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REVISÃO

FUNCTIONAL ANALYSIS OF THE MURINE T LYMPHOCYTE IMMUNE RESPONSE TO A PROTOZOAN PARASITE, *LEISHMANIA TROPICA*

H.D. ENGERS
S.G. COUTINHO
G.M.C. DE ARAÚJO LIMA
J.A. LOUIS

The results presented in this review summarize a series of experiments designed to characterize the murine T cell imune response to the protozoan parasite Leishmania tropica. *Enriched T cell populations and T cell clones specific for* L. tropica *antigens were derived from lymph nodes of primed mice and maintained in continuous culture* in vitro. *These T lymphocytes were shown (A) to express the Lyt $1^+ 2^-$ cell surface phenotype, (B) to proliferate specifically* in vitro *in response to parasite antigens, together with a source of irradiated syngeneic macrophages, (C) to transfer antigen-specific delayed-type hypersensitivity (DTH) responses to normal syngeneic mice, (D) to induce specific activation of parasitized macrophages* in vitro *resulting in the destruction of intracellular parasites, (E) to provide specific helper activity for antibody responses in vitro in a hapten-carrier system. Protection studies using these defined T cell populations should allow the characterization of parasite antigens(s) implicated in the induction of cellular immune responses beneficial for the host.*

Leishmanias are protozoan parasites which infect several mammalian species, including man (Faust, Russel & Jung, 1974). The parasite is transmitted from one animal to another by sandflies, which upon biting an infected host acquire parasitized mononuclear phagocytes (macrophages) containing the amastigote (non-flagellated) form of the parasite. The amastigote then transforms into the promastigote flagellated form of the parasite in the alimentary tract of the vector, and thus the cycle of trnasmission is perpetuated by the infected fly.

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Immunology Department, Swiss Institute for Experimental Cancer Research, and W.H.O. Immunology Research and Training Center, Institute of Biochemistry, University of Lausanne, CH-1066, Epalinges, Switzerland.

Correspondence: Sergio G. Coutinho, Instituto Oswaldo Cruz, Caixa Postal 926 – 20000 Rio de Janeiro, Brazil.

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The type and intensity of immune response exhibited by the host has been suggested to determine the clinical manifestations of the various forms of leishmaniasis observed in humans (Turk & Bryceson, 1971). In fact, there are several observations which emphasize the importance of cell-mediated responses (including the development of delayed-type hypersensitivity (DTH) reactions) in the healing of leishmanial lesions in man (Adler, 1964; Bryceson, 1970; Convit, Pinardi & Rondon, 1972). For example, DTH reactions to leishmanial antigens are absent during the active phase of Kala-Azar, but are detectable after healing and correlate with protection against a subsequent reinfection.

The fact that infection of mice with *L. tropica* induces cutaneous lesions analogous to those observed in human leishmaniasis provides an animal model in which one may confirm the role of cellular immunity with regards to the healing of lesions and subsequent immunity to reinfection (Handman, Ceredig & Mitchell, 1978; Behin, Mauel & Sordat, 1979). In several studies it has been shown that mice deficient in thymus-derived (T) lymphocytes were highly susceptible to infection with *L. tropica* (Preston et al, 1972; Handman, Ceredig & Mitchell, 1978) and that resistance to infection could be restored by adoptive transfer of syngeneic T lymphocytes (Mitchell et al, 1980). However, the precise mechanism(s) by which T cells contribute to the eventual destruction of parasites remain to be delineated.

In this review, a series of results are presented which demonstrate that homogeneous populations of leishmania-specific murine T lymphocytes can be generated *in vitro* following an initial priming *in vivo*, and that these enriched T cell populations exhibit several parasite-specific immunological functions, including (1) antigen-specific proliferation, (2) T cell helper function for hapten-carrier responses using TNP-leishmania, (3) specific DTH reactions and (4) activation of macrophages to yield destruction intracellular leishmania. In addition, parasite-specific Lyt 1^+ T cell clones have been derived from the T cell enriched populations and these clones were also observed to perform the above mentioned immunological functions.

Generation of a specific T cell-dependent proliferative response to *L. tropica in vitro*.

The activation of antigen-specific murine T lymphocytes to yield specific T cell proliferative responses *in vitro* has been reported by several laboratories (Schwartz, Yano & Paul, 1978; Corradin, Ethinger & Chiller, 1977; Rosenwasser & Rosenthal, 1978). We have recently adapted the method of Corradin, Ethinger & Chiller (1977) to the study of murine lymph node T cell activation by various parasite antigens, including *L. tropica* (Louis et al, 1979, 1981, 1982) and *T. brucei* (Gasbarre, Hug & Louis, 1980, 1982). As shown in Table I, when cells from the draining lymph nodes of *L. tropica*-injected mice were reexposed to *L. tropica in vitro*, an intense proliferative response was obtained. This proliferative response was T cell-dependent since (a) lymph node cells from nu/nu mice immunized with *L. tropica* did not exhibited a proliferative response *in vitro* (Table I) and (b) pretreatment of primed lymph node cells with anti-T cell antibodies and complement greatly reduced a subsequent proliferative response *in vitro* when challenged with *L. tropica* (Louis et al, 1979).

The observed proliferative responses were specific for the immunizing antigen, and independent of the source of serum used either to grow the parasites (Louis et al, 1979) or used in the actual T cell proliferation assay (Table II). This was an important point, since several *in vitro* responses had been shown previously to be affected by fetal calf serum (Forni & Green, 1976; Golstein et al, 1978).

In experiments using nylon wool passed lymph node populations, i.e. purified T cell preparations, it was found that immune T cells alone did not respond to *L. tropica* unless a source of macrophages was readded to the cultures (Louis et al, 1979). As observed in Figure 1, purified T cells plus antigen plus macrophages yielded an optimal

TABLE I

L. tropica-induced proliferation of the draining lymph node cells from *L. tropica* injected mice[a]

Mouse strain	*[^3H] thymidine incorporation (cpm x 10^{-3})*	
	medium	*L. tropica*
C57BL/6	4.8 ± 2.4	86.0 ± 14.6
CBA/T6	0.7 ± 0.2	102.8 ± 10.6
DBA/2	1.7 ± 0.2	30.1 ± 1.8
BALB/c nu/+	1.0 ± 0.1	64.2 ± 2.4
BALB/c nu/nu	0.2 ± 0.1	1.1 ± 1.8

[a] Mice were primed with 10^6 *L. tropica* in CFA subcutaneously at the base of the tail and 6-9 days later the draining lymph node cells were tested *in vitro* for antigen specific proliferation 4×10^5 cells were stimulated with 10^6 *L. tropica* for 5 days and [^{3}H] thymidine incorporation measured in a 16 hr pulse (mean cpm ±SD for triplicate cultures).

TABLE II

Specificity of the *in vitro* antigen-induced proliferation of *in vivo* primed lymph node cells[a]

Priming in vivo	*[^3H] thymidine incorporation (cpm x 10^{-3})*			
	medium	*L. tropica*	*HGG*	*FBS*
Expt. 1				
L. tropica	0.4 ± 0.2	133.4 ± 3.7	ND[b]	0.5 ± 0.1
FBS	0.9 ± 0.1	3.8 ± 1.4	ND	71.2 ± 15.8
None	0.2 ± 0.1	0.6 ± 0.1	ND	1.1 ± 0.4
Expt. 2				
L. tropica	4.3 ± 0.9	50.1 ± 3.6	10.6 ± 1.2	ND
FBS	2.3 ± 0.3	6.9 ± 1.1	38.3 ± 3.8	ND
None	1.9 ± 0.3	5.8 ± 0.7	3.6 ± 0.3	ND

[a] CBA/T6 mice were primed with 10^6 *L. tropica*, or 100 μg HGG or 20 μl FBS, all in CFA, and then lymph node cells were tested 8 days later for antigen-induced proliferation *in vitro*. (Reproduced with permission from Eur. J. Immunol. 9:841, 1979).

[b] ND = not determined

response. In addition, macrophages which had been "pulsed" (i.e. incubated with *L. tropica* for 24 hr and then washed free of parasites) were also able to induce a very good T cell proliferative response in this system (Fig. 1).

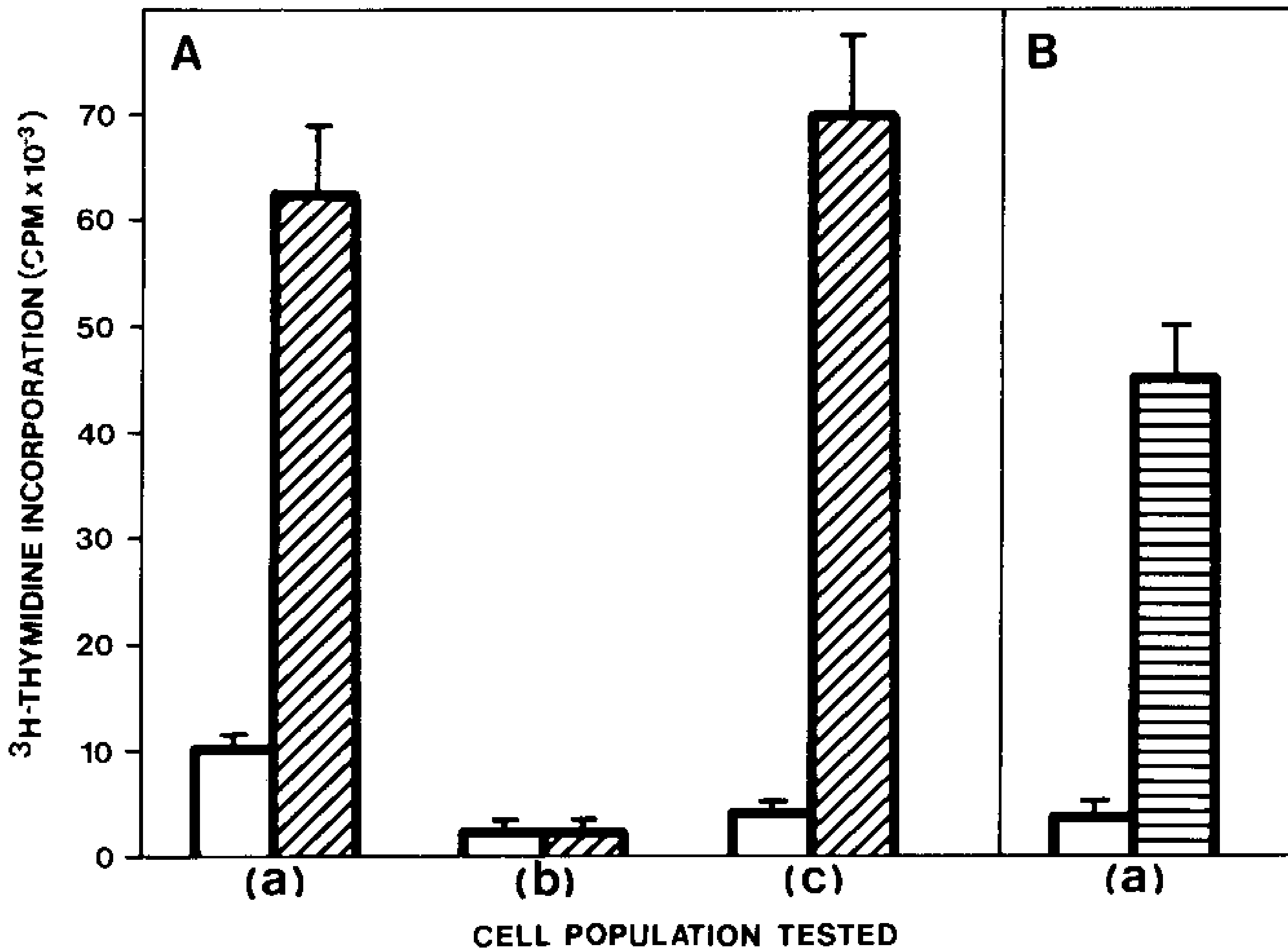

Fig. 1 – **Proliferative response of unfractionated and nylon wool-purified immune lymph node cells cultured with *L. tropica*.** Primed lymph node cells from CBA mice injected with 10^6 *L. tropica* s.c. at the base of the tail 8-10 days earlier were pooled and passed over nylon wool columns. (A) Cultures contained 10^6 *L. tropica* plus (a) 4×10^5 unfractionated lymph node cells (b) 4×10^5 nylon wool purified (i.e. T cells) lymph node cells (c) 4×10^5 nylon wool purified lymph node cells *plus* 10^5 irradiated (2,000 rads) CBA peritoneal exudate cells as a source of macrophages. Proliferation was measured 6 days later using a 16 hr [^{3}H] thymidine pulse. Open bars represent the response in the absence of added *L. tropica* antigen. (B) Ability of parasitized (i.e. antigen pulsed) macrophages to trigger proliferation of primed lymph node cells. 10^5 *L. tropica* pulsed CBA immune lymph node cells and 6 days later proliferation assessed. The open bar represents the proliferation observed using normal, non-parasitized macrophages. (Reproduced from Eur. J. Immunol. 9 :841 (1979), with permission).

However, when the nylon wool purified lymph node cells were investigated for a possible genetic restriction with regards to the source of macrophages used to reconstitute the culture system, no such restriction was seen (Louis et al, 1981). These results were attributed to the presence of suboptimal numbers of residual syngeneic macrophages contaminating the nylon wool purified T cell populations. In subsequent attempts to obtain immune T cell populations functionally devoid of macrophages, we first purified T cell blasts harvested from day 5 *in vitro* cultures similar to those described in Table 1 (Louis et al, 1981). When these T cell blasts were maintained in culture for a further 4-5 days in 25% mixed leukocyte culture (MLC) supernatant (a source of T cell growth factor (TCGF)), they could then be specifically restimulated by *L. tropica* antigens *only* in the presence of syngeneic macrophages. Figure 2 illustrates the phenomenon of genetic restriction as observed in the T cell proliferative response to *L. tropica* antigens in this system. As can be seen using congenic recombinant strains of mice, the requirement for homology within the H-2 region of the major histocompatibility complex mapped to the I-A region.

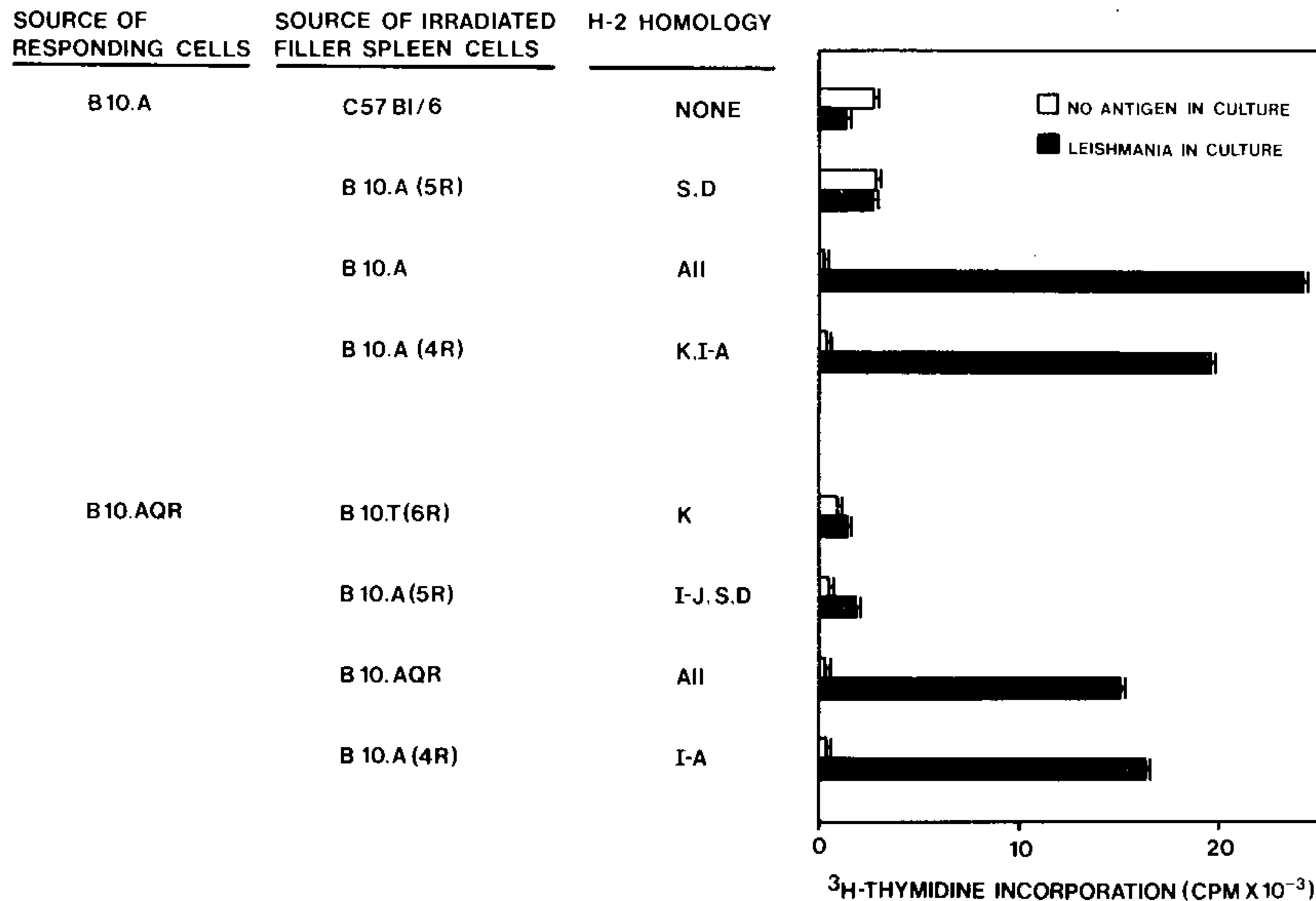

Fig. 2 – Antigen-specific proliferative responses of *Leishmania*-immune lymph node blasts as a function of the haplotype of added spleen cells. *L. tropica*-specific blasts derived from immune B10.A or B10.AQR mice were depleted of functional macrophages by maintenance in 2° MLC supernatant for 5 days. The blasts were then restimulated with 10^6 *L. tropica* together with 5 x 10^6 irradiated spleen cells from mice of the indicated strains. Proliferation was assessed 5 days later using a 16 hr ^3H thymidine pulse, with the values given representing 1/10 of the initial cultures. (Reproduced from J. Immunol. *126* :1661 (1981), with permission).

The requirement for cells bearing Ia antigens was further supported by experiments showing that prior treatment of the irradiated syngeneic filler spleen cells with a monoclonal anti-Ia antibody and complement greatly reduced their ability to support a parasite-specific proliferation of syngeneic lymph node T-cell blasts (Louis et al, 1981).

Again, when T cell blasts which had been purified from *in vitro* cultures and subsequently maintained in TCGF-containing medium were restimulated in the presence of the immunizing antigen, the observed T-cell proliferative response was antigen-specific (Table III).

TABLE III

Specificty of the proliferative response of antigen-indaced blast cells following propagation in 2° MLC SN[a]

Antigen added to cultures	*[^3H] thymidine incorporation (cpm x 10^{-3})*	
	OVA-induced blasts	*L. tropica-induced blasts*
OVA	13.4 ± 0.2	0.3 ± 0.1
L. tropica	0.8 ± 0.1	24.8 ± 1.0
None	0.9 ± 0.1	0.3 ± 0.1

[a] Antigen-induced BDF_1 blast cells were maintained for 5 days in medium containing 2° MLC SN. Then 10^5 blasts were cultured with either 10^6 *L. tropica* or OVA (100 μg/ml) together with 5 x 10^6 irradiated BDF_1 filler spleen cells. Proliferation was estimated 5 days later using a 16 hr [^{3}H] thymidine pulse, with the values given representing 1/10 of the initial cultures. (Reproduced from J. Immunol. 126 :1961, 1981, with permission).

Immunological functions displayed by *L. tropica*-specific T-cell blast populations.

Since various functions have been attributed to distinct subpopulations of T lymphocytes, as distinguished by their Lyt phenotype (Cantor & Boyse, 1976), the populations of *L. tropica* specific T-cell blasts were further characterized with regard to their surface phenotype and various functional activities.

The Lyt phenotype of parasite-specific T cell blasts was determined by flow microfluorometry (Cerottini & MacDonald, 1981) and it was found that in several strain combinations tested, more than 90% of the blast cells expressed Lyt-1 antigens (Louis et al, 1981). For example, in Figure 3, the majority of DBA/2 T cell blasts examined exhibit Lyt-1 antigens with a minority positive for Lyt-2 antigens. Thus, after maintenance in TCGF-containing medium, the majority of parasite-specific T cells exhibited the Lyt-1^+, Lyt-2^- phenotype.

Prompted by the fact that the parasite specific T cell blasts were Lyt 1^+, the potential helper activity of *L. tropica*-specific blasts was examined using a secondary *in vitro* anti-trinitrophenyl (TNP) antibody response (Zubler & Louis, 1981). In this system, *L. tropica*-immune T cells were cultured for 5 days together with purified B cells from spleens of mice immunized with TNP-keyhole lympet hemocyanin (TNP-KLH) and TNP-*L. tropica* as a source of antigen. Then IgG anti-TNP plaque-forming cells were enumerated by the Jerne plaque assay, using TNP-horse red blood cells as indicator cells. As shown in Table IV significant anti-TNP PFC responses were obtained only when an optimal number of *L. tropica*-immune T cell blasts were added to the culture system.

Additional experiments showed that the PFC response was specific and that linkage of the TNP hapten to the carrier (*L. tropica*) was required (Zubler & Louis, 1981).

TABLE IV

Helper activity exhibited by *L. tropica*-specific T cell blasts[a]

L. tropica-specific blasts added to the culture	*Anti-TNP PFC per culture (day 5)*
None	<20
10^2	83
10^3	1180
10^4	910

[a] 2.5×10^4 TNP-KLH primed B cells from C57BL/6 mice were cultured together with 10^5 TNP-*L. tropica*, 5×10^3 irradiated syngeneic PEC and varying numbers of *L. tropica*-specific C57BL/6 T cell blasts. Anti-TNP IgG PFC were determined 5 days later.

As several recent reports suggest that DTH responses may represent an important effector mechanism by which the host may eliminate foreign cells (Liew & Simpson, 1980; Loveland et al, 1981), we then tested the possibility that the enriched populations of *L. tropica*-immune T cells could mediate DTH responses *in vivo*. Indeed, when normal syngeneic mice were injected in the footpad with varying numbers of *L. tropica*-immune T-cell blasts, together with *L. tropica* antigen, a significant DTH response was elicited, as measured by footpad swelling 24 hr later (Table V). In additional experiments, transfer of DTH was shown to be antigen-specific and could only be achieved in syngeneic recipient mice, suggesting that these DTH responses were also restricted by genes within the MHC (Lima et al, in preparation).

TABLE V

Transfer of delayed type hypersensitivity (DTH) with *L. tropica*-specific T cell blasts[a]

T cell blasts transferred ($\times 10^{-6}$)	*Increase in footpad thickness (mm)*
0	0.03 ± 0.01
0.1	0.30 ± 0.11
1.0	0.65 ± 0.03
15.0	1.22 ± 0.05

[a] Varying numbers of *L. tropica*-specific DBA/2 T cell blasts were injected together with *L. tropica* antigen in the footpads of normal syngeneic mice. 24 hours later, the average increase in footpad thickness was measured.

Finally, based on the fact that macrophages have been shown to be activated by various stimuli and subsequently become capable of efficiently killing and digesting leishmania and other intracellular microorganisms (Mauel, Buchmuller & Behin, 1978; Behin, Mauel & Sordat, 1979; Nacy & Meltzer, 1979), we investigated the potential activation of macrophages by parasite-immune T cell populations *in vitro*. These experiments were based on an experimental model recently described by Mauel, Behin & Louis (1981), whereby murine macrophages infected with *L. enriettii* (which crossreacts extensively with *L. tropica* and is killed and eliminated more efficiently than *L. tropica*) were cultured together with *L. tropica*-immune T-cell blasts and the resultant destruction of intracellular *L. enriettii* monitored after 24, 48 and 72 hr of culture. As shown in Table VI, using T-cell blasts from two different mouse strains there was a dramatic destruction of intracellular parasites by infected macrophages which were activated following the addition of *L. tropica*-immune T-cell blasts. In additional experiments, the activation of macrophages was confirmed to be parasite-specific, since ovalbumine-specific T-cell blasts failed to activate *L. enriettii*-infected macrophages (Louis et al, 1981; Coutinho et al, in preparation). Thus it is conceiveable that *in vivo*, macrophage activation may represent a major effector mechanism by which infected hosts are able to eliminate the parasites in question.

TABLE VI

Destruction of intracellular parasites by infected macrophages activated following the addition of *L. tropica*-immune T cell blasts[a]

Cells added to macrophage cultures (5 x 10⁶)	*Parasites per 100 macrophages*		
	24 hr	*48 hr*	*72 hr*
Expt. 1			
CBA/T6 normal lymph node cells	315	321	317
CBA/T6 L. tropica-specific blasts	295	102	11
Expt. 2			
NZB normal lymph node cells	313	295	289
NZB L. tropica-specific blasts	303	27	3

[a] Monolayers of *L. enriettii*-infected PEC macrophages were prepared on glass coverlips, and at the specified intervals, the intracellular destruction of parasites as a result of macrophage activation was determined microscopically.

Another possible effector mechanism related to the generation of parasite-immune T-cell blasts would be that of direct cytolysis of parasite-infected macrophages by cytolytic T lymphocytes (CTL) specific for parasite antigens expressed on the macrophage cell surface. However, as observed in Figure 3, only a minority of T cells resulting from parasite stimulation *in vitro* expressed Lyt-2 alloantigens, a marker for CTL, and therefore these populations might not be expected to demonstrate considerable cytolytic activity. Indeed, when tested using ^{51}Cr-labeled syngeneic *L. tropica*-infected macrophages as potential targets, parasite-immune T-cell populations did not express cytolytic activity, over the 4 hr assay interval (Fig. 4). The infected macrophages were capable of being lysed however, since allogeneic CTL directed against H-2 antigens were capable of killing quite efficiently both infected and non-infected macrophages of the appropriate strain (Fig. 4). These latter results would suggest that in fact, infected and non-infected macrophages expressed roughly the same levels of cell surface antigens (i.e., K/D H-2 determinants) recognized by the allogeneic CTL effector cells.

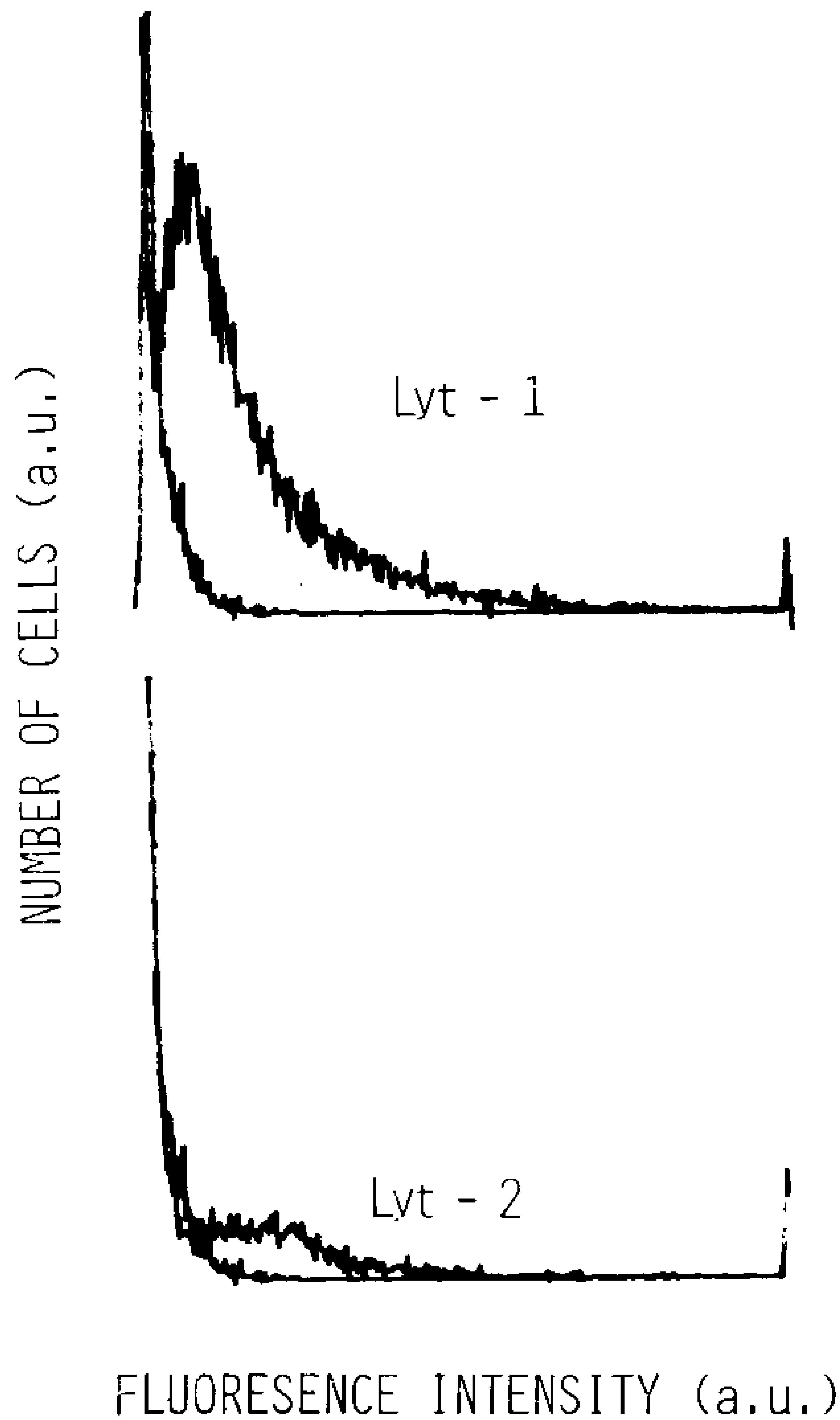

Fig. 3 – The Lyt phenotype of DBA/2 *L. tropica*-stimulated lymph node blasts. Immune lymph node cells from DBA/2 mice previously primed with parasites were cultured together with *L. tropica* for 5 days. The resulting T cell blasts were analyzed for expression of Lyt-1 and Lyt-2 cell surface alloantigens using monoclonal antibodies and a fluorescence-activated cell sorter. The lower curve in each tracing represents the negative control value i.e. cells stained with the fluorescent indicator reagent in the absence of monoclonal antibody.

Analysis of *L. tropica*-specific T-cell responses at the clonal level.

Utilizing recent advances made in the area of the derivation and subsequent propagation of antigen-specific murine T cell clones *in vitro* (Nabholz et al, 1978; Schreier & Tees, 1979; MacDonald et al, 1980; Glasebrook et al, 1981), we were successful in deriving and maintaining in short-term culture (up to two months) more than a dozen clones of T cells specific for *L. tropica* antigens. The basic approach was to first restimulate parasite-immune lymph node cells *in vitro* for 5-6 days and then derive independent clones of *L. tropica*-specific T cells, either by limiting dilution analysis techniques (MacDonald et al, 1980) or by direct micromanipulation (Zagury et al, 1980).

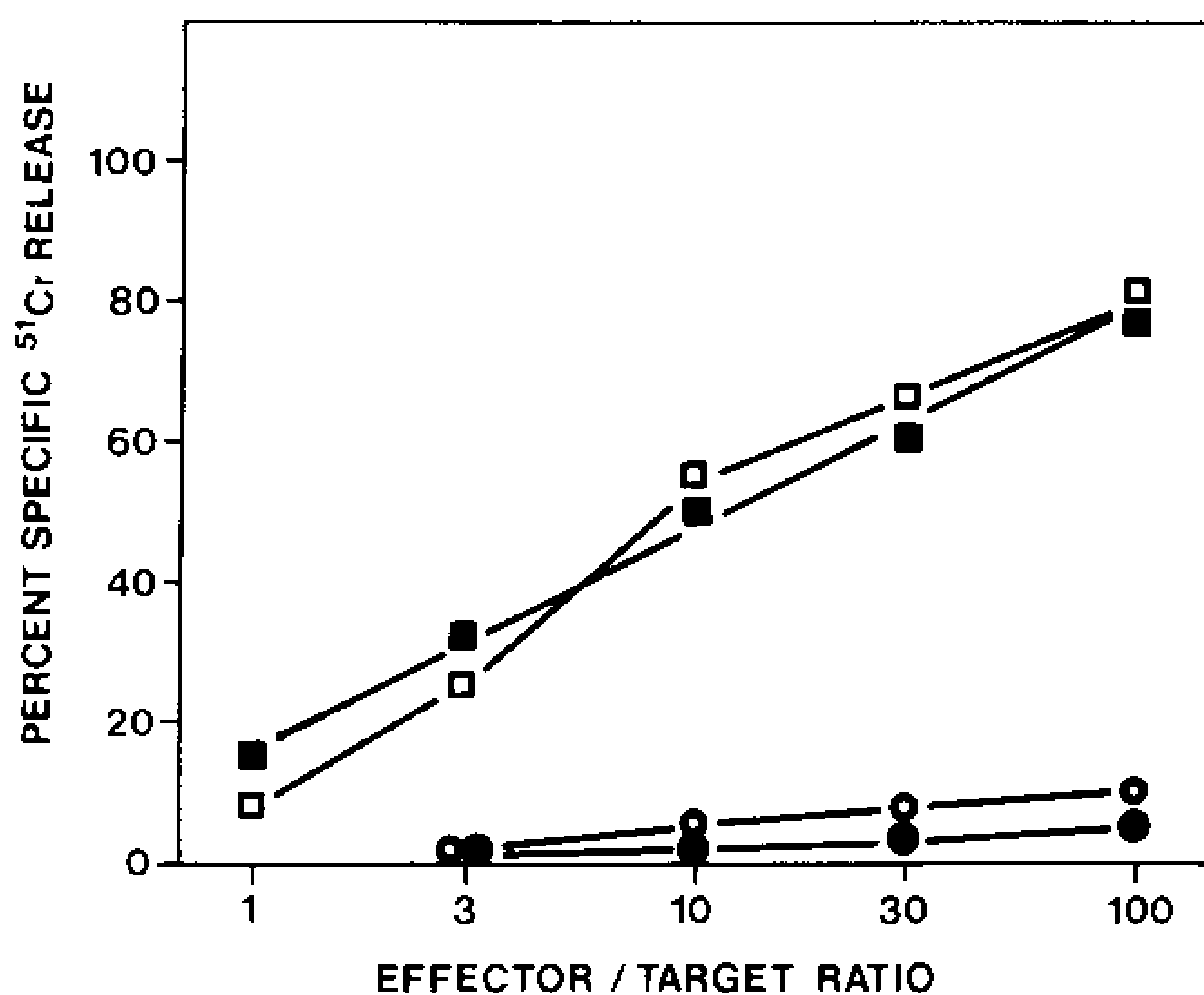

Fig. 4 – Inability of *L. tropica*-specific T cell blasts to lyse *L. tropica*-infected peritoneal macrophages. ^{51}Cr-labeled peritoneal exudate macrophages from CBA mice were incubated for 4 hr with either *L. tropica*-stimulated CBA T cell blasts (●–●, infected macrophages; O– O, normal macrophages) or C57BL/6 anti-CBA MLC cells as a source of CTL directed against H–2^k antigens (■ – ■, infected macrophages; □· · □, normal macrophages).

The frequency of *L. tropica*-specific T cells present in immune lymph nodes was determined by limiting dilution analysis to be in the range of 1/1500 (Fig. 5A) whereas this frequency was increased dramatically following *in vitro* propagation of the same immune lymph node cells, i.e. to a frequency of 1/25 (Fig. 5B). Clones were established from microcultures set up under limiting conditions (< 0.5 cells/well) and maintained in continuous culture by the addition of irradiated syngeneic spleen cells, a source of TCGF and *L. tropica* antigens. After removal from TCGF-containing medium, these T cell clones exhibited specific proliferative responses upon challenge with *L. tropica* antigens, together with irradiated syngeneic spleen cells as a source of adherent cells.

The surface phenotype of three such clones as determined by FACS analysis using monoclonal antibodies is given in Figure 6. The majority of clones obtained either by limiting dilution or by direct micromanipulation expressed high levels of Lyt-1 alloantigen, whereas virtually no Lyt-2 antigen-bearing cells were detected.

As an alternative method for the derivation of *L. tropica*-specific T cell clones, single blast cells were isolated by micromanipulation and then expanded into long-term T cell lines by culture together with irradiated syngeneic spleen cells, *L. tropica* antigen, and a source of TCGF. Then, those clones which proliferated specifically in response to stimulation with parasites plus irradiated spleen cells alone (in the absence of added TCGF) were tested for their capacity of function as helper T cells in a hapten-carrier antibody response *in vitro*. As presented in Table VII, in two separate experiments a total of eight micromanipulated clones demonstrated both *L. tropica*-specific proliferation and helper activity using TNP-*L. tropica* as antigen.

Certain *L. tropica*-specific T cell clones were also tested for the capacity of transferring DTH to syngeneic mice. In one experiment presented in Table VIII, T cell clone A-5 was capable of transferring DTH reactions in a dose-dependent manner when varying numbers of cloned T cells were injected together with *L. tropica* antigens into footpads of syngeneic DBA/2 mice. Thus, clone A-5 is an example of a T cell clone which can proli-

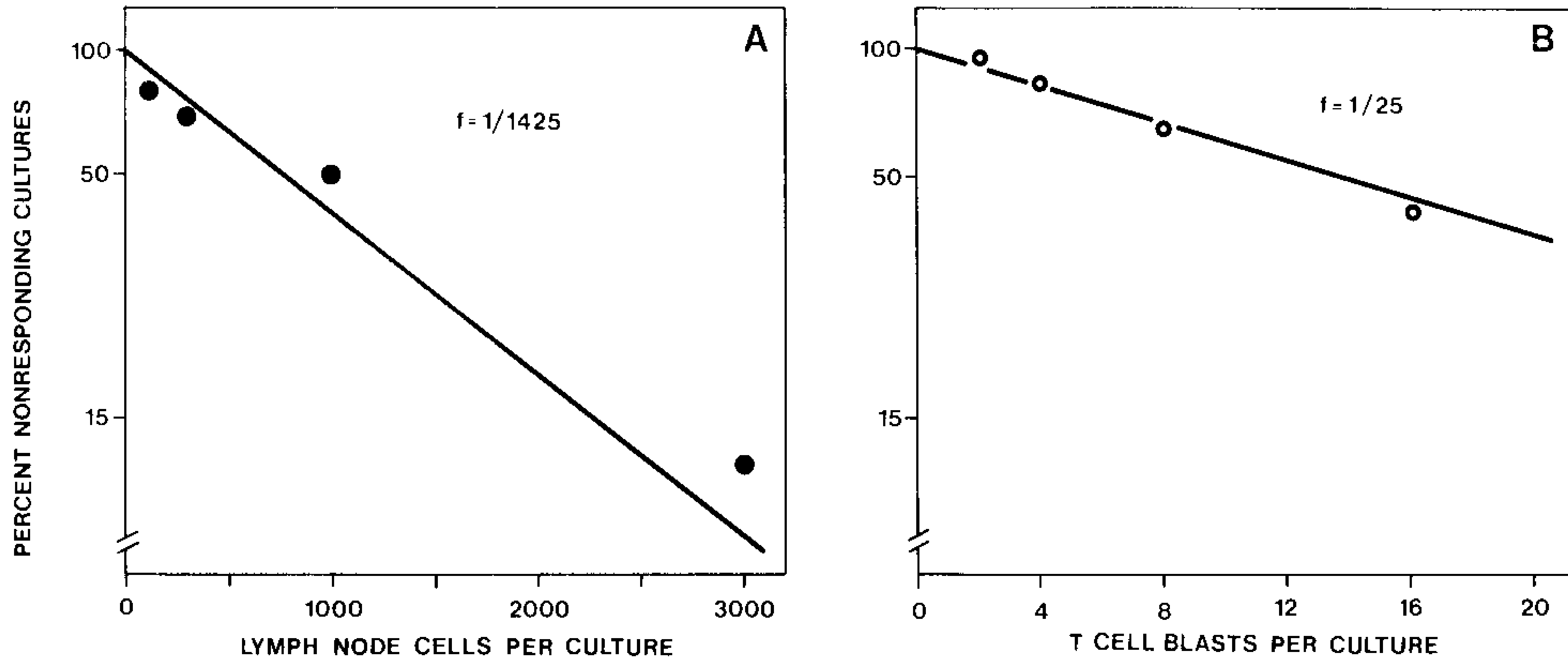

Fig. 5 - Frequency of lymph node cells which proliferate in response to stimulation by *L. tropica* parasites. (A) Lymph node cells from (C57BL/6 x DBA/2) F_1 mice injected with 10^6 *L. tropica* s.c. at the base of the tail 8 days earlier were cultured under limiting dilution conditions in microwells with 10^5 parasites, 10^6 irradiated spleen cells and 2° MLC supernatant as a source of TCGF. The frequency of cells proliferating in response to parasite antigens was determined as described by MacDonald *et al.* (1980). (B) *L. tropica*-specific T cell blasts isolated after specific restimulation *in vitro* of F_1 immune lymph node cells for 5 days were tested by limiting dilution analysis as described above.

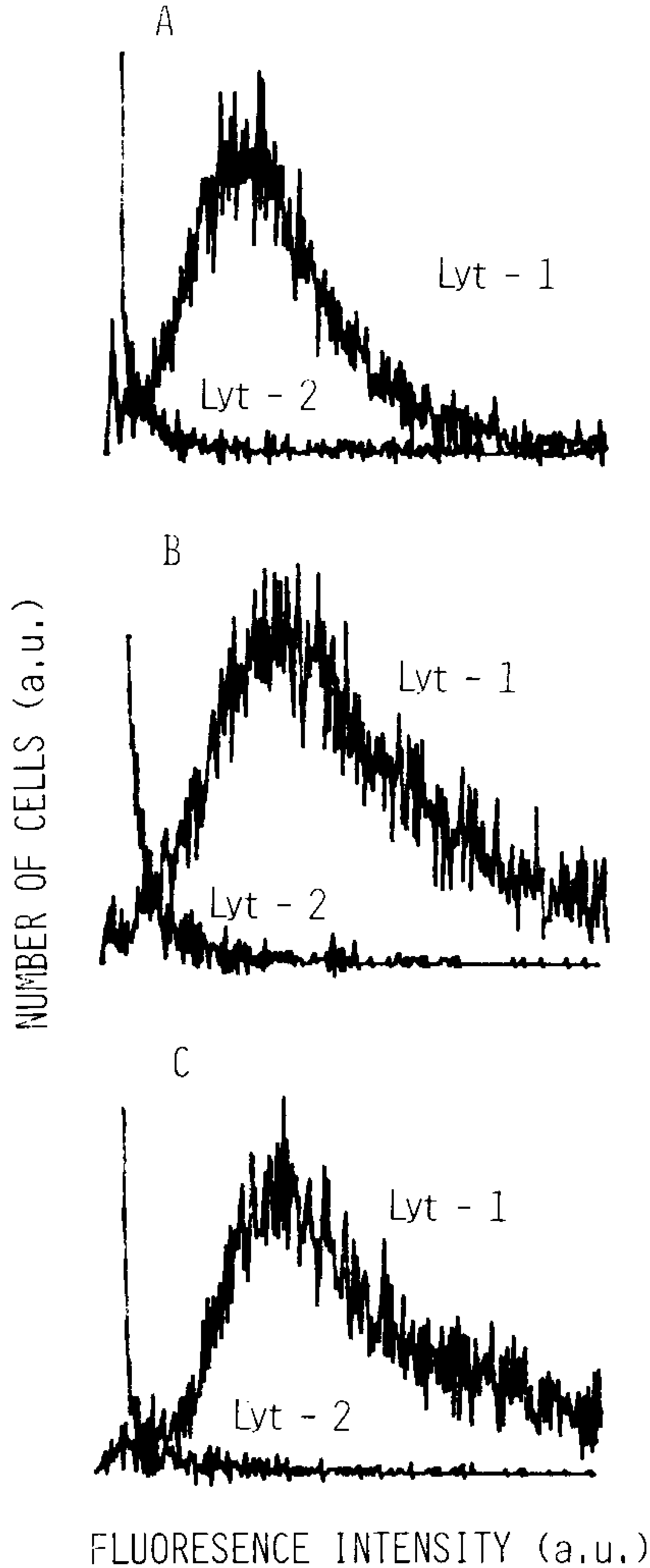

Fig. 6 – Lyt phenotype of three lymph node T cell clones specific for *L. tropica* antigens. Clones derived from (C57BL/6 x DBA/2)F_1 mice were analyzed by flow cytometry using monoclonal antibodies, as described in Figure 3. All 3 clones which were strongly positive for Lyt-1 antigens and virtually negative for Lyt-2 antigens yielded specific proliferative responses when stimulated with *L. tropica* antigens.

TABLE VII

Parasite-induced proliferation and helper cell activity exhibited by *L. tropica*-specific T cell clones[a]

Clone tested	*Proliferation [^3H]-Thymidine incorporation (cpm x 10^{-3})*	*Helper activity anti-TNP PFC per 10^6 B cells*
Expt. 1		
None	0.8 ± 0.3	<20
A5	3.3 ± 0.4	538
E9	10.7 ± 0.7	770
F2	5.5 ± 0.3	163
Expt. 2		
None	0.6 ± 0.2	<20
A4	7.7 ± 0.3	2,120
B6	13.1 ± 0.4	1,482
C4	4.6 ± 0.2	1,156
C6	15.8 ± 0.5	210
D7	1.9 ± 0.2	240

[a] *L. tropica*-specific DBA/2 T cell clones derived by micromanipulation were tested for proliferative responses by culturing together with *L. tropica* antigen and irradiated DBA/2 spleen cells, or tested for helper activity by culturing together with TNP-HGG primed DBA/2 spleen B cells and TNP-*L. tropica* as antigen.

ferate specifically in response to *L. tropica*, can function as a helper T cell and also can transfer DTH reactions *in vivo*. We are currently testing such T cell clones for the ability to activate parasite-infected macrophages to yield intracellular killing of the parasites, and preliminary results suggest that indeed certain clones are capable of activating syngeneic macrophages to yield parasite destruction. Therefore, these results suggest that either the same T cell clone can perform three different immunological functions or perhaps DTH responses are an *in vivo* correlate of macrophage activation and induction of humoral antibody responses *in vivo*.

TABLE VIII

Transfer of delayed type hypersensitivity (DTH) with *L. tropica*-immune T cell clones[a]

Number of cloned T cells transferred (x 10^{-5})	*Increase in* footpad thickness (mm)
0	0.04 ± 0.02
0.1	0.22 ± 0.04
1.0	0.58 ± 0.02
10.0	0.75 ± 0.03

[a] Varying numbers of a DBA/2 *L. tropica*-specific T cell clone were injected together with *L. tropica* antigen into the footpads of syngeneic mice and the mean increase in footpad thickness measured 24 hours later.

In the future, the above described parasite-specific T cell populations and clones will be used in protection studies *in vivo*, in an attempt to define the possible role of T cells in the healing of cutaneous lesions and in the development of protective immunity to *L. tropica*. In addition, the availability of cloned T cell lines which could afford protection against challenge with parasites would aid in the identification of parasite antigens(s) implicated in the generation of cell-mediated responses beneficial for the infected host.

RESUMO

Os resultados apresentados nesta revisão, sumariam uma série de experimentos planejados no sentido de caracterizar a resposta imune de linfócitos T de camundongos, para o protozoário parasita *Leishmania tropica*. Populações enriquecidas de linfócitos T e clones de linfócitos T específicos para antígenos de *L. tropica* foram derivados de gânglios linfáticos de camundongos primados e a seguir mantidos em cultura contínua *in vivo*. Ficou demonstrado que estes linfócitos T eram capazes de: A) Expressar o fenótipo de superfície celular Lyt $1^{+} 2^{-}$, B) Proliferar *in vitro* especificamente em resposta aos antígenos parasitários quando em presença de macrófagos singênicos irradiados, C) Transferir uma resposta tipo hipersensibilidade retardada antígeno específico à camundongos normais singênicos, D) Induzir ativação específica de macrófagos parasitizados *in vitro* resultando em destruição dos parasitas intracelulares, E) Providenciar uma atividade "helper" específica para uma resposta com produção de anticorpos *in vitro* em um sistema hapteno-carreador. Estudos de proteção utilizando estas populações definidas de linfócitos T poderão permitir a caracterização de antigeno(s) parasitários implicados na indução de uma resposta imune celular com benefícios para o hospedeiro.

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