Response of inbred mice to infection with a new isolate of *Trypanosoma musculi*

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SUMMARY

A new isolate of *Trypanosoma musculi* has been derived from organisms recovered from *Mus spretus* captured in Lisbon, Portugal. The time-course and profiles of infection with this isolate in inbred mice have been compared with those obtained with the existing Partinico II isolate. Infections with the Portuguese isolate are less intense, and controlled more quickly than those with the Partinico isolate. As with the latter, there are marked mouse strain-dependent influences on infection with the Portuguese isolate, but these strain-dependent characteristics differ considerably with each isolate. For example, NIH mice were the most susceptible to the Partinico II isolate, but virtually refractory to the Portuguese isolate. Mice exposed to infection with one isolate show complete immunity to both homologous and heterologous challenge infections. These striking interactions between host and parasite genotype are discussed in terms of immunological influences on infection.

Key words: *Trypanosoma musculi*, Partinico II isolate, Portuguese isolate, inbred mice, parasitaemia, genetic influences.

INTRODUCTION

Trypanosoma musculi is a natural, highly host-specific stercorarian trypanosome parasite of mice in which, typically, it produces a self-limiting and subclinical infection (Viens *et al.* 1974). However, under certain circumstances it can produce significant pathology which includes ascites, impaired erythropoiesis, immunodepression, lymphoid hyperplasia and thrombocytosis (Albright, Holmes & Albright, 1990*a*; Albright & Albright, 1991).

Qualitatively, the time-course of infection in all strains of mice so far studied is remarkably consistent and reproducible (e.g. Viens *et al.* 1974; House & Dean, 1988; Albright & Albright, 1989; Albright, Pierantoni & Albright, 1990*b*) and consists of the following four phases. (1) A pre-patent period of 3–5 days; (2) an exponential, multiplicative or growth phase of approximately 5 days; (3) a relatively long plateau phase lasting for 7–10 days and (4) an exponential immune elimination phase of 5–7 days duration, giving an overall patent period of 20–25 days. Recovery from infection is accompanied by strong long-lasting resistance to re-infection, although small numbers of the parasite invariably persist in the vasa recta of the kidneys (Viens *et al.* 1974).

Although the kinetics of infection are qualitatively similar in all mice, there are quantitative differences in the relative susceptibility of strains to the parasite, as judged by the magnitude of parasitaemia and the severity of the associated pathological and immunological responses. For example, peak parasitaemia in A and C3H mice, which experience the most severe infections, is nearly 10 times greater than in the relatively more resistant C57BL/6 strain, which is also one of the most efficient in the immune elimination of the parasite (Bell, Adams & Ogden, 1984*a*; Albright & Albright, 1991).

Although a great deal is known about the influence of host strain on response to the trypanosome infection very little is known about the influence of parasite genotype on the course of infection. Indeed, it would appear from published reports that, until recently, the only available isolate of the parasite was the Partinico II, which, according to Viens *et al.* (1974) was collected from *Mus musculus* in Sicily in 1962. This species of mouse is considered to be the original and natural host of the parasite (Kendall, 1906). A trypanosome which is morphologically indistinguishable from *T. musculi*, but which was isolated from *Mus spretus*, has been used in a series of experiments to establish base-line parameters in laboratory mice. The time-course of infection with this isolate, and the pattern of responses seen in mouse strains, show marked differences from those described for the Partinico isolate.

MATERIALS AND METHODS

Animals

BALB/c, C57BL/10 and NIH mice were obtained from Harlan Olac, Bicester, Oxon., or bred in the

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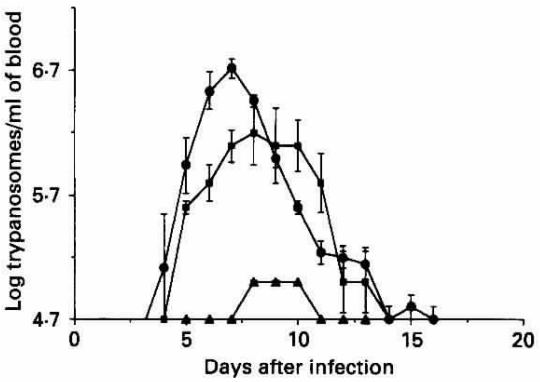

Fig. 1. Time-course of parasitaemia following infection with 3×10^4 *Trypanosoma musculi* (Portuguese isolate) in inbred mice. Values are means ± S.E. of log trypanosomes/ml of blood. Log 4·7 represents the limit of detection used in these experiments. (●) C57BL/10; (■) BALB/c; (▲) NIH.

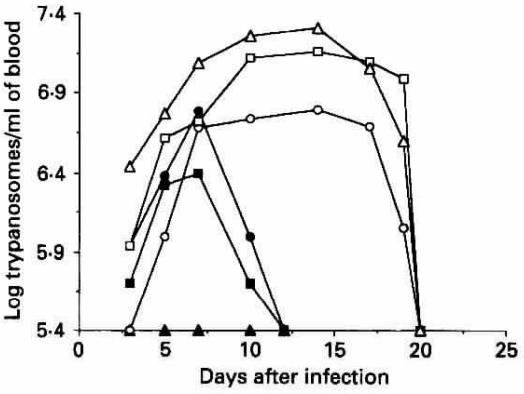

Fig. 2. Time-course of parasitaemia following infection with *Trypanosoma musculi* in inbred mice. Infections were established using 3×10^5 trypanosomes (Partinico II isolate – open symbols; Portuguese isolate – closed symbols). Values are means of log trypanosomes/ml of blood. Log 5·4 represents the limit of detection in these experiments. (●) C57BL/10; (■) BALB/c; (▲) NIH; (○) C57BL/10; (□) BALB/c; (△) NIH.

laboratory. Male mice were used in the majority of experiments and were usually 6–8 weeks of age at infection. Mice were treated with the anthelmintic piperazine (Sigma, 1 g/l) in drinking water prior to infection.

Trypanosomes

The new isolate (designated Portuguese isolate) was originally recovered from *Mus spretus* in Lisbon, Portugal by Dr J. M. Behnke, in 1988. Its morphological identity with *T. musculi* was confirmed by Professor D. H. Molyneux. The parasite has been maintained by weekly passage in susceptible mice. The initial passage was contaminated by organisms identified as *Grahamella* spp. No *Grahamella* has been seen subsequently. The Partinico II isolate was obtained from Dr R. A. Matthews in 1990 and similarly maintained.

Experimental infections were established in groups of 5 or 6 mice by intraperitoneal inoculation of 3×10^4 or 3×10^5 trypanosomes. Challenge infections of 3×10^5 trypanosomes were given 4 weeks after the initial infection.

Parasitaemia was quantified for 3–4 weeks after each infection using the method of Herbert & Lumsden (1976), which was found to give accurate and consistent results and was more convenient than use of a haemocytometer. A slight modification of this matching method was also used especially in infections with the Portuguese isolate in highly resistant NIH mice whose parasitaemias were consistently well below log 5·4/ml, the lower limit of detection with this technique. Approximately 20 μl of heparinized blood was obtained from BALB/c mice with parasitaemias of log 6·8 and log 7·0 trypanosomes/ml, respectively, as determined using the standard haemocytometer counting technique. A drop of Hanks' balanced salt solution containing 0·25 % (w/v) sodium azide was added. The sodium azide slowed the otherwise very rapid and erratic movements of the trypanosomes. Two series of doubling dilutions of each sample were prepared in Hanks' solution, up to 1:256 and 1:512 respectively, equivalent to log 4·6 and log 4·7 trypanosomes/ml. A wet film was prepared from each dilution according to the method of Herbert & Lumsden (1976) and the number of trypanosomes counted in 20 microscope fields at × 400 magnification. If none was present, an area of 40 fields or, if necessary, the entire preparation, was examined. From these counts it was determined that 2–3 trypanosomes and 1 trypanosome in 40 microscope fields were equivalent to log 5·2 and log 5·0 trypanosomes/ml respectively. Dilutions which were negative for trypanosomes in this number of fields but were positive when the entire preparation was examined, were equivalent to log 4·7 trypanosomes/ml. No trypanosomes were found at lower counts. The accuracy of these dilutions, and the corresponding counts, were verified from parallel haemocytometer counts carried out on dilutions up to 1/32. Higher dilutions were unsuitable for counting by this technique.

RESULTS

Primary infections

Primary infections of the three strains of mice with the Portuguese isolate produced three distinctive response phenotypes. C57BL/10 and BALB/c mice were fully susceptible to infection and developed significantly higher ($P < 0{\cdot}05$) peak parasitaemias than NIH mice which were virtually refractory. These patterns of parasitaemia were consistent in a large number of experiments carried out over a 3-year period, during which the isolate was passaged weekly. Fig. 1 shows details of parasitaemia in the three strains of mice in a representative experiment

Table 1. Time-course of *Trypanosoma musculi* infection in inbred strains of mice

	Mean ± S.D. and range ( ) of duration in days				
T. musculi isolate	Pre-patent period	Exponential growth phase	Plateau phase	Immune elimination	Patent period
Portuguese	3·41 ± 0·50 (3–4)	2·79 ± 0·42 (2–3)	4·07 ± 0·90 (3–5)	5·71 ± 0·98 (5–7)	12·89 ± 0·89 (12–14)
Partinico II	3·33 ± 0·75 (3–5)	3·42 ± 1·08 (2–6)	9·96 ± 2·67 (5–14)	2·88 ± 0·88 (2–5)	16·17 ± 2·09 (10–19)

from 1989. The patterns of parasitaemia were also essentially similar whether infections were initiated with 3×10^4 or 3×10^5 trypanosomes, NIH mice remaining refractory at both levels of infection. Infections with the Partinico II isolate revealed completely different response phenotypes. The three strains of mice infected with this isolate were all fully susceptible to infection, with NIH being the most susceptible. Fig. 2 shows the general patterns of parasitaemia with the two isolates in a representative experiment from 1992. The parasitaemia profile of the Partinico II isolate differed from that of the Portuguese in having significantly longer ($P < 0·05$) plateau phase and patent period but shorter immune elimination phase (Table 1).

Challenge infections

All strains of mice were completely resistant to homologous and heterologous challenge given 1 month after the primary infection. The course of challenge infections in age-matched naive controls was found to be identical with that of primary infections in the respective strains of mice (see Fig. 2).

DISCUSSION

Infections in inbred strains of mice provide some of the most useful laboratory models for the study of host–parasite interactions in both rodent and African trypanosomiases (Anderson & Banks, 1982; Morrison & Murray, 1985; Pinder, Chassin & Fumoux, 1986). *T. musculi* infections, in particular, have proved convenient in the study of immunological and genetic factors responsible for the host strain-dependent variability in susceptibility and ability to control infections (Albright & Albright 1981, 1989; Bell *et al.* 1984*a*, *b*; Albright *et al.* 1990*a*). In all these studies the profile of parasitaemia and its intensity were among the important parameters used to assess host susceptibility or resistance to infection. It was therefore of interest to evaluate these two parameters with respect to the new Portuguese isolate from *M. spretus*, an 'aboriginal' species of mice which is more closely related to *M. abbotti* and *M. hortulanus* than to the 'commensal' species, *M. musculus* (Sage, 1981), the original source of the well-known Partinico II isolate.

The pronounced host strain-related influence on susceptibility to infection with the Portuguese isolate and the development of solid acquired immunity to homologous challenge are consistent with previous descriptions of *T. musculi* infections in mice (Viens *et al.* 1974; Albright & Albright, 1981; Albright *et al.* 1990 *b*). However, the sharply contrasting response phenotypes demonstrated for the two trypanosome isolates in 3 strains of mice, which was most evident in NIH mice, the marked differences in the magnitude and duration of peak parasitaemia following infections with the two isolates, and the complete protection produced by each trypanosome infection against heterologous challenge, clearly indicate that the two isolates, though immunologically related, are distinct from one another, and the differences are not a consequence of the recent isolation of the Portuguese trypanosome. The cross-protection between these strains also suggests that the strong resistance of NIH mice to the Portuguese strain is immunologically mediated, a view which is supported by the course of infection in immune-suppressed mice, which are fully susceptible (unpublished data).

It has been suggested (Albright & Albright, 1991) that the maintenance of the relatively long plateau phase in Partinico isolate-induced infections is achieved by a combination of several immunological mechanisms which include (1) depression of the immune response of the host by the direct action of trypanosome-derived substances on B and possibly T helper lymphocytes, (2) impaired cytokine production and activity, and (3) blockage of 'crucial receptors' involved in immune elimination of trypanosomes. The net result is to prolong the survival of the trypanosome population. Similar mechanisms are probably involved in the regulation of infections with the Portuguese isolate, but it would appear that they are far less effective than those operative in infections with the Partinico II isolate, thus enabling immune elimination of the parasite to commence much earlier. The immune clearance of *T. musculi*,

which is believed to require the cooperation of trypanosome-specific antibodies, notably IgG1, IgG2a and IgG2b isotypes, complement, rheumatoid-like factors and activated effector cells such as macrophages and neutrophils (Viens *et al.* 1974; Wechsler & Kongshavn, 1986; Albright & Albright, 1991) is likely to be similar in both infections. The 5–7 days taken for this phase to be completed in infections with the Portuguese isolate is similar to published data on the Partinico II isolate (Viens *et al.* 1974; House & Dean, 1988; Albright & Albright, 1991), although in our model the clearance of this isolate is more rapid.

One of the most striking and most interesting differences between the two isolates of *T. musculi* is seen when infections are established in NIH mice. This strain is fully susceptible to the Partinico isolate, but almost refractory to the Portuguese isolate, parasitaemia being consistently well below log 5·4 trypanosomes/ml. The ability of this inbred strain to respond so effectively to the Portuguese isolate (apparently immunologically mediated) and to express complete cross-immunity against heterologous challenge, suggests that Portuguese trypanosomes may express more of the common immunodominant antigens involved in immunity, express additional antigens, or interfere less effectively with the development of protective immunity, all of which can be tested experimentally. The lower parasitaemias seen in BALB/c and C57BL/10 mice infected with the Portuguese isolate support this view, but clearly there is some unique factor influencing the host–parasite relationship between NIH mice and *T. musculi*. The availability of the new isolate therefore enhances the value of this immunologically and genetically well-defined model for analysis of factors influencing host-protective responses to trypanosome infections.

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