Mechanisms of Acquired Immunity in Leishmaniasis

J. G. Howard and F. Y. Liew

Email alerting service	Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

Mechanisms of acquired immunity in leishmaniasis

By J. G. Howard, F.R.S., and F. Y. Liew

*Biomedical Research Division, The Wellcome Research Laboratories, Beckenham,
Kent BR3 3BS, U.K.*

Self-curing cutaneous leishmaniasis depends on T cell-mediated immune activation of infected macrophages. Failure of immune control in inbred mouse models of metastasizing mucocutaneous and visceralizing forms of the disease involves, respectively, insusceptibility of the parasite and the generation of T cells that suppress a potentially curative response. Prophylactic immunization in man has so far been restricted to cutaneous leishmaniasis and based on inducing infection under controlled conditions with virulent *Leishmania tropica major* promastigotes. The feasibility of immunization against visceral leishmaniasis merits reconsideration. BALB/c mice are genetically vulnerable to *L. tropica major*, which produces a fatal visceralizing type of disease involving specific suppression of cell-mediated immunity. Potent and lasting protection can be induced by repeated intravenous immunization with irradiated promastigotes. The efficacy of this 'vaccine' is relatively heat-stable (1 h at 56 °C). Immunity is not attributable to antibody but to the generation of Lyt-1$^+$2$^-$ T cells which, although possessing helper and macrophage-activating functions, do not express classical delayed-type hypersensitivity. The immunological features of this system and its relevance to the possibility of protection against human *Leishmania donovani* infection are considered.

Introduction

All leishmanial species are obligate intracellular protozoon parasites of the mononuclear phagocyte (macrophage) system. Each of them displays selectivity towards both a restricted range of vertebrate hosts and the phlebotamine sandflies which are their vector. Animal reservoirs of species pathogenic for man include dogs, rodents and, in South America, arboreal mammals. The promastigote (flagellated) stage develops in the sandfly (and in cell-free cultures) while transformation into the amastigote stage occurs within the macrophage.

Three main categories of leishmaniasis are recognized:

(i) Cutaneous disease, due to *L. tropica* (*L. major*) and *L. mexicana* species, evolves chronically and heals slowly. Less common non-healing, diffuse disseminating and relapsing (recidiva) forms are due to the same parasites and indicate the involvement of a host component in determining the outcome.

(ii) Mucocutaneous disease (espundia), due to *L. braziliensis braziliensis*, involves delayed metastasis to the nasal and oropharyngeal regions with progressive tissue destruction.

(iii) Systemic leishmaniasis (Kala-azar), due to *L. donovani*, involves progressive widespread parasitization of the macrophage system and, when untreated, a very high mortality rate within two years.

Self-cure in this disease is due to the immune response it evokes. This was clearly demonstrated experimentally in guinea-pigs when immunosuppressive measures were shown to abrogate normal healing with *L. enrietti* infection (Bryceson *et al.* 1972). The reasons for failure

of the response to control various manifestations of the disease are considered in this paper. The only immunization strategy against leishmaniasis used so far in man with any success has been restricted to the cutaneous disease. It is based on convalescent immunity following controlled induction of a lesion with viable *L. tropica* (see Greenblatt 1980). The feasibility of vaccination with a killed 'cocktail' vaccine against South American cutaneous leishmaniasis is currently being re-evaluated.

Understanding of immunological control in leishmaniasis has been greatly advanced by the use of inbred mouse strains that are susceptible to most species pathogenic for man. A wide spectrum of disease patterns can be obtained according to the genetic background of the host. The classic studies of Bradley and his colleagues (Bradley 1977; Bradley *et al.* 1979; Blackwell *et al.* 1983) established two levels of genetic expression affecting *L. donovani* infection: (i) innate susceptibility based on the relative resistance of the macrophage during the non-immune phase and determined by the *Lsh* gene; (ii) the efficacy of the immune stage for which three regulatory genes have been identified so far. The genetics of susceptibility to *L. tropica* are not yet as well defined, but are clearly not identical to those for *L. donovani*. This infection is, nevertheless, very well suited for immunological analysis, since all grades of disease ranging from a self-healing cutaneous lesion to uniformly fatal visceral leishmaniasis can be obtained with the same organism according to host genetic constitution (figure 1). It will feature largely in considering the following three aspects of immune control in leishmaniasis:
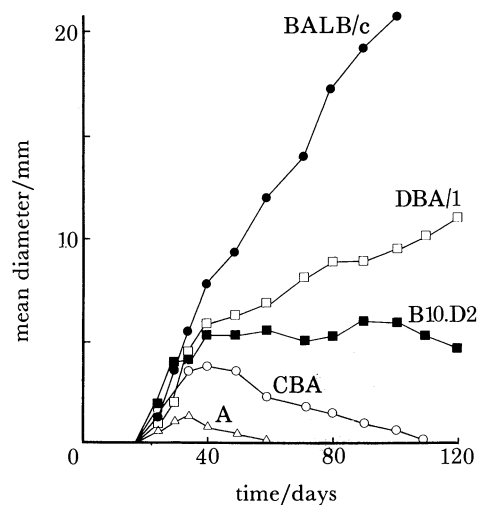

FIGURE 1. Differences in capability of inbred mouse strains to control infection with *L. tropica* (2×10^5 promastigotes subcutaneously) as shown by the course of lesion development. Uniformly fatal visceralization develops in BALB/c only. From Howard (1984).

(i) the nature of the healing response in infection;

(ii) the basis for failure of this response;

(iii) attempted induction and the nature of protection induced by prophylactic immunization.

NATURE OF CURATIVE OR CONTROLLING IMMUNITY INDUCED DURING LEISHMANIAL INFECTIONS

The case for acquired resistance to leishmaniasis being attributable to cell-mediated immunity (c.m.i.) is based on an impressive range of experimental and clinical observations. This c.m.i. response is generally associated with delayed-type hypersensitivity (DTH) reactivity, although recently an exception to this correlation has been observed (Howard *et al.* 1982; Liew *et al.* 1984). Humoral antibody responses, predominantly IgG, are directly related to the severity of infection, being weakest with self-healing cutaneous lesions and greatest in disseminated progressive disease. They reflect the size and duration of antigenic stimulation provided by the parasite load. Studies *in vitro* with anti-leishmanial antibodies have demonstrated three activities – complement-mediated lysis of promastigotes (Pearson & Steigbigel 1980), promotion of their phagocytosis by opsonic and cytophylic mechanisms (Herman 1980) and induction of surface patching and capping on promastigotes and amastigotes (Dwyer 1976). Nevertheless, there is no evidence of any corresponding *in vivo* role for antibody in determining the outcome of infection. In fact, the case is strongly to the contrary:

(i) the appearance of antibody is relatively late and low in titre during self-cure at which time the parasite would be protected by its intracellular location;

(ii) the antibody response bears no relation in either titre or isotype to outcome of disease in different inbred mouse strains (Olobo *et al.* 1980);

(iii) mice selected genetically for low antibody responses (Biozzi Ab/L selection 1) are highly resistant to *L. tropica* and develop minimal self-healing lesions (Hale & Howard 1981);

(iv) even more impressively, C3H mice rendered wholly antibody deficient by 'μ-suppression' treatment control *L. tropica* infection indistinguishably from normal mice (Sacks *et al.* 1984).

The possibility has been considered that leishmania-infected macrophages could be destroyed by lysis induced by contact with cytotoxic (Tc) lymphocytes (Bray & Bryceson 1968). No satisfactory evidence has yet been produced for the implication of this mechanism. Furthermore, expression of the Lyt-2 antigen is characteristic of Tc cells in the mouse, whereas adoptive transfer of immunity to leishmania has consistently involved Lyt-2 negative T cells (Liew *et al.* 1982, 1984).

The prime mechanism in acquired resistance seems clearly to be the induction of an Lyt-1^+2^- T cell response which leads to lymphokine-mediated activation of infected macrophages. Supportive evidence includes:

(i) CBA mice rendered relatively T cell deficient by adult thymectomy followed by irradiation and syngeneic bone marrow reconstitution show pronounced delay in their ability to control *L. tropica* infection (Preston *et al.* 1972). More rigorous T cell deprivation in CBA *nu/nu* (nude) mice leads to a total inability to control the disease which can be restored to normality by as few as 10^6 Lyt-1^+2^- T cells (Mitchell *et al.* 1980).

(ii) Immunity acquired during *L. tropica* and *L. donovani* infections can be transferred with lymphoid cells (Preston & Dumonde 1976; Rezai *et al.* 1980). This is a property of T cell and not B cell fractions. In the case of *L. tropica*, the immune effector cells have been delineated as Lyt-1^+2^- phenotype (Liew *et al.* 1982). Recently, *L. tropica* specific Lyt-1^+2^- T cell clones have been isolated which possess helper, DTH and macrophage activating activities (Louis *et al.* 1982). It is the latter of these properties which plays an effector role (Nacy *et al.* 1981; Scott *et al.* 1983).

(iii) Evidence has accumulated that associates oxygen-dependent mechanisms with a key role in the intracellular killing of leishmania by macrophages (Murray 1981, 1982; Haidaris & Bonventre 1982). This involves the generation of unstable toxic intermediates, especially hydrogen peroxide and superoxide anion, that are potentially capable of killing promastigotes and amastigotes. However, the recent finding that effective leishmanicidal activity can be induced by lymphokines in the IC-21 macrophage cell line without any accompanying respiratory burst establishes the existence of a non-oxidative killing mechanism (Scott et al. 1984). Whatever this may be, the Lyt-1^+2^- T cell response can undoubtedly lead to an effective parasiticidal response by infected macrophages.

Failure of immunological control in leishmaniasis

Two mechanisms have been delineated experimentally in mice:

(i) resistance of the parasite to macrophage leishmanicidal activity in a model which resembles espundia;

(ii) specific suppression of cell-mediated immunity which is relevant to the situation in Kala-azar and diffuse cutaneous disease.

(a) Resistance to macrophage leishmanicidal activity

C57BL/6 mice are innately resistant to *L. mexicana amazonensis*, which induces small regressing lesions. Despite an apparent cure and prolonged retention of strong DTH, low numbers of parasites persist. Many months later they increase locally and lead to destructive metastases which develop in the nasal region (Barrall et al. 1983). The implication here is that, despite continued exposure to lymphokine activation via a persisting c.m.i. response, macrophages fail to effect adequate cytocidal activity against the parasite. This has recently been verified experimentally by Scott et al. (1983). *L. mexicana amazonensis* was found to resist killing within lymphokine-activated C57BL/6 macrophages, whereas *L. tropica* and *Toxoplasma gondii* were wholly susceptible (the latter even in double infections with the resistant protozoa). The extent to which this mechanism of evasion is more widely operative in leishmaniasis is currently being investigated. An important question which arises from these observations is whether such refractoriness to the normal c.m.i. effector mechanism would create an obstacle to prophylactic immunization with the relevant species.

(b) Suppression of cell-mediated immunity

The most striking immunological feature of fatal disseminating *L. tropica* infection, which develops uniformly in BALB/c mice, is profound leishmania-specific suppression of DTH in the presence of a normal antibody response. Such anergy is not due to any intrinsic failure in the initial induction or expression of c.m.i. BALB/c mice develop quite strong levels of DTH within a week of infection, but this disappears within three to four weeks as the disease progresses, unless they are subjected shortly before infection to sublethal irradiation (Howard et al. 1981), cyclophosphamide or adult thymectomy, X-irradiation and bone marrow reconstitution (Howard et al. 1980). Mice in which DTH reactivity is sustained as a consequence of these manoeuvres are capable of controlling or healing the disease. This argues against any intrinsic defect in the ability of their macrophages either to present antigen effectively to T cells or to kill the parasite in response to lymphokine activation. The failure of BALB/c mice

to contain *L. tropica* infection is a consequence of the generation of potent specific suppressor T (Ts) cells which abrogate a potentially curative c.m.i. (Howard *et al.* 1981). The main evidence for this is as follows: (i) T cells transferred from infected DTH-negative mice can suppress both the induction and expression of leishmania-specific DTH in the recipient; (ii) the majority of BALB/c mice can control the disease and retain DTH if they are subjected to 550×10^{-2} Gy irradiation before infection; this can be entirely reversed, however, if irradiated mice are injected with as few as 10^6 Lyt-1^+2^- T cells isolated from suppressed donors with progressive disease (Howard *et al.* 1982; Liew *et al.* 1982). The course of infection reverts indistinguishably to that in a normal mouse (figure 2). B cells from such donors are ineffective, whereas T cells from normal donors can also reverse the irradiation effect, despite a transient period of disease arrest. T cells from healed donors transfer protective immunity (see figure 2). The Ts cells with Lyt-1^+2^- phenotype from BALB/c mice with progressive disease are analogous to Ts cells found to suppress DTH in other systems (Liew 1982). They are distinct from the Lyt-1^-2^+ Ts cells regulating antibody responses. A cloned Ts cell line has recently been obtained from a BALB/c mouse with active *L. tropica* infection (Liew 1983). These cells or their culture supernatant manifest specific suppression against lymphocyte proliferation *in vitro* and induction of DTH to *L. tropica* antigens *in vivo*. Furthermore they can also enhance lesion development of *L. tropica*-infected BALB/c mice, suggesting a causal role in the pathogenesis of the disease. This cloned Ts cell line is Lyt-1^+2^-I-J$^-$ and devoid of cytotoxic or helper activities.
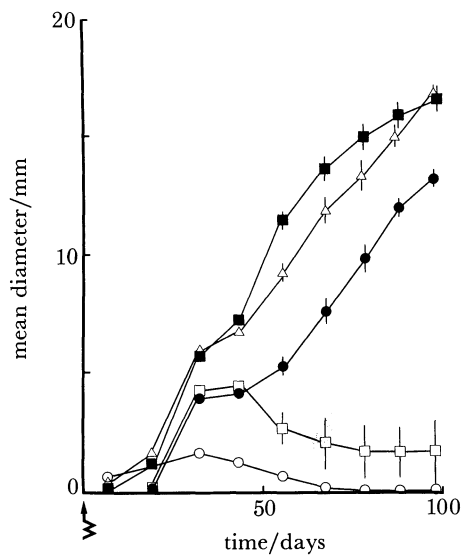

FIGURE 2. Prophylactic effect of 550×10^{-2} Gy irradiation 1 d before infection with *L. tropica* (2×10^7 promastigotes) in BALB/c mice ($\square$) compared with normal mice ($\triangle$). This effect is abrogated by injection with 2×10^7–5×10^7 T cells from DTH-suppressed, infected ($\blacksquare$) or normal ($\bullet$) donors. T cells from convalescent immune donors transfer immunity ($\circ$), ($n = 6$). From Howard *et al.* (1981).

Generation of Ts cells in highly vulnerable hosts seems likely to be a direct consequence of innate susceptibility at the macrophage level (see figure 3). Much greater amastigote proliferation occurring within the cutaneous lesion (Gorczynski & MacRae 1981) and in subsequent visceralization (Hill *et al.* 1983) would lead to a higher systemic antigen load which is known to favour suppressor cell induction (Asherson & Zembala 1975). It is also relevant
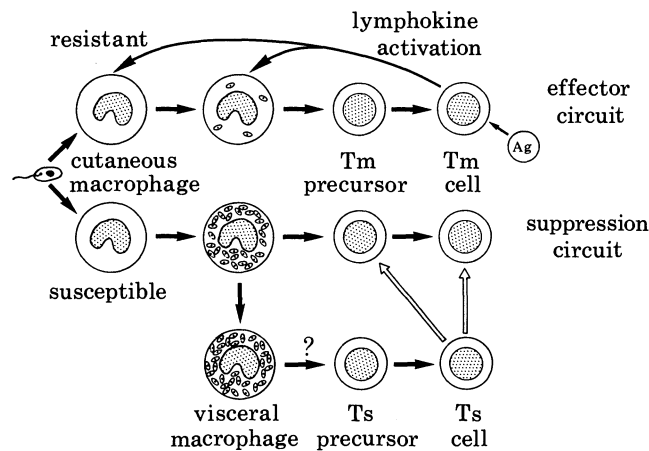
FIGURE 3. Schematic representation of Ts cell generation and impairment of cell-mediated immunity in mice genetically susceptible to *L. tropica* infection. Tm, Macrophage activating T cells; Ts, Suppressor T cells; Ag, antigen; ?, no direct evidence available. From Howard (1984).

that exposure of virgin lymphocytes *in vitro* to *L. tropica*-infected BALB/c (but not resistant CBA) cutaneous macrophages induced cells capable of inhibiting subsequent sensitization of other lymphocytes by *L. tropica* (Gorczynski & MacRae 1981). Analogous Ts cell generation with a parallel effect on the outcome of infection has now been demonstrated in *L. donovani* infection of innately susceptible B10.D2 mice (Ulczak & Blackwell 1983; Blackwell & Ulczak 1984). Although antibody-deficient (μ-suppressed) BALB/c mice can also arrest progression of *L. tropica* infection, this can be reversed completely by Ts cells alone (Sacks *et al.* 1984). Thus antibody is clearly not responsible for direct suppression of c.m.i. On the other hand, μ-suppression treatment seems in some way, so far unexplained, to interfere with Ts cell generation.

EXPERIMENTAL PROPHYLACTIC IMMUNIZATION

Until recently, experimental and clinical experience of attempted immunization against leishmaniasis with non-viable vaccines had been discouraging. Although some protection against *L. tropica* was shown to be induced in resistant mice with ultrasonicated promastigotes (Preston & Dumonde 1976) and crude antigen–antibody complexes (Handman *et al.* 1977), attempts to protect similarly against more severe disseminating disease caused by *L. donovani* or by *L. tropica* in BALB/c mice with variously killed promastigotes met with little real success. Our recent studies with lethally irradiated promastigotes in BALB/c mice have, however, established the feasibility of inducing substantial protection against otherwise fatal *L. tropica* infection (Howard *et al.* 1982; Howard *et al.* 1984; Liew *et al.* 1984). Since the causative organism of Kala-azar, *L. donovani*, does not itself produce fatal disease in mice, this model seems highly relevant to human visceral leishmaniasis which has so far not been amenable to immunological control.

BALB/c mice given repeated intravenous (i.v.) doses of heavily irradiated (1500 Gy) *L. tropica* promastigotes develop substantial resistance to a challenge infection with *L. tropica*, such that 100% mortality due to visceralization is reduced to 0–20%. About 50% of mice heal completely while the remaining survivors retain non-progressive local lesions only. Four weekly

injections are more effective than a single immunization, and the optimal dosage is 2×10^7 organisms with activity extending down to 2×10^4. Intravenous immunization is superior to the intraperitoneal route in protecting against higher infecting doses, whereas both intramuscular and subcutaneous administration (even in conjunction with a range of adjuvants) and i.v. immunization subsequent to infection are totally ineffective (figure 4). This prophylactic effect is *not* dependent on continuing viability or cellular invasiveness of the irradiated parasites, since their effective immunogenicity is retained following exposure to 56 °C for 1 h (figure 5) or even ultrasonication (unpublished data), indicating the relative stability of the protective antigen(s). The immunity induced is long lasting (more than 150 days), effective against both promastigote and amastigote challenges and is detectable against some other leishmanial species (*L. mexicana mexicana*, *L. mexicana amazonensis* and *L. braziliensis panamensis*), but not against unrelated infections with *Babesia rodhaini* (D. Snary, personal communication) or *Salmonella typhimurium* (D. Maskell, personal communication). Substantial protection can also be induced by immunization with heterologous irradiated *L. donovani* promastigotes (figure 6). Although this immunity does not extend to i.v. challenge with homologous *L. donovani* amastigotes (J. Blackwell and P. A. Scott, personal communications), the possibility of more relevant protection against homologus promastigotes introduced via the cutaneous route cannot be tested in mice.
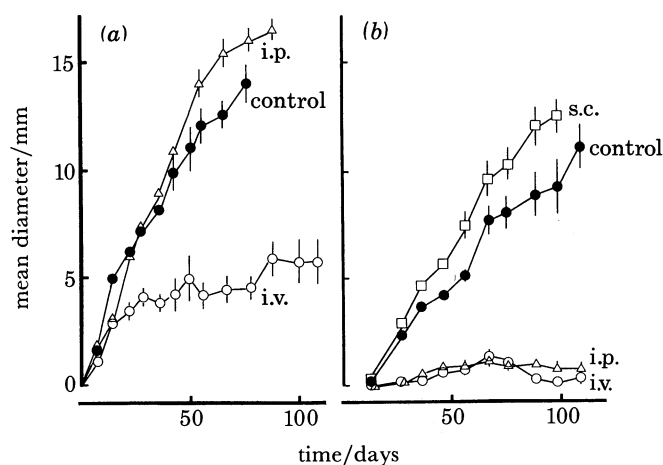

FIGURE 4. Comparison of four immunizations with 2×10^7 irradiated *L. tropica* promastigotes intravenously (i.v.), intraperitoneally (i.p.) and subcutaneously (s.c.) against infection with (*a*) 2×10^7 and (*b*) 2×10^5 promastigotes. Note lack of effect with s.c. route and inability of i.p. route to protect against the higher dose. $n = 6$. From Howard *et al.* 1982.

The immunological features of mice protected by repeated i.v. injection with irradiated *L. tropica* promastigotes are strikingly different from those characteristic of convalescent immunity. Predictably, specific antibody responses (in the isotype sequence M → G1/G3 → G2a/G2b →A) are substantially higher than are found in mice either with cured infection or with progressive disease. Nevertheless, no evidence for a causal association between antibodies and the prophylactic immunity induced could be found. First, while splenectomy before immunization drastically reduces the antibody response, it does not impair the extent of protection. Second, passive transfer of large amounts (up to 9 ml) of hyperimmune serum (or isotype fractions thereof) throughout the first eight weeks of infection fails to arrest disease progression.
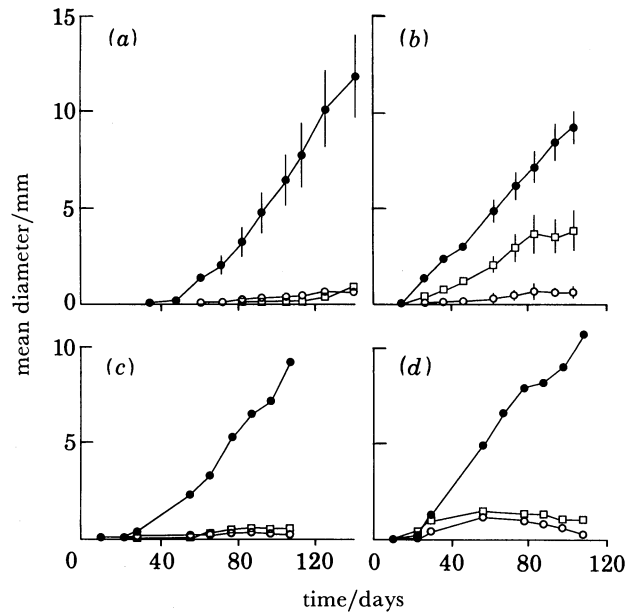
FIGURE 5. Prophylactic immunization of BALB/c mice with irradiated *L. tropica* promastigotes killed by heating at 56 °C for 1 h. Groups of mice were immunized (weekly) four times, i.v. with 2×10^7 1500 Gy irradiated promastigotes heated (□) or not heated (○). Together with non-immunized mice (●) they were challenged nine days later with 2×10^5 (*a–c*) or 2×10^6 (*d*) *L. tropica* promastigotes s.c.; $n = 7$ (*a*), *8* (*b*), *5* (*c, d*) (for clarity, not all standard errors of the mean are shown). From Howard *et al.* 1984.
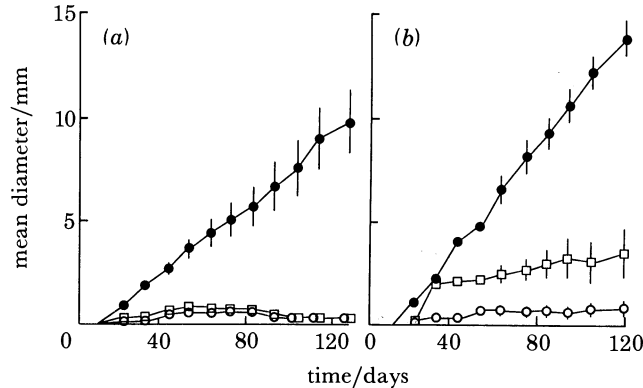

FIGURE 6. Comparison of protection induced in BALB/c mice by irradiated *L. donovani* and *L. tropica* promastigotes against subsequent *L. tropica* infection. Groups of mice were injected four times i.v. with 2×10^7 1500 Gy irradiated *L. donovani* (□) or *L. tropica* (○) promastigotes and, together with unimmunized controls (●), were challenged s.c. with 2×10^5 *L. tropica* promastigotes either 10 days (*a*) or 62 days (*b*) after immunization. (Bars = 1 s.e.m., $n = 10$ (*a*) or 8 (*b*). From Howard *et al.* 1984.

The prophylactic immunization does not induce any detectable cytotoxic T cell response. A paradoxical feature, in the light of the foregoing, is the failure of protected mice to show any cutaneous DTH or its early memory recall in response to live or killed promastigotes or a soluble *L. tropica* antigen preparation. This is in striking contrast with mice recovered from infection which are equally protected yet express strong DTH to all *L. tropica* antigens tested (table 1). Spleen, lymph node and peritoneal exudate cells from protectively immunized donors

similarly fail to transfer any DTH systemically or even locally (table 2). These cells also lack any demonstrable suppressive activity against the induction or expression of DTH to *L. tropica*. DTH in the immunized mice emerges with non-immune kinetics, in response to infection and is sustained in parallel with disease control. The generation of Ts cells thus appears to be prevented.

TABLE 1. FAILURE TO ELICIT DTH RESPONSE IN IMMUNIZED BALB/c MICE BEFORE OR FOUR DAYS AFTER INFECTING WITH 2×10^7 PROMASTIGOTES

	specific DTH ($\times 10^{-2}$ m) elicited by			
mice	protein soluble antigen	formalin fixed parasite	irradiated promastigotes	live promastigotes
before infection				
immunized	0	6 ± 2‡	5 ± 2‡	0
cured	65 ± 5†	78 ± 5†	70 ± 5†	30 ± 13†
normal	0	0	0	0
after infection				
immunized	0	3 ± 3	7 ± 2	8 ± 5‡
cured	37 ± 9†	57 ± 11†	77 ± 12†	67 ± 8†
normal	0	0	3 ± 3	25 ± 6

From Liew *et al.* 1984.
† $p < 0.005$ compared with respective normal controls ($n = 5$).
‡ Not significantly different from normal controls.

TABLE 2. PASSIVE TRANSFER OF DTH TO *L. TROPICA*

	specific DTH ($\times 10^{-2}$ mm)		
	local transfer (footpad)		systemic transfer (i.v.)
donors	5×10^6 spl. +l.n.	5×10^5 p.e.c.	10^7 p.e.c.
immunized	6 ± 5	13 ± 3‡	0
cured	28 ± 2†	30 ± 3†§	24 ± 3†§
normal	0	8 ± 3‡	0
immunized + cured	NT	23 ± 3†§	18 ± 4†§

spl., Spleen cells; l.n., lymph node cells; p.e.c., peritoneal exudate cells.
From Liew *et al.* 1984
  † $p < 0.005$ compared with their respective normal cell controls ($n = 5$).
  ‡,§ Not significantly different from each other.
  n.t., Not tested.

A series of adoptive transfer experiments have established that T cells and not B cells play a causal role in the protection induced by prophylactic immunization. Protection is apparent at the onset of challenge infection and is sustained in sublethally irradiated (200–550×10^{-2} Gy), but not non-irradiated, recipients (figure 7). Cell surface marker studies have delineated effector cells as Lyt-1^+2^-, a phenotype consistent with effector cells belonging to the helper/DTH lineage, rather than cytotoxic T cells that characteristically express Lyt-2 antigen.

The effector T cell population contains T helper cells (Th) for antibody synthesis. This is predictable in view of the Lyt phenotype and the high levels of *L. tropica*-specific antibody found in immunized mice. The dissociation found between helper and DTH activities in the present
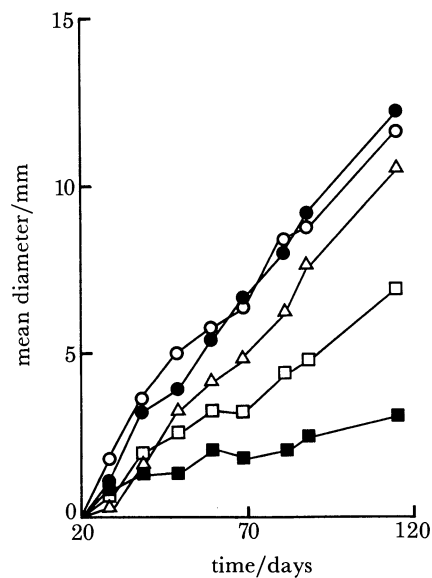
FIGURE 7. Adoptive transfer of protective potential of T cells from immunized BALB/c mice in 200×10^{-2} Gy irradiated syngeneic recipients. Donor spleen cells were enriched for T cells by an anti-Ig column and were transferred i.v. into recipients with the following dosage: 10^8 (■), 5×10^7 (□), 5×10^6 (△) or nil (○). Together with normal controls (●), they were infected s.c. with 2×10^5 L. tropica promastigotes 24 h after cell transfer, $n = 5$. From Liew et al. 1984.

system, however, is notable. The question as to whether or not Th and DTH-mediating T cells are indeed the same cell has long been controversial (discussed in the present context by Liew et al. 1984). Evidence presented here implies that they *can* be dissociable functions.

The protective effector T cells induced via the parental route in the present system are not cytotoxic T cells and must differ in either lineage or differentiation stage from the classical helper and DTH-mediating T cell subset. Evidence that these T cells are nevertheless capable of engaging in lymphokine-mediated activation of normal macrophages against ingested L. tropica has recently been obtained (Scott et al. 1984).

In conclusion, these studies demonstrate experimentally the feasibility of protective immunization against fatal visceral leishmaniasis and that this can be achieved with a non-viable vaccine. They also show that the protective Lyt-1^+2^- T cells which activate macrophages for parasite elimination contain Th activity but no demonstrable DTH reactivity, a finding which may have wider immunological implications. Thus protective immunity induced by immunization with non-replicating antigen is dissimilar in cellular detail from that of convalescent immunity. The extensive correlation previously observed between DTH reactivity and control of L. tropica infection is imperfect, since T cells involved in the effector function need not mediate cutaneous DTH. Finally, it remains to be determined whether a requirement for the i.v. route of administration would present a deterrent to applied vaccination or whether this restriction, described here, might not extend to molecularly defined antigens which are likely to become available in due course.

References

Asherson, G. L. & Zembala, M. 1975 Inhibitory T cells. *Curr. Top. Microbiol. Immunol.* **72**, 55–100.

Barral, A., Petersen, E. A., Sacks, D. L. & Neva, F. A. 1983 Late metastatic leishmaniasis in the mouse. A model for mucocutaneous disease. *Am. J. trop. Med. Hyg.* **32**, 277–285.

Blackwell, J. M. & Ulczak, O. M. 1984 Immunoregulation of genetically controlled acquired responses to *L. donovani* infection in mice: demonstration and characterisation of suppressor T cells in non-cure mice. *Infect. Immun.* **44**, 97–102.

Blackwell, J. M., Ulczak, O. M. & Channon, J. Y. 1983 Immunogenetics and immunoregulation of parasitic infection in mice with specific reference to leishmaniasis. In *Experimental bacterial and parasitic infections* (ed. G. Keusch and T. Wadstrom), pp. 365–373. New York: Elsevier.

Bradley, D. J. 1977 Regulation of *Leishmania* populations within the host. II. Genetic control of acute susceptibility of mice to *Leishmania donovani* infection. *Clin. exp. Immunol.* **30**, 130–140.

Bradley, D. J., Taylor, B. A., Blackwell, J., Evans, E. P. & Freeman, J. 1979 Regulation of *Leishmania* populations within the host. III. Mapping of the locus controlling susceptibility to visceral leishmaniasis in the mouse. *Clin. exp. Immunol.* **37**, 7–14.

Bray, R. S. & Bryceson, A. D. M. 1968 Cutaneous leishmaniasis of the guinea pig. Action of sensitized lymphocytes on infected macrophages. *Lancet* ii, 898–899.

Bryceson, A. D. M., Preston, P. M., Bray, R. S. & Dumonde, D. C. 1972 Experimental cutaneous leishmaniasis. II. Effects of immunosuppression and antigenic competition on the course of infection with *Leishmania enriettii* in the guinea pig. *Clin. exp. Immunol.* **10**, 305–335.

Dwyer, D. M. 1976 Antibody-induced modulation of *Leishmania donovani* surface membrane antigens. *J. Immunol.* **117**, 2081–2091.

Gorczynski, R. M. & MacRae, S. 1981 Analysis of subpopulations of glass-adherent mouse skin cells controlling resistance/susceptibility to infection with *Leishmania tropica* and correlation with the development of independent proliferative signals to Lyt-1.2$^+$/Lyt-2.1$^+$ T lymphocytes. *Cell. Immunol.* **67**, 74–89.

Greenblatt, C. L. 1980 The present and future of vaccination for cutaneous leishmaniasis. In *New developments with human and veterinary vaccines*, pp. 259–285. New York: Alan R. Liss.

Haidaris, C. G. & Bonventre, P. F. 1982 A role for oxygen-dependent mechanisms in killing of *Leishmania donovani* tissue forms by activated macrophages. *J. Immunol.* **129**, 850–855.

Hale, C. & Howard, J. G. 1981 Immunological regulation of experimental cutaneous leishmaniasis. 2. Studies with Biozzi high and low responder lines of mice. *Parasit. Immunol.* **3**, 45–55.

Handman, E., El-On, J., Spira, D. T., Zuckerman, A. & Greenblatt, C. L. 1977 Protection of C3H mice against *L. tropica* by a non living antigenic preparation. *J. Protozool.* **24**, 20A–21A.

Herman, R. 1980 Cytophilic and opsonic antibodies in visceral leishmaniasis in mice. *Infect. Immun.* **28**, 585–593.

Hill, J. O., North, R. J. & Collins, F. M. 1983 Advantages of measuring changes in the number of viable parasites in murine models of experimental cutaneous leishmaniasis. *Infect. Immun.* **39**, 1087–1094.

Howard, J. G. 1984 Host immunity to leishmaniasis. In *Leishmaniasis* (ed. K.-P. Chang and R. S. Bray), Amsterdam: Elsevier/North-Holland Biomedical Press (In the press.)

Howard, J. G., Hale, C. & Liew, F. Y. 1980 Immunological regulation of experimental cutaneous leishmaniasis. III. Nature and significance of specific suppression of cell-mediated immunity in mice highly susceptible to *Leishmania tropica*. *J. exp. med.* **152**, 594–607.

Howard, J. G., Hale, C. & Liew, F. Y. 1981 Immunological regulation of experimental cutaneous leishmaniasis. IV. Prophylactic effect of irradiation as a result of abrogation of suppressor T cell generation in mice genetically susceptible to *Leishmania tropica*. *J. exp. Med.* **153**, 557–568.

Howard, J. G., Liew, F. Y., Hale, C. & Nicklin, S. 1984 Prophylactic immunization against experimental leishmaniasis. II. Further characterization of the protective immunity against fatal *Leishmania tropica* infection induced by irradiated promastigotes. *J. Immunol.* **132**, 450–455.

Howard, J. G., Nicklin, S., Hale, C. & Liew, F. Y. 1982 Prophylactic immunization against experimental leishmaniasis. I. Protection induced in mice genetically vulnerable to fatal *Leishmania tropica* infection. *J. Immunol.* **129**, 2206–2212.

Liew, F. Y. 1982 Regulation of delayed-type hypersensitivity to pathogens and alloantigens. *Immunol. Today* **3**, 18–23.

Liew, F. Y. 1983 Specific suppression of responses to *Leishmania tropica* by a cloned T-cell line. *Nature, Lond.* **305**, 630–632.

Liew, F. Y., Hale, C. & Howard, J. G. 1982 Immunologic regulation of experimental cutaneous leishmaniasis. V. Characterization of effector and specific suppressor T cells. *J. Immunol.* **128**, 1917–1922.

Liew, F. Y., Howard, J. G. & Hale, C. 1984 Prophylactic immunization against experimental leishmaniasis. III. Protection against fatal *Leishmania tropica* infection induced by irradiated promastigotes involves Lyt-1$^+$2$^-$ T cells which do not mediate cutaneous DTH. *J. Immunol.* **132**, 456–461.

Louis, J. A., Zubler, R. H., Coutinho, S. G., Lima, G., Behin, R., Mauel, J. & Engers, H. D. 1982 The *in vitro*

generation and functional analysis of murine T cell populations and clones specific for a protozoan parasite, *Leishmania tropica. Immunol. Rev.* **61**, 215–243.

Mitchell, G. F., Curtis, J. M., Handman, E. & McKenzie, I. F. C. 1980 Cutaneous leishmaniasis in mice: disease patterns in reconstituted nude mice of several genotypes infected with *Leishmania tropica. Aust. J. exp. Biol. med. Sci.* **58**, 521–532.

Murray, H. W. 1981 Susceptibility of *Leishmania* to oxygen intermediates and killing by normal macrophages. *J. exp. Med.* **153**, 1302–1315.

Murray, H. W. 1982 Cell-mediated immune response in experimental visceral leishmaniasis. II. Oxygen-dependent killing of intracellular *Leishmania donovani* amastigotes. *J. Immunol.* **129**, 351–357.

Nacy, C. A., Meltzer, M. S., Leonard, E. J. & Wyler, D. J. 1981 Intracellular replication and lymphokine-induced destruction of *Leishmania tropica* in C3H/HeN mouse macrophages. *J. Immunol.* **127**, 2381–2386.

Olobo, J. O., Handman, E., Curtis, J. M. & Mitchell, G. F. 1980 Antibodies to *Leishmania tropica* promastigotes during infection in mice of various genotypes. *Aust. J. exp. Biol. med. Sci.* **58**, 595–601.

Pearson, R. D. & Steigbigel, R. T. 1980 Mechanism of lethal effect of human serum upon *Leishmania donovani. J. Immunol.* **125**, 2195–2201.

Preston, P. M., Carter, R. L., Leuchars, E., Davies, A. J. S. & Dumonde, D. C. 1972 Experimental cutaneous leishmaniasis. III. Effects of thymectomy on the course of infection of CBA mice with *Leishmania tropica. Clin. exp. Immunol.* **10**, 337–357.

Preston, P. M. & Dumonde, D. C. 1976 Experimental cutaneous leishmaniasis. V. Protective immunity in subclinical and self-healing infection in the mouse. *Clin. exp. Immunol.* **23**, 126–138.

Rezai, H. R., Farrell, J. & Soulsby, E. L. 1980 Immunological responses of *L. donovani* infection in mice and significance of T cell in resistance to experimental leishmaniasis. *Clin. exp. Immunol.* **40**, 508–514.

Sacks, D. L., Scott, P. A., Asofsky, R. & Sher, F. A. 1984 Cutaneous leishmaniasis in anti-IgM treated mice: enhanced resistance due to functional depletion of a B cell dependent T cell involved in the suppressor pathway. *J. Immunol.* **132**, 2072–2077.

Scott, P., James, S. & Sher, A. 1984 The respiratory burst is not required for killing of intracellular and extracellular targets by a lymphokine-activated macrophage cell line. *Science, Wash.* (In the press.)

Scott, P., Sacks, D. & Sher, A. 1983 Resistance to macrophage-mediated killing as a factor influencing the pathogenesis of chronic cutaneous leishmaniasis. *J. Immunol.* **131**, 966–971.

Scott, P. A., Sher, A. & Howard, J. G. 1984 (In preparation.)

Ulczak, O. M. & Blackwell, J. M. 1983 Immunoregulation of genetically controlled acquired responses to *Leishmania donovani* infection in mice: the effects of parasite dose, cyclophosphamide and sublethal irradiation. *Parasit. Immunol.* **5**, 449–463.