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IMMUNOSUPPRESSION DURING TRYPANOSOMIASIS

L. G. GOODWIN, D. G. GREEN, M. W. GUY AND A. VOLLER

From the Nuffield Institute of Comparative Medicine, The Zoological Society of London, Regent's Park, London, N.W.1

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SUMMARY.—Mice and rabbits infected with *Trypanosoma brucei* developed much lower agglutinin levels than uninfected animals when injected with sheep erythrocytes. The immunosuppression became more marked as the infection progressed. The infected rabbits produced heterophile agglutinins but the mice did not.

IT has long been known that some protozoal infections provide a massive antigenic load to the hosts immune system, but it is only recently that the effects of this on the immune response to other antigens has been investigated. Salaman, Wedderburn and Bruce-Chwatt (1969) showed that malaria-infected mice at the peak of parasitaemia, had a much depressed response to sheep erythrocytes, and this has been confirmed by Barker (1971) and Greenwood, Playfair and Torrigiani (1971). The only comparable work employing haemoflagellates is that of Clinton, Stauber and Palczuk (1969) who found that hamsters infected with *Leishmania donovani* showed a diminished response to injected chicken ovalbumin.

The purpose of the present investigation was to determine whether immunodepression occurs in mice and rabbits infected with *Trypanosoma brucei*. The results of one of our experiments with mice has been reported briefly by Goodwin (1970).

MATERIAL AND METHODS

The strain of *T. brucei* used was Lister Institute S/42. Infected mouse blood was distributed into capillary tubes, frozen and stored at − 70°. When an inoculum was required the contents of a capillary were melted and injected into a mouse. After about 6 days, when a heavy parasitaemia had developed, the blood of the mouse was collected and the trypanosomes counted. A suitable dilution was prepared and used for infecting groups of mice (1000 trypanosomes, given intraperitoneally) and rabbits (6 million trypanosomes given subcutaneously).

Washed sheep erythrocytes (5×10^9 cells/mouse) were injected into groups of mice which had been infected with trypanosomes 7, 14 or 21 days earlier. Groups of uninfected control mice were challenged in the same way. All the mice were killed 7 days after injection of the sheep erythrocytes and the separated serum was stored at − 20°.

Four rabbits were inoculated with *T. brucei* and injected 15 days later with 5×10^9 sheep erythrocytes. Two uninfected control rabbits were also given a sheep erythrocyte challenge at this time. Serum was separated from blood collected from the ear of each rabbit at various intervals before and after infection. The serum was stored at − 20° until tested.

Agglutinins to sheep erythrocytes were measured by means of a micro-agglutination test. All sera were inactivated at 56° for 30 min before use and 2-fold dilutions were made in 0·15 mol/l phosphate buffered saline, pH 7·2. To the 20 μl of serum dilution in each well of a haemagglutination plate 20 μl of 0·8 per cent washed sheep erythrocyte suspension were added. The pattern of the settled cells was observed after 18 hr at room temperature. The end-point was recorded as the last dilution of serum which caused complete agglutination of all the red cells.

RESULTS

The sheep erythrocyte agglutinin levels for individual animals and the geometric means of the groups of uninfected and *T. brucei* infected mice are shown in Fig. 1. Twenty-six of the total of 27 control mice developed agglutinin titres of 1 : 256 or higher.

Mice infected with *T. brucei* 1 week earlier showed a lower agglutination titre than the controls; the difference was much more marked in the groups of animals with infections of 2 and 3 weeks' duration. All the mice infected with *T. brucei* died during the 3rd or 4th week after inoculation.

Fig. 2 shows agglutinin titres of the sera of rabbits infected with *T. brucei* and

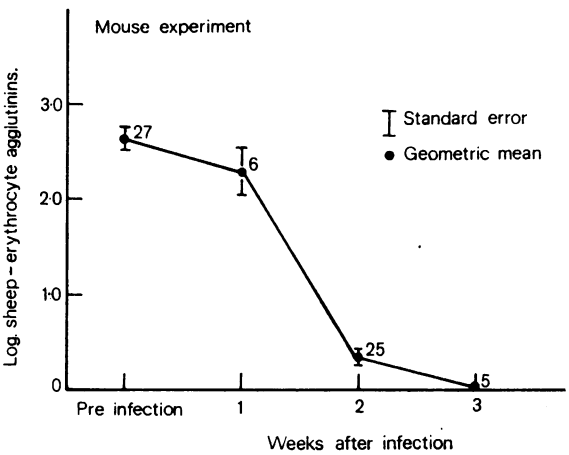

FIG. 1.—Haemagglutination titres of mice infected with *T. brucei* at various intervals before being injected with sheep erythrocytes.

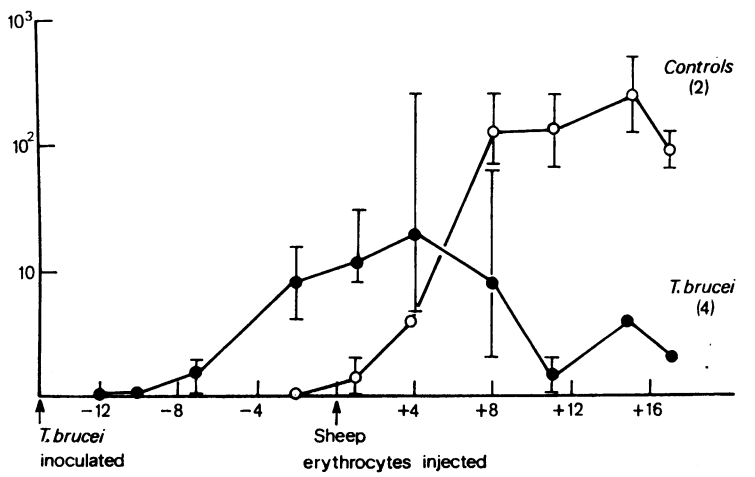

FIG. 2.—Haemagglutination titres of uninfected and *T. brucei* infected rabbits injected with sheep erythrocytes. (Vertical lines indicate the range of the observations.)

those of uninfected controls. All the rabbits infected with *T. brucei* had developed detectable antibody titres to sheep erythrocytes before any of them were challenged with the sheep cells. After challenge, no further increase in mean titre was observed, and in some animals the antibody levels decreased. In contrast the uninfected control rabbits only developed agglutinins to sheep erythrocytes after they had been challenged with the cells, and the titres reached higher levels than in the animals infected with trypanosomes.

DISCUSSION

The observations reported here show that trypanosomiasis can be added to the rapidly lengthening list of diseases during which there is a depression of the antibody response of the host to unrelated antigens. Earlier workers (reviewed by Salaman, 1970) have shown that this phenomenon is not uncommon during viral infections. More recently a similar but transient immunodepression has been observed during peak parasitaemia in rodent malaria infections (Salaman *et al.*, 1969; Barker, 1971; Greenwood *et al.*, 1971). The strain of *T. brucei* we used in our experiments was invariably fatal to mice and rabbits so that it was not possible to determine whether the immunosuppressive effect was temporary or permanent. Over the 3–4 week course of the infection in mice, the immunosuppression became progressively greater and accompanied the increasing parasitaemia.

Following the example of earlier workers we used sheep red cells as the indicator antigen for detection of immunosuppression. The results do not necessarily imply that alterations occur in the immune response to all other antigens; Barker (1971) has shown that there is normal antibody response to a bacteriophage. Similarly Greenwood *et al.* (1971) noted that normal antibody levels were produced against haemocyanin injected during malarial infections and they suggest that immunodepression is brought about by a failure of the antigen processing mechanism of the macrophages. This view is consistent with the lowered response to sheep red cells observed in our experiments and with the diminished response to injected ovalbumin in *L. donovani* infections (Clinton *et al.*, 1969).

The similarity of the immunosuppressive effects of experimental malarial, trypanosomal and leishmanial infections would suggest a similar mechanism in all these protozoal infections.

Most attention has hitherto been directed to alterations in the humoral response. However, Allt, Evans, Evans and Targett (1971) have noted that concurrent trypanosome infections reduce the severity of induced experimental allergic neuritis in rabbits, suggesting that cell-mediated responses are also affected.

The spontaneous development of heterophile agglutinins to sheep red cells which we have seen in rabbits infected with *T. brucei* have been reported to occur in infected human patients (Houba and Allison, 1966) and in African trypanosome infections in monkeys (Houba, Brown and Allison, 1969). The time course of the development of heterophile antibodies parallels that of IgM and rheumatoid factor in *T. equiperdum* infections in rabbits (Klein, Mattern and Kormann-Bosch, 1970). These indications of antiglobulin reactivity may reflect the extensive tissue damage which occurs during infection resulting in the alteration of modified host tissue proteins which act as antigens.

The phenomenon of immunosuppression may be of more than academic

interest. Patients with chronic leishmaniasis or trypanosomiasis show little resistance to concurrent bacterial pneumonias and septicaemias, and Wedderburn (1970) has shown that rodents infected with malaria are highly susceptible to virus-induced malignant lymphomata. On the other hand, it has been reported that autoimmune disease is rare in tropical countries where multiple parasitaemia is widespread (Greenwood, 1968; Greenwood, Herrick and Voller, 1970).

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