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# Immune depression in African trypanosomiasis: the role of antigenic competition

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## SUMMARY

The capacity of trypanosome-infected cattle to mount an immune response to a simultaneous or subsequent challenge with other trypanosomes was investigated using various clones of *Trypanosoma congolense* and *T. brucei*. In animals infected simultaneously with equal numbers of trypanosomes of two different clones, the variant-specific antibody response to one clone was severely depressed while the response to the other was not affected. In cattle infected with one clone and then subsequently challenged with another, the variant-specific antibody response to the challenge clone was or was not severely depressed depending on the time interval between the two inoculations. These observations were consistent regardless of whether the clones of trypanosomes used were derived from the same or different species. The characteristics of these responses would suggest that the inability of trypanosome-infected cattle to respond well to a simultaneous or subsequent challenge with other trypanosomes or other antigens may be due to antigenic competition.

## INTRODUCTION

Man and animals infected with African trypanosomes respond poorly to a subsequent challenge with other antigens such as microbial antigens (Greenwood, Whittle & Molyneux, 1973; Rurangirwa *et al.*, 1979) and sheep red blood cells (Goodwin *et al.*, 1972). This immunodepression is thought to result partly from a depletion of antigen-reactive lymphocytes of the infected host due to a polyclonal B-cell activation (Greenwood, 1974; Hudson *et al.*, 1976; Corsini *et al.*, 1977) and partly from a generation of suppressor T lymphocytes and suppressor macrophages in the host (Eardley & Jayawardena, 1977; Jayawardena & Waksman, 1977; Corsini *et al.*, 1977; Pearson *et al.*, 1979; Wellhausen & Mansfield, 1979).

In all these studies, however, the immune response to trypanosome-infected hosts has been tested using non-trypanosomal antigens. The work reported here was designed to establish whether the infected host is also unable to respond well to a superinfection with other trypanosomes, and, if so, to determine the mechanism by which such depression may occur.

## MATERIALS AND METHODS

*Animals.* Cattle (Borans and crosses between Borans and Charolais) were obtained from areas

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known to be free from African trypanosomiasis and were screened for antibodies to *T. congolense*, *T. vivax* and *T. brucei* by immunofluorescence (Wilson, 1969) and enzyme-linked immunosorbent assay (Luckins, 1977) before use. BALB/c mice and Wistar rats were bred at ILRAD.

*Parasites. T. brucei*, Clone ILTat 1.3 (previously known as RM-1, Barbet & McGuire, 1978) was derived from Stock 227; MITat 1.2 (previously called Clone 221, Cross, 1977) was derived from Stock 427 isolated in Uganda in 1960 from a sheep (Cunningham & Vickerman, 1962); and ILTat 2.1 is a clone derived in lethally irradiated (900 rad) BALB/c mice from Stock 247 isolated in the Serengeti area, Tanzania (Geigy & Kauffmann, 1973). *T. congolense* Stock 212 is an isolate from a lion in the Serengeti area, Tanzania (Geigy & Kauffmann, 1973), while ILNat 2.1, previously known as X4 (Rovis, Barbet & Williams, 1978), is a clone derived from an isolate originally collected in 1966 from Southern Kenya (Wellde *et al.*, 1974). All parasites were grown in lethally irradiated (900 rad) mice and isolated from infected mouse blood following the method described by Lanham (1968).

*Indirect immunofluorescent antibody test (IFAT).* IFAT on live trypanosomes was performed according to Barbet & McGuire (1978) and that on formaldehyde-fixed parasites was done as described by Nantulya & Doyle (1977).

*Fixation of trypanosomes with formaldehyde.* A sample of each trypanosome population used to infect cattle was washed four times in phosphate-buffered saline–glucose (PSG), pH 8·0, and immediately fixed by suspension overnight at 4°C in 1% formaldehyde in phosphate-buffered saline (PBS), pH 7·2 (Fisher Scientific Co., Fair Lawn, New Jersey, USA), with continuous stirring, to stabilize the variable surface glycoproteins (VSGs) on the parasites (Nantulya & Doyle, 1977). Formol-treated parasites were washed three times in PBS and resuspended in the same buffer containing 2 mg/ml bovine serum albumin (A-4503, Sigma Chemical Co., St Louis, Missouri, U.S.A). The suspension was then applied onto the pre-marked areas of Cooke's microscope slides (Dynatech, Sussex, UK), the slides dried at 37°C for 15 min, and then used in the IFAT.

*Radioimmunoassay (RIA).* Antibody activity to *T. brucei* VSGs was tested by the conventional radioimmunoassay (Barbet & McGuire, 1978) while antibodies to *T. congolense* VSGs were detected by the solid-phase radioimmunoassay.

The solid-phase radioimmunoassay was performed as described by Sendashonga & Black (1981) with some modifications. Briefly, formol-treated trypanosomes were washed four times in phosphate-buffered saline (PBS, pH 7·2) and then resuspended in the same buffer to give a final concentration of $2 \times 10^6$ parasites/ml. Aliquots of 50 $\mu$l of the trypanosome suspension were then added to each well of a microtitre plate and centrifuged for 5 min at 500 *g*. The plate was then kept at room temperature for 1 hr and the supernatant discarded. The wells were then filled with radioimmunoassay (RIA) buffer (0·01 M PBS, 1% normal rabbit serum and 0·02% sodium azide) and incubated at room temperature for 1 hr to saturate free-binding sites. Thereafter, the buffer was drained off, 50 $\mu$l test serum (diluted in RIA buffer) added to each well and the plates incubated for 1 hr at room temperature. The wells were then drained and washed three times with RIA buffer before addition of 50 $\mu$l $^{125}$I-labelled goat anti-bovine IgM or IgG, containing 20,000 c.p.m. The plates were incubated for another hour at room temperature, washed three times with RIA buffer and dried. The wells were cut apart with a hot wire, placed into tubes and the bound radioactivity measured in a Packard 5360 scintillation spectrometer.

*Antisera.* Antisera against bovine immunoglobulins were prepared in goats as described by McGuire, Musoke & Kurtti (1979) and labelled with $^{125}$I following the method of Tsu & Herzenberg (1980).

*Experiments A and B.* In experiments A and B, groups of cattle were infected with one clone of *T. brucei* or *T. congolense* and then subsequently challenged with a different clone of *T. brucei* on different days to assess the ability of the initial infection to depress the immune response of the host to the second clone. The full protocol for these two experiments is summarized in Table 1.

*Experiment C.* Three test animals were inoculated simultaneously with equal amounts (200 $\mu$g) of variable surface glycoproteins from *T. brucei* clones MITat 1.2 and ILTat 1.3 in Freund's complete adjuvant by the intramuscular route to determine whether isolated antigens would interfere with the immune response to each other. Controls received either MITat 1.2 alone (three

**Table 1.** The design of experiments A and B

| Group number | Number of cattle | Initial infection with $1 \times 10^8$ | Challenge with $1 \times 10^8$ ILTat 1.3 on day: | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 2 | 4 | 6 |
| Experiment A | | | | | | |
| I | 3 | MITat 1.2 | + | − | − | − |
| II | 3 | MITat 1.2 | − | + | − | − |
| III | 3 | MITat 1.2 | − | − | + | − |
| IV | 3 | MITat 1.2 | − | − | − | + |
| V (control) | 3 | MITat 1.2 | − | − | − | − |
| VI (control) | 3 | ILTat 1.3 | − | − | − | − |
| | | | Challenge with $1 \times 10^8$ MITat 1.2 on day: | | | |
| | | | 0 | 5 | 8 | |
| Experiment B | | | | | | |
| I | 3 | *T. congolense* | + | − | − | |
| II | 3 | *T. congolense* | − | + | − | |
| III | 3 | *T. congolense* | − | − | + | |
| IV (control) | 3 | *T. congolense* | − | − | − | |
| V (control) | 3 | MITat 1.2 | − | − | − | |

animals) or ILTat 1.3 (three animals). All animals were bled twice a week for 4 weeks and sera tested for variant-specific antibody activity.

*Experiment D.* Three animals were inoculated simultaneously with equal numbers ($1 \times 10^8$ trypanosomes) of *T. congolense* Clone ILNat 2.1 and *T. brucei* Clone ILTat 2.1 and then sera obtained from them at 3-day intervals to determine the relative levels of antibodies produced against each clone. Controls received either ILTat 2.1 alone (three animals) or ILNat 2.1 (three animals).

## RESULTS

In animals infected simultaneously with equal numbers of trypanosomes of each of the two clones of *T. brucei* (experiment A), the peak titre of variant-specific antibody response to one, MITat 1.2, was comparable to that of the control animals infected with this clone alone (Fig. 1a), although there was a lag of 3 days before peak titres of antibody were attained in the test animals. The humoral response to the other clone (ILTat 1.3), however, was severely depressed (Fig. 1b), in that the peak titre of antibody in test animals was 82% lower than that in the control animals.

We postulated that the capacity of MITat 1.2 to depress the immune response to ILTat 1.3 depended on the relative immunogenicity of the corresponding surface antigens in that MITat 1.2 could be more immunogenic than ILTat 1.3. This hypothesis was tested by immunizing a group of animals with equal doses of surface antigens prepared from the two clones (experiment C). The immune response to ILTat 1.3 (Fig. 2a) was comparable to that of the control animals which had

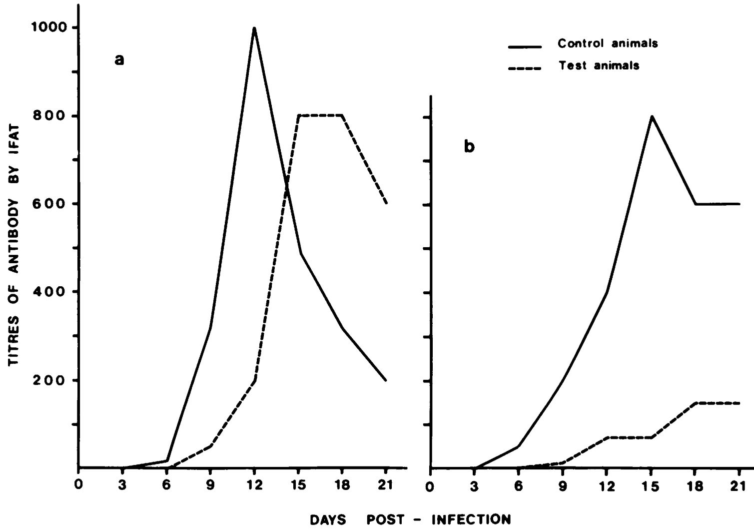


**Fig. 1.** Variant-specific antibody responses to MITat 1.2 (a) and ILTat 1.3 (b) in cattle infected simultaneously with equal numbers of organisms of the two *T. brucei* clones. Each point represents the geometric mean of antibody titres in three animals.

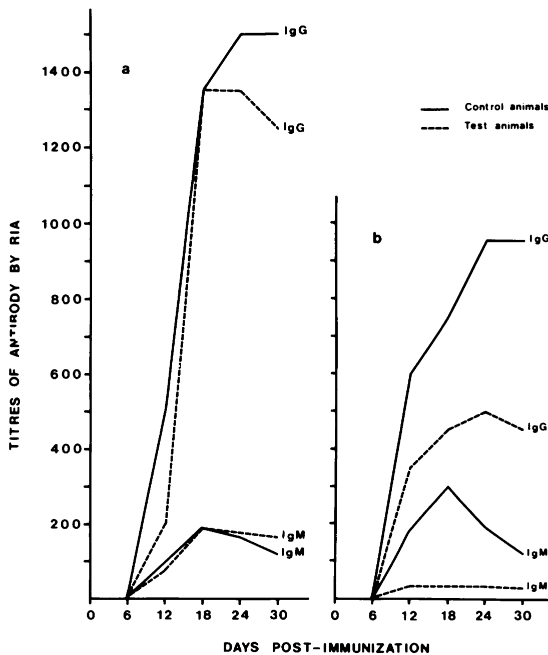


**Fig. 2.** Variant-specific antibody responses to ILTat 1.3 (a) and MITat 1.2 (b) in cattle immunized with equimolar amounts of variable surface antigens from the two *T. brucei* clones. Each point represents the geometric mean of antibody titres in three animals.

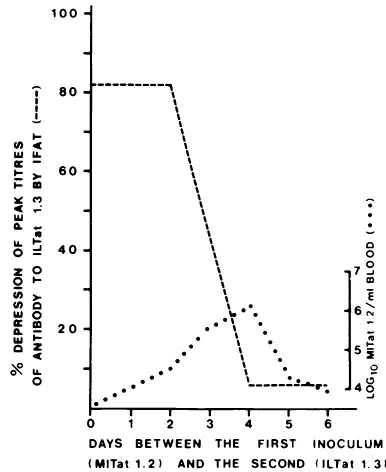


**Fig. 3.** The depression of variant-specific antibody responses to ILTat 1.3 in groups of cattle challenged with this clone at various time intervals after initial infection with a different *T. brucei* clone, MITat 1.2. Each point represents the percentage reduction in the geometric mean of peak titres of antibody in three test animals as compared to three controls.

received ILTat 1.3 surface antigen only. In contrast, the response to MITat 1.2 was depressed by 47% in the IgG antibody class and 90% in the IgM class (Fig. 2b), indicating that there was no direct correlation between the immunodepressive role of MITat 1.2 and the immunogenicity of its surface antigen. This then led us to investigate the possibility that MITat 1.2 might depress the immune response to ILTat 1.3 in cattle infected simultaneously with both clones by outgrowing it and thereby presenting a larger antigenic mass to the immune system of the host than ILTat 1.3. It was found that the proportion of MITat 1.2 parasites isolated from the first-peak parasitaemia in cattle infected simultaneously with both clones always exceeded that of ILTat 1.3 by a factor of 10–20 as

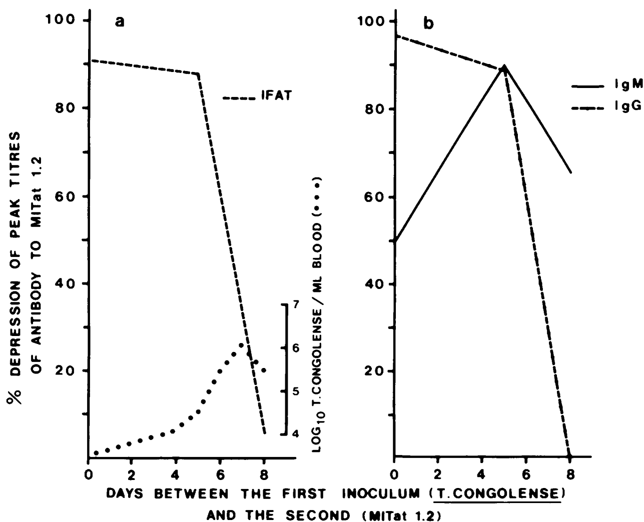


**Fig. 4.** The depression of variant-specific antibody response to MITat 1.2 in groups of cattle challenged with this clone at various time intervals after initial infection with *T. congolense*, as measured by IFAT (a) and RIA (b). Each point represents the percentage reduction in the geometric mean of peak titres of antibody in three test animals as compared to three controls.

assessed by indirect immunofluorescence using antisera prepared against the purified surface glycoproteins of these clones, indicating that MITat 1.2 indeed outgrew ILTat 1.3.

In this same experiment, groups of animals were infected with MITat 1.2 and then assessed for their ability to respond to a subsequent challenge dose of ILTat 1.3 given on the second, fourth and sixth day of infection. It was found that the variant-specific antibody response to ILTat 1.3 was severely depressed (82%) in animals which were challenged with this clone 2 days after infection with MITat 1.2 (Fig. 3). Interestingly, antibody response to ILTat 1.3 was not depressed in animals challenged with this clone on the fourth and sixth day after infection with MITat 1.2 (Fig. 3). The immune response to MITat 1.2 was itself not affected in all groups of test animals.

In experiments C and D, groups of cattle were infected simultaneously or sequentially with mixtures of *T. congolense* and *T. brucei* to assess whether or not prior infection with *T. brucei* could also depress antibody response to *T. congolense* and vice versa. The results of these experiments were similar to those of experiment A. In the case of experiment C, *T. congolense* (Stock 212) outgrew *T. brucei* MITat 1.2 and also depressed the antibody response of the test animals to MITat 1.2 (Fig. 4a) and this depression affected both the IgM and IgG antibody classes (Fig. 4b). In experiment D, *T. brucei* Clone ILTat 2.1 outgrew and also depressed the antibody response to *T. congolense* Clone ILNat 2.1 in animals infected simultaneously with equal numbers of trypanosomes of the two clones, whereas responses to ILTat 2.1 were not affected (Fig. 5a & b). Interestingly, however, the variant-specific antibodies produced against *T. congolense* in both test and control animals were of the IgM class only (Fig. 5b).

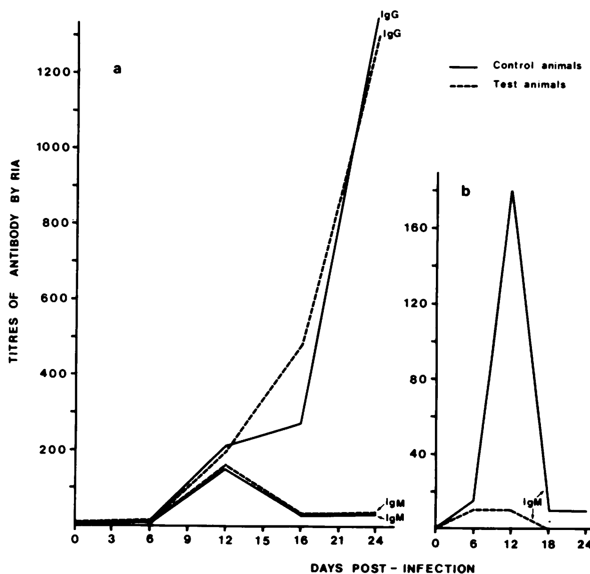


**Fig. 5.** Variant-specific antibody responses to ILTat 2.1 (a) and ILNat 1.2 (b) in cattle infected simultaneously with equal numbers of organisms of the two trypanosome clones. Each point represents the geometric mean of antibody titres in three animals.

## DISCUSSION

The results of our studies have demonstrated that in cattle infected simultaneously with equal numbers of trypanosomes of different variable antigen types (VATs), the variant-specific antibody response to one may be severely depressed while the response to the other is not affected. This relationship was consistent regardless of whether the VATs in question were derived from the same or different trypanosome species and the depression affected both IgM and IgG antibody classes.

The main factor determining the role played by each VAT was the relative growth rate of the VATs in the mixture in that the faster grower depressed antibody responses to the slower grower.

The characteristics of these responses are similar to those observed in animals immunized with mixtures containing a variety of other antigens in which it has consistently been shown that the immune response to one can be severely depressed while the response to the other is unaffected. This depression of the response is thought to be due to antigenic competition (Taussig, 1973; Liacopoulos & Ben-Efraim, 1975), also known as non-specific antigen-induced suppression (Pross & Eidinger, 1974). The principal factor governing this relationship is the molar ratio between the competing antigens in the mixture in that, should the amount of the suppressed antigen be increased to an appropriate level, this antigen can abolish or even reverse the immunodepressive role of the dominant antigen (Taussig, 1971; Taussig *et al.*, 1972). It is therefore suggested that the reason why the faster growing VAT depresses the response to the slower growing one in animals infected simultaneously with both VATs is that the former ultimately presents a larger antigenic mass to the bovine immune system. This would readily explain why in combinations like that of MITat 1.2 and ILTat 1.3, the faster growing MITat 1.2 was able to depress the variant-specific antibody response to the slower growing ILTat 1.3 despite the fact that in immunized animals the VSG of ILTat 1.3 was the dominant antigen when given in equal amounts.

Our work has further demonstrated that if two VATs are inoculated in sequence, the immune response to the first inoculum is not affected while the response to the second VAT is depressed. However, the ability of the first VAT to depress the immune response to the second VAT depended on the time interval between the two inoculations. If animals were inoculated with the second VAT later than 2 days after initial infection with the first VAT (in the case of *T. brucei*) or 5 days (in the case of *T. congolense*), the immune response to the second VAT was not depressed.

Thus these results are also suggestive of antigenic competition where a prior immunization of the host with one antigen can lead to the depression of its response to the second antigen. In this form of competition, 'sequential' type, the ratio of the amounts of antigens used is not the critical factor. Instead, it is the time interval that is critical in that the response to the second antigen is only depressed if this antigen is administered during a specific interval after the first (Taussig, 1973; Liacopoulos & Ben-Efraim, 1975). Terry (1977) also speculated on the possibility of a sequential type of antigenic competition as a cause of immunodepression in African trypanosomiasis.

There are several other mechanisms which have been postulated to explain the immunodepression observed in African trypanosomiasis. We would like to suggest that some of these mechanisms, namely, suppressor T cells (Eardley & Jayawardena, 1977; Jayawardena & Waksman, 1977; Pearson *et al.*, 1979), suppressor macrophages (Corsini *et al.*, 1977; Wellhausen & Mansfield, 1979) and suppressor factors (Moulton & Coleman, 1978) are part and parcel of antigenic competition. This possibility can readily be envisaged considering that several studies on the mechanism of antigenic competition, summarized by Liacopoulos & Ben-Efraim (1975), have indeed indicated that suppressor T cells, suppressor macrophages and suppressor factors could be the final effector mechanisms of antigenic competition.

The immune interference phenomenon described here could take place at the level of the macrophages, possibly because those macrophages that are already engaged in the processing of antigens of one trypanosome population may be unable to handle appropriately antigens of other trypanosomes. Alternatively, such macrophages could elaborate a factor that inhibits the proper lymphocyte responses of the infected host to the antigens of a different trypanosome population. The results of Corsini *et al.* (1977), Eardley & Jayawardena (1977) and Wellhausen & Mansfield (1979) have made this hypothesis particularly attractive. These authors have shown that macrophages collected from mice infected with *T. brucei* or *T. rhodesiense* depress profoundly the ability of normal spleen cell cultures to proliferate and secrete immunoglobulins in the presence of *E. coli* lipopolysaccharide. Such macrophages also depress the response of normal mouse spleen cells to sheep red blood cells *in vitro*. In addition, the work by Eardley & Jayawardena (1977) has shown that macrophages may not be the primary source of suppression but rather that trypanosome-induced suppressor T cells secrete a factor which arms macrophages to carry the suppressive messages to other T and B cells.

The implications of our findings are manifold. Firstly, since in this disease several different

variable antigen types (VATs) of the parasite continuously arise both simultaneously and sequentially in the same host during the course of a single infection (Doyle, 1977), the primary immune response to some VATs may be severely depressed, thereby facilitating their survival. Hudson & Terry (1979) have also suggested that early trypanosome populations in the host may effectively depress antibody responses to the VATs arising in subsequent waves. Depression of the primary response could lead to a defective secondary response to the suppressed VATs (Hanna & Peters, 1970; Rhodes & Larsen, 1972; Taussig *et al.*, 1972) so that when such VATs reappear in the same host (Nantulya *et al.*, 1979) they are not quickly eliminated.

Secondly, although in our experiments cattle with long-standing infections were not tested for their capacity to respond to a superinfection with unrelated trypanosomes, it is quite possible that such response would depend on the ability of the challenge VATs to compete with VATs from the continuing infection. Since under field conditions animals are exposed to multiple trypanosome challenges, this may be an important consideration because the initial infections may impair the response of the host to subsequent challenge with different strains and species, thereby leading to mixed infections. This possibility has also been alluded to by de Gee & Shah (1979).

Finally, on the basis of antigenic competition, one can explain the observations of McGuire, Barbet & Musoke (unpublished) that in a natural infection with African trypanosomes, as well as in animals immunized with isolated trypanosome variable surface glycoproteins (VSGs), antibodies to the cross-reacting antigenic determinants in the C-terminal region of the VSGs (Barbet & McGuire, 1978; Cross, 1979) are not readily detectable in contrast to the high levels of antibodies to those determinants in the N-terminal region. This preferential response to the determinants in one region of the VSG molecule may be due to antigenic competition, 'intramolecular' type (Taussig, 1973), in that the immune response to the determinants in the N-terminal region could dominate and depress the response to those in the C-terminal region.

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