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INFECTIVITY OF *TRYPANOSOMA (TRYPANOZOON) BRUCEI GAMBIENSE* FOR BABOONS (*PAPIO HAMADRYAS, PAPIO PAPIO*)

by

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Summary — In order to study sensitivity or resistance of *T.b. gambiense* to baboon serum, two species of baboons, *P. hamadryas* and *P. papio* were inoculated with *T.b. gambiense clone LiTat 1.1.* Both species were receptive to infection but, parasitological and immunological parameters showed that *P. papio* was more trypanotolerant than *P. hamadryas.*

The VAT-specific trypanolysis test and the ELISA, using MoAb for circulating antigen detection may be appropriate for the diagnosis of human trypanosomiasis due to *T.b. gambiense.*

KEYWORDS: *Trypanosoma brucei gambiense*; Trypanolytic Factor; Baboon Serum; Serum Incubation Infectivity Test; Immunolysis Test; Radio-Immunoprecipitation; Self-Cure; Antigenic Variation; Antibodies; Trypanotolerance; *Papio hamadryas*; *Papio papio.*

Introduction

Contradictory results have been reported with respect to susceptibility of baboons to trypanosome infections (1, 11, 17, 18).

In human and in baboon an innate trypanolytic serum factor effective on *T.b. brucei* but not on *T.b. gambiense* has been demonstrated (3, 4, 10, 22).

The trypanocidal factor present in normal human serum, and identified as a high density lipoprotein fraction (20), is currently used to characterize *T. brucei spp.* (19, 21, 13, 7). Comparative studies on the trypanolytic activity of human and baboon (*Papio papio* and *P. hamadryas*) serum on salivarian trypanosomes demonstrated that *T.b. gambiense* stocks and clones from different areas in Africa were consistently resistant. *T.b. brucei* was consistently sensitive to human and *P. papio* sera but variable results were obtained with serum from *P. hamadryas* (9).

The purpose of the present experiment was to compare the infectivity of *T.b. gambiense* to baboons, *P. papio* and *P. hamadryas.*

Material and methods

Four adult baboons, two *Papio hamadryas* male and female and two *males Papio papio* were inoculated with LiTat 1.1, a rodent-adapted clone of *T.b. gambiense* (Lille, Trypanozoon, variable Antigen Type 1/1). Each animal received intramuscularly 0,5 ml of blood from mice with antilog 9 of parasitaemia (5).

Follow-up of parasitaemia was done by wet blood film, thick blood smear and buffy coat examination and by mice sub-inoculation. If these techniques

gave negative results, mini-Anion Exchange Centrifugation Technique (m-AECT) was done (12, 14).

Circulating trypanosomal antigen was measured by ELISA using a *T. brucei*-group specific monoclonal antibody (15, 16).

The antibody response was measured by a radio-immunoprecipitation test (2) using an extract of *T. evansi* Variable Antigen Type (VAT) Antat 3/3 (8) and by VAT-specific immune trypanolysis test (21) using *T.b. gambiense* clones of LiTat 1.1., the VAT inoculated, and LiTat 1/3 to 1/9, seven other VATs of the same antigen repertoire.

Results

As far as the parasitaemia is concerned, there was a clear difference between the two species. *P. hamadryas* developed patent parasitaemia which persisted for more than three months after inoculation (Table 1, 2). *P. papio* remained negative throughout the observation period.

TABLE 1

Sensitivity of parasitological methods for detecting *T.b. gambiense* infection in *Papio hamadryas*, ♂

Days p.i.	Fresh blood preparation	Thick blood smear	Buffy coat	Mouse subinoculation: Number inoculation	Number positive	Prepatent period (days)	Survival time (days)
7	–	–	–	2	2	4	6
14	–	–	–	3	3	4.7	11
21	–	–	+	3	3	4.7	8.3
29	–	–	+	3	3	3.3	6
36	–	–	–	3	3	5.3	8
43	–	–	+	3	3	4.3	8
50	–	–	+	3	3	5	10
57	–	–	–	3	3	12.3	16.7
64	–	–	+	3	3	10	15.3
78	–	+	+	3	3	4.3	11
100	–	–	–	3	3	7	17.3
150	–	–	–	3	0	—	58***

***: negative untill the end of the experiment

TABLE 2

Sensitivity of parasitological methods for detecting *T.b. gambiense* infection in *Papio hamadryas*, ♀

Days p.i.	Fresh blood preparation	Thick blood smear	Buffy coat	Mouse subinoculation: Number inoculation	Number positive	Prepatent period (days)	Survival time (days)
7	–	–	–	2	2	5	6.5
14	–	–	–	3	2*	7	11
21	–	–	–	3	3	7.7	1.3
29	–	–	–	3	3	4	7
36	–	–	–	3	1*	6	99
43	–	–	+	3	2*	6	15
50	–	–	+	3	3	8	12
57	–	–	–	3	2	14.5	19
64	–	–	–	3	3	15	18
78	–	–	–	3	0	–	53 ***
100	–	–	–	2	1	7	20
150	–	–	–	3	0	–	58

\+ : positive
– : negative
* : one mouse accidentally died
** : two mice accidentally died
*** : negative until the end of the experiment.

Serum antibodies were detected in the two species by radioimmunoprecipitation. Up to 15 antigenic components of molecular weight ranging from 26 to 150 kd, and two major components with molecular weight 33 and 60 kd were recognized by the sera of *P. hamadryas*. Only 5 antigenic components were picked up by *P. papio* sera (Figure 1).

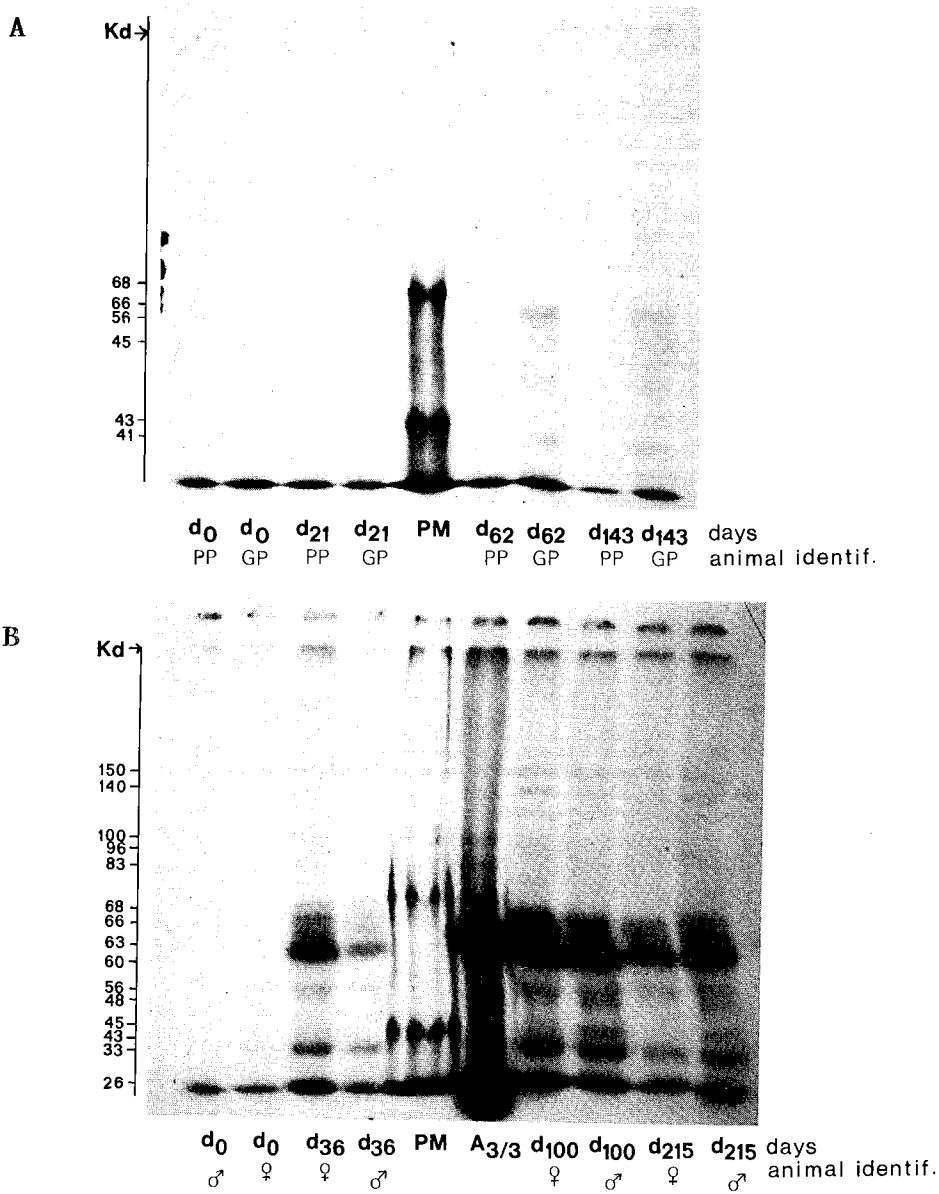

Figure 1.
Trypanosomal antigen recognition patterns as revealed by the radio-immunoprecipitation technique. — A. *Papio papio* B. *Papio hamadryas*. A 3/3 : *T. evansi* AnTat 3/3.

Lytic antibodies against the inoculated LiTat 1/1 trypanosomes and against other VATs of the same repertoire appeared in sera from both species. *P. hamadryas* developed the highest antibody titers, finally recognized all VATs tested and remained positive for more than two years post-inoculation (Table 3, 4). In *P. papio* antibody titers were low and only 5 VATs were recognized. The antibodies decreased more rapidly and finally disappeared completely (Table 5, 6).

TABLE 3

***T.b. gambiense* infection in *Papio hamadryas* (Animal PH ♂)**
Evolution of VAT-Specific trypanolytic antibody titers

days post infection	trypanolytic end-titers against VATs							
	LiTat 1/1*	LiTat 1/3	LiTat 1/4	LiTat 1/5	LiTat 1/6	LiTat 1/7	LiTat 1/8	LiTat 1/9
0	0	0	0	0	0	0	0	0
7	8	0	0	0	0	0	0	0
21	256	32	128	16/32	⩾1024	0	128/256	⩽4
36	64/128	256	128	13/32	128	32	64	8/16
50	32/64	ND	ND	ND	ND	ND	ND	ND
64	64	256	128	16	ND	16	4-8	16/32
100	32/64	64/128	128	8/16	64	8-16	4	32
136	32	ND	ND	ND	ND	ND	ND	ND
175	32	ND	ND	ND	ND	ND	ND	ND
193	32	32	64	8	16-32	8-16	0	16
306	32	16/32	32	8	16	8	0	16
907	4/8	⩽4	4	0	4	0	0	0

0 = ⩽1/4 ND = not done
* = VAT inoculated

TABLE 4

***T.b. gambiense* infection in *Papio hamadryas*. (Animal PH ♂)**
Evolution of VAT-Specific trypanolytic antibody titers

days post infection	trypanolytic end-titers against VATs							
	LiTat 1/1*	LiTat 1/3	LiTat 1/4	LiTat 1/5	LiTat 1/6	LiTat 1/7	LiTat 1/8	LiTat 1/9
0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
21	256	>1024	64	128	128	0	32	8
36	128/256	512	128	256	64	32/64	16	32
50	128	ND	ND	ND	ND	ND	ND	ND
64	128/256	256	128	16	ND	16	4/8	32
100	128/256	256	256	16/32	64	8/16	4/8	64/128
136	64/128	ND	ND	ND	ND	ND	ND	ND
175	64	ND	ND	ND	ND	ND	ND	ND
193	64	128	128	8	16/32	8	⩽4	16/32
306	64	64/128	64	8	16/32	8	⩽4	16
903	8/16	16	8	0	⩽4	⩽4	0	0

0 = ⩽1/4 ND = not done
* = VAT inoculated

TABLE 5

***T.b. gambiense* infection in *Papio papio* (Animal PP_2)**
Evolution of VAT-Specific trypanolytic antibody titers

days post infection	trypanolytic end-titers against VATs							
	LiTat 1/1*	LiTat 1/3	LiTat 1/4	LiTat 1/5	LiTat 1/6	LiTat 1/7	LiTat 1/8	LiTat 1/9
0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
21	8/16	0	0	0	16	0	0	0
39	8/16	0	4	0	16	0	0	0
62	4	0	64	0	48	0	0	0
78	4	0	64	0	0	0	0	0
109	⩽4	0	32	0	0	0	0	0
143	⩽4	0	32	0	0	0	0	0
744	0	0	0	0	0	0	0	0

0 = ⩽1/4
* = VAT inoculated

TABLE 6

***T.b. gambiense* infection in *Papio papio* (Animal PP_1)**
Evolution of VAT-Specific trypanolytic antibody titers

days post infection	trypanolytic end-titers against VATs							
	LiTat 1/1*	LiTat 1/3	LiTat 1/4	LiTat 1/5	LiTat 1/6	LiTat 1/7	LiTat 1/8	LiTat 1/9
0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
21	32/64	0	0	0	0	0	0	0
39	32/64	0	4-8	0	64	0	16	0
62	16	0	⩽4	16	16/32	0	32	0
78	16	0	0	16/32	16/32	0	8/16	0
109	4-8	0	0	⩽4	4-8	0	⩽4	0
143	⩽4	0	0	⩽4	⩽4	0	⩽4	0
744	0	0	0	0	0	0	0	0

0 = ⩽1/4
* = VAT inoculated

Circulating trypanosomal antigen was detected by ELISA using *T. brucei* group-specific monoclonal antibody (MoAb). End titers ranged from 1/4 to 1/64. Maximum highest titer was obtained 3 to 4 weeks post-inoculation. Afterwards they declined progressively. With a limited number of serum samples a quantitative ELISA was performed. An optical density (OD) < 0.100 was considered being negative (Table 7).

TABLE 7

Detection of circulating trypanosomal antigens in *T.b. gambiense* infected baboons using *T. brucei* group-specific MoAb

Weeks post-inoculation	Papio hamadryas		Papio papio	
	♂	♀	PP	GP
0	0.098	0.066	0.076	0.028
1	0.722	0.542	0.824	ND
2	0.295	0.219	0.335	0.335
3	0.227	0.335	0.971	0.567
5	0.261	0.335	0.481	0.179
11	0.294	0.294	ND	0.227

N.D. = not done

Discussion and conclusions

According to its serum resistance *T.b. gambiense* proved infective to both species of baboons, *P. hamadryas* and *P. papio.* Parasitological and immunological parameters show clear differences between both species. *P. hamadryas* being more sensitive shows a patent though transient parasitaemia, which lasted for more than three months after inoculation. During the stage of patent parasitaemia the infectivity and virulence of the parasites for mice remained stable as judged by subinoculation. After this period the trypanosomes were no longer detectable and the animals survived. Trypanosomes were never observed in *P. papio*.

Radio-immunoprecipitation and immunolysis-tests showed that all the baboons developed a variety of specific antibodies against *T.b. gambiense*. In radio-immunoprecipitation using protein extract of *T. evansi*, AnTat 3/3, the sera from *P. hamadryas* recognized more components than *P. papio*.

In the immunolysis test the sera of both species recognized not only the VAT inoculated, LiTat 1/1 but also other VATs of the LiTAR 1 repertoire. The finding that VAT specific antibodies are produced suggests that trypanosomes develop in both species. However, at an early stage, *P. papio* is capable of controlling parasitaemia and eliminating the parasites.

Although no parasitaemia was detected in *P. papio*, the circulating trypanosomal antigen in this host reached a peak level two weeks after inoculation and declined after 3 months. This is further evidence that *T.b. gambiense* can develop in *Papio papio* giving a serological response without detectable parasitaemia and clinical symptoms.

It is concluded from these studies that both species of baboons, *P. hamadryas* and *P. papio*, are receptive but tolerant to *T.b. gambiense.* The trypanotolerance trait is characterized by: the ability to control parasitaemia, spontaneous self-cure, limited expression of the VAT-specific antibodies. The last parameter is the most significant immunological evidence of self-cure. It appears also that the trypanotolerant trait developed by baboons has no immunological basis, as is generally assumed, because the more trypanotolerant *P. papio* developed subpatent parasitaemia, lower trypanolytic antibody titers towards a small number of VATs and earlier disappearance of these variant specific antibodies.

Since human trypanosomiasis is usually characterised by parasitaemia of extremely low grade, the present studies are suggestive that for the diagnosis of *T.b. gambiense* infection also, trypanolytic test using an ubiquitary VAT and ELISA, using *T. brucei* group specific MoAb, for the detection of circulating parasite antigen may be appropriate.

Infectivité de *Trypanosoma* (*Trypanozoon*) *brucei gambiense* pour les babouins (*Papio hamadryas, Papio papio*).

Résumé — Afin de rechercher une éventuelle corrélation entre l'infectivité et la résistance de *T.b. gambiense* au sérum de babouin et de vérifier les résultats contradictoires rapportés antérieurement, deux espèces de babouins, *P. papio* et *P. hamadryas* ont été inoculées. Les deux espèces se sont montrées réceptives mais trypanotolérantes. Les paramètres parasitologiques et immunologiques ont prouvé que *P. papio* est plus trypanotolérant que *P. hamadryas*.

Le test de trypanolyse VAT-spécifique et ELISA utilisant un MoAb pour la mise en évidence de l'antigène circulant, sont aussi applicables au diagnostic de la trypanosomiase humaine à *T.b. gambiense*.

Infectiviteit van *Trypanosoma* (*Trypanozoon*) *brucei gambiense* voor bavianen (*Papio hamadryas, Papio papio*).

Samenvatting — Ter bepaling van de gevoeligheidsgraad van *T.b. gambiense* voor bavianenserum werden twee soorten bavianen, *P. hamadryas* en *P. papio* besmet met *T.b. gambiense* clone LiTat 1.1. Beide bavianensoorten waren gevoelig aan de besmetting, maar parasitologische en immunologische parameters wezen in de richting van een hogere trypanotolerantiegraad voor *P. papio* in vergelijking met *P. hamadryas.*

De VAT-specifieke trypanolysetest en de ELISA test, die cirkulerend antigeen aantoont uitgaande van MoAb, zijn ook bruikbaar voor de diagnose van humane trypanosomiasis door *T.b. gambiense.*

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