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Host-parasite interactions which influence the virulence of *Trypanosoma (Trypanozoon) brucei brucei* organisms

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Summary

Subclones were prepared in mice from *T. b. brucei* ILTat 1.4 parasites. Subclones which did not differentiate to stumpy forms in mice were highly virulent and did not stimulate detectable antibody responses. A subclone which did give rise to stumpy forms in mice, was less virulent and did stimulate an antibody response specific for the trypanosome surface glycoprotein. Clones and subclones of *T. b. brucei* parasites which did not give rise to stumpy forms in mice, did give rise to stumpy forms in Bovidae. Plasma from cattle infected with those parasites did not stimulate differentiation of *T. b. brucei* parasites in mice. Murine pleomorphic and monomorphic *T. b. brucei* parasites retained their respective phenotypes in co-infected mice. Both types of parasites were equally pleomorphic in Bovidae. We conclude that some clones of *T. b. brucei* remain monomorphic in mice as a result of a high avidity interaction between slender forms and host molecules which inhibit differentiation of *T. b. brucei* parasites.

Key words: *T. b. brucei;* slender; stumpy; mice; cattle; differentiation; regulation.

Introduction

The rate of differentiation of blood stream *T. b. brucei* parasites from rapidly dividing slender forms through intermediate and stumpy forms to senescent parasites (Black et al., 1982 a) influences the parasite population growth rate and the kinetics of the host antibody responses (Sendashonga and Black, 1982): Antibody responses are induced by senescent parasites derived from the stumpy form population but not by dividing parasites. Recent studies suggest that

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the host plays a role in regulating the rate of parasite differentiation: The rate of differentiation of the same *T. b. brucei* parasites differs between inbred strains of mice because a host mechanism, which influences the rate of parasite differentiation, is activated to different degrees in these mice during the first parasitaemic wave (Black et al., 1982 b). In this presentation we further investigate the role of the host in regulating parasite differentiation and speculate on the mechanisms involved.

Materials and Methods

Mice. – Male and female BALB/c mice, bred and maintained at ILRAD and aged between 3 and 6 months were used in the study.

Cattle. – Female Hereford cattle, aged 6 months, were bred and maintained at ILRAD under trypanosome-free conditions and were serologically negative for anti-trypanosome antibody prior to use in experiments.

Trypanosomes. – *T. b. brucei* ILTat 1.4 and 1.2 were derived from Lump 227 stock (UHEMBO/64/EATRO/795). *T. b. brucei* GUTat 3.1 was derived from Treu 667 stock and cloned in BALB/c mice. The properties of these clones of *T. b. brucei* in mice have been described (Sendashonga and Black, 1982). Methods for the purification of trypanosomes from blood have been described (Lanham and Godfrey, 1970). Slender and stumpy differentiation forms were identified in air-dried, methanol-fixed, Giemsa-stained, thin films of infected blood by the method of Wijers (1959). At least 100 parasites were examined per slide. Parasitaemia in mouse blood was assessed by counting parasites in a haemocytometer. Parasitaemia in bovine blood was assessed by the dark ground method of Murray et al. (1977).

Solid phase radioimmunoassay (SRIA). – Serum antibodies to variable surface glycoprotein (VSG) were measured in a SRIA which has been described (Black et al., 1982 a). Briefly, parasites which had been centrifuged onto wells in a flexible polystyrene microtiter plate (10^5 organisms/well, 500 *g* for 10 min) and fixed with 0.25% glutaraldehyde for 5 min were used as target antigen. The assay was conducted as for SRIA against other antigens using affinity labelled ^{125}I anti-mouse IgM or IgG as a second step reagent (Tsu and Herzenberg, 1980). The $\log_{10}$ reciprocal titer of antibodies was calculated by comparing the 50% binding points of pre-infection and post-infection sera. Results are presented as the arithmetic mean $\pm$ 1 standard deviation (SD).

Indirect immunofluorescence. – Purified ILTat 1.4 trypanosomes were spread as a thin film on glass slides, air-dried for 10 min, fixed in cold (4° C) acetone for 10 min and incubated with a staining buffer containing 0.01 M $NaPO_4$ (pH 8.1), 0.15 M NaCl and 1% bovine serum albumin for 30 min. The parasites were reacted for 30 min with monoclonal antibodies V3/16.15.1 and V3/66.1.1 which recognize internal and external epitopes of ILTat 1.4 VSG respectively (Pearson et al., 1980; Black et al., 1982 a). The slides were washed with 20 ml of staining buffer and exposed to staining buffer containing fluorescein conjugated anti-mouse Ig (Nordic Immunological Laboratories B.V., Tilberg, The Netherlands) for 30 min. The slides were washed and stained trypanosomes were examined on a UV microscope (Leitz, Wetzlar, W. Germany) using a 63/1.3 oel Phaco 3 Fluoresz objective and GW 6.3 $\times$ eyepieces.

Analyses of ILTat 1.4 trypanosomes in thin smears prepared from the buffy coat of washed mouse blood (3 $\times$ in RPMI 1640 medium) were conducted in the same manner. Trypanosomes present in the preparations which did not express the ILTat 1.4 VSG were identified by examining the slides under UV and phase contrast illumination. Results are presented as the arithmetic mean $\pm$ 1 SD and at least 200 parasites were examined in each preparation.

Irradiation. – BALB/c mice were given 800 rads of γ irradiation delivered by a ^{137}Cesium source. This dose depletes the B- and T-cell dependent areas of the spleen in 6 days and kills the recipients in 10 to 14 days.

Results

Subclones of T. b. brucei ILTat 1.4 Parasites are heterogeneous with respect to differentiation rates and their capacities to induce detectable antibody responses in BALB/c mice

BALB/c mice, inoculated with 50 or more *T. b. brucei* ILTat 1.4 parasites die during the first parasitaemic wave with a parasitaemia of $>10^9$ parasites/ml. At the time of death of the host, the parasite population in the blood is composed of approximately 95% slender form, 4.8% intermediate form and 0.2% stumpy form parasites. Seventeen subclones of *T. b. brucei* ILTat 1.4 parasites were prepared in lethally irradiated BALB/c mice. All of the subclones expressed the ILTat 1.4 VSG to a level greater than 99.9% of the parasites examined, using the monoclonal antibodies V3/16.15.1 and V3/66.1.1.

Sixteen of the subclones, represented by No. 1 in Table 1, did not give rise to stumpy form parasites in BALB/c mice and did not stimulate a VSG-specific antibody response. In contrast, subclone 17 (Table 1) did give rise to stumpy form parasites in BALB/c mice and stimulated a VSG-specific antibody response. The BALB/c mice were inoculated intravenously with 50 parasites. Recipients of subclone 1 died after 8 days whereas recipients of subclone 17 died after 15 days.

T. b. brucei ILTat 1.4 subclone 1, ILTat 1.2 and GUTat 3.1 parasites give rise to stumpy forms in Bovidae

Two calves were inoculated intravenously with either 10^5 *T. b. brucei* ILTat 1.4 subclone 1 or 10^5 *T. b. brucei* GUTat 3.1 parasites (a mouse pleomorphic parasite which when recloned only gives rise to pleomorphic parasites in mice). The blood parasitaemia and morphological types were assessed daily and results are presented in Table 2. Both sets of parasites differentiated at a similar rate in cattle although the pre-patent period of the ILTat 1.4 subclone 1 parasites was shorter than that of the GUTat 3.1 parasites. Differences in the pre-patent periods might have resulted from the proportionally greater number of slender form parasites present in the *T. b. brucei* ILTat 1.4 subclone 1 inoculum. Similar results were obtained using another mouse monomorphic clone of *T. b. brucei*, ILTat 1.2 (Sendashonga and Black, 1982). *T. b. brucei* GUTat 3.1, ILTat 1.4 subclone 1 and ILTat 1.2 parasites also gave rise to stumpy forms in sheep, domestic cattle, waterbuck, eland and african buffalo (Grootenhuis, Whitelaw and Black, unpublished).

The same T. b. brucei ILTat 1.4 and 1.2 parasites which are pleomorphic in Bovidae, are monomorphic in mice

T. b. brucei ILTat 1.4 subclone 1 parasites which were harvested at 5 days from the Hereford calf described in Table 2, were cloned into lethally irradiated BALB/c mice. All of the 10 subclones which were obtained expressed the ILTat

Table 1. Immune responses to and differentiation of subclones of *T. b. brucei* ILTat 1.4

T. b. brucei ILTat 1.4 Subclone No.	Day of analysis	Log_{10} parasites/ml blood ± 1SD	% ± 1SD of parasite morphological types		Log_{10} reciprocal titer ± 1SD antibodies on *T. b. brucei* ILTat 1.4	
			Intermediate	Stumpy	IgM	IgG
1	4	5.0 ± 0.3	0	0	0	0
	6	7.5 ± 0.2	0	0	0	0
	7	8.8 ± 0.3	0.5 ± 0.2	0	0	0
17	4	4.0 ± 0.2	0	0	0	0
	6	6.2 ± 0.3	3.0 ± 2.0	0.5 ± 0.3	0.5 ± 0.2	0
	8	8.2 ± 0.3	19.0 ± 3.0	3.0 ± 2.0	1.0 ± 0.5	0.5 ± 0.2
	9	8.9 ± 0.2	32.0 ± 4.0	5.0 ± 3.0	1.5 ± 0.2	0.7 ± 0.2
	10	8.1 ± 0.4	nt	nt	2.8 ± 0.4	1.5 ± 0.2

50 parasites were inoculated i.v.
No antibody binding was detected on *T. b. brucei* ILTat 1.2.
nt = not tested

Table 2. *T. b. brucei* ILTat 1.4 subclone 1 and GUTat 3.1 parasites differentiate at similar rates in Hereford calves

T. b. brucei	Day after infection	Log_{10} parasites/ ml blood	% of parasite morphological types:	
			Intermediate	Stumpy
ILTat 1.4	4	5.0	7	0
Subclone 1	5	6.1	19	5
	6	6.4	38	50
	7	6.0	11	85
	8	3.0	nt	nt
GUTat 3.1	5	5.1	10	0
	6	6.2	24	6
	7	5.6	36	55
	8	5.9	13	82
	9	3.0	nt	nt

6-month-old female Hereford calves were inoculated intravenously with 10^5 parasites.
nt = not tested

1.4 VSG as detected by indirect immunofluorescence with monoclonal antibodies V3/16.15.1 and V3/66.1.1 and did not differentiate from slender to stumpy form parasites in intact or irradiated BALB/c mice. *T. b. brucei* parasites inoculated into mice from the third parasitaemic wave of the calf also did not give rise to stumpy form trypanosomes. The studies were repeated using ILTat 1.2 parasites and similar results were obtained.

Plasma from T. b. brucei infected cattle does not contain molecules which stimulate differentiation of T. b. brucei ILTat series parasites in mice

Plasma prepared from normal cattle, from cattle in which ILTat 1.4 parasites had switched to stumpy forms or from cattle which had just cleared the first parasitaemic wave of *T. b. brucei* ILTat 1.4 parasites, did not induce *T. b. brucei* ILTat 1.2 parasites to switch to intermediate or stumpy forms in intact or irradiated BALB/c mice. The mice were inoculated intraperitoneally with 10^2 *T. b. brucei* ILTat 1.2 parasites and after the first day were inoculated intravenously with 0.25 ml of the bovine plasma every day until death which occurred between 6 and 7 days after infection. The reciprocal experiment was performed using mice infected with *T. b. brucei* ILTat 1.4 and plasma from cattle infected with *T. b. brucei* ILTat 1.2 or GUTat 3.1. No stumpy form parasites were detected nor was there any increase in the percentage of intermediate form parasites detected.

Table 3. Influence of co-infection on differentiation of *T. b. brucei* ILTat 1.4 and GUTat 3.1 in mic

T. b. brucei inoculation	Day after infection	Log_{10} parasites/ ml blood ± 1SD	% GUTat 3.1 ± 1SD	% stumpy forms
10^2 ILTat 1.4*	4	6.3 ± 0.2	0	0
	5	8.0 ± 0.2	0	0
	6	9.3 ± 0.2	0	0
5×10^3 GUTat 3.1	4	6.0 ± 0.1	100	0
	5	7.6 ± 0.1	100	8 ± 2
	6	7.4 ± 0.1	100	58 ± 17
	7	< 5.0		
10^2 ILTat 1.4*	4	6.5 ± 0.1	41 ± 10	0
and	5	8.2 ± 0.2	40 ± 16	0
5×10^3 GUTat 3.1	6	9.2 ± 0.2	0.9 ± 0.7	0.6 ± 0.4

* ILTat 1.4 parasites were identified by indirect immunofluorescence using monoclonal antibodie reactive with ILTat 1.4 VSG. No fluorescent stumpy form parasites were observed. GUTat 3. parasites were identified under phase contrast and UV illumination as those trypanosomes whic did not express ILTat 1.4 VSG. All recipients of ILTat 1.4 died between 6 and 7 days after inocula tion.

Pleomorphic and monomorphic T. b. brucei parasites retain their respectiv phenotypes in co-infected BALB/c mice

BALB/c mice were inoculated intraperitoneally with 10^2 *T. b. brucei* ILTa 1.4, 10^3 *T. b. brucei* GUTat 3.1 or 10^2 *T. b. brucei* ILTat 1.4 and 10^3 *T. b. bruce* GUTat 3.1. Each group contained 10 mice. After 4, 5 and 6 days, the parasitae mia in each mouse was counted, thin blood films were prepared for analysis o parasite morphology and blood was collected from the retro-orbital plexus o each mouse into heparinized pipettes. The blood was washed and thin smears o buffy coat were prepared. The smears were stained for indirect immunofluores cence with antibodies reactive with ILTat 1.4 VSG and were observed under UV and phase contrast illumination. Parasites with ILTat 1.4 VSG were taken to be ILTat 1.4 parasites whereas those without ILTat 1.4 VSG were taken to be GUTat 3.1 parasites. Results are presented in Table 3 which shows that ILTa 1.4 parasites in co-infected mice continued to multiply and remained as slende forms whereas the GUTat 3.1 parasites switched to stumpy forms and were overgrown. Differentiation of *T. b. brucei* GUTat 3.1 parasites to stumpy form was delayed by 1 day in co-infected hosts. In the co-infected hosts, the propor tional representation of GUTat 3.1 parasites by 6 days after infection was ap proximately 1% of the total parasites detected (i.e. about 2×10^7/ml) and most if not all, of these parasites had switched to stumpy forms. The experiment wa performed several times in both intact and irradiated (650 rads) BALB/c mice with similar results.

Discussion

The studies show that a cloned population of *T. b. brucei* parasites can contain members with the same VSG which are heterogeneous in virulence, rates of differentiation to intermediate and stumpy forms and capacities to induce detectable antibody responses in mice. The studies confirm previous observations which show that the degree of pleomorphism of *T. b. brucei* parasites is not linked to VSG type (Barry et al., 1979) and that antibody responses might be induced by senescent parasites derived from the stumpy form population but not by actively dividing parasites (Sendashonga and Black, 1982).

T. b. brucei parasites which were monomorphic in mice, were pleomorphic in cattle and when cloned back into mice from the first or third parasitaemic wave in a calf, were monomorphic. The results confirm previous observations (Black et al., 1982 b) which suggest that differentiation of *T. b. brucei* parasites is under host control. Plasma collected from cattle in which the murine monomorphic *T. b. brucei* parasites had differentiated to stumpy forms, did not induce differentiation of those parasites when injected into mice. The observations suggest that the bovine plasma did not contain molecules which stimulate differentiation *T. b. brucei* parasites. We tentatively suggest that differentiation of *T. b. brucei* parasites is negatively regulated by the host, i. e. there are variable concentrations of molecules which encourage growth of the parasites and prevent differentiation.

Murine monomorphic and pleomorphic *T. b. brucei* parasites were shown to retain their respective phenotypes in co-infected mice although differentiation of the pleomorphic parasites was slightly retarded. This suggests that murine molecules which encourage growth of *T. b. brucei* parasites are more efficiently perceived or utilized by monomorphic than pleomorphic parasites. Because murine monomorphic *T. b. brucei* were pleomorphic in cattle and did not grow more quickly than murine pleomorphic parasites when inoculated into cattle, it is likely that murine and bovine molecules which encourage *T. b. brucei* growth differ in some respect.

The rate of differentiation of *T. b. brucei* parasites directly influences parasitaemia (Black et al., 1982 b) and the kinetics of development of the host protective antibody response (Sendashonga and Black, 1982). Elucidation of the host molecules which inhibit *T. b. brucei* differentiation and the trypanosome receptor site for those molecules, might suggest new methods for control of infections with trypanosomes and new ways of examining the trait of trypanotolerance.

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