Effects of the timing of antigen stimulation on parasitaemia profile and subsequent immunodepression in an experimentally induced *Trypanosoma brucei* infection

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IKEME (M.M.), ADELAJA (A.O.). Effet du moment de la stimulation antigénique sur le déroulement de la parasitémie et immunodépression consécutive lors d'une infection expérimentale à *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (3) : 331-336

L'influence de l'administration de globules rouges de mouton comme antigène, avant ou après l'exposition trypanosomienne, sur le profil parasitémique et la réponse immunitaire de rats albinos Wistar est étudiée. Des taux élevés de parasitémie, en association avec une diminution significative des anticorps et de la valeur de l'hématocrite (PCV), ont été observés lorsque l'infection trypanosomienne a précédé la stimulation antigénique. A l'inverse, un net retard a été observé dans l'apparition et le développement de la parasitémie quand l'imprégnation antigénique précédait l'infection. Au début, les valeurs de l'hématocrite et la réponse immunitaire à l'antigène ont soutenu favorablement la comparaison avec les taux décelés sur les rats témoins ; mais, au cours de l'infection, la parasitémie a augmenté et une réponse de type hypoimmunitaire est apparue. Avec le temps, elle a rejoint les taux trouvés chez les rats qui avaient été touchés par l'infection trypanosomienne avant la stimulation antigénique. Les auteurs suggèrent que de tels résultats soient retenus lors de l'évaluation des tests sérologiques pour mettre en évidence les réponses à des vaccinations spécifiques ou pour le diagnostic d'infections basé sur l'élévation des titres d'anticorps chez l'hôte. *Mots clés* : Trypanosomose - *Trypanosoma brucei brucei* - Réponse immunitaire - Immunodépression - Antigène - Nigeria.

INTRODUCTION

In the last two decades, considerable attention has been focused on the phenomenon of immunodepression during trypanosomiasis. This phenomenon results in the impaired immune response of the trypanosome infected host to a variety of antigens including sheep red blood cells (3, 4), virus (11, 13, 15) and bacteriae (5, 7, 9, 12, 13). With the increasing use of vaccines to control various epizootics in domestic animals, this immunodepression is of relevance as subsequent inadequate immune responses following vaccination render animals susceptible to infections by the bacterial or viral agent against which they have been vaccinated. However, the immunodepression may not be total, but may only be at a level whereby vaccinated animals may still retain enough antibodies capable of conferring adequate protection against the disease for which a particular vaccine was administered (13, 14).

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In trypanosome endemic areas of tropical Africa, susceptible domestic animals in the field are usually subjected to intermittent trypanosome challenge throughout their life and unless prophylactically protected, may contract trypanosomiasis. Some of such animals may become immunized through vaccinations against the various endemic epizootic diseases. Therefore, while some animals may be vaccinated after infection with trypanosomes, others may be immunized before exposure to natural trypanosome challenge.

The present study was designed to assess the effect of the timing of antigen stimulation (immunization) on the immune response during trypanosomiasis in order to elucidate whether immunization before trypanosome challenge or *vice versa* has any influence in the parasitaemia profile of the host and the subsequent antibody response to the antigen.

MATERIALS AND METHODS

Experimental rats

Forty-five albino Wistar rats weighing between 100 to 120 g were used in the experiment. They were kept in cages, fed and watered *ad libitum*.

Trypanosomes

The organisms were *Trypanosoma brucei brucei*, previously isolated from the brain of a pig in Nsukka, Eastern Nigeria, and stabilized by three serial passages in Wistar albino rats. They were characterized by the production of high parasitaemia and chronic infection lasting for up to thirthy days before death of the rat.

Estimation of parasitaemia and PCV percentage

Parasitaemia was estimated from tail blood of the experimental rats by the rapid matching method of

HERBERT and LUMSDEN (6) and trypanosome population expressed as $\log_{10}$. Blood for the estimation of packed cell volume (PCV) was collected with a heparinized capillary tube through the retrobulbar plexus behind the eye. The cells were packed by centrifuging in a Hawksley haematocrit centrifuge at 30 000 rpm for five minutes and the PCV read with a Hawksley haematocrit reader.

Immunization of rats against SRC

Immunization was achieved by an initial intraperitoneal injection of 0.3 ml of a 10 % sheep red cells (SRC) suspension in normal saline, followed by a second injection fourteen days later.

Antibody assay against SRC

Antibody response to SRC was assayed by direct haemagglutination employing a 2 % SRC suspension in normal saline and carried out in WHO perspex microtitre plates. The test serum was initially diluted (1:80) to exclude non-specific isoagglutination of SRC by normal rat serum. Twenty-five microlitres of saline were pipetted into all the wells of each row except the first and penultimate wells. A 25 microlitre volume of the diluted test serum was pipetted into the first well, another, used for doubling the dilutions, was pipetted into the second well. The last well in each row contained 25 microlitres of saline control while the penultimate well contained 25 microlitres of 1 in 80 normal rat sera as serum controls. To each well was added 25 microlitres of a 2 % SRC suspension.

The contents of the wells were mixed for 1 min by gentle rocking of the plate and incubated at room temperature (30 °C) for 3 h after which the resulting agglutination titres were read and expressed as geometric titres.

Experimental design

The rats were divided into three groups of fifteen rats each (table I). Rats of group A were each infected with 1×10^3 *Trypanosoma brucei brucei* on day 0, given a primary immunizing dose of 0.3 ml of a 10 % SRC suspension as antigen on day 7 and a second dose of 0.3 ml of a 10 % SRC suspension on day 21. Rats of group B were given a primary immunizing dose of 0.3 ml of a 10 % SRC suspension on day 0, infected with 1×10^3 *T. brucei brucei* on day 7 and received a second dose of 0.3 ml SRC suspension on day 14. Rats of group C served as uninfected controls, but received a primary immunizing dose of antigen on day 0 and a second on day 14.

TABLE I Experimental design.

Group	Day 0	Day 7	Day 14	Day 21
A	*Trypanosoma*	Antigen	—	Antigen
B	Antigen	*Trypanosoma*	Antigen	—
C	Antigen	—	Antigen	—

After infection with *T. b. brucei*, tail blood from rats of groups A and B was examined daily to establish the onset of parasitaemia. Parasitaemia profile in both groups was subsequently followed every third day. Rats of groups A, B and C were bled every third day for PCV estimation and for sera. The various parameters were all observed until day 36.

Statistical analysis

Conventional analysis of variance (ANOVA) and correlation methods were used, parasitaemia being converted to absolute trypanosome populations per ml of blood before the analysis.

RESULTS

All rats of groups A and B showed patent infections with *T. b. brucei* irrespective of the time of infection. Similarly, all rats immunized with SRC developed antibody to this antigen.

Parasitaemia profile

Parasitaemia profiles in rats of groups A and B are shown in figure 1. Parasitaemias were observed in rats of groups A and B first on the third and ninth day of infection, respectively. In rats of group A the first peak appeared on day 9 of infection with a mean trypanosome population of $10^{8.2}$ per ml of blood and in rats of group B on day 18 of infection with a mean trypanosome population of $10^{8.4}$. The second peak appeared in group A and B on the days 21 and 36 respectively, the mean trypanosome populations per ml of blood being $10^{8.73}$ and 10^9.

A third peak was attained in rats of group A on the day 36 of infection, with a mean trypanosome population of 10^9 per ml of blood. Mean parasitaemias of rats of group A were significantly higher than in rats of group B on days 3 ($F = 19.5053$, $P < 0.001$), 6 ($F = 6.2095$, $P < 0.05$), 9 ($F = 5.5433$, $P < 0.05$) and 12 ($F = 4.7520$, $P < 0.05$), respectively, of infection, after which the respective values became comparable.

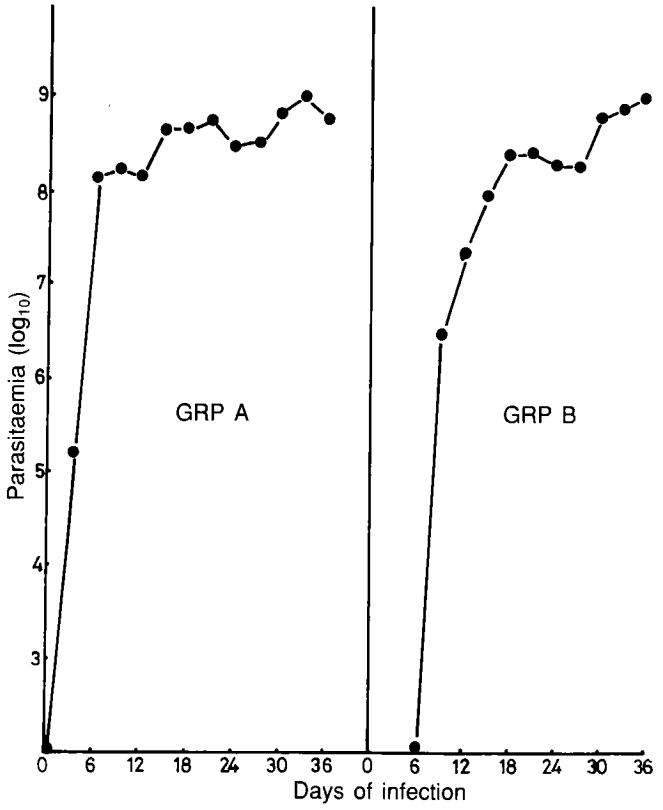

Fig. 1 : Parasitaemia profile in rats infected with 1 × 10^3 T. brucei brucei.

Packed cell volume

The mean PCV values of rats of groups A, B and C from day 0 to day 36 of the experiment are shown in figure 2. The rising parasitaemia in rats of group A was associated with a corresponding decline in PCV values which showed a significant difference when compared with rats of group C from day 6 ($F = 19.0156$, $P < 0.001$) to the end of the experiment and with rats of group B from day 6 ($F = 10.4156$, $P < 0.001$) to day 15 inclusive, all being lower in group A. From day 18 onwards, PCV values of rats in group B dropped sharply, became comparable with values of group A rats and showed significant differences with values of uninfected controls (group C). Prior to this, there were no significant differences between the values of the two groups.

Significant correlation occurred between mean parasitaemia and mean PCV values of rats in both groups A ($r = -0.7403$, $P < 0.05$) and B ($r = -0.8778$, $P < 0.01$).

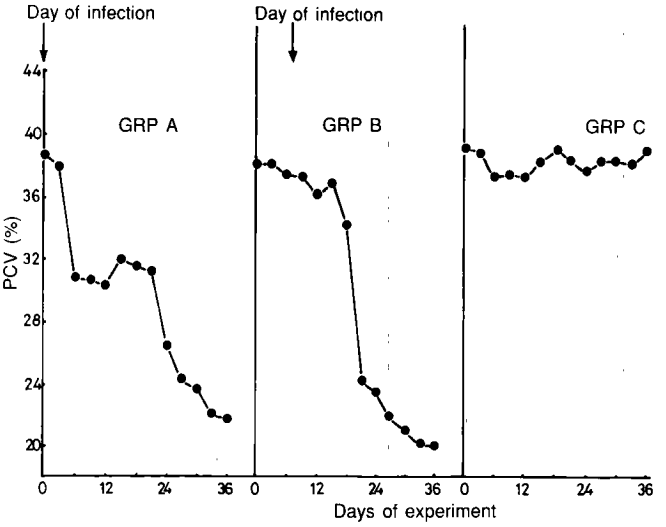

Fig. 2 : Mean PCV values of rats infected with 1 × 10^3 T. brucei brucei.

Antibody titres

They were detected in rats of groups A, B and C on day 3 following antigen priming irrespective of whether priming occurred before (group B) or after (group A) inoculation with *T. brucei brucei*. Antibody responses to SRC in groups A, B and C are shown in figure 3.

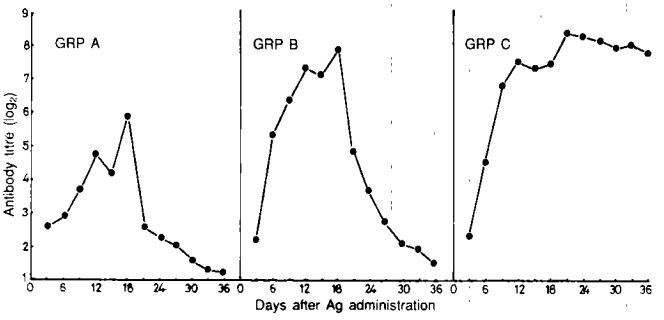

Fig. 3 : Mean antibody titre of rats infected with 1 × 10^3 T. brucei brucei.

Antibody levels were generally of higher magnitude in rats of group C than in rats of either group A or B and higher in rats of group B than in rats of group A. The first peak of antibody response was noticed on day 12 following antigen priming in the 3 groups. A significant difference ($F = 10.7054$, $P < 0.001$) occurred between mean of this first peak titres in the 3 groups, the titres being 4.7143, 7.40 and 7.5333, respectively for rats of groups A, B and C. The differences were significant

between titres of groups A and B, A and C, but not between B and C.

The second peak of antibody response occurred on day 18 following the initial antigen administration in rats of groups A and B and on day 21 in rats of group C. Significant differences ($F = 13.2465$, $P < 0.001$) occurred between mean titres of this second peak in the 3 groups, the titres being 5.8462, 7.9286 and 8.4, respectively for rats of groups A, B and C. The differences were significant between mean titres of groups A and B, A and C, but not between B and C. Significant differences in the mean titres of groups B and C appeared from day 21 following antigen priming and continued till the end of the experiment. With the exception of days 3 and 6 and from days 13 to 36 inclusive following antigen priming, significant differences occurred between the mean titres of rats of groups A and B in favour of rats of group B.

The performance of groups A and B rats in comparison with control group C rats are summarised in table II.

TABLE II Comparative performance of rate of groups A, B and C at specific time periods.

Time period	First antibody peak			Second antibody peak			Day 21			Day 24			Day 30			Day 36		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
Group A	63	8.7	30.9	70	8.4	26.6	30	8.5	25.2	27.4	8.8	23.8	13	8.3	21.2	19	8.8	21.4
Group B	98	0	36.0	94	7.4	33.1	57	7.9	24.2	45	8.4	23.8	28	8.3	21.2	28	9.0	21.0
Group C	100	—	37.2	100	—	38.4	100	—	38.4	100	—	37.6	100	—	38.3	100	—	38.0

X : Mean antibody titre as a percentage of control group C titre. Y : Parasitaemia (Log_{10}) at the corresponding period. Z : PCV levels at the corresponding period.

DISCUSSION

This study indicates a clear state of immunological hyporesponsiveness in rats infected with *T. brucei brucei* and gives some insight into the influence of the time of antigen priming on parasitaemia profile, PCV values and subsequent immunodepression.

In the rats of group A where infections with *T. brucei brucei* preceded antigen priming, an early onset of parasitaemia occurred, which gave rise to discernable peaks of increasing magnitude. It was associated with a significant generalized depression of antibody response to SRC in comparison with responses of control group C. At the peak of primary antibody response, this group produced only 63 % of control group C titres and 70 % during the secondary response period. Subsequently, rats of group A attained much lower responses in comparison with rats of group C (table II).

In rats of group B where antigen priming preceded infection with *T. brucei brucei*, there was a clear delay in the onset of parasitaemia which did not reach levels found in group A until day 24 following infection. This was associated with a minimal depression in antibody response with mean titres comparable to those of control group C until after the secondary antibody response.

In both groups A and B, parasitaemias were significantly but negatively correlated with PCV values, which can therefore be used as a reliable measure of pathogenicity of the infection in the two groups. The experimental design has the advantage in that crucial events taking place at the time of significant immunological hyporesponsiveness in both groups A and B rats can be clearly established (table II). Some of the important deductions would appear to be that immunodepression is dependent on the presence of noticeable parasitaemia and that its severity increases with rising parasitaemia and subsequent increase in pathogenicity as shown by lowered PCV values. While rats of group A, which exhibited high parasitaemias and corresponding low PCV values, showed significant immunological hyporesponsiveness, those of group B, which showed an extended prepatent period, an initial low parasitaemia and near normal PCV values, reacted to the antigen similar to control group C rats. It was only when the rats showed rising parasitaemias with decrease in PCV values that significant immunological hyporesponsiveness similar to that found in group A was exhibited. This is in accordance with findings of GRIFFIN *et al.* (5), BALTZ *et al.* (1) and IKEME *et al.* (8) using various trypanosome species.

The timing of antigen stimulation is essential for the phenomenon of immunodepression as it appears to have a profound effect on both the onset and level of

parasitaemia resulting from a trypanosome challenge. Thus, rats of group B, which received the antigen 7 days before trypanosome challenge exhibited an ability to partially depress the development of the trypanosome infection as evidenced by the extended prepatent period of infection and initial low parasitaemias so that the rats could develop an immune response initially comparable to the performance of control group C.

It is difficult to explain the reasons for this phenomenon. However, it could be speculated that antigen priming before trypanosome challenge activates the mononuclear phagocytic system of the host, expanding and increasing its activity in such a way that it results in a non-specific induction of « increased resistance » to any antigen. When the new antigen is *T. brucei brucei*, the « non-specific resistance » would result in a delay in the onset of parasitaemia as well as in a depression of the level of parasitaemia resulting from the infection. This sounds reasonable as the initial low parasitaemias in rats of group B led to a maintenance of PCV levels comparable to control group C. Later in the course of the infection, the rats lost this « non-specific resistance » and began to exhibit rising parasitaemias, lowered PCV values and immunological hyporesponsiveness similar to rats of group A. BROOKS *et al.* (2), working with *Trypanosome musculi* reported normal antibody responses to SRC by mice primed with SRC prior to infection with the trypanosome. The present study indicates that immunodepression occurs in Albino rats primed with SRC as antigen whether before or after infection with *T. brucei brucei*. Initial priming before trypanosome infection, however, significantly delays the build-up of parasitaemia and the development of immunological hyporesponsiveness to the antigen. It is doubtful whether this has any real significance in the field. However, in trypanosome endemic areas, where the diagnosis of infections or response to specific vaccination may be based on the detection of rising antibody titres in a host, the importance of these findings should be borne in mind when interpreting the results of such serological tests. Furthermore, any delay in the development of immunological hyporesponsiveness in the host must be of some benefit to the animal.

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IKEME (M.M.), ADELAJA (A.O.). Effect of the timing of antigen stimulation on parasitaemia profile and subsequent immunodepression in an experimentally induced *Trypanosoma brucei* infection. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (3) : 331-336

The influence of administering sheep red cells (SRC) as antigens, either after or before a trypanosome challenge, on parasitaemia profile and antibody response was assessed in albino Wistar rats. High levels of parasitaemia associated with significantly depressed antibody response and packed cell volume (PCV) values were observed when trypanosome challenge preceded antigen stimulation. In contrast, a clear delay in the onset and development of parasitaemia occurred when antigen priming preceded trypanosome challenge. At the beginning, PCV values and antibody response to the antigen were in the range of levels found in control rats. However, as infection progressed, parasitaemia rose and significant immunological hyporesponsiveness developed which at least reached levels found in rats that had received trypanosome challenge prior to antigen stimulation. These findings should be taken into consideration when evaluating serological tests used for assessing responses to specific vaccinations, or for the diagnosis of infections based on rising antibody titres in the host. *Key words* : Trypanosomiasis - *Trypanosoma brucei brucei* - Antibody response - Immunodepression - Antigen - Nigeria.

IKEME (M.M.), ADELAJA (A.O.). Efecto del momento de la estimulación antigenica sobre el desarrollo de la parasitemia e inmunodepresión consecutiva durante una infección experimental por *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (3) : 331-336

Se estudia la influencia de la administración de globulos rojos de oveja como antigeno, antes y después de infección por tripanosomas, sobre la parasitemia y la reacción inmunitaria de ratas albinos Wistar. Se observaron tasas elevadas de parasitemia, asociadas con una disminución significativa de los anticuerpos y del valor del hematocrita cuando la infección por tripanosomas precedió la estimulación antigenica. Al contrario, se observó un retraso claro en la aparición y el desarrollo de la parasitemia cuando la impregnación antigenica precedía la infección. Al principio, los valores del hematocrita y la reacción inmunitaria al antigeno fueron al nivel de los encontrados en ratas testigos ; pero, durante la infección, la parasitemia aumentó y una respuesta de tipo hipoinmunitario apareció, la que llegó al nivel encontrado en ratas infectadas por tripanosomas antes de la estimulación antigenica. Se debería tener en cuento estos resultados cuando se evaluan las pruebas serologicas para evidenciar las respuestas a vacunaciones específicas o para el diagnóstico de infecciones a partir de la aumentación de las dosificaciones de anticuerpos en el huesped. *Palabras claves* : Tripanosomosis - *Trypanosoma brucei brucei* - Respuesta inmunitaria - Inmunodepresión - Antigeno - Nigeria.

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