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Specific Suppressor T Cells for Delayed-Type Hypersensitivity in Susceptible Mice Immunized against Cutaneous Leishmaniasis

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BALB/c mice injected intravenously with 10^6 or higher doses of formaldehyde-fixed promastigotes (ffp) of *Leishmania major* developed significantly lower levels of delayed-type hypersensitivity (DTH) compared with uninjected control mice when they were subsequently immunized intradermally with ffp. The suppression of DTH was antigen specific and was also inducible with lethally irradiated promastigotes or soluble parasite antigens. The suppressive effect was adoptively transferable with splenic T cells which express the Lyt-1^+2^+ and L3T4^+ phenotypes. These specific suppressor T cells were active against both the inductive and expressive phases of DTH. They were sensitive to 200 rads of γ-irradiation in vitro and appeared to manifest the suppressive activity via soluble factors. In spite of this profound suppression of DTH, BALB/c mice injected intravenously with 4×10^7 ffp were substantially protected against a challenge infection with *L. major* promastigotes. The possible relationship between the suppressor T cells for DTH and prophylactic immunization against fatal cutaneous leishmanial infection in susceptible BALB/c mice is discussed.

BALB/c mice infected with *Leishmania major* develop uncontrolled cutaneous disseminating and fatal visceralizing disease (10, 12, 16). Susceptibility of BALB/c mice to *L. major* is controlled by a single autosomal non-H-2 linked gene (5, 12) designated *scl* (1) (susceptibility to cutaneous leishmaniasis), apparently unrelated to the *Lsh* gene that controls mouse susceptibility to *Leishmania donovani* (2, 3), and is associated with the formation of a potent T-cell population that suppresses cell-mediated immunity (13, 14). Adult thymectomy (13) or sublethal γ-irradiation (550 rads) (14) before infection reverses the susceptibility of BALB/c mice by preventing the development of suppressor T (TS) cells which interfere with the generation of immunity associated with the eventual resolution of the infection. Recovery is independent of antibody (20) but correlates with the development of leishmanial-specific delayed-type hypersensitivity (DTH) (13). Diminution of DTH is often observed with progressive disease (21, 22).

BALB/c mice can be protected from fatal disease by one to four weekly intravenous (i.v.) immunizations with lethally γ-irradiated or heat-killed promastigotes (15), the level of protection being similar to that observed in convalescent immunity resulting from 550 rads of prophylactic irradiation. However, in contrast to convalescent immunity, DTH reactivity was not discernible even though no role could be ascribed to antibody (15). The T cells responsible for this protection express the Lyt-1^+2^- phenotype and were found not to interfere with the development of specific DTH in recipient mice after *L. major* infection (19).

Suppressor cells for DTH to horse erythrocytes, sheep erythrocytes (SRBC), lysozyme, picryl chloride, allogeneic cells, and viruses have been reported (reviewed in reference 18). In all these systems, TS cell induction is determined to a large extent by the route and the amount of antigen administered. In this paper, we report the characterization of the TS cells induced by the i.v. administration of formaldehyde-fixed promastigotes (ffp) and examine the role it may play in mice protected from *L. major* infection.

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MATERIALS AND METHODS

Mice. Male and female BALB/c mice aged 7 to 10 weeks were obtained from an inbred colony maintained at The Wellcome Research Laboratories, Beckenham, Kent, United Kingdom.

Parasite. *L. major* PLV39 was kindly supplied by R. A. Neal, and *L. donovani* L82 (Ethiopian strain) was supplied by J. Blackwell, both of the London School of Hygiene and Tropical Medicine. *L. major* was grown by passaging a piece of nonulcerated lesion into Schneider *Drosophila* medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 30% fetal calf serum (Flow Laboratories, Inc., McLean, Va.). The parasite cultures were expanded by passaging into fresh medium every 4 to 5 days, harvested by centrifugation, washed once with saline, and counted in a hemocytometer after dilution in a 10% solution of Lugol iodine.

ffp were prepared by incubating parasites (*L. major* or *L. donovani*) in 0.5% formaldehyde (BDH Chemicals, Poole, England) in saline at a concentration of 2×10^8 parasites per ml for 5 min at room temperature. After incubation, parasites were washed three times in saline, recounted, and stored at 4°C at a concentration of 2×10^8/ml.

Irradiated parasites were prepared by 150,000-rad irradiation of live promastigotes in phosphate-buffered glucose saline at a concentration of 2×10^8 parasites per ml. Irradiation was carried out using a cesium (^{137}Cs) source at a rate of 143.7 rads per min.

Sonicated promastigote antigen (SPA) was prepared by sonicating 10^9 parasites per ml for 6 min at 25,000 cycles per s in an MSE sonicator (MSE Ultrasonic Power Unit). The resulting suspension was centrifuged at 33,000 rpm for 1 h at 4°C in a Beckman zonal ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.) and filter sterilized with a

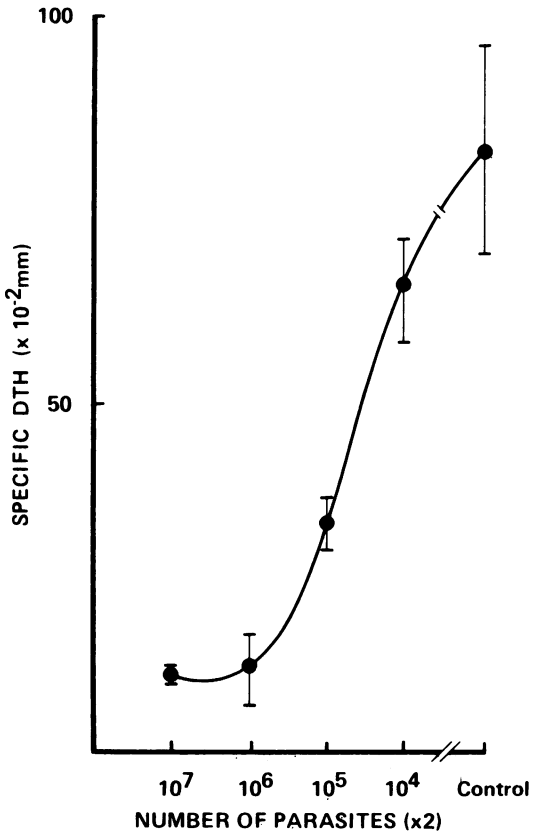

FIG. 1. Effect of i.v. administration of ffp on DTH. Groups of five mice were given 2×10^7, 2×10^6, 2×10^5, or 2×10^4 ffp i.v. After 4 days they were immunized for DTH by an intradermal administration of a total of 2×10^7 ffp given in a 0.05-ml volume per flank. Six days later they were elicited for DTH by injecting 10^7 ffp in 0.05 ml into the left hind footpad. Normal BALB/c mice were injected in the footpad with the same amount of eliciting antigen to determine background swelling. Specific DTH = experimental − background (mean ± standard error).

membrane filter (0.22 μm; Millipore Corp., Molsheim, France). The protein content was estimated by UV spectrophotometry at 280 nm. SPA was stored in aliquots at −70°C until used.

Frozen-thawed epimastigotes of the Y strain of *Trypanosoma cruzi* (2×10^8/ml) in phosphate-buffered saline, kindly supplied by M. T. Scott, Wellcome Research Laboratories, were used for sensitization and elicitation of DTH. Mice were injected subcutaneously into each thigh region with 0.25 ml of this suspension, and DTH was elicited by injecting 0.05 ml of the same preparation into the left hind footpad.

Mice were infected by injecting 0.1 ml of the appropriate concentration of live promastigotes subcutaneously into their shaved rumps. The lesions that developed were measured at intervals up to 120 days with a direct reading caliper gauge (GMH-390-T; Gallenkamp, London, England) in two perpendicular diameters. The average diameter (millimeters) was recorded and corrected by subtracting 1 mm for the thickness of the skin at the same site of an uninfected mouse. Results were expressed as group mean ± standard error.

Induction of TS cells. TS cells were induced by injecting mice with 4×10^7 ffp i.v. in 0.2 ml of phosphate-buffered saline, and mice were routinely tested 4 days after induction, unless otherwise stated.

Adoptive cell transfer. Spleens were collected in phosphate-buffered saline (pH 7.2). A single-cell suspension was obtained by homogenizing spleens and passing the resulting suspension through a fine stainless steel sieve. The cells were then washed, the percent viability was estimated by the trypan blue dye exclusion assay, and the cells were suspended at a concentration of 10^8 spleen cells per ml. A total of 5×10^7 cells were routinely transferred in cell transfer experiments unless otherwise stated.

Treatment of cells. 10^8 cells per ml were treated with the various antisera as follows: Thy-1.2 (F7D5, monoclonal immunoglobulin M [IgM] antibody; Olac, Ltd., Oxon, United Kingdom; 1:500 dilution); Lyt-1.2 and Lyt-2.2 (monoclonal IgG2b and IgM antibodies; Cedarlane Laboratories, Ltd., Ontario, Canada; 1:50 and 1:25 dilutions, respectively); and GK-1.5 (monoclonal IgG2b antibody; 1:30) dilution) (7). After incubation for 30 min at room temperature, the cells were sedimented by centrifugation and incubated for 45 min at 37°C with a 1:10 dilution of complement (C′) (Low-Tox-M Rabbit complement; Cedarlane Laboratories, Ltd.) that had been previously absorbed with normal mouse spleen cells for 1 h at 4°C. Cells were then washed, their viability was estimated, and they were made up to the original cell concentration.

Anti-IgG column fractionation of spleen cells. T-cell-enriched fractions were prepared according to the method described by Shand (23). Briefly, this was done by coupling ammonium sulphate-precipitated mouse immunoglobulin to plastic Diakon beads (MG101; Imperial Chemical Industries, Welwyn Gardens City, United Kingdom) at pH 7.4 by incubation for 1 h at 45°C, followed by overnight incubation at 4°C. The coated beads were loaded onto a column (1 by 20 cm) and washed extensively. A 1:3 dilution of rabbit anti-mouse IgG serum was added to the column and allowed to react for 90 min at room temperature. The column was again washed extensively with phosphate-buffered saline (pH 7.2), and 20 ml of spleen cell suspension at 5×10^7 cells per ml was passed through at a rate of 4 ml/min at room temperature. This procedure reduced the immunoglobulin-positive

TABLE 1. Suppression of DTH by different preparations of *L. major* antigen

i.v. treatment (day 0)[a]	No. of mice	Specific DTH[b] ($\times 10^{-2}$ mm)	% Suppression[c]
Expt 1			
4×10^7 ffp	6	14 ± 3[d]	71.4
4×10^7 irradiated parasites	4	16 ± 4[d]	67.3
2 mg of SPA	4	14 ± 1[d]	71.4
Control	8	49 ± 1	
Expt 2			
4×10^7 ffp	5	8 ± 3[d]	80.5
4×10^7 irradiated parasites	5	12 ± 3[d]	70.7
Control	5	41 ± 3	
Expt 3			
2 mg of SPA	5	1 ± 1[d]	96.3
Control	5	27 ± 3	

[a] All mice were immunized on day 4 with 2×10^7 ffp intradermally and elicited on day 10 with 1×10^7 ffp in the footpad.

[b] Normal BALB/c mice were injected in the footpad to determine background DTH. Specific DTH = experimental − background (mean ± standard error).

[c] % Suppression = [(DTH control − DTH experimental)/DTH control] × 100.

[d] $P < 0.001$.

cells from 40 to 50% to <0.5% as assesed by an indirect immunofluoresence assay.

Induction, elicitation, and measurement of DTH. DTH was induced by injecting each flank intradermally with 10^7 ffp in 0.05 ml of saline (total amount, 2×10^7 ffp) and elicited six days later with 10^7 ffp in 0.05 ml of saline injected into the left hind footpad. Footpad swelling was measured by using a dial caliper (Pocotest, reverse spring-loaded caliper; Carobronze, England). DTH was expressed as the increase in footpad thickness, in 10^{-2} mm at 24 h, after subtracting the background due to antigen alone.

Statistical analysis. All experiments were performed two to four times and representative results are shown. Standard errors of the mean were calculated, and the statistical significance was analyzed by Student's *t* test.

RESULTS

Concommitant induction of suppression of DTH and protection against *L. major* infection. To examine the effect of administrating large amounts of *L. major* antigen on the induction of DTH, groups of five mice were injected i.v. with various amounts of ffp. Four days later they were immunized intradermally with ffp for the induction of specific DTH which was elicited in the footpads with 10^7 ffp 6 days after immunization. Mice given the i.v. injections developed significantly lower levels of DTH to ffp compared with control mice not receiving the i.v. injections. This apparent suppression was dose dependent and ranged from 88% for mice injected with 2×10^7 ffp to 22% for mice injected with 2×10^4 ffp (Fig. 1). Suppression of DTH can also be induced with γ-irradiated parasites or sonicated SPA injected i.v. (Table 1). The DTH suppression caused by ffp given i.v. was long lasting and was still present at comparable levels 12 weeks after the injection (data not shown).

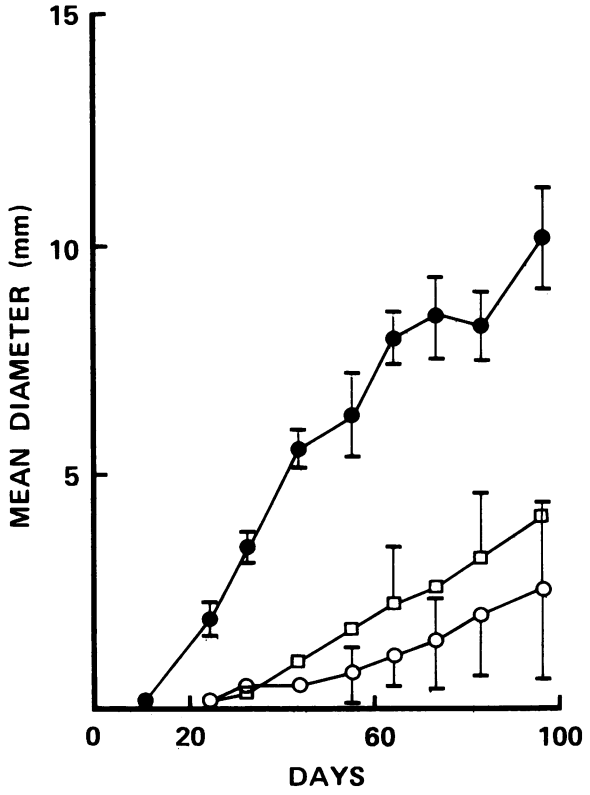

FIG. 2. Comparison of protective effects induced by ffp and irradiated *L. major* promastigotes. Group of mice were injected i.v. with 4×10^7 ffp (○) or 150,000-rad-irradiated promastigotes (□) and 7 days later, together with unimmunized controls (●), were infected subcutaneously with 2×10^7 viable promastigotes. The lesions that developed were measured as described in the text (mean lesion diameter ± standard error; $n = 5$)

TABLE 2. Phenotype of suppressor cells[a]

Treatment of cells	Specific DTH ($\times 10^{-2}$ mm)	% Suppression
Expt 1		
Immunoglobulin-negative spleen cells	16 ± 4	60[b]
Whole spleen cells	15 ± 5	62.5[b]
Normal spleen cells	40 ± 4	
Expt 2		
Anti-Thy-1.2 + C′	52 ± 3	13.3
Anti-Lyt-1.2 + C′	62 ± 3	−3.3
Anti-Lyt-2.2 + C′	49 ± 5	18.3
C′ only	21 ± 4	65[b]
Normal cells	60 ± 5	
Expt 3		
GK-1.5 + C′	41 ± 6	14.5
C′ only	20 ± 4	58.3[b]
Normal cells	48 ± 3	
Expt 4		
Anti-Lyt-1.2 + C′	49 ± 3	5.8
Anti-Lyt-2.2 + C′	43 ± 3	17.3
Anti-Lyt-1.2 + C′ + Anti-Lyt-2.2 + C′	50 ± 4	4
C′ only	30 ± 4	42.3[c]
Normal cells	52 ± 2	

[a] For definitions of specific DTH and % suppression, see Table 1, footnotes *b* and *c*.
[b] $P < 0.001$ compared with controls.
[c] $P < 0.005$ compared with controls.

Mice given one i.v. injection of 4×10^7 ffp were significantly protected against a challenge infection with 2×10^7 promastigotes. The level and characteristics of protection induced was similar to that developed after one i.v. immunization with 4×10^7 γ-irradiated promastigotes (Fig. 2).

Specificity of suppression. The specificity of suppression induced by ffp injected i.v. was tested against DTH to related and unrelated antigens. Mice were injected i.v. with 4×10^7 ffp and then immunized intradermally for DTH to *L. major*, *L. donovani*, *T. cruzi*, or SRBC. i.v. priming with *L. major* completely suppressed the induction of DTH to *L. major* and partially suppressed the DTH to *L. donovani*. In contrast, there was no discernible suppression of DTH to the non-cross-reacting antigens *T. cruzi* and SRBC (Fig. 3).

Transfer of suppression by spleen cells. Experiments were carried out to determine whether the suppression of DTH induced by the i.v. administration of ffp could be adoptively transferred by cells. Donor mice were injected i.v. with 4×10^7 ffp, and graded numbers of spleen cells, harvested 4 days later, were transferred i.v. into normal syngeneic recipients which were immunized for DTH to ffp. Substantial suppression of DTH was found in recipients injected with 5×10^7 spleen cells from suppressed donors compared with mice receiving the equivalent number of normal spleen cells (Fig. 4). This suppression decreased linearly and was not significant with a dose of 10^7 cells. Subsequently, 5×10^7 cells were routinely used in adoptive transfer experiments.

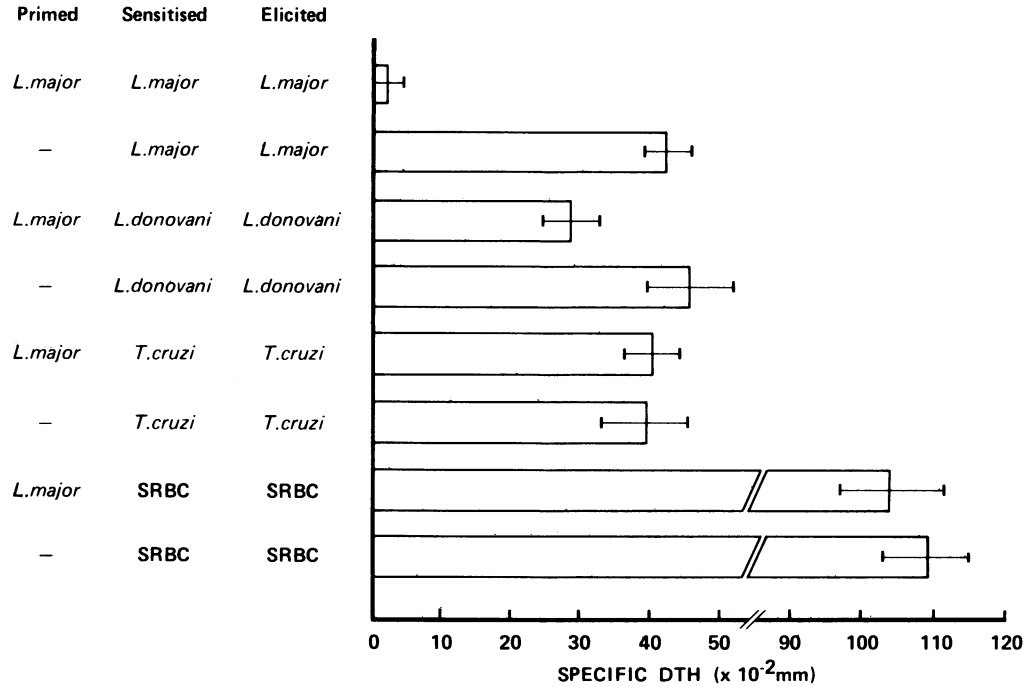

FIG. 3. Specificity of suppression. Mice were injected i.v. with 4×10^7 ffp of *L. major* and tested for DTH against SRBC, *T. cruzi*, *L. donovani*, or ffp. DTH to SRBC was induced by subcutaneous injection of 0.1 ml of 5% SRBC in each flank. It was elicited by footpad injection of 0.02 ml of 50% SRBC. DTH to *L. donovani* was obtained in the same way as *L. major*. For each eliciting antigen, normal BALB/c mice were injected in the footpad to determine the background DTH. Specific DTH = experimental − background (mean ± standard error; $n = 5$)

Phenotype of suppressor cells. After establishing that suppression could be transferred by cells, the phenotype of the cell involved was determined. Spleen cells from DTH-suppressed donor mice were depleted of B cells by passing through an anti-immunoglobulin column. The immunoglobulin-negative cells retained all the suppressive activity when adoptively transferred into normal recipients, indicating that the suppression was mediated by T cells. The TS cells were further characterized by treatment with various antisera against the T-cell surface markers and complement. The specific DTH response in mice given TS cells treated with C′ alone were significantly suppressed compared with controls. Treatment with anti-Thy-1.2 plus C′, anti-Lyt-1.2 plus C′, anti-Lyt-2.2 plus C′, or GK-1.5 plus C′ completely abrogated

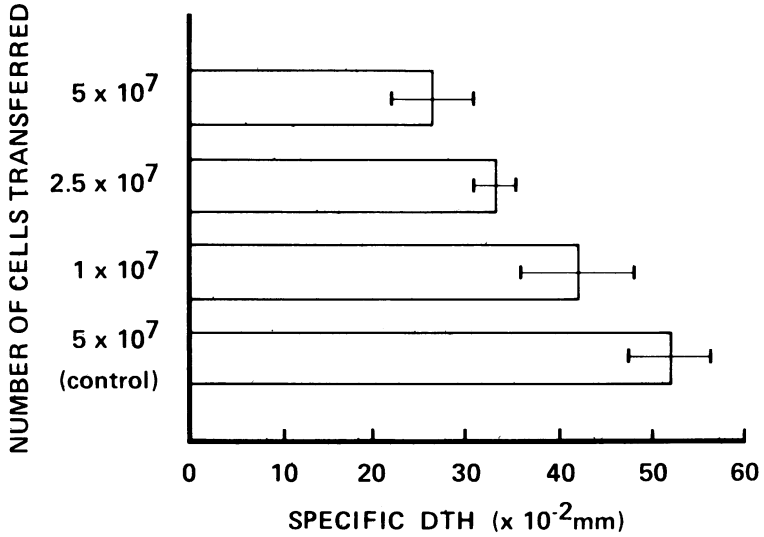

FIG. 4. Titration of the number of cells required to transfer suppression. Mice were injected i.v. with 4×10^7 ffp. Four days later spleen cells were harvested and transferred in graded numbers into groups of five normal recipients, which were then sensitized and elicited for DTH as described in the legend to Fig. 1.

the suppressive activity of the TS cells (Table 2). Thus, the TS cells are Thy-1^+, Lyt-1^+2^+, and L3T4^+. The possibility that the suppression was caused by a synergistic effect of Lyt-1^+2^- and Lyt-1^-2^+ cells were excluded by the demonstration that cotransfer of anti-Lyt-1.2 plus C'-treated and anti-Lyt-2.2 plus C'-treated TS cells did not reverse the abrogation of suppression (Table 2).

Suppression of induction and expression of DTH. To determine whether the TS cells were acting on the induction or the expression of DTH, spleen cells from suppressed donor mice were transferred into recipients at the induction or the elicitation phase of DTH. Results shown in Table 3 demonstrate that the TS cells were effective against the induction as well as the expression of DTH. Transfer of TS cells on day 0 or 6 did not affect the induction and expression of DTH to SRBC or *T. cruzi* (Table 4).

Effect of irradiation on TS cells. The radiosensitivity of activated TS cells was also investigated. Suspensions of spleen cells from mice primed for suppression were irradiated in vitro with various doses of γ-irradiation before being transferred into normal recipients which were then immunized and elicited for DTH in the normal way. Irradiation at 100 rads had no effect on the suppressive activity, which was, however, completely abrogated by 200 rads of irradiation (Fig. 5).

Suppressor cells may act through a suppressive factor. To test the possibility that the effect produced by suppressor cells could also be obtained by a suppressor factor, spleen cells from DTH-suppressed mice were incubated for 48 h at 37°C and 5% CO_2 at a concentration of 10^8 cells per ml in RPMI medium. Supernatant was collected, and 1 ml was injected intraperitoneally injected into normal mice that were then immunized and elicited for DTH. Table 5 demonstrates that the culture supernatant of spleen cells containing TS cells significantly suppressed the induction of DTH compared with the supernatant of normal spleen cells. This was confirmed in two other separate experiments. However, the degree of suppression obtained with the supernatant was generally lower than that mediated by adoptive transfer of suppressive spleen cells.

TABLE 3. Effect of suppression on induction and expression of DTH[a]

Cell transfer[b]	Specific DTH ($\times 10^{-2}$ mm)	% Suppression
Expt 1		
Day 0		
Primed donors	11 ± 4[c]	77.1
Normal donors	48 ± 1	
Day 6		
Primed donors	14 ± 4[c]	68.8
Normal donors	45 ± 2	
Expt 2		
Day 0		
Primed donors	26 ± 5[c]	60.6
Normal donors	66 ± 4	
Day 6		
Primed donors	37 ± 10[d]	39.3
Normal donors	61 ± 2	

[a] For definitions of specific DTH and % suppression, see Table 1, footnotes *b* and *c*.

[b] All recipients were injected i.v. with 5×10^7 spleen cells from mice primed 4 days before with 4×10^7 ffp i.v. All mice were sensitized on day 0 with 2×10^7 ffp intradermally and elicited for DTH on day 6 with 1×10^7 ffp in the footpad.

[c] $P < 0.001$.

[d] $P < 0.05$.

TABLE 4. Specificity of induction and expression of DTH[a]

Cell transfer[b]	No. of mice	Antigen used for induction and elicitation of DTH	Specific DTH ($\times 10^{-2}$ mm)	% Suppression
Day 0	5	*L. major*	24 ± 5[c]	55.1
Day 6	4	*L. major*	38 ± 6[d]	28.9
Control	10	*L. major*	54 ± 5	
Day 0	5	SRBC	95 ± 3	−3.2
Day 6	5	SRBC	84 ± 9	8.6
Control	10	SRBC	92 ± 8	
Day 0	5	*T. cruzi*	47 ± 4	4.4
Day 6	5	*T. cruzi*	45 ± 4	0
Control	5	*T. cruzi*	45 ± 11	

[a] For definitions of specific DTH and % suppression, see Table 1, footnotes *b* and *c*.

[b] Recipients were injected with 5×10^7 spleen cells from mice primed 4 days before with 4×10^7 ffp i.v. Control mice were injected with 5×10^7 normal spleen cells on day 0.

[c] $P < 0.001$.

[d] $P < 0.05$.

DISCUSSION

Our results indicate that mice given large i.v. doses of ffp not only did not develop detectable levels of DTH, but in fact generated specific TS cells which were demonstratable on adoptive transfer. In spite of this strong specific suppression of DTH, mice injected i.v. with ffp were protected against *L. major* infection (Fig. 2). In an earlier report, such TS cells were not demonstrated by passive transfer in a similar system used for prophylactic immunization (19), and this may be because of the time between TS cell induction and cell transfer. Suppression can only be successfully and consistently transferred provided it is carried out optimally

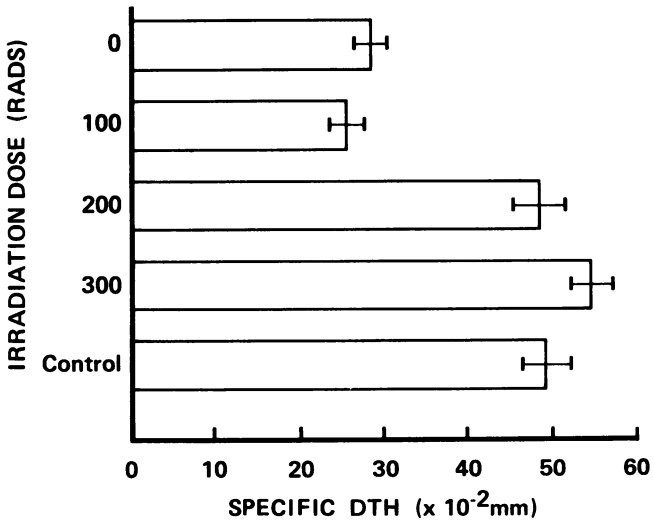

FIG. 5. Radiosensitivity of activated suppressor cell. Mice were primed for suppression by the administration of 4×10^7 ffp i.v. Four days later spleen cells were harvested and irradiated in vitro with 100, 200, or 300 rads of γ-irradiation. A total of 5×10^7 irradiated or control cells were injected i.v. into groups of normal recipients that were then sensitized and elicited for DTH. Specific DTH = experimental-background (mean ± standard error; $n = 5$).

TABLE 5. Suppression of DTH by suppressor cell supernatant[a]

Treatment (1 ml, intraperitoneal)	No. of mice	Specific DTH ($\times 10^{-2}$ mm)	% Suppression
Expt 1			
Supernatant from TS cells	4	36 ± 4[b]	41.9
Supernatant from normal cells	3	62 ± 12	
Control	4	60 ± 6	
Expt 2			
Supernatant from TS cells	4	3 ± 3[c]	95.9
Supernatant from normal cells	4	73 ± 3	
Control	4	78 ± 8	
Expt 3			
Supernatant from TS cells	4	22 ± 4[c]	53.2
Supernatant from normal cells	4	47 ± 8	
Control	9	53 ± 2	

[a] For definition of specific DTH and % suppression, see Table 1, footnotes *b* and *c*.
[b] $P < 0.05$.
[c] $P < 0.001$.

at 3 to 4 days postinduction (data not shown). However, in the earlier experiment (19), donor cells were harvested 6 days after i.v. injection of irradiated promastigotes.

The cell surface phenotype of TS cells is controversial. Most effector TS cells for humoral response appear to be Lyt-1^-2^+ and L3T4$^-$ (7), whereas Lyt-1^+2^- TS cells are featured predominantly in cell-mediated immunity either as suppressor inducer or effector TS cells (reviewed in references 7 and 18). The secondary TS cells for DTH to alloantigens (17) and the TS cells for the proliferative response to *L. major* (9) are both Lyt-1^+2^+. The TS cells in the present system are also Lyt-1^+2^+. Furthermore, they appear to bear the L3T4 antigen as defined by GK-1.5, a marker thought not to coexpress with Lyt-2 antigen (6). Thus, the present data compound the doubt that has been raised about the consistency of cell surface markers and the premise of classifying cellular functions according to them (11).

TS precursors specific for DTH to SRBC have been shown to be abrogated by 400 rads of γ-irradiation but were resistant to greater than 600 rads after antigen activation (8). On the other hand, it was demonstrated that the activity of in vitro-activated SRBC-specific TS cells was significantly reduced at 200 rads and abolished at 400 rads. The TS cells induced by i.v. injection of ffp in the present system were sensitive to 200 rads of irradiation. This is somewhat surprising, as they were at least three times more radiosensitive than the TS cells for DTH to SRBC induced under comparable conditions. The reason for this difference is unclear. However, the radiosensitivity of the suppressor system is likely to be complex, as the suppression observed may involve a series of interacting subsets of T cells, each of which could have distinct replicative requirements. Furthermore, it is uncertain whether irradiation carried out in vitro, as in the present system, is directly comparable to the in vivo irradiation of TS cells for DTH to SRBC.

That TS cells manifest their suppressive activity through soluble factors appears to be a general phenomenon (24). This has been demonstrated in a variety of systems, including lysozyme, SRBC, picryl chloride, and dinitrophenylated syngeneic lymphoid cells. In the present system, the suppressor activity was detectable in the supernatant of suppressive spleen cells cultured in vitro for 24 to 48 h in the absence of antigen. The nature, relevance, and specificity of this suppressor factor remains to be determined.

The development of diffuse cutaneous leishmanias has been associated clinically and experimentally with the impairment of cell-mediated immunity (5), and the relative susceptibility of some inbred mouse strains has been shown to correlate directly with reduced DTH reactivity (21, 22). Sublethally irradiated BALB/c mice that recover from *L. major* infection develop high and sustained levels of specific DTH. This is in contrast to normal unirradiated controls, whose initial DTH is strongly suppressed as the infection progresses to uniform fatality (14). However, direct evidence for a causal role of DTH in cutaneous leishmaniasis is lacking. Earlier work has shown that i.v. immunizations with γ-irradiated promastigotes protected mice against lethal *L. major* infection and that the cells responsible for this protection did not mediate DTH (19). Furthermore, it has been shown that the transfer of T-cell populations (Lyt-1^+2^-, L3T4$^+$) capable of mediating specific DTH exacerbated the *L. major* infection (25). Thus, a direct protective role for DTH in cutaneous leishmaniasis is in doubt. Indeed, it is possible that the DTH reactivity recruits permissive macrophages to the percutaneous tissue and that this provides an increased number of targets for parasitic infection. Protection could be mediated by another subset of T cells, also of the Lyt-1^+2^- phenotype, as reported for mice given four weekly i.v. immunizations with γ-irradiated promastigotes (19).

The protective effect induced by ffp injected i.v. was similar to that induced by γ-irradiated promastigotes (Fig. 2). Mice prophylactically immunized with irradiated parasites do develop high and sustained levels of DTH after challenge infection with *L. major* (19). Thus, it appears that the DTH suppression mediated by TS cells described here, though effective against immunization with killed parasites, could be overridden by infection. However, the neutralization of the suppressive effect was probably irrelevant to the subsequent outcome of the disease, as the determining factor would be at the onset of infection, where the availability of target macrophages is crucial.

In conclusion, specific Lyt-1^+2^+ L3T4$^+$ TS cells for DTH to *L. major* could be readily induced in mice with ffp administered i.v. However, mice with such profound DTH suppression were substantially protected against a challenge infection with *L. major* promastigotes. These data therefore do not support a causal role for DTH in immunity against cutaneous leishmaniasis. Furthermore, they lend credence to the notion that suppression of cutaneous DTH at the outset of infection may be important in prophylactic immunity.

ACKNOWLEDGMENT

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