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Cure of *Trypanosoma musculi* Infection by Heat-Labile Activity in Immune Plasma

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Passive transfer of plasma from a mouse cured of parasitemia to a *Trypanosoma musculi*-infected host rapidly eliminates parasitemia; this curative activity, presumably mediated by an immunoglobulin, is sensitive to heat treatment (56°C, 30 min). In addition, pretreatment with immune plasma, even after heat treatment, prevents the development of a patent parasitemia in a naive host (protective activity).

Trypanosoma musculi, a natural parasite of mice, produces a characteristic, self-limiting infection which lasts for ca. 3 weeks and comprises a growth phase, a plateau phase, and an elimination phase (15, 16, 19). Previous observations in immunodeprived mice have indicated that both T-cell (4, 12, 19) and B-cell (17) function must be intact to allow development of the process which brings about elimination of parasitemia. The most obvious deduction is, therefore, that this is an immune process in which a T-cell-dependent anti-trypanosomal antibody plays a critical role. If so, the passive transfer of serum from an immune host to an infected mouse should have the capacity to terminate the infection. Reports on the success of this procedure, however, have been equivocal (2, 14, 16, 18, 19), and it has clearly been ineffective in the hands of recent investigators. Therefore, elimination of *T. musculi* by a humoral antibody-dependent process has been seriously questioned (2). The present communication demonstrates that passive transfer of plasma from a cured mouse brings about rapid and complete elimination of parasitemia from an infected host; this curative activity is lost by heat treatment. A naive host is also protected from developing a patent parasitemia by pretreatment with immune plasma, even when heat treated (protective activity).

Groups of four to five adult male or female C57BL/6 mice (Charles River Breeding Laboratories, Inc., St. Constant, Québec, Canada) were infected intraperitoneally with 10^4 trypanosomes in 0.2 ml of buffered saline, and parasitemia was determined in heparinized retro-orbital blood samples (19). Individual counts were determined and expressed as $\log_{10}$ values of the number of parasites per milliliter of blood, giving the mean value of each group ±1 standard error of the mean.

Immune plasma was prepared from *T. musculi*-infected C57BL/6 retired breeders which were bled out by cardiac puncture 28 to 30 days postinfection (p.i.) with heparin. The plasma was separated by centrifugation and stored at −20°C. Normal mouse plasma was prepared similarly from noninfected mice. Where desired, plasma was heat inactivated by incubation in a 56°C water bath for various times as indicated below. A 0.4-ml injection of plasma was given to each mouse intravenously unless stated otherwise.

Immune plasma was first tested for its curative effect when administered to mice during the course of infection. All mice were infected with trypanosomes, and the normal course of parasitemia was followed in one group of mice which received no further treatment (Fig. 1a). The typical phases of parasitemia were seen, namely, the growth period (0 to 8 days p.i.), the plateau phase (8 to 18 days p.i.), and the elimination phase (18 to 22 days p.i.). Two other groups of infected mice were treated with immune plasma or normal mouse plasma, respectively, on day 13 p.i. (Fig. 1b). The parasitemia cleared completely within 24 h of injection of immune plasma, and the blood remained aparasitemic. A 0.2-ml dose of immune plasma was ineffective, whereas a 0.8-ml dose produced a result similar to that seen with a 0.4-ml dose (data not shown). Intraperitoneal administration of immune plasma was as effective as intravenous administration, but subcutaneous administration was significantly less effective (Fig. 1c). When the immune plasma was heat inactivated for as little as 30 min., its ability to eliminate parasitemia in an infected mouse was abolished (Fig. 1d). Even 0.8 ml of heat inactivated immune plasma was ineffective in curing parasitemia.

Intravenous administration of immune plasma at any time during the plateau phase cured the parasitemia. Thus, a single 0.4-ml dose of immune plasma was given intravenously to individual groups of mice 7, 10, 12, 14, and 16 days p.i.; 24 h later, the parasitemia counts fell from mean values which ranged between 6.3 and 6.6 $\log_{10}$ *T. musculi* per ml of blood to zero values, and counts remained at zero in all cases. Normal mouse plasma given to control groups had no effect.

Immune plasma was then tested for its ability to protect a naive mouse from developing trypanosomal infection when the plasma was given before inoculation of the parasite. Accordingly, groups of mice were either nontreated or treated with immune plasma, normal mouse plasma, or 30-min heat-inactivated immune plasma; all of the mice were inoculated with *T. musculi* 1 h later. Immune plasma, both nontreated and heat inactivated, completely protected the mice from developing parasitemia (Fig. 2). Heat inactivation for up to 2 h was equally ineffective in abolishing this protective activity of immune plasma (data not shown).

These findings demonstrate that passive transfer of immune plasma from cured mice, when given in adequate amounts (0.4 ml) at any time during the plateau phase, brings about a permanent cure of the blood parasitemia in infected recipients. This curing activity presumably represents the same immune process whereby elimination of parasitemia develops in the final phase of the normal infection. As mentioned above, indirect evidence strongly suggests that this elimination process is brought about by an immunoglob-

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ulin, particularly since parasitemia is not eliminated in B-lymphocyte-deprived mice; such mice have normal T-cell function but lack B-cell function and fail to develop specific anti-trypanosomal antibodies (17). Thus, it seems reasonable to assume that the curative activity observed in immune plasma is due to a specific anti-trypanosomal antibody; yet, the curative mechanism apparently is not a complement-mediated lytic process, since trypanosomal elimination occurs normally in C5-deficient mice (6). Other evidence from our laboratory (P. A. L. Kongshavn, C. St.-Charles, D. Wechsler, and W. Rapattoni, Fed. Proc. 41:1821, 1982) suggests that a cell also participates in the curative process. The latter could, therefore, be an antibody-dependent cellular cytotoxic mechanism involving immunoglobulin and an effector cell (neutrophil, eosinophil, platelet, or macrophage) similar to that seen in defense against metazoan parasites (reviewed in reference 8). Alternatively, the elimination process may comprise an antibody-mediated phagocytic clearance of trypanosomes within the liver and spleen of infected hosts, resembling that proposed for the closely-related *Trypanosoma lewisi* infection in rats (7).

The class of immunoglobulin purportedly mediating cure of parasitemia is not known but is apparently T-cell dependent (4, 12, 19). Immunoglobulin M (IgM) seems an unlikely candidate, particularly since its production was found to be normal in T-cell-deprived infected mice (5, 19). Moreover, initial partial purification of immune plasma by molecular sieve chromatography has indicated that the curative activity resides in a fraction with a molecular weight of less than 200,000 (unpublished data), i.e., within the range of IgG or IgE. The curative activity is unstable when heated and less effective when administered subcutaneously, possibly because it is homocytotropic. Reaginic antibody of the IgE class is distinctive in being thermolabile and retained in the tissues (9). However, until purified, it cannot be assumed that the putative immunoglobulin in question is intrinsically thermolabile. It is also possible that the curative activity is mediated by one of the subclasses of IgG, all of which have been implicated in antibody-dependent cellular cytotoxicity and antibody-promoted phagocytosis (11). Certain subclasses of human (13) and mouse (3) IgG are sensitive to the proteolytic activity of proteases; for example, mouse IgG_1 and IgG_3 are cleaved by macrophage-derived elastase (3). The loss of curative activity observed upon heating or subcutaneous administration of immune plasma may be a result of proteolytic cleavage of an IgG subclass, rendering it incapable of eliminating parasites, e.g., through an F_c receptor-mediated mechanism.

Two reasons for the success of our experiments in curing parasitemia may be suggested. First, in some of the earlier experiments, heat-inactivated serum reportedly was used (16); also, the serum was given subcutaneously (16, 18). Second, our experiments were done in genetically resistant C57BL/6 mice (1, 10) in which the parasitemia plateau is ca. 10-fold lower than in the genetically susceptible mouse strains used by others (2, 16, 18, 19). Indeed, we have

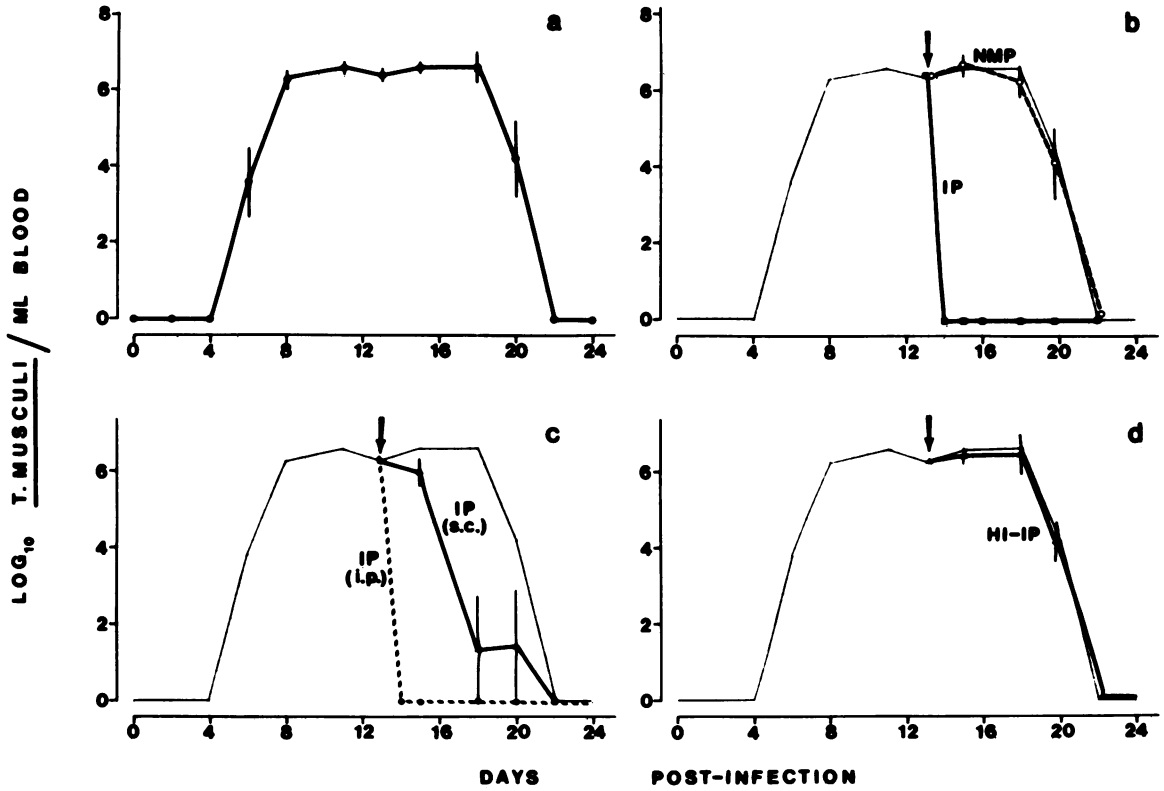

FIG. 1. Course of parasitemia in C57BL/6 mice inoculated on day 0 with 10^4 *T. musculi*. (a) Mice received no further treatment. (b) On day 13 (arrow) each mouse received intravenously 0.4 ml of immune plasma (IP, ■) or normal mouse plasma (NMP, ○); shaded line shows no-treatment group. (c) On day 13 (arrow) each mouse received 0.4 ml of immune plasma subcutaneously (IP [s.c.], ▲) or intraperitoneally (IP [i.p.], ●); shaded line shows no-treatment group. (d) On day 13 (arrow) mice received intravenously 0.4 ml of immune plasma, heat inactivated for 30 min at 56°C (HI-IP, △); shaded line shows no-treatment group. Each point represents mean of four to five mice ±1 standard error of the mean.

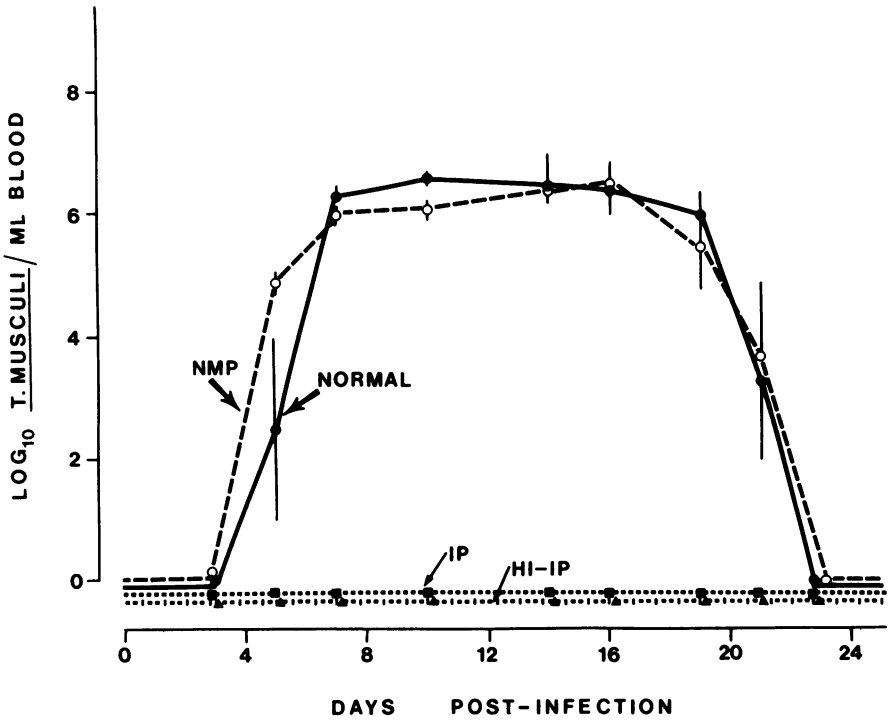

FIG. 2. Course of parasitemia in C57BL/6 mice inoculated on day 0 with 10^4 *T. musculi* 1 h after the following treatment: no treatment (normal, ●), 0.4 ml of normal mouse plasma intravenously (NMP, ○), 0.4 ml of immune plasma intranvenously (IP, ■), 0.4 ml of heat-inactivated (56°C for 30 min) immune plasma intravenously (HI-IP, ▲).

evidence that the ability of immune plasma to cure *T. musculi*-infected mice varies with the strains of mice employed; C3H/HeN cannot be cured, for example, whereas DBA/2 mice are cleared of parasitemia as easily as C57BL/6 mice (unpublished data).

The protective activity observed in immune plasma (Fig. 2) is presumably the same as that reported previously by others and is attributed to the activity of specific anti-trypanosomal antibody. Immune serum administered shortly before inoculation of parasites provided either partial protection (14, 19) or else completely prevented the establishment of an infection (2). This putative immunoglobulin is stable to heat treatment, suggesting that it is different from the curative activity.

In summary, immune plasma contains a curative activity, presumably an immunoglobulin (not IgM), which is sensitive to heat treatment. A heat-stable protective activity, also observed in immune plasma, confirms the findings of others and is presumably also immunoglobulin in nature.

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