Ablastin: Control of *Trypanosoma musculi* Infections in Mice

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*Trypanosoma musculi* infections in CBA mice consist of a phase of increasing parasitemia during which dividing forms of the parasite are present in the blood, followed by a period when only nondividing trypomastigotes are seen. A second crisis terminates the blood infection and leaves the host immune, but small numbers of trypanosomes, including multiplicative forms, persist in the kidneys for many months. Studies were made involving infections in T-lymphocyte deprived mice, the effects of passive transfer of serum and cells, measurement of DNA synthesis by the parasite, serological responses, and *in vitro* effects of serum on the trypanosomes. These indicated that the initial check on the increase in blood parasitemia is due in part to two humoral factors. One of these has a trypanocidal effect (this is thought to be an IgM antibody) while the other, which may be an IgG antibody, is the ablastin that inhibits further reproduction by the parasite. Both trypanocidal and ablasic effects were demonstrable in the serum of immune mice yet the parasite was still able to survive and multiply in the kidneys.

**INDEX DESCRIPTORS:** Ablastin; *Trypanosoma musculi*; Immunity; Mice; *Herpetosoma*; T-lymphocyte deprivation; Agglutination; X-irradiation; Cyclophosphamide; Opsonin; Neutralization; Kidneys; DNA Synthesis.

**INTRODUCTION**

The development of acquired immunity in rats to *Trypanosoma* (*Herpetosoma*) *lewisi* has been studied extensively and, in his review of the immunology of the rodent stercorarian trypanosomes, D’Alesandro (1970) considered that *T. lewisi* is a valid model for the group as a whole. This was justifiable on the basis of the limited observations that had been made on the host responses to the other species, but it was in part this fact—that so little is known about immunity to other *Herpetosoma* species—that led us to start our present investigations. We are making a detailed study of the immunological responses of mice to *T. musculi* in order to establish the role and interactions of the different components of the immune system and the functional sequence of events responsible for initial control of infection and elimination of parasites from the blood. Full details of the results we have obtained so far are published (Viens et al. 1974; Targett and Viens 1975; Viens, Targett, and Lumsden 1975), but we shall consider here our observations that are important in relation to the possible role of a reproduction-in-
hibiting factor (ablastin) in control of the infection.

**Experimental Results**

*T. musculi* Infections in Normal CBA Mice

The parasite closely resembles *T. lewisi* both morphologically (Hoare 1972) and in its reproductive characteristics (Deane 1969). After a latent period, which is determined by the size of the parasite inoculum, there is a phase of rapidly increasing parasitemia with multiplicative forms (mostly epimastigotes with a few dividing trypomastigotes) present in the blood, although the most active reproduction occurs in the kidney capillaries (Wilson 1971). The level of parasitemia becomes stabilized after 6–7 days and gives rise to a plateau phase lasting about 10 days, during which the blood forms are monomorphic, consisting entirely of long slender “adult” trypomastigotes (Taliaferro et al. 1931). The onset of the plateau phase with the elimination from the blood of all reproductive forms of the parasite we call the first crisis, by analogy with a similar response in *T. lewisi*-infected rats (Taliaferro 1932). A second crisis at the end of the plateau phase produces an abrupt fall in parasitemia, and parasites disappear from the blood within a few days (Fig. 1); subinoculation into clean, irradiated C3H mice of blood taken 7 days after infected mice had become aparasitemic by direct examination revealed that the blood was in fact parasite-free. Such mice do not show a relapse or recrudescence of infection, even after immunodepressive treatment such as T-lymphocyte deprivation, and are immune to homologous challenge. We have shown, however, that multiplicative, infective parasites are still present in the vasa recta of the kidneys of mice which had recovered from *T. musculi* infection about one year previously (Viens et al. 1972). The infectivity of these parasites was demonstrated by inoculation of infected kidney tissue intraperitoneally into normal mice. The pattern of infection obtained was similar to that described above, and illustrated in Fig. 1, although the maximum parasitemias obtained were generally lower.

**Infections in CBA Mice Immunodepressed by T-lymphocyte Deprivation**

CBA mice were T-cell deprived when 6–8 wk old by thymectomy followed, one week later, by lethal X-irradiation (850 rad) and injection with syngeneic bone marrow cells (Leuchars 1966). The prepatent period after inoculation and the phase of increasing parasitemia in these mice were similar to those in intact, infected CBA mice, but the plateau phase was established at a higher level. Thereafter, the parasitemia remained relatively constant or increased slowly until shortly before the mouse died, which has so far proved to be the inevitable outcome with more than 100 deprived mice, when it rose.

![Graph showing parasitemia](image)
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Fig. 2. Trypanosoma musculi infection in deprived CBA mice. Parasitemias are expressed as numbers of parasites (in thousands) per mm² of pooled blood. The "deprived" group initially consisted of 14 mice. D = death of one mouse.

Fig. 3. The parasitemias and proportions of multiplication forms in the blood of two deprived mice infected with Trypanosoma musculi. D = death of mouse.

The time after inoculation when death occurred varied from about 40 days to more than 350 days (Fig. 2). Multiplicative forms of the parasite persisted throughout the infection without breaking the equilibrium until just before the animal died (Fig. 3). A similar pattern of infection was obtained in mice treated with antithymocyte serum (ATS), although recovery eventually occurred about 70 days after ATS treatment.

Monospecific fluorescent antibody titers were measured in intact and in deprived infected mice. In intact mice, IgG1, IgG2, and IgM antibodies appeared simultaneously and quickly reached high levels which were maintained for a long period of time. In infected, deprived mice, IgM titers were similar to those of the intact controls, but IgG levels were reduced, production of IgG1 being suppressed completely for 35 days and reaching only low levels thereafter.

Passive Transfer of Immunity

Immune serum (obtained from blood collected 7 days after donor mice had become aparasitemic) inoculated intravenously into recipients just prior to intraperitoneal injection of a mixed population of dividing and adult forms prolonged the prepatent period, and the subsequent parasitemia was reduced. This was also true when the inoculum consisted of $3 \times 10^4$ adult trypomastigotes. Treatment of intact infected mice, shortly after their infections had become patent, with serum collected during the plateau phase of the infection checked the rise in parasitemia, reduced the numbers of multiplicative
forms in the blood, and inhibited DNA synthesis by the parasite (Viens and Taggett 1972). Immune serum had a similar though less marked effect, and serum from deprived mice had a partial inhibiting effect on the parasitemia but no effect on DNA synthesis. Transfer of these sera to infected deprived mice produced similar though less dramatic results. Inoculation of immune serum into infected mice at a stage when only "adult" nondividing forms were present did not influence the course of infection, but simultaneous inoculation of immune serum (given intravenously) and a population consisting solely of adult forms ($10^9$ organisms given intraperitoneally) resulted in a delay in the onset of patency, when compared with control infections, which may be explained as a temporary inhibition of parasite multiplication, as a trypanocidal effect against adult trypanosomes, or as a combination of the two.

The adoptive transfer of immune lymphocytes ("nonadherent cells") from the spleen or from peritoneal exudates to mice in which the infection had reached the plateau (nondividing) phase accelerated the elimination of parasites from the blood, and this effect was not influenced by the simultaneous administration of immune serum (or, incidentally, of immune peritoneal macrophages). Lymphocyte transfer carried out at the time of parasite inoculation produced an extension of the prepatent period and a reduction of the subsequent parasitemia—a result similar to that obtained by transfer of immune serum. It appears, therefore, that the transferred lymphoid cells act on dividing parasites by synthesizing the serum factors responsible for the "first crisis," but that they may be effective in some other way against adult parasites.

Agglutination and Neutralization Tests

Agglutinating antibodies could not be demonstrated in sera from infected, immune, or immunized mice, although rabbits and guinea pigs immunized with a parasite extract in Freund's complete adjuvant developed high agglutinin titers. Parasites obtained from X-irradiated or from cyclophosphamide-treated mice (containing about 20% multiplicative forms) or from normal infected mice (adult parasites only) were compared in these tests and gave similar results. Antiglobulin tests also failed to reveal "monovalent" agglutinating antibodies. It is interesting that, in his original description of this parasite, Kendall (1906) reported failure to produce agglutination.

Sera from mice which had recovered from infection did, however, have a marked neutralizing effect in vitro on the infectivity of the homologous parasites, although the numbers of live organisms were not reduced during the period of in vitro incubation. The neutralization test did not reveal antigenic differences between the original population of parasites and populations isolated from deprived mice, passively immunized animals, or from mice in which infection was initiated from persisting kidney forms. The neutralizing effect is not due to ablastin, which is effective only when present in high concentration in the blood. We do not rule out the possibility that it is due to an opsonin since phagocytosis of the parasites by immune adherent cells (macrophages) occurs in vitro in the presence of the immune serum.

**Discussion**

**Inhibition of Parasite Reproduction**

Trypanosoma musculi infection in CBA mice is characterized by a series of distinct phases. After the prepatent period there is a phase of rapidly increasing parasitemia with dividing trypanosomes present in the circulation. The level of parasitemia then becomes stabilized abruptly and multiplicative forms of the parasite are cleared.
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from the blood. This "first crisis" is the stage in the infection we are concerned with here, and we shall consider separately the reproducing forms that persist in the kidneys of immune animals.

The passive transfer experiments showed that the first crisis is due, at least in part, to humoral factors. By measuring levels of parasitemia, proportions of multiplicative forms, and DNA synthesis, it was possible to distinguish between two effects on the infection at this stage, one producing a reduction in total numbers of organisms and the other an inhibition of reproduction. The ablastic effect was more marked with serum collected after the first crisis, but at a time when the animals were still infected, than with serum obtained after the mice had become aparasitemic. This is in line with the observation by Coventry (1925) that ablastin titers in T. lewisi infections reached a peak after the first crisis but then declined. The fact that passive transfer was apparently less effective in deprived than in normal mice would indicate that the recipients' own immune mechanisms must be intact to extend or supplement the effect due to the transferred immunoglobulin.

Our conclusion that control of the infection at the first crisis is due to two serum factors, one having a trypanocidal effect and the other inhibiting reproduction, is in agreement with Taliaferro's concept of control of T. lewisi infections at this stage (Taliaferro 1932), which is widely accepted (see D'Alesandro 1970). Alternative ideas that have been put forward would not explain our results as well. Thus, Ormerod (1963) postulated that trypanocidal antibody alone was responsible for control of T. lewisi infection at this stage (but see Ormerod 1975), and Chandler (1958) thought that ablastin was entirely responsible for the first crisis. In T-cell deprived mice, T. musculi continues to show multiplication throughout the infection, and this implies that deprivation has removed the factor responsible for inhibition of reproduction of the parasite (ablastin). Ablastin production is thus thymus dependent. However, blood of deprived mice must contain an antibody or other factor with a trypanocidal effect; otherwise a fulminating and fatal infection would result in these animals (as occurs in irradiated mice) rather than the equilibrium that is established. The presence of such a factor was indicated in passive transfer experiments with serum from deprived, infected mice where there was a transitory but nevertheless clearly demonstrable effect on parasitemia in the recipient animals (Viens et al. 1974).

D'Alesandro (1970) considers that ablastin produced during T. lewisi infections is an IgG antibody which has peculiar properties of low avidity and nonadsorbability. He believes, too, that the first trypanocidal antibody is also IgG and is directed against division forms only, these being antigenically different from the succeeding monomorphic "adult" population; antigenic variation is thought to be limited to the production of only two antigenic types. Our results with T. musculi infections are also best explained in terms of a difference in antigenicity between dividing parasites and the adult forms. We have as yet no direct evidence on the nature of the trypanocidal antibody which removes dividing forms other than the fact that it appears to be a thymus independent antibody. The antiparasitic antibody, which was unaffected by T-cell deprivation (see Taylor and Wortis 1968; Davies et al. 1970) and is therefore thymus independent, is IgM, and on this basis we suggest that the first trypanocidal antibody is IgM.

We have avoided describing ablastin as an antibody since, at this stage, we have little information about its physicochemical characteristics. However, its production appears to be thymus dependent since it did not appear to be present in infected,
deprived mice and, in such mice, IgG production was suppressed with a particularly marked reduction in the production of IgG1 (see Torrigiani 1972).

Thus, in summary, we suggest that dividing parasites (first antigenic variant) stimulate two systems: (1) B-lymphocytes synthesize antibody (IgM?) which has either a direct trypanocidal effect or an indirect action involving macrophages. D'Alesandro's review (D'Alesandro 1970) provides fairly strong evidence for a purely humoral response in T. lewisi infections, and this is supported by some recent studies (e.g., Greenblatt et al. 1972) although the possible involvement of cellular elements cannot be ruled out (see Greenblatt and Tyroler 1971). The trypanocidal antibody production is thymus independent but is suppressed by irradiation. (2) The second system is thymus dependent, sensitized T-cells activating B-cells to produce a reproduction-inhibiting ablastin (IgG1?).

We are comparing serum fractions collected at the “first crisis” in intact infected mice and at a corresponding time in T-cell deprived, infected mice in an attempt to define the immunochemical characteristics of ablastin and of the trypanocidal antibody.

**Persistence of T. musculi in the Immune Host**

Since we are concerned here only with the role of ablastin in the immune response we shall not discuss the sequence of events leading to elimination of parasites from the blood. There remains, however, the problem of the survival of T. musculi in the vasa recta of the kidneys of mice which have recovered from infection and are immune to homologous challenge (Viens et al. 1972). In his hypothesis on the nature of immunity to T. lewisi, Ormerod (1963) suggested that there was replenishment of parasites removed from the blood by low-grade reproduction occurring in the blood vessels of the kidneys outside the peripheral circulation—in other words, in the site where we have demonstrated the persistence of T. musculi. Although we have not examined the kidneys of parasitemic rats for evidence of continued division after the first crisis, we have made an extensive study of the kidneys and other organs of rats which have recovered from T. lewisi to see whether anything comparable to the survival of T. musculi occurs. We have not so far been able to find persisting forms (Wilson et al. 1973). Recently, Lee and Lincicome (1972) studied the duration of immunity to T. lewisi by challenging rats with a small inoculum (100 organisms) at intervals up to 505 days after an initial immunizing infection. Challenge infections given up to 234 days after the initial inoculum did not lead to the production of demonstrable parasitemias but, in rats challenged after 324, 414, or 505 days, dead intact trypanosomes were seen in the peripheral blood. They suggest that development of the parasites must have occurred somewhere outside the bloodstream (see Ormerod 1963; Ormerod and Killick-Kendrick 1956) and that there are tissue developmental forms which appear in peripheral blood only under special circumstances. It seems likely that, in rats that had recovered from infection 324-505 days previously, a challenge inoculum would be able to develop to some extent before being controlled by the secondary immune (anamnestic) response. In experiments in which we gave a homologous challenge inoculum intravenously to mice which had recovered from infection 1 or 11 mo previously, two quite different responses were seen. In the first group, the inoculated parasites were removed from the blood very rapidly, most of the parasites having disappeared within a few hours. In the mice which had recovered 11 mo previously, it was 3-4 days before a dramatic fall in parasite numbers
occurred, leading again to complete elimination of the infection.

D'Alesandro (1970) criticized Ormerod's concept of parasites persisting and reproducing in the kidney capillaries, partly on the grounds that "it is not explained how the cryptic division forms would escape destruction, since they would still be bathed by antibody-containing blood." However, this is the situation that exists in mice immune to T. musculi. Parasites in all stages of division and development occur in the vasa recta of the kidneys despite the presence in the blood of ablastin and of trypanocidal antibody. An immune response requiring cell-to-cell interaction could well be ineffective in certain anatomical sites and this may in part explain the survival of the parasites: our results do not rule out the participation of such a mechanism in the ablasic phenomenon. It is wrong, too, to consider that humoral factors will operate equally effectively in all parts of the circulatory system; the protection of lymphocytes from the effect of antilymphocyte serum while they are within lymph nodes is one example (Mitchison 1970; Levey 1970). These parasites are present in relatively small numbers, and multiplication by the parasite includes the formation of large rosette-forms which might block the vasa recta so effectively as virtually to exclude the parasites from the general circulation. We have failed, so far, to demonstrate that the survival of the parasites could be effected by repeated antigenic variation, although we are still investigating it. If it does occur, then a mechanism similar to that proposed by Brown (1971) to explain the persistence of Plasmodium knowlesi in immune monkeys might operate.

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