et IL-10 dans les phénomènes de séropositivité sans confirmation parasitologiques. Ces observations suggèrent fortement l'implication de l'immunité dans cet aspect particulier de l'épidèmiologie de la THA.

mots clés trypanosomiase humaine Africaine, TNF-α, interleukine, interféron gamma, *Trypanosoma brucei gambiense*