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Failure To Demonstrate a Major Role for Kupffer Cells and Radiosensitive Leukocytes in Immunoglobulin-Mediated Elimination of *Trypanosoma musculi*

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Previous studies have indicated that elimination of parasitemia in *Trypanosoma musculi* infection is brought about by immunoglobulin G2a antibodies, C3, and an effector cell. Experiments were designed to identify the putative effector cell by using several approaches. Infected C5-deficient or C5-sufficient mice treated with silica particles or given 900 rads of radiation 3 days earlier effectively eliminated trypanosomes following administration of immune plasma (IP). Silica-treated, noninfected mice given *T. musculi* preincubated with IP also cleared the parasites. Radiolabeling studies revealed that uptake of the cleared trypanosomes by the liver in normal mice was relatively low (24%) and fell only slightly (19%) in silica-treated mice. In contrast, uptake of radiolabeled sheep erythrocytes by the liver was normally much higher (47%) and fell drastically (7%) in silica-treated mice. Mice were then immunocompromised by 900 rads of radiation, silica particles, and anti-platelet serum combined before IP-sensitized trypanosomes were given. Leukocyte and platelet counts were both reduced by 95% and sheep erythrocyte uptake by the liver fell from 77 to 5%; however, >99% of the injected trypanosomes were cleared in these mice and uptake of radiolabeled trypanosomes by the liver was similar to that of normal mice. Lastly, in anesthetized mice in which Kupffer cells were excluded surgically from the circulation, >99% of the IP-sensitized trypanosomes disappeared rapidly from the blood. Only 7% of the radiolabel was found in the liver versus 60% in sham-operated mice. The results are interpreted as showing that hepatic Kupffer cells play a minor role in the immune elimination of *T. musculi*. Likewise, radiosensitive leukocytes and platelets are unlikely to be sole candidates for the putative effector cell that mediates a cure of murine trypanosomiasis.

Trypanosoma musculi is a natural parasite of mice. It produces a characteristic infection lasting for approximately 3 weeks and comprises an exponential growth phase, a plateau phase, and an elimination phase (10). Recent studies in our laboratory have demonstrated that parasitemia can be eliminated in various strains of infected mice by passive transfer of immune plasma (IP) from cured mice (12, 15) and that this curative activity is associated with the immunoglobulin G2a fraction of IP (14, 15). A requirement for component 3 of complement (C3) in the elimination process has also been demonstrated (13). Mouse strains deficient in component 5 of complement (C5) are able to cure the natural infection (4, 15), and passive transfer of C5-deficient IP into infected C5-deficient mice brings about elimination of parasites as effectively as in normocomplementemic strains (15). In view of the absence of activated late-acting complement components to mediate trypanolysis in C5-deficient mouse strains, it is logical to conclude that antibody and C3 act in concert with an effector cell to destroy trypanosomes in this situation. This idea is supported by our recent observations that when *T. musculi* organisms are cultured in vitro with IP (with C3), the parasites are not destroyed when IP from C5-deficient animals is used (15). In all likelihood, the putative effector cell is not present (or not present in sufficient numbers to be effective) in the culture system. It should be noted that in the presence of C5, the parasites are killed in this in vitro system (15). Thus, it appears that the major mechanism of trypanosome elimination, and indeed, the only one in C5-deficient mice, is an immunoglobulin- and C3-dependent, cell-mediated event.

One likely candidate for the effector cell is the fixed liver macrophage or Kupffer cell. Earlier studies by Holmes et al. and MacAskill et al. have shown that the liver plays a major role in immune clearance of *T. brucei* from the circulation in mice (3, 5). Similarly, Dempsey and Mansfield reported that the liver was the primary organ of clearance in mice infected with *T. rhodesiense* (1). Further, Vincendeau et al. (11) and Ferrante (2) have shown in vitro that *T. musculi* is phagocytosed and killed by peritoneal macrophages in the presence of immune serum, suggesting a role for mononuclear phagocytes in trypanosome elimination. Vincendeau et al. showed that antibodies of the immunoglobulin G2a and G1 isotypes were responsible for the phagocytic event.

The present experiments were done to identify the putative effector cell in the cure of *T. musculi* infection. The approach used was to immunodeprive mice of one or more cell types and assess their ability to overcome infection following passive transfer of IP or to eliminate IP-sensitized *T. musculi* from the circulating blood. Particular attention was paid to the role of Kupffer cells, but the data obtained failed to support the idea that these are the major effector cells.

MATERIALS AND METHODS

Mice, parasites, and infection. Mice used for experiments were 6- to 10-week-old male and female animals obtained from Charles River Breeding Laboratories Inc., St. Constant, Quebec, Canada, or Jackson Laboratory, Bar Harbor, Maine, or bred in our laboratory from breeders purchased

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from Jackson Laboratory. Retired female breeders were usually used for preparation of IP. Mice were infected intravenously (i.v.) with an inoculum of 10^4 *T. musculi* (Partinico II strain) organisms, and parasitemias were monitored as described previously (13).

Plasma preparation. IP was obtained from C5-deficient B10.D2/oSn or A/J mice 28 days postinoculation (p.i.) approximately 1 week after clearance of infection (13). Normal mouse plasma (NMP) was obtained from noninfected mice of the same strain. When required, plasma was heat treated by incubation at 56°C for 30 min (13).

Preparation of [^{75}Se]methionine-labeled trypanosomes. Donor A/J strain mice were irradiated (550 rads) and infected with 10^4 trypanosomes. When the parasitemia was 10^8 (usually 7 to 9 days p.i.), the mice were injected i.v. with 10 μCi of [^{75}Se]methionine (specific activity, 20 to 50 Ci/mmol). At 18 h later, the mice were exsanguinated with heparin as the anticoagulant. The pooled blood was diluted 1:10 with phosphate-buffered saline (PBS)–glucose (pH 8.0), and the trypanosomes were separated from the blood cells by being washed four or five times with PBS-glucose with slow-speed centrifugation (200 × *g* for 5 min). The supernatants from the washings were pooled, and the parasites were pelleted by centrifugation at 1,200 × *g* for 15 min. The pellet was washed with PBS-glucose after which the trypanosomes were enumerated and their radioactivity was determined. The trypanosomes were suspended in PBS-glucose to the desired concentration. In most experiments, the trypanosomes were sensitized by incubation with a 1:4 dilution of IP in a shaking water bath at 37°C for 30 min. Trypanosomes for control groups were incubated similarly in NMP. The parasites were checked to determine that no agglutination had occurred and then injected into mice in a volume of 0.2 to 0.3 ml without washing away of the mouse plasma.

^{51}Cr-labeled SRBC. One milliliter of saline-washed, pelleted sheep erythrocytes (SRBC; Institut Armand Frappier, Laval, Quebec, Canada) was incubated in a shaking water bath for 1 h at 37°C with 100 μCi of ^{51}Cr (Dupont, NEN Research Products, Lachine, Quebec, Canada) per ml (specific activity, 200 to 900 Ci/g) and washed three times with PBS. The SRBC were sensitized by incubation as described above, with rabbit anti-sheep hemolysin at a subagglutinating titer. After being washed three times with PBS, the cells were adjusted to the required concentration and 0.2 ml was given to each mouse.

Injection of labeled trypanosomes or SRBC and sampling. The suspension of ^{75}Se-labeled trypanosomes or ^{51}Cr-labeled SRBC was injected i.v. into the tail veins of mice. The number of parasites or SRBC given varied in different experiments. Except when specified otherwise, 1 h later the animals were bled from the retroorbital sinus under ether anesthesia and livers, kidneys, and lungs were removed for radioactivity determination. The total blood radioactivity was estimated by multiplying the radioactivity of a known volume (200 or 500 μl) of blood by a correction factor (0.067 ml/g of body weight) (3). The radioactivity of each organ was expressed as a percentage of the total injected radioactivity. Radioactivity determination was done with a Gamma 310 counter (Beckman Instruments, Inc., Fullerton, Calif.). The ^{75}Se isotope was counted on the ^{125}I channel.

Irradiation. Mice were irradiated in a Plexiglas (Rohm & Haas Co., Philadelphia, Pa.) box with a Gamma-cell 40 (cesium 137 source) irradiator (Atomic Energy of Canada Ltd.) by giving a dose of 550 or 900 rads at a rate of 145 rads/min.

Silica treatment. Silica particles less than 5 μm in diameter were prepared for inoculation by sonicating a washed, dried, autoclaved suspension of 10 to 12 mg of silica (no. 216 min-u-sil; Whittaker, Clarke, and Daniels, Inc., Plainfield, N.Y.) per ml in saline immediately before administration to prevent particles settling. Mice received 3- or 5-mg doses i.v. on the days indicated in the footnotes to Tables 1 and 3.

CVF. Cobra venom factor (CVF; Cedarlane Laboratories, Hornby, Ontario, Canada) was administered intraperitoneally to mice in two 10-U doses at 24 h and 1 h before injection of trypanosomes into the mice.

Occlusion of hepatic circulation. Mice were anesthetized with pentobarbital sodium (Nembutal; 50 mg/kg intraperitoneally), diazepam (5 mg/kg intraperitoneally) and atropine sulfate (0.02 to 0.05 mg/kg subcutaneously). The peritoneal cavity was opened, and the hepatic portal vein and artery were clamped with a hemostat. Other control mice underwent sham operations in which the peritoneal cavity was opened and the liver was disturbed.

Leukocyte and platelet enumeration. Total leukocyte and platelet counts were performed by standard techniques.

Anti-platelet serum. Rabbit anti-platelet serum was prepared by immunizing two rabbits intradermally each with 6 × 10^8 mouse platelets separated from whole blood, followed by a booster injection of 1.5 × 10^9 platelets 2 weeks later. After a further week, the rabbits were bled by cardiac puncture, the serum was collected, and small samples were absorbed three times with equal volumes of a mixture of washed mouse spleen, thymus, and liver cells. Mice received 0.2-ml doses of the absorbed antiserum intraperitoneally.

RESULTS

Effect of irradiation or silica treatment on elimination of trypanosomes following IP treatment. Groups of B10.D2/nSn and B10.D2/oSn strain mice were infected with 10^4 parasites and were irradiated (900 R) on day 10 p.i.; given three 3-mg doses of silica on days 10, 11, and 12 p.i.; or left untreated. On day 12 p.i., all of the mice received 0.4 ml of IP. Another group of untreated infected mice received heat-treated IP as a control, since heat treatment destroys the curative activity (12). Parasitemia was measured before plasma administration on day 12 p.i. and 24 and 48 h after plasma administration.

In mice that received IP, the number of trypanosomes in the blood fell to undetectable levels, despite prior exposure to either irradiation or silica particles (Table 1). Thus, mice immunodeprived by these methods retained the ability to eliminate *T. musculi* in the presence of IP.

To confirm the effectiveness of silica treatment in depressing mononuclear phagocyte function, another group of identically treated mice were tested for sensitivity to infection with *Listeria monocytogenes* by using methods described previously (8). The number of bacteria measured in the liver 48 h after injection of the listerias was over $\log_{10}$ 3 times higher in the silica-treated group than in a nontreated control group, thus confirming the effect of silica treatment on mononuclear phagocyte function (data not shown).

Similar experiments were performed with C57BL/6 and DBA/2 strain mice and other regimens of silica treatment (up to 5-mg doses) with essentially the same findings, namely, that silica treatment does not impair clearance of *T. musculi* following IP treatment. It was also possible to irradiate mice for up to 5 days before IP treatment without interfering with the ability of mice to eliminate parasitemia following plasma transfer.

TABLE 1. Cure of parasitemia with IP in irradiated or silica-treated mice infected with *T. musculi*[a]

Treatment	No. (10^4) of *T. musculi* organisms/ml of blood[b]			
	B10.D2/nSn		B10.D2/oSn	
	Before IP	24 h after IP[c]	Before IP	24 h after IP[c]
IP[d]	41, 39, 35, 60	0, 0, 0, 1	58, 37, 53, 42	0, 0, 0, 0
Silica[e]–IP[d]	30, 44, 64, 35	0, 0, 0, 0.5	42, 48, 46[f]	0, 0, 0, 0
900 rads[g]–IP[d]	42, 31, 29, 46	0, 0, 0[f]	49, 52, 41, 35	0, 0, 0, 0
Heat-treated IP[h]	40, 51, 39, 70	36, 57, 38, 32	39, 44, 34, 36	9, 33, 3, 35

[a] Trypanosomes (10^4) were given on day 0.
[b] Individual values are shown.
[c] Similar values were obtained 48 h after IP.
[d] IP (0.4 ml) from syngeneic donors on day 12 p.i. was used.
[e] Silica (3 mg per mouse) was given on day 2, day 1, and the day of IP administration.
[f] One mouse died.
[g] On day 2 before IP, 900 rads was administered.
[h] IP (0.4 ml) heat treated at 56°C for 30 min was used.

It should be noted that B10.D2/oSn and DBA/2 strain mice are deficient in C5. Thus, even if complement-mediated lysis of *T. musculi* could account for the elimination of parasitemia following IP treatment in silica-treated and irradiated B10.D2/nSn and C57BL/6 mice, this mechanism is unable to account for the findings obtained with C5-deficient strains.

Determination of the best method to measure IP-mediated clearance of ^{75}Se-labeled *T. musculi* in normal and silica-treated mice. Since trypanosomes are cleared from the blood even in immunodeprived mice, the next objective was to ascertain the fate of the cleared organisms in both normal and immunodeprived animals and, in particular, to examine the putative role of the liver in this event. Initially, the experiments were performed by introducing ^{75}Se-labeled trypanosomes into naive mice (pretreated with silica) or normal animals shortly before giving IP or NMP and measuring the distribution of radiolabel in various organs after a suitable interval. In these experiments, the trypanosomes were always cleared from the blood by IP, even in immunodeprived hosts, but the method was laborious and the variation in the radiolabel values within a single group was rather high. Therefore, a different protocol was adopted in which ^{75}Se-labeled trypanosomes were sensitized in vitro with IP and then injected into noninfected normal or immunodeprived mice and their fate was investigated. The results were essentially the same as those of the earlier experiments with preinfected mice, but the radiolabel values within each group were more consistent, so that this method was adopted for the subsequent experiments. Since trypanosomes presensitized with IP are eliminated while those incubated with NMP are not (see below), it seems reasonable to assume that the pathway of clearance of these presensitized trypanosomes in noninfected mice is likely to be the same as that which occurs when infected mice are treated with IP and the parasitemia is cleared (our previous protocol) or when the parasites are eliminated in the normal course of infection.

Organ distribution of ^{75}Se-labeled *T. musculi* pretreated with IP or NMP and injected into normal or silica-treated mice. Groups of B10.D2/oSn strain mice were given 5 mg of silica particles per mouse i.v. or left untreated. One day later, the mice each received approximately 10^6 trypanosomes that had been preincubated with either IP or NMP. As a comparison, other groups of mice were given the same number of ^{51}Cr-labeled, sensitized SRBC. One hour later, the mice were bled under ether anesthesia through the retroorbital sinus and killed. Various organs were collected, and the radioactivity was measured. Parasitemias were enumerated in infected mice on wet smears prepared from the blood.

When normal mice were injected with trypanosomes that had been sensitized with IP, hepatic uptake of radiolabel was higher (24%) than in mice injected with trypanosomes preincubated with NMP (12%) (Fig. 1). This indicates that immune clearance of sensitized trypanosomes by the liver occurred to some extent. However, uptake of sensitized ^{51}Cr-labeled, sensitized SRBC by the liver was, by comparison, much greater (47%; Fig. 2), suggesting that the liver plays a somewhat minor role in immune clearance of trypanosomes. Moreover, when Kupffer cell function was impaired by silica treatment, the uptake of IP-sensitized trypanosomes by the liver was not greatly affected (19 versus 24%; Fig. 1); in contrast, uptake of sensitized SRBC by the liver was almost eliminated under these conditions (7 versus 47%; Fig. 2). The percentage of radioactivity found remaining in the blood changed in the reverse direction, as expected.

When the mice were injected with trypanosomes sensitized with IP, no parasites were detected in the blood at the time of sacrifice (Table 2). In contrast, trypanosomes preincubated in NMP remained in the blood in large numbers.

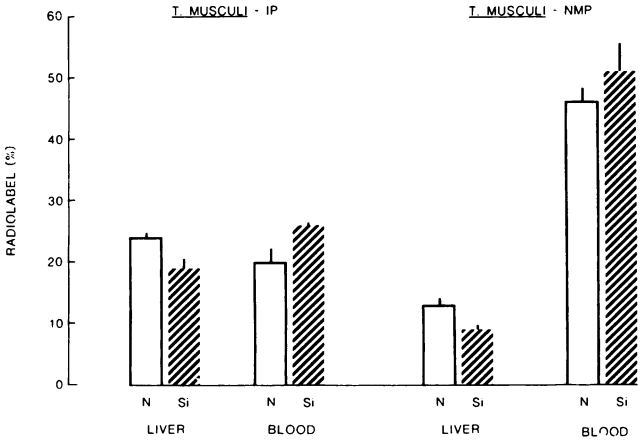

FIG. 1. Hepatic uptake and clearance from the blood of ^{75}Se-labeled *T. musculi*. Mice were either normal (N) or pretreated with 5 mg of silica particles (Si) 1 day before receiving 10^7 trypanosomes that had been preincubated with IP or NMP. The total radioactivity injected into each mouse was 5,526 or 6,144 cpm, respectively. Each value represents the mean of four or five mice ± 1 standard error of the mean.

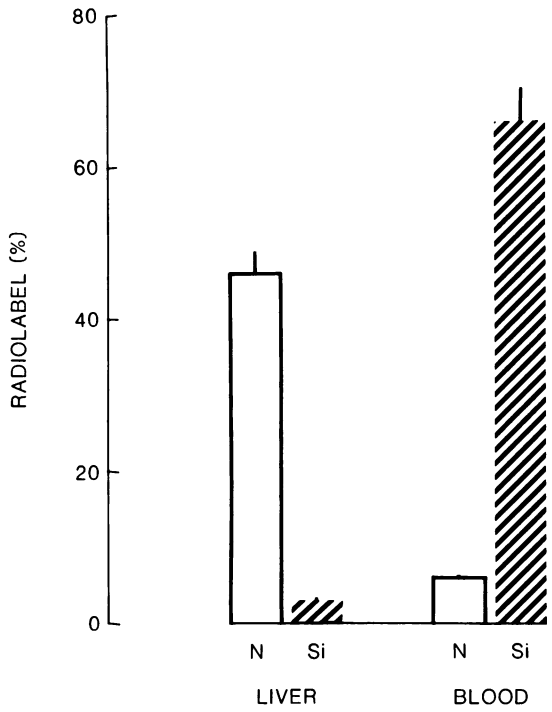

FIG. 2. Hepatic uptake and clearance from the blood of ^{51}Cr-labeled, sensitized SRBC. Mice were either normal (N) or pretreated with 5 mg of silica particles (Si) 1 day before receiving 10^7 SRBC. The total radioactivity injected into each mouse was 29,652 cpm. Each value represents the mean of three to five mice ± 1 standard error of the mean.

These differences were reflected in the greater amount of radioactivity remaining in the blood of mice given trypanosomes preincubated with NMP (46 to 50%) rather than IP (20 to 26%) (Fig. 1).

To confirm and extend these observations, the same experiment was repeated with B10.D2/oSn strain mice with a range of trypanosome inocula from 4×10^6 to 10^8 per mouse and was also done with a C5-sufficient mouse strain, C57BL/6 (but with incubation of the parasites in strain B10.D2oSn mouse IP to preclude the possibility of trypanolysis in vitro). The data obtained (Table 3) were comparable to the earlier findings; i.e., immune clearance of trypanosomes by the liver was modest (30 to 35% of the total radioactivity was taken up) and was only moderately reduced in silica-treated mice (17 to 22% uptake); however, the parasites were cleared from the circulation. Not surprisingly, the parasitemia was not completely cleared when the highest dose (10^8) of trypanosomes was given; however, it

TABLE 2. Parasitemia in silica-treated and normal mice at time of sacrifice in the experiment shown in Fig. 1

Treatment of mice	Treatment of *T. musculi*	No. of *T. musculi* organisms injected (10^7)	Log_{10} no. of *T. musculi* organisms/ml of blood at sacrifice[a]
None	IP	1.1	0
Silica	IP	1.1	0
None	NMP	1.4	6.15 ± 1.0
Silica	NMP	1.4	6.27 ± 1.43

[a] Means of four or five mice ± 1 standard error of the mean.

was markedly lower in mice that received IP-sensitized parasites than in the control group. In these experiments, radiolabel uptake was also measured in the spleen and found to be higher in silica-treated mice; this is in contrast to the reduced uptake by the livers of silica-treated mice. Values for the lungs and kidneys were also measured. They ranged from 1 to 5% and were of no particular interest (data not shown). Radiolabel uptake in the skin was not measured, although perhaps it should have been, in view of the substantial numbers of phagocytes in this organ.

The clearance of radiolabeled, sensitized SRBC from blood and their uptake by organs were measured in the normal and silica-treated groups of mice used in each of the above-described experiments. The data (Table 4) confirmed that clearance of foreign cells by Kupffer cells was virtually eliminated in silica-treated mice, and this was true even when the injected inoculum was small.

Clearance and distribution in organs of ^{75}Se-labeled, sensitized *T. musculi* in mice treated with silica, irradiation, and anti-platelet serum. An experiment was performed in which combined treatments were used to deplete mice of leukocytes, Kupffer cells, and platelets. Thus, B10.D2/oSn mice were irradiated with 900 rads of radiation on day −6 and given 5 mg of silica particles on day −1 and two doses of 0.2 ml each of anti-platelet serum, on days −1 and 0, respectively. The normal control groups were either nontreated mice or mice treated as described above and given, in addition, two doses of 0.2 ml of CVF on days −1 and 0. All mice received 6.6×10^7 ^{75}Se-labeled trypanosomes preincubated with IP on day 0. The percentages of radiolabel in various organs were measured 1 h after injection of the trypanosomes, and parasitemia was estimated. To assess the immune status of the immunocomprised mice, leukocyte and platelet counts were measured before injection of the parasites and afterward, at the time of sacrifice. In addition, the uptake of radiolabeled, opsonized SRBC by the liver was measured as before in other normal and immunocompromised groups of mice for comparison.

No detectable trypanosomes remained in the blood of normal mice. In immunocompromised mice also, most (>99%) of the trypanosomes disappeared from the circulation. The number of *T. musculi* organisms injected was log_{10} 6.6 per mouse, of which log_{10} 2.8 ± 1.4 remained. In mice that had received CVF, almost all of the trypanosomes, namely, log_{10} 6.04 ± 0.05, remained in the blood. This was to be expected since, as shown previously, clearance of *T. musculi* by i.v. injection of IP fails to occur if the mice have been pretreated with CVF (13). These mice thus provided an excellent negative control group in the present experiments.

Assessment of the immune status of the mice is shown in Fig. 3. In immunocomprised mice, the leukocyte and platelet counts were reduced by 95% and the ability of the liver to take up SRBC was largely destroyed. Differential counts of leukocytes were not done at the time but were performed later in a separate experiment with mice given an identical treatment regimen. In normal and immunocompromised mice, respectively, the values (10^5) for polymorphonuclear leukocytes were 5.5 and 0.11, for mononuclear phagocytes they were 2.3 and 0.14, and for lymphocytes they were 49 and 3.03. The mononuclear phagocytes appeared to be mostly mature macrophages, and monocytes were very rare. (Values for the CVF-treated group resembled the normal values.)

Uptake of radiolabeled *T. musculi* by various organs in the different groups of mice is illustrated in Fig. 4. In normal mice in which trypanosomes were cleared from the blood,

TABLE 3. Distribution of radiolabel in normal and silica-treated mice injected with ^{75}Se-labeled trypanosomes preincubated in IP or NMP

Expt no.[a] (no. of *T. musculi* organisms injected)	Preincubation plasma[b]	Treatment of host	% Distribution of radiolabel (mean ± SEM)[c]			Clearance or $\log_{10}$ no. of *T. musculi* organisms/ml of blood[d]
			Blood[e]	Liver	Spleen	
1 (10^8)	IP	Normal	25.4 ± 4.4	34.7 ± 2.6	2.2 ± 0.2	Not cleared[f]
	IP	Silica[g]	42.3 ± 4.8	17.3 ± 1.9	5.7 ± 0.6	Not cleared[f]
	NMP	Normal	52.0 ± 2.3	14.6 ± 0.5	2.1 ± 0.3	Not cleared[f]
	NMP	Silica	52.3 ± 2.3	10.1 ± 0.2	4.8 ± 0.4	Not cleared[f]
2 (2×10^7)	IP	Normal	9.7 ± 0.9	33.3 ± 0.3	2.4 ± 0.3	Cleared
	IP	Silica	15.4 ± 1.0	19.7 ± 2.7	10.3 ± 2.2	Cleared
	NMP	Normal	57.3 ± 2.8	12.1 ± 0.5	1.8 ± 0.1	Not cleared
	NMP	Silica	58.8 ± 0.8	11.4 ± 0.7	4.5 ± 0.2	Not cleared
3 (4×10^6)	IP	Normal	32.4 ± 2.9	30.8 ± 1.4	4.0 ± 0.4	0, 0, 0, 0[h]
	IP	Silica	38.4 ± 8.4	22.5 ± 0.8	16.8 ± 0.5	0, 0, 0, 0
	NMP	Normal	48.4 ± 6.0	11.2 ± 1.0	2.8 ± 0.4	5.60 ± 0.03
	NMP	Silica	44.7 ± 8.2	10.6 ± 1.4	6.4 ± 0.8	5.30 ± 0.08
4 (4×10^6)	IP	Normal	19.8 ± 2.1	31.8 ± 0.8	2.4 ± 0.7	0, 0, 0, 10^4
	IP	Silica	13.5 ± 1.8	17.2 ± 2.2	5.9 ± 1.0	0, 0, 0, 10^4
	NMP	Normal	51.0 ± 12.2	7.5 ± 0.3	1.6 ± 0.2	5.98 ± 0.08
	NMP	Silica	35.5 ± 1.9	9.6 ± 1.4	4.1 ± 0.3	5.63 ± 0.14

[a] Experiments 1 to 3 were done with B10.D2/oSn strain mice. Experiment 4 was done with C57BL/6 strain mice. There were four to six mice per group.
[b] Trypanosomes were incubated with a 1:4 dilution of IP or NMP at 37°C for 30 min.
[c] The radioactivity of each organ was measured 1 h after injection of ^{75}Se-labeled trypanosomes; it is expressed as a percentage of the total injected radioactivity. Values for the latter were 15,887 and 15,746 cpm (experiment 1), 2,237 and 2,102 cpm (experiment 2), 1,156 and 1,218 cpm (experiment 3), and 6,316 and 6,525 cpm (experiment 4) for the IP- and NMP-treated groups, respectively.
[d] Numbers of trypanosomes in blood at 1 h postinjection are shown.
[e] Total blood radioactivity was estimated by multiplying the radioactivity of a sample (200 to 500 μl) of blood by a correction factor (0.067 ml/g of body weight).
[f] However, the IP group had markedly fewer trypanosomes than the NMP group.
[g] Silica (5 mg per mouse) was administered 1 day before trypanosomes.
[h] Individual values are shown.

accumulation of radiolabel in the liver was relatively low (29%) compared with that seen for SRBC uptake (77%; Fig. 3). Furthermore, since CVF treatment prevents trypanosome clearance, it can be assumed that the uptake value for the liver obtained with this group (18%; Fig. 4) represents the amount of radiolabel that would be found in the liver when there is no specific uptake of trypanosomes. Thus, in normal mice, approximately 11% (18 to 29%) of the total radioactivity was apparently taken up specifically by the liver. In the experimental group of mice in which Kupffer cell function, as measured by SRBC uptake, was severely immunocompromised, there was no marked difference in the amounts of radiolabel found in the livers of these animals versus those of normal mice. These results confirm the earlier findings and suggest a relatively minor role of hepatic fixed macrophages in the immune clearance of *T. musculi*. In these same mice, depletion of leukocytes and platelets by 95% of their normal value was also apparently ineffective in preventing clearance of most of the parasites. Either the residual Kupffer cell and leukocyte functions remaining were sufficient to cause trypanosome clearance or another mechanism was responsible.

TABLE 4. Distribution of radiolabel in normal and silica-treated mice injected with ^{51}Cr-labeled SRBC[a]

Expt no. (no. of SRBC injected)	Treatment of host	Mean (±1 SEM) % of total radiolabel in:[b]		
		Blood[c]	Liver	Spleen
1 (5×10^8)	Normal	0.7 ± 0.1	42.7 ± 1.5	6.4 ± 2.0
	Silica[d]	16.5 ± 3.2	1.7 ± 0.5	24.0 ± 3.2
2 (5×10^8)	Normal	0.6 ± 0.1	48.1 ± 1.3	6.8 ± 0.5
	Silica	33.2 ± 5.7	2.2 ± 0.2	26.6 ± 3.8
3 (10^7)	Normal	2.4 ± 0.1	65.7 ± 1.5	1.4 ± 0.1
	Silica	22.0 ± 11.8	4.5 ± 0.8	36.0 ± 11.3
4 (6×10^6)	Normal	1.8 ± 0.2	83.4 ± 2.0	1.4 ± 0.2
	Silica	30.8 ± 3.6	5.6 ± 0.6	29.1 ± 0.1

[a] The data shown are from experiments described in Table 3.
[b] There were three or four mice in each group. The radioactivity of each organ was measured 1 h postinjection of ^{51}Cr-labeled SRBC and is expressed as a percentage of the total injected radioactivity. Values for the latter were 191,100, 218,737, 26,993, and 2,730 cpm for experiments 1 to 4, respectively.
[c] Calculated as described in Table 3, footnote *e*.
[d] Administered as described in Table 3, footnote *g*.

Clearance and distribution in organs of ^{75}Se-labeled, sensitized *T. musculi* in mice with occluded hepatic circulation. To try to distinguish between the two possibilities outlined above, a different approach was taken in which the hepatic portal vein and artery were occluded before inoculation with trypanosomes so that parasite clearance could be measured under conditions in which Kupffer cells were excluded from the circulation. Accordingly, groups of B10.D2/oSn strain mice in which the hepatic portal vein and artery had been clamped or sham-operated control mice were injected through the retroorbital sinus while still under anesthesia with 6×10^6 IP-sensitized or 8×10^6 NMP-treated trypanosomes and killed 15 min later. The percentages of radiolabel in various organs and parasitemia were measured.

Over 99% of the IP-sensitized trypanosomes were cleared from the blood in 15 min, even when the circulation to the

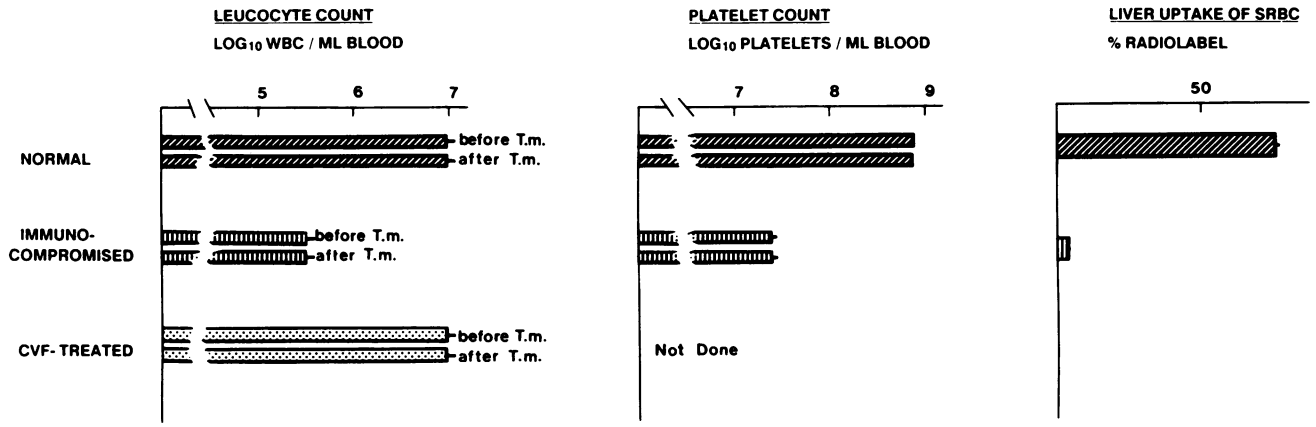

FIG. 3. Measurements of immune deprivation of mice used in the experiment shown in Fig. 4. Leukocyte and platelet counts were measured before injection of *T. musculi* (before T.m.) and 1 h later, at the time of sacrifice (after T.m.). Hepatic uptake of ^{51}Cr-labeled SRBC was measured in other groups of mice. The total radioactivity injected into each mouse was 44,310 cpm. Each value represents the mean of three or four mice ± 1 standard error of the mean.

liver had been occluded (Fig. 5). The radiolabeling data (Fig. 6) confirmed that very few parasites reached the liver in the experimental groups, the value being 7% for mice receiving IP-treated trypanosomes compared with 60% of the radiolabel found in the sham-operated group. The distribution of radiolabel among organs in this experiment was somewhat different from that seen in the previous experiments, possibly because the animals were under anesthesia and/or because the values were taken 15 min rather than 1 h after injection of the parasites. In particular, it is interesting that there was substantial accumulation of radiolabel in the lungs of experimental mice that received IP-treated trypanosomes. As expected, uptake of radiolabeled, NMP-treated trypanosomes was lower and of no particular interest (data not shown).

DISCUSSION

The experiments described in this report were done to identify the effector cell which is inferred to mediate immune destruction of *T. musculi*. For this reason, the experiments were always done with C5-deficient mouse strains (and with some C5-sufficient strains as well, for comparison) to ensure that disappearance of parasites from the circulation was not due to a trypanolytic event mediated by late-acting complement components.

As shown by us, passive transfer of IP to infected mice of the C57BL background results in rapid and complete elimination of trypanosomes (12). It was reasoned, therefore, that if radiosensitive leukocytes, such as monocytes or neutrophils, were the effector cells, prior exposure of infected mice to 900 rads of radiation several days before IP transfer should prevent the trypanosomes from being eliminated. Similarly, multiple treatments with silica particles were given to impair the function of the fixed hepatic macrophages (6, 7). However, administration of IP was able to cure irradiated or silica-treated infected mice, even in C5-deficient mice (Table 1). This was a surprising finding, and it prompted us to devise further experiments to locate the site

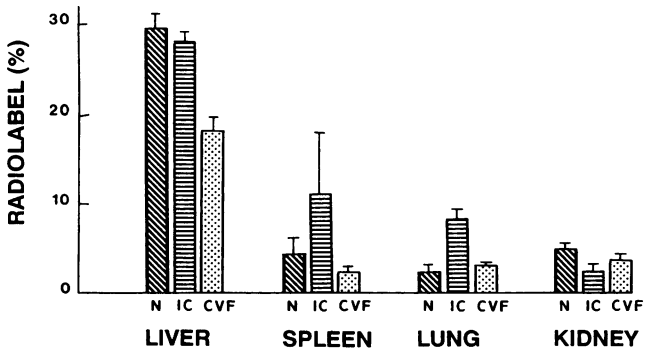

FIG. 4. Uptake of ^{75}Se-labeled *T. musculi* by various organs. Mice were normal (N), immunocompromised (IC), or CVF treated (CVF). The total radioactivity injected into each mouse was 1,580 cpm. Each value represents the mean of three or four mice ± 1 standard error of the mean.

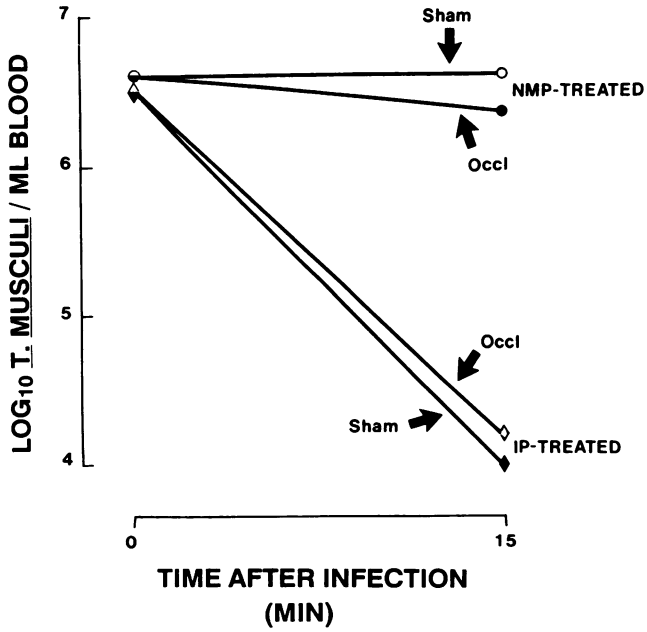

FIG. 5. Clearance of parasitemia following injection of trypanosomes preincubated with IP (IP-treated) in mice with hepatic circulation occluded (Occl) or sham operated (Sham). Each value represents the mean of three to five mice ± 1 standard error of the mean.

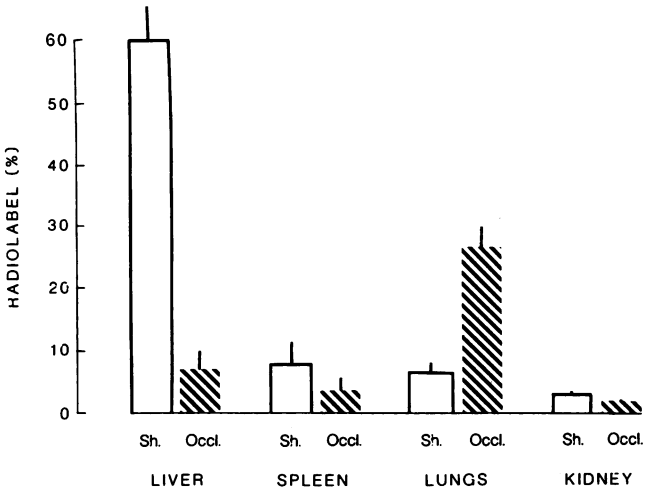

FIG. 6. Uptake of ^{75}Se-labeled trypanosomes by various organs of mice in experiment shown in Fig. 5. The total radioactivity injected into each mouse was 6,852 cpm Sh., Sham; Occl., occluded.

of elimination of the trypanosomes. In these studies, it was more feasible to measure the immune clearance of trypanosomes preincubated with IP in noninfected mice. As outlined in Results, we believe that the mechanism of clearance in this case was essentially the same as that responsible for bringing about a cure of the normal infection.

The first point to emphasize is that in every experiment, whatever method of deprivation was used, the immune elimination mechanism remained essentially intact and the trypanosomes were removed from the circulation (except when a very large inoculum was given). This could mean that none of the cell populations affected by the deprivation treatments is involved in the clearance process. Another possibility could be that more than one cell type is able to effect immune elimination so that in the absence of one population (e.g., Kupffer cells), another cell population (e.g., neutrophils or platelets) is responsible. When combined treatments were used to deplete the host of both Kupffer cells and radiosensitive circulating leukocytes and platelets, this argument became less tenable. However, none of the cell populations depleted by any of the treatments was entirely eradicated. Certainly, some platelets were still present in the blood and small numbers of neutrophils were observed in the immunocompromised mice. The differential counts showed that a small number (~2%) of neutrophils were left in mice 3 days postirradiation. Monocytes were very rare, but a considerable number of mononuclear phagocytes, which appeared to be mostly mature macrophages, were present. The question of whether these elements remaining in the blood could have been adequate to mediate trypanosome clearance remains.

With regard to the efficacy of the treatments used to impair Kupffer cell function in the mice, the evidence for this is quite convincing. (i) When the mice were treated with silica, hepatic uptake of radiolabeled SRBC was always drastically reduced; uptake in normal mice ranged between 43 and 83%, while for silica-treated mice the range was 2 to 7%. Also, silica-treated mice became highly sensitive to infection with *L. monocytogenes*. Both of these observations demonstrate the inadequacy of Kupffer cell activity in silica-treated mice. (ii) When surgical interruption of hepatic circulation was used, the very low amounts of radiolabel found in the livers of these mice compared with those in their sham-operated counterparts (Fig. 6) provided direct evidence that Kupffer cells had been excluded from the circulation and therefore could not have been responsible for removal of the trypanosomes that disappeared from the blood (Fig. 5).

Since in other models of trypanosome infections in mice, the liver was found to be the principal site of phagocytic uptake of radiolabeled parasites (1, 3, 5), the experiments were directed primarily at examining the role of the fixed liver macrophages in *T. musculi* infection. The data showed that the liver specifically takes up a certain proportion of the radiolabeled, sensitized trypanosomes. Thus, the results of all of the experiments (except the last one in which the mice were anesthetized) show that the range of values for normal uptake of IP-sensitized trypanosomes by the liver was substantially higher (24 to 35%) than that for uptake of NMP-treated trypanosomes (7 to 19%). For example, in Fig. 1, the respective values are 24 versus 12%. In the experiment in which IP-sensitized *T. musculi* organisms were given to normal mice and CVF-treated control mice, uptake by the liver was 11% higher in the normal group (Fig. 4). This value is probably a fair representation of the amount of radiolabeled parasites taken up specifically by the liver. In experiments reported by others who used *T. brucei* (3, 5) or *T. rhodesiense* (1), antibody-mediated hepatic uptake of radiolabeled trypanosomes was 50 to 60% or more; also, as mentioned above, in the present studies, hepatic uptake of sensitized SRBC was 43 to 83%. The liver does not appear to be a major site of *T. musculi* clearance, therefore, when hepatic uptake of this trypanosome in our experiments is compared with that observed for other trypanosomes or other opsonized foreign particles, such as SRBC. Furthermore, hepatic uptake of radiolabeled, sensitized *T. musculi* was only moderately reduced in silica-treated mice; i.e., the range was 17 to 22% in these mice compared with 24 to 35% in normal mice. In contrast, SRBC uptake was drastically reduced in silica-treated mice (i.e., 2 to 6% from 43 to 83%). Lastly, when the hepatic circulation was interrupted, the percentage of radiolabel found in the liver was very low (7%) (Fig. 6), confirming that hepatic uptake of trypanosomes was largely prevented. The fact that the parasites were cleared from the blood shows that elimination of the trypanosomes can occur quite readily without the participation of liver cells.

The question of the identity of the putative effector cell(s) remains. As mentioned above, one possibility could be that killing *T. musculi* is not the sole property of one particular cell type but that a variety of different cells can all act as effector cells in the presence of C3 and immunoglobulin. Certainly, in vitro experiments have shown that macrophages can ingest and kill *T. musculi* intracellularly (2). The fact that uptake of radiolabel by the spleen always increased in silica-treated mice lends support to this idea (Table 3). However, splenic uptake of not only IP-sensitized trypanosomes but also NMP-treated trypanosomes increased under these circumstances, so that this phenomenon may not be associated with a specific immune clearance mechanism. It seems likely, however, that a major effector cell exists which is not affected by the immunodeprivation methods used. If so, it can be concluded that this cell is radioresistant and silica resistant. Also, it must bear Fc and C3 receptors. One cell type present in the vascular system that may have these characteristics is the endothelial cell that lines the vascular sinuses and small blood vessels. This possibility is currently being investigated.

In conclusion, immune elimination of *T. musculi* by a

specific antibody, complement component C3, and a putative effector cell is a process in which hepatic Kupffer cells appear not to play an essential role, despite being an obvious choice. Under normal circumstances, these cells probably take up a certain proportion of the trypanosomes, but if this is prevented, the immune clearance mechanism remains intact. Radiosensitive leukocytes and platelets are also in this category.

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