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Synthesis and secretion of immunoglobulin by spleen cells from resistant and susceptible mice infected with *Trypanosoma brucei brucei* GUTat 3.1

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Summary By 6 days after infection of susceptible C3H/He and resistant C57BL/6 mice with *Trypanosoma brucei brucei* GUTat 3.1, splenic plasma cell responses of both strains of mice were similar in terms of plasma cell number, intracellular Ig, Ig secretion, Ig class and Ig specificity for surface-accessible variant surface glycoprotein (VSG) epitopes on the infecting organisms, despite higher parasitaemia in the C3H/He mice. By 7 days after infection, however, although splenic plasma cells from both strains of mice had greatly amplified their Ig responses, those from C57BL/6 mice (which cleared parasites from their bloodstream between 6 and 7 days after infection) contained and secreted 3–5 times more Ig specific for exposed VSG epitopes on the infecting organisms than those from C3H/He mice which did not clear parasites from their bloodstream. *In vitro*, trypanosomes can absorb significant amounts of VSG-specific antibody produced by splenic plasma cells. However, differences in the detection of VSG-specific antibodies present in, and secreted by, splenic plasma cells of 7-day infected C3H/He and C57BL/6 mice were shown not to result from the presence of parasites in the cultures of C3H/He spleen cells. It is argued that between 6 and 7 days after infection, the C3H/He mice selectively lose the capacity to support development of plasma cells specific for exposed VSG epitopes on the infecting organisms and that this is a consequence rather than a cause of differences in the peak levels of first wave parasitaemia.

Keywords: *Trypanosoma brucei*, immunoglobulin secretion, splenic plasma cells, C57BL/6 mice, C3H/He mice

Introduction

Mice of different inbred strains differ in their abilities to control infections with salivarian trypanosomes (Black *et al.* 1983, 1986, Clayton 1978, De Gee, Shah & Doyle 1982,

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Jennings *et al.* 1978, Mahan, Hendershot & Black 1986, Morrison & Murray 1985, Morrison *et al.* 1978). Generally the most susceptible mice fail to cause remission of the first wave of parasitaemia after needle challenge with parasites homogeneous for variable antigenic type. Genetic analyses showed that the ability or inability of a strain of mice to cause remission of the first wave of parasitaemia following infection with African trypanosomes is not linked to the major histocompatibility complex (Levine & Mansfield 1984), and may be under the control of several genes (De Gee, Levine & Mansfield 1988). Further studies using chimaeras constructed between H-2-compatible resistant and susceptible strains of mice indicated that the ability or inability to cause remission of the first wave of parasitaemia is a property of cells whose progenitors reside in the spleen or bone marrow (De Gee & Mansfield 1984, Greenblatt, Potter & Rosenstreich 1985).

Comparative serological analyses of trypanosome-infected resistant and susceptible strains of mice have shown that clearance of the first parasitaemic wave in the former correlated with serologically detectable antibodies specific for exposed epitopes on parasite-attached variant surface glycoprotein (VSG). The parasite VSG-specific antibody responses in resistant mice were accompanied by a wide variety of bystander antibody responses (Black *et al.* 1983, Hudson & Terry 1979, Sendashonga & Black 1982) concomitant with a rapid rise in the level of serum Igs, particularly of the IgM class (Black *et al.* 1983, Dempsey & Mansfield 1983a, Hudson *et al.* 1976, Mitchell & Pearson 1983). In contrast, susceptible mice produced little or no serologically detectable parasite-VSG specific antibody response during infection, few if any bystander antibody responses and showed little or no change in overall serum Ig levels (Black *et al.* 1983, Hudson & Terry 1979, Morrison & Murray 1985, Sendashonga & Black 1982). The capacity of inbred mice to cause remission of the first wave of parasitaemia after infection with African trypanosomes, therefore, appears to correlate with the kinetics of development of serologically detected parasite VSG-specific and bystander antibody responses.

Although inbred mice differ in their ability to mount serologically detectable antibody responses after infection with African trypanosomes, they mounted similar antibody responses when challenged with lethally irradiated trypanosomes (Black *et al.* 1983). Furthermore, following trypanocidal drug treatment of susceptible mice infected with African trypanosomes, there was a rapid parasite-VSG specific antibody response (Black *et al.* 1986, Mahan *et al.* 1986), which arose from spleen cells in the infected animals. The susceptible mice are therefore competent to respond to African trypanosomes and during infection appear to initiate immune responses which fail to reach serologically detectable levels in the absence of chemotherapeutic intervention.

Investigations of B-cell responses at the cellular level in susceptible and resistant mice infected with African trypanosomes showed that the mice mounted similar B-cell DNA synthetic responses with similar kinetics (Mahan *et al.* 1986). Both strains of mice developed similarly elevated levels of splenic plasma cells with similar kinetics, and the splenic plasma cells contained similar amounts of intracellular antibody specific for the bystander antigen TNP or the VSG of the infecting trypanosome (Black *et al.* 1986). However, serum antibodies against those antigens were detected in the resistant mice only. This led to the idea that trypanosome-infected susceptible mice developed plasma cells with an impaired ability to secrete Ig (Black *et al.* 1985, 1986, Mahan *et al.* 1986). Investigations of plasma cell morphology by light microscopy, immunofluorescence and immunoelectron micrography, however, revealed no structural differences in the parasite-

induced plasma cells of trypanosome-infected resistant and susceptible mice (Black *et al.* 1986).

In this paper we continue the above investigations by examining *in vitro* the Ig synthetic and secretory capacities of populations of spleen cells, collected from *Trypanosoma brucei brucei* GUTat 3.1-infected susceptible C3H/He and resistant C57BL/6 mice on the day at which major divergence of levels of parasitaemia occurs.

Materials and methods

EXPERIMENTAL ANIMALS AND PARASITES

Female C3H/He and C57BL/6 mice, 3–6 months old, bred and maintained at ILRAD, were used in all studies. Blood, collected from anaesthetized mice, was left at room temperature for 1 h, then at 4°C for 16 h before centrifugation and collection of serum. For irradiation, mice were given 500 rad from a Caesium-137 source.

Trypanosoma brucei brucei GUTat 3.1 (Black, Hewett & Sendashonga 1982) and *T. b. brucei* IL 1785 (Black *et al.* 1985) have been described, as have methods for purification of the parasites from the blood (Lanham & Godfrey 1970). Trypanosomes from cryopreserved stabilate were injected intraperitoneally at 5×10^3 parasites per mouse.

PREPARATION OF CELLS

Mice were killed by ether anaesthesia, spleens and lymph nodes excised and placed into complete medium: RPMI-1640 (GIBCO, UK) containing 10% heat-inactivated fetal bovine serum (FBS) (Flow, UK), 10 μg/ml DNase, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 5×10^{-5} M 2-mercaptoethanol. They were gently disrupted using a glass pestle. Released cells were collected after allowing cell debris to settle. Peritoneal exudate cells were collected by injecting intraperitoneally 5 ml of complete medium containing 10 iu/ml heparin, which was then aspirated. Subsequent treatment of cells differed according to the assay.

For immunoblots

Spleen cells were washed twice in 10 ml complete medium at 200 ***g***, and were then diluted to 5×10^4 or $2{\cdot}5 \times 10^4$/ml.

For ELISA

Spleen cells, to be used for producing supernatants and cell lysates for determining total and parasite-specific Ig by ELISA, were washed three times in 20–50 ml of complete medium at 200 ***g***, underlaid each time with 1–2 ml FBS. The cells were resuspended at 10^8/ml. One-millilitre samples were distributed in 2-ml tubes (Nunc, Denmark), which were placed on a test-tube rotator (Cenco, The Netherlands) at 37°C for up to 3 h. Before rotation, one sample (time 0 sample) was harvested by spinning for 1 min in an Eppendorf centrifuge (Eppendorf, West Germany). The supernatant was collected, and 1 ml lysis buffer, consisting of 50 ml 0·15 M phosphate-buffered saline (PBS) (pH 7·2), 50 ml water,

0·5% sodium deoxycholate, and 0·5 ml Nonidet P-40 was added to the cell pellet. After further centrifugation to remove precipitated material, the cell lysate was collected. Samples after 1, 2 and 3 h of culture were harvested similarly.

To kill trypanosomes in spleen cell suspensions with antibody and complement, cells were counted after one wash and resuspended at 10^8 cells/ml in complete RPMI-1640 lacking FBS. One-millilitre aliquots were placed in wells of 24-well plates (Costar, Cambridge, MA, USA), and to these were added 50 μl of ascitic fluid containing an IgM monoclonal antibody (MoAb) raised against *T. b. brucei* GUTat 3.1, a kind gift from Dr D.Russo, ILRAD, and 100 μl of fresh guinea-pig serum. Untreated control wells received guinea-pig serum only. After 45 min incubation at 37°C in air with 10% CO_2, cells from treated and untreated wells were harvested, by repeated rinsing of the wells to remove adherent cells. The cells were washed three times in 20-ml volumes of complete medium, underlaid each time with 1–2 ml FCS, before harvesting the time 0 sample and setting up rotating cultures for 2 h as described above.

ASSAYS

Immunogold blot assay for the detection of cells secreting immunoglobulin

A technique has been described (Walker & Dawe 1987) for visualizing the secretion products of individual cells. In preliminary experiments this technique was modified and optimized (Newson 1990). Briefly, flat-bottomed 96-well Immulon plates (Dynatech, West Germany) were coated with 1 μg/well of goat anti-mouse IgM or IgG (Southern Biotechnology, Birmingham, AL, USA) in 0·05 M carbonate buffer, pH 9·6, and left overnight at 4°C. The coated wells were washed three times in 20 mM Tris-HCl buffer (pH 8·2) containing 0·15 M NaCl, 20 mM NaN_3 and 0·1% casein hydrolysate (Sigma, St Louis, MO, USA). Uncoated sites were blocked with the same buffer containing 5% casein hydrolysate for at least 30 min at 37°C. After washing, the wells were rinsed with complete medium. Washed spleen cells were added to duplicate wells (5000 cells at day 7, and 2500 at day 9 of infection) and were incubated for 40 min at 37°C in air with 10% CO_2. The cells were then washed away, and biotinylated sheep anti-mouse Ig (Amersham, UK) was added for 1 h at room temperature. After washing, colloidal gold streptavidin (5 nm particles, Amersham), diluted in PBS, was added and left overnight at 4°C. After three washes in distilled water, silver enhancement was carried out according to the supplier's instructions (Janssen, Belgium). Blots were counted using an inverted microscope at ×40 magnification with a ruled graticule in the eye-piece.

ELISA for measuring IgM and IgG in supernatants and cell lysates

A sandwich enzyme-linked immunosorbent assay (ELISA) (Voller & Bidwell 1986) was used for measuring the concentration of IgM or IgG in supernatants and cell lysates. Immulon plates (Dynatech) were coated with goat anti-mouse IgM or IgG (Southern Biotechnology) at 0·5 μg/well diluted in 0·05 M carbonate buffer, pH 9·6. Serial dilutions in duplicate were made for each sample, using PBS containing 0·05% Tween 20. A mouse immunoglobulin reference standard (Meloy, Springfield, VA, USA) was titrated on each plate. The conjugate, alkaline phosphatase-labelled goat anti-mouse IgM or IgG (Southern Biotechnology), was used at 0·01 μg/well, and the substrate was *p*-nitrophenyl

phosphate (Sigma). Optical densities (OD) were read at 405 nm using a Titertek Multiskan micro-ELISA reader (EFLAB, Finland). Samples from the two strains of mice which were to be compared were always put on the same plate, so that ODs were read from the same standard curve.

ELISA for measuring parasite VSG-specific IgM and IgG

Immulon plates (Dynatech) were coated with glutaraldehyde-fixed whole trypanosomes, *T. b. brucei* GUTat 3.1 or *T. b. brucei* IL 1785, as described for radioimmune assay (Black *et al.* 1982). Plates were stored at 4°C with PBS containing 1% heat-inactivated goat serum and 0·2% sodium azide in the wells until required. Serial dilutions in duplicate were made for each sample and, as above, samples to be compared were always included on the same plate. The alkaline phosphatase conjugate and the substrate were as described above.

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Cytoplasmic immunofluorescence

Thin films were prepared from spleen, lymph node and peritoneal exudate cells and were stained for cytoplasmic Ig as described (Mahan *et al.* 1986).

Surface immunofluorescence of trypanosomes

Blood (50 μl) from trypanosome-infected mice was immediately fixed in 1 ml of ice-cold 1% formalin in heparinized PBS. The fixed blood suspensions were kept on ice for at least 1 h, then were spun in an Eppendorf centrifuge, resuspended in PBS and underlaid with 100 μl FCS. After a further wash the cells were resuspended in 10 μl FCS and smears were made on glass microscope slides. The smears were air-dried, rehydrated and stained by adding fluorescein isothiocyanate-conjugated sheep anti-mouse Ig (made by C.Hinson and S.A.Khaushal, ILRAD) for 30 min. After washing with PBS and mounting in PBS-glycerol, coverslips were placed on the smears and sealed with nail varnish, and the slides were coded and scored for immunofluorescence by two investigators using a Leitz Orthoplan microscope, before decoding and tabulating results.

Results

PARASITAEMIC PROFILES AND ANALYSES OF PLASMA CELLS IN C3H/He AND C57BL/6 MICE INFECTED WITH *T. B. BRUCEI* GUTat 3.1

The parasitaemic profile given in Table 1 is representative of those in the experiments described. The infecting dose of parasites (5×10^3) resulted in peak parasitaemias being reached 1 day earlier than in previous studies where 10^3 organisms were used (Black *et al.* 1983, 1986). C57BL/6 mice caused remission of the first parasitaemic wave on day 7, whereas C3H/He mice did not. As before, the level of parasitaemia reached in C3H/He mice was 2·5–10 times higher than that in C57BL/6 mice.

Table 1. Parasitaemias of C3H/He and C57BL/6 mice infected with 5×10^3 *T. b. brucei* GUTat 3.1

Mouse strain	Day after infection	Log_{10} parasites/ml of blood (mean* ± s.d.)
C3H/He	4	6·9 ± 0·2
	5	8·0 ± 0·2
	6	8·3 ± 0·2
	7	8·2 ± 0·1
	8	8·1 ± 0·4
	9	8·1 ± 0·3
C57BL/6	4	7·0 ± 0·1
	5	7·7 ± 0·2
	6	7·5 ± 0·5
	7	Occ/none†
	8	None seen
	9	None seen

* Mean of 20 mice.

† Occasional parasites seen in 25% of mice, none seen in remainder.

Although infected C3H/He mice did not control the first parasitaemic wave, they developed similar numbers of plasma cells to C57BL/6 mice which did control the first parasitaemic wave. Plasma cells, as defined by high levels of cytoplasmic Ig, were predominantly detected in the spleen in both strains of mice. The numbers of plasma cells in lymph node and peritoneal exudate did not exceed 2% even in infected mice, whereas in the spleen they increased from 0–1% in uninfected mice to 12–18% in infected mice of both strains. Thus ensuing studies of parasite-induced plasma cell function were restricted to analysis of spleen cell populations.

ANALYSIS BY IMMUNOGOLD BLOTS OF SPLEEN CELL Ig SECRETION

Blots made by Ig-secreting spleen cells from C3H/He and C57BL/6 mice infected for 7 and 9 days were individually visualized and counted. The quality of the blots is shown in Figure 1. Since the size and shape of the blots were much more variable than those produced by hybridoma cells in preliminary experiments, tests were done to determine whether the immunoblots represented secretion or bursting of cells. Briefly, blot size was considerably diminished by including 0·2% sodium azide in the incubation medium and blots were essentially abrogated by carrying out the assay at 4°C in the presence of 0·2% sodium azide. It was therefore assumed that blots arose as a result of active secretion of Ig.

There were no statistically significant differences between the two strains of mice in the

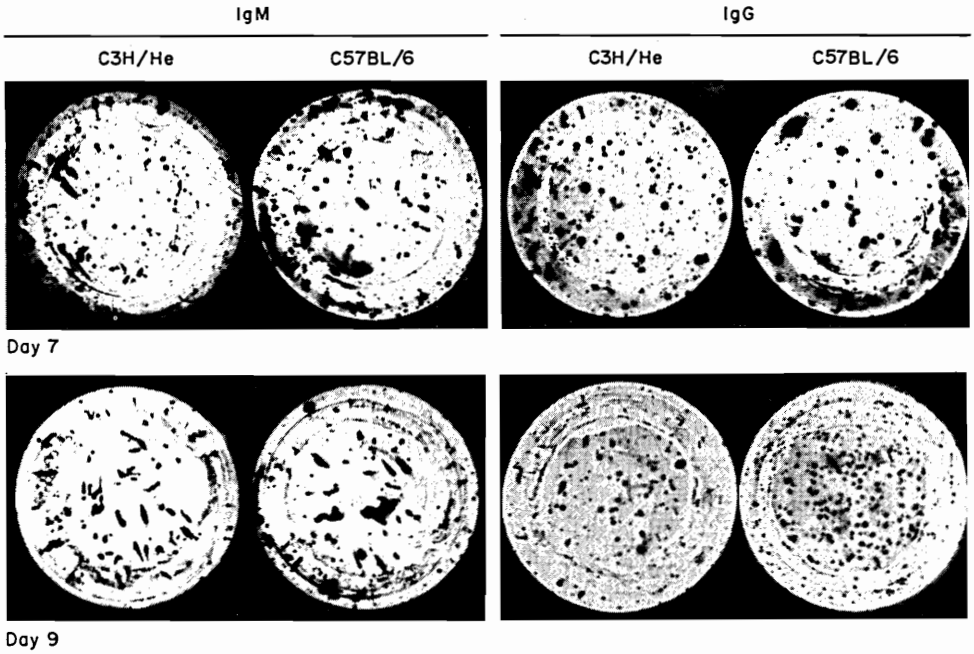

Figure 1. Microtitre wells containing immunoblots made by spleen cells from C3H/He and C57BL/6 mice after infection for 7 and 9 days with *T. b. brucei* GUTat 3.1.

percentages of spleen cells secreting IgM or IgG on day 7 after infection, i.e. the day of parasite clearance from the bloodstream of infected C57BL/6 mice, or at day 9 (Table 2). There were also no obvious differences in the amounts of Ig secreted per cell (i.e. differences in blot size, Figure 1) between the two strains of mice. By day 9 after infection, the proportion of IgM- and IgG-secreting cells in the C57BL/6 spleen cell population had

Table 2. Percentages of spleen cells from C3H/He and C57BL/6 mice secreting IgM and IgG after infection with *T. b. brucei* GUTat 3.1, as measured by immunoblots

	Percentage of cells secreting*			
	IgM		IgG	
Days after infection	C3H/He	C57BL/6	C3H/He	C57BL/6
7	3·4±1·3	4·2±2·9	2·6±1·9	2·4±1·6
9	3·1±1·7	7·7±4·7	8·5±5·0	11·2±6·2

* Mean±s.d. from five experiments, each using pooled cells from five mice.

increased, but only those secreting IgG had increased in the C3H/He population. The increases were variable, as is shown by the high standard deviations of the means.

ANALYSIS BY ELISA OF SPLEEN CELL Ig SYNTHESIS AND SECRETION

It was possible that the blot assay only detected release of preformed Ig. Synthesis and secretion of Ig were therefore quantitated separately by ELISA. Preliminary studies showed that in static cultures of spleen cells there was marked inhibition of secretion at those cell densities where cell confluence was exceeded. However, in rotated cultures, secretion of Ig increased in proportion to increased cell density up to 10^8 cells/ml of culture medium (Newson 1990). At the latter density, rotated cell cultures secreted up to 10 times more Ig than stationary ones. In-vitro cultures were therefore always rotated. Hourly sampling of rotated cultures showed that there was no reduction of cell viability, or of secreted Ig, or of the capacity of cells to make blots during three successive hours of culture (data not shown).

Spleen cells from C3H/He and C57BL/6 mice infected for 7 and 9 days were set up at 10^8 cells/ml in rotating cultures. Cell pellets and culture supernatants were collected at 0 and 3 h after initiation of the cultures for quantitative analysis of preformed and *de novo* formed cellular Ig and secreted Ig. The results (Table 3) showed only minor differences in IgM and IgG synthesis and secretion by spleen cells from C3H/He and C57BL/6 mice at 7 days after infection. In different replicate experiments, intracellular and secreted Ig in

Table 3. Synthesis and secretion of immunoglobulins by 10^8 spleen cells from C3H/He and C57BL/6 mice infected with *T. b. brucei* GUTat 3.1

Day after infection	Ig†	IgM (μg/ml)*		IgG (μg/ml)*	
		C3H/He	C57BL/6	C3H/He	C57BL/6
7	Secreted	5·6	6·7	4·5	4·3
	Preformed	5·6	5·0	3·3	2·7
	New	6·0	7·7	3·6	4·0
9	Secreted	4·5	14·3	30·5	45·7
	Preformed	7·7	24·0	23·7	23·5
	New	2·1	5·7	29·6	47·6

* Measured by ELISA on culture supernatants and cell pellets collected after rotation of 10^8 spleen cells/ml medium for 3 h at 37°C. Mean of two experiments, each using pooled cells from five mice.

† Secreted Ig = Ig in culture supernatant at 3 h, less Ig in culture supernatant at 0 h; preformed Ig = Ig in lysed cell pellet at 0 h; new Ig = Ig in culture supernatant plus lysed cell pellet at 3 h, less Ig in the same at 0 h (i.e. Ig formed *de novo*).

C57BL/6 mice was up to two times higher than in C3H/He mice. (The results in Table 5 show an experiment with the maximum twofold difference between the mouse strains.)

The differences between the two strains of mice were amplified at 9 days after infection and closely matched the results obtained using the immunoblotting technique (Table 2). It was clear that under these in-vitro conditions plasma cells from both strains of mice were secreting Igs, and also synthesizing Igs *de novo*.

ANALYSIS BY ELISA OF SPLEEN CELL SECRETION OF *T. B. BRUCEI* GUTat 3.1 VSG-SPECIFIC ANTIBODIES

Despite the similarity in total Ig synthesis and secretion by spleen cells from C3H/He and C57BL/6 mice infected for 7 days (Table 3), the absence of a serologically detectable antibody response at that time in the C3H/He mice (Black *et al.* 1983) suggested that there might be a failure to induce antibodies reactive with the parasite surface coat. Specific suppression has also been suggested by other investigators (Dempsey & Mansfield 1983b, Inverso & Mansfield 1983). A parasite VSG exposed epitope-specific ELISA was used, therefore, to analyse both intracellular and secreted Ig generated by spleen cells from mice infected for 6 and 7 days, and also Ig levels in serum since these had been measured by radioimmune assay in previous studies. Specificity of the assay was initially checked against three other *T. b. brucei* strains. Of these, IL 1785 gave the highest binding and so it was routinely used for checking specificity. Binding to IL 1785 by IgM in supernatants and pellets always gave OD less than 0·1 at sample dilutions of 1/10. Little parasite-specific IgG was detected in either strain of mice at this stage of infection and so IgG has been ignored in further experiments.

As before, serum levels of exposed VSG-specific antibodies were hardly detectable in C3H/He mice (Figure 2a). Furthermore, even in C57BL/6 mice, serum levels did not rise

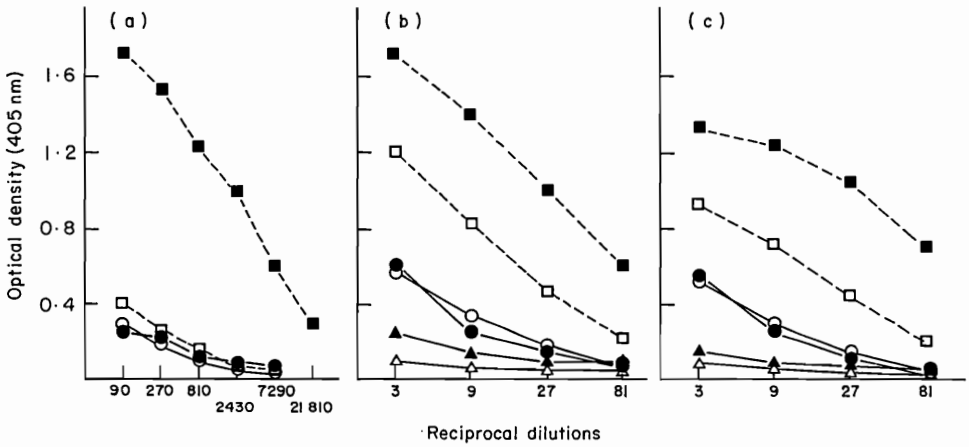

Figure 2. Parasite VSG-specific IgM in (a) serum and in (b) spleen cell lysates and (c) supernatants after 2 h in rotating culture. C3H/He mice at day 0 (▲——▲), day 6 (○——○) and day 7 (□- - -□) and C57BL/6 mice at day 0 (△——△), day 6 (●——●) and day 7 (■- - -■) after infection. Each point represents the mean for four individual mice. In the serum (a), results for day 0 were so close to those for day 6 that they have been omitted for the sake of clarity.

above uninfected background levels until day 7, in contrast to spleen cell output which was already amplifying at day 6. Although parasite VSG-specific IgM was amplified in the splenic plasma cells of both strains of mice at day 7 after infection, much more was detected in C57BL/6 mice than in C3H/He mice (Figure 2b & c).

ADDITION OF TRYPANOSOMES TO SPLEEN CELL CULTURES FROM INFECTED C57BL/6 MICE AND REMOVAL OF TRYPANOSOMES FROM SPLEEN CELL CULTURES FROM INFECTED C3H/He MICE

The apparently reduced production of parasite VSG-specific antibodies by spleen cells from C3H/He, as compared to C57BL/6, mice might have resulted from in-vitro absorption of this antibody by parasites present in the C3H/He spleen cell population. The C3H/He mice have a higher parasitaemia at day 6 after infection than the C57BL/6 mice and maintain this level of parasitaemia at day 7 after infection, which is when C57BL/6 mice clear the parasites from their bloodstream. Two procedures, adding trypanosomes to cultures of C57BL/6 spleen cells or removing them from the cultures of C3H/He spleen cells, were therefore used to examine the contribution of trypanosomes to the level of total Ig and parasite VSG-specific antibody detected in culture supernatants.

Trypanosomes were counted in washed spleen cell preparations from mice infected for 7 days. None was seen in the C57BL/6 spleen cell population, whereas 7×10^6 trypanosomes were counted per 10^8 C3H/He spleen cells. Trypanosomes were isolated from the blood of intact C3H/He mice which had been infected for 7 days or from irradiated C3H/He and C57BL/6 mice infected for 6 days with *T. b. brucei* GUTat 3.1, and 0, 7×10^6 and $1{\cdot}4 \times 10^7$ of the purified trypanosomes were added to cultures of 10^8 spleen cells from C57BL/6 mice infected for 7 days. Cultures were also set up with 10^8 C3H/He spleen cells (which contained 7×10^6 trypanosomes). The addition of 7×10^6 or $1{\cdot}4 \times 10^7$ trypanosomes to the C57BL/6 spleen cells resulted in a reduction in detectable

Table 4. The effect of adding trypanosomes on the amount of IgM secreted by spleen cell cultures of C57BL/6 mice infected with *T. b. brucei* GUTat 3.1 for 7 days

		IgM secreted*	
Mice	Treatment	Total (μg/ml)	Parasite-specific (reciprocal titre to give OD 0·5)
C3H/He	None (7×10^6 trypanosomes present)	5·4	< 10
C57BL/6	None (no trypanosomes seen)	11·8	78
	Added 7×10^6 trypanosomes	7·3	< 10
	Added $1{\cdot}4 \times 10^7$ trypanosomes	5·6	< 10

* In supernatants from 10^8 spleen cells/ml of medium (pooled from five mice) in rotating culture for 2 h, measured by ELISA. Background IgM levels, from similar supernatants at 0 h, have been subtracted. Results given are the means for two experiments.

Table 5. The effect of the removal of trypanosomes on the amount of IgM produced by spleen cell cultures of C3H/He and C57BL/6 mice infected with *T. b. brucei* GUTat 3.1 for 7 days

	C3H/He		C57BL/6	
	Untreated	Treated*	Untreated	Treated*
a) Total IgM (μg/ml)				
Secreted†	2·0	2·6	4·8	4·7
Intracellular‡	5·3	6·7	12·5	13·1
b) Parasite-specific IgM (reciprocal titre to give OD 0·5)				
Secreted†	0§	9	39	30
Intracellular‡	19	22	110	120

* Treatment with MoAb and complement.

† In supernatant from 10^8 spleen cells/ml of medium (pooled from five mice) in rotating culture for 2 h, measured by ELISA. Background IgM levels from similar supernatants at 0 h have been subtracted.

‡ From the lysed cell pellets from the same cultures as above after 2 h rotation.

§ None detectable.

total IgM and parasite VSG-specific IgM in the culture supernatants to levels similar to those detected in similar supernatants from infected C3H/He mice (Table 4). The reductions were similar whether the trypanosomes originated from intact or irradiated mice, and so the results of the two experiments were pooled. The lower levels of parasite VSG-specific antibody detected in spleen cell cultures of 7-day infected C3H/He as compared to C57BL/6 mice therefore possibly resulted from absorption of specific antibody by trypanosomes present in the cultures of C3H/He spleen cells.

The other approach, removal of trypanosomes from the C3H/He spleen cell suspensions, was achieved by incubating the cells with a *T. b. brucei* GUTat 3.1-specific MoAb and guinea-pig complement. More than 95% of the trypanosomes were killed. This treatment and the extra washing involved (necessary because the MoAb was both GUTat 3.1-specific and IgM) did not result in the loss or damage of plasma cells, as was shown by the ability of samples of pretreated, treated (MoAb and complement) and control (complement only) cell samples to make Ig blots. The removal of trypanosomes from the spleen cell suspensions of C3H/He mice had only a minor effect on the total and parasite VSG-specific IgM detectable in the cell supernatants (Table 5). Comparison of the C57BL/6 treated and control samples, which differed only in that one was treated with the MoAb, showed no differences, indicating that no residual MoAb remained in the treated samples. The treated cell pellets from both strains also showed no increase in IgM. Thus there was no evidence of residual MoAb in the supernatant or adhering to the cells. This experiment was repeated twice, with pooled samples from five mice on each occasion, with similar results.

Thus, addition of trypanosomes to C57BL/6 spleen cell cultures had a marked effect on parasite VSG-specific IgM levels, but removal of trypanosomes from C3H/He cell cultures did not affect the difference in parasite VSG-specific antibody between the two strains of mice.

ANALYSES OF Ig BOUND ON THE SURFACE OF BLOODSTREAM *T. B. BRUCEI* GUTat 3.1 *IN VIVO*

The in-vitro studies reported above suggested that spleen cells from infected C57BL/6 and C3H/He mice produced similar amounts of total and parasite VSG-specific Ig, up to 6 days after infection and presumably close to, if not up to, the time of clearance of the parasites from the bloodstream of the infected C57BL/6 mice. Assuming that the cells also produce antibody *in vivo*, it should be possible to detect antibody on the surface of bloodstream parasites. Examination of parasites in the blood from C3H/He and C56BL/6 mice infected for 6 and 7 days, stained with fluorescein-conjugated anti-mouse Ig, showed that most trypanosomes from both strains of mice were fluorescent. Fluorescence scores were higher on the parasites from C57BL/6 than from C3H/He mice.

Discussion

Whereas infected C57BL/6 mice cleared *T. b. brucei* GUTat 3.1 from the bloodstream between 6 and 7 days after infection, similarly infected C3H/He mice did not. Both strains of mice mounted identical plasma cell responses up to 6 days after infection. The responses occurred predominantly in the spleen and were mainly of IgM. By 6 days after infection, spleen cell populations from both strains of mice were shown to have similar numbers of Ig-containing cells, similar numbers of Ig-secreting cells, similar amounts of intracellular Ig, both total and specific for exposed epitopes on *T. b. brucei* GUTat 3.1 VSG, and similar secretory capacities to produce both total and parasite-specific Ig. By 7 days after infection, considerable amplification of both intracellular and secreted Ig responses had taken place. However, despite amplification in both strains of mice, they differed in one important respect: the C57BL/6 spleen cell population both contained and secreted approximately 3–5 times more parasite VSG-specific Ig than the C3H/He spleen cell population.

We have shown that addition of trypanosomes to spleen cell cultures of C57BL/6 mice can reduce the levels of exposed VSG-specific IgM to the levels found in C3H/He mice. The higher numbers of trypanosomes present at day 6 after infection can presumably absorb enough parasite VSG-specific antibody to render serum levels undetectable. Removal of trypanosomes from spleen cell cultures of C3H/He mice did little to affect the parasite VSG-specific Ig output, consistent with the observation that the intracellular content of splenic plasma cells from these mice is lower than that of C57BL/6 mice.

These studies supplement previous serological investigations into the nature of the immunological defect in trypanosome-infected C3H/He mice (Black *et al.* 1983, De Gee *et al.* 1982, Levine & Mansfield 1984, Mahan *et al.* 1986, Morrison & Murray 1985) and support the contention of Levine & Mansfield (1984) that parasite-induced suppression is initially manifested at the B-cell clonal level. Our results suggest that this form of suppression operates on the terminal stage of development of exposed VSG epitope-

specific plasma cell responses. We have previously shown (Black *et al.* 1986) that infected C3H/He mice also fail to mount antibody responses on challenge with rabbit erythrocytes. Both the kinetics and nature of the suppression which prevented the development of rabbit erythrocyte specific responses in C3H/He mice were similar to that observed with the exposed VSG epitope-specific response. Thus, 1 to 2 days after *T. b. brucei* GUTat 3.1 organisms reach a plateau parasitaemia in infected C3H/He mice, the mice also fail to support terminal maturation of other specific antigen-induced B-cell responses. This does not occur in infected C57BL/6 mice which can support terminal maturation of antigen-specific B-cell responses, at least up to the time of remission of the first parasitaemic wave.

T. b. brucei GUTat 3.1-infected C57BL/6 and C3H/He mice differ in a second respect: the peak level of first wave parasitaemia is 2·5 to 10 times higher in infected C3H/He mice (Black *et al.* 1983, 1985). The higher level of parasitaemia in the C3H/He mice results from an apparently slower switch from dividing slender forms to non-dividing stumpy forms in the bloodstream. Both slender and stumpy form *T. b. brucei* GUTat 3.1 are cleared from the bloodstream of infected C57BL/6 mice at essentially the same time (Black *et al.* 1983). Hence clearance probably occurs when a critical amount of antibody per trypanosome is reached in the bloodstream. This was borne out by our immunofluorescence studies. Presumably the higher numbers of trypanosomes in the C3H/He mice, and the lower output of parasite exposed VSG epitope-specific antibody (occurring between 6 and 7 days after infection in the present study), result in a failure of the C3H/He mice to achieve an adequate concentration of plasma antibody for parasite clearance.

Our previous investigations (Black *et al.* 1983) have shown that the differentiation of *T. b. brucei* from dividing slender to non-dividing stumpy forms is not driven by the developing antibody response. This is confirmed in the present study. Parasitaemia was higher in C3H/He than in C57BL/6 mice by 6 days after infection, at which time the antibody responses were identical in both strains of mice. Thus the timing of the developing antibody response is not responsible for initial limitations on levels of parasitaemia. In contrast we suggest, therefore, that differences in levels of parasitaemia may be responsible for differences in the final maturation of antibody responses in infected C3H/He and C57BL/6 mice.

Seed & Sechelski (1988) have shown that susceptible intact C3H mice are able to clear *T. b. rhodesiense* from their bloodstream during first wave parasitaemia if the height of that wave is artificially controlled by administration of difluoromethylornithine (DFMO). DFMO, which inhibits trypanosome division, is trypanostatic rather than trypanocidal and requires an immune response if it is to be effective chemotherapeutically. Because parasites were cleared from the bloodstream of *T. b. rhodesiense*-infected DFMO-treated C3H and untreated resistant B10 mice with similar kinetics, it is reasonable to conclude that both strains of mice achieve a similar critical level of parasite VSG exposed epitope-specific antibodies, i.e. the DFMO-treated C3H mice can maintain terminal development of antigen-induced plasma cell responses.

Based on the argument above, we propose that the suppression in infected C3H/He mice is associated with a critical parasite load or a critical load of a parasite-released or induced host component. We conclude that the most significant difference between susceptible C3H/He and resistant C57BL/6 mice, with respect to control of first wave *T. b. brucei* GUTat 3.1 parasitaemia, lies in non-immunological responses which regulate parasite differentiation from dividing slender to non-dividing stumpy forms. The failure

to develop full immunological competence in the infected C3H/He mice is a consequence rather than a cause of the higher level of parasitaemia in the C3H/He mice.

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