VISCERAL LEISHMANIASIS IN THE GOLDEN HAMSTER AS A MODEL FOR HUMAN KALA-AZAR

Annual/Final Report

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Leishmania donovani infection in the golden hamster was studied as a model for human kala azar. Following intradermal inoculation of L. donovani amastigotes, hamsters developed positive DTH responses to parasite antigens and expressed resistance to reinfection. Lymphoid cells from these hamsters responded to antigens in in vitro proliferation assays and transferred DTH reactivity to normal recipients. In contrast, hamsters inoculated intracardially with live amastigotes developed progressive visceral infections and failed to respond to skin-test antigens. Spleen cells, lymph node cells and peripheral blood cells (PBL) were unresponsive to parasite antigens in vitro and spleen cells failed to transfer DTH to recipient animals. Spleen and lymph node cells but not PBLs, also displayed depressed responses to mitogens. The addition of indomethacin or catalase failed to reconstitute proliferative responses. However, removal of adherent cells from populations of spleen but not lymph node and peripheral blood cells restored responsiveness to parasite antigens. Non-adherent spleen cells also-

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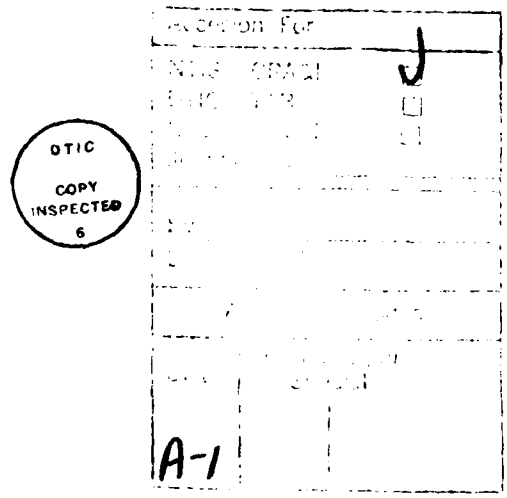
transferred DTH to normal hamsters. Addition of adherent cells of their supernates suppressed antigen specific responses of cultured cells from intradermally inoculated hamsters. In addition, serum from chronically infected hamsters suppressed proliferative responses by antigen-reactive cells. The adherent cells which have the characteristics of macrophages, appear to be localized to the spleen and are apparently not responsible for the failure of peripheral blood or lymph node cells to respond to antigen. These studies provide evidence that hamsters with visceral infections develop a population of antigen-reactive cells and that in the absence of suppression, these cells may express functional activities including DTH reactivity.

Additional studies characterized the course of infection of a canine isolate of L. donovani (WR 503) in the gold hamster. While the 2S strain of L. donovani routinely increases 50 fold or more during the first two weeks of infection, the WR 503 increased by only 1.5 fold. Whether this low increase in parasite numbers represents a low reproductive rate was not determined. Also, in contrast to infection with other L. donovani isolates which were invariably fatal, hamsters inoculated with WR 503 spontaneously recovered from infection. Recovered hamsters were shown to express some resistance to a challenge infection with a virulent isolate.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).



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Experimental Infections with Geographical Isolates of Leishmaniasis

During the course of this study, we maintained and characterized various Leishmania isolates of both human and animal origin. Almost all of the isolates originated from visceral infections. A number of these parasites were routinely carried in hamsters including two L. infantum strains from France and Greece, two L. chagasi strains from Brazil, and a L. donovani strain from the Sudan. In a previous progress report (January, 1983), we also reported extensively on infections with a Honduran strain (WR116; Santos Herrera) isolated from the bone marrow of a human with visceral leishmaniasis. This organism was interesting in that we could produce fulminating dermal lesions in hamsters, but not systemic infections. Inoculation of WR116 intradermally into mice produced cutaneous, ulcerating lesions, some of which appeared to heal and then subsequently relapse to produce chronic, ulcerative lesions. C57BL/6 mice infected with WR116 were not protected against L. donovani inoculated IV; however, cross-protection was observed between WR116 and a strain of L. mexicana amazonensis. This last observation made questionable, previous characterizations of WR116 as L. chagasi. We have subsequently studied the behavior of WR116 in mouse macrophage cultures and observed that the parasite grows intracellularly in large, vaculated phagolysosomes in a manner similar to L. mexicana. The organism was sent to Dr. Phillip Scott (NIH) to utilize in intracellular killing assays and was observed to produce two distinct types of infection in macrophages, one of which is characteristic of L. mexicana. It is now our opinion that WR116 contains two parasite species, one L. mexicana and the orther a possible L. braziliensis strain. Whether this organism is the same one isolated from human bone marrow is now in

doubt.

By far the most intersting visceral isolate we have studied is strain WR503 isolated from a dog during a recent outbreak of leishmaniasis in Oklahoma. This parasite is presumably a strain of L. chagasi or L. infantum and produced classical visceral infections in dogs. Our initial attempts to infect hamsters utilized cultured promastigotes inoculated either intracaridally or intradermally into the nose or shaved flank. Although no parasites were recovered from cutaneous sites, we were successful in culturing promastigotes from the spleen of an IC inoculated animal. Approximately 9 passages in hamsters were required before the first microscopically patent visceral infection was observed. Subsequent passages, often using a dose as high as 1-2 x 10^8 promastigotes injected IC, have yielded low level infections. Since our early objective was merely to establish this strain in hamsters, we often sacrificed animals at random time intervals to determine visceral parasite burdens. Analysis of the numerical data from specific groups of animals, however, turned up an interesting trend in the course of infection. In contrast to all other strains of L. donovani (chagasi or infantum) studied in this laboratory, the WR503 strain appeared to spontaneously resolve following IC inoculation into hamsters. The data in Figure 1 represent hepatic parasite burdens in 4 groups of hamsters receiving this parasite. Splenic parasite burdens are not graphed, but were found to be low in most animals with peak numbers only about 2-5 x 10^4 parasite/spleen.

In an attempt to boost parasite burdens in WR503 infected hamsters, animals were treated twice weekly with 2.5 mg/100g cortisone acetate during the first 2 weeks of infection. Cortisone treated, as

well as non-treated, hamsters were sacrificed at 4 weeks and parasite burdens were determined. In contrast to control animals, most of which did not harbor microscopically patent infections, cortisone-treated animals averaged over 10^9 hepatic amastigotes. The results from two separate groups of animals are shown in Figure 2. The dramatic effect of cortisone on the course of infection suggests that a host response is controlling parasite numbers in vivo.

We were also able to produce low-level visceral infections in mice with WR503. Inoculation of 2×10^7 promastigotes IV into BALB/c mice led to the establishment of approximately 7% of the inoculum of hepatic amastigotes as compared to approximately 15% for promastigotes of the 2S strain of L. donovani. While the 2S strain routinely increases 50-fold or more during the first 14 days of infection, the WR503 increased by only 1.5 fold (Figure 3). Whether this low increase in parasite numbers represents a low reproductive rate of this strain or is a relection of parasite susceptibility to a host response was not determined.

One additional experiment was performed with the WR503 strain. Individual animals from groups of hamsters which had been shown to resolve infection were pooled and challenged IC, along with controls, with approximately 5-10 millior amastigotes of the 2S strain of L. donovani. These animals were then sacrificed 1 week later. The control hamsters averaged greated than 10^8 hepatic parasites while the "WR503 healed" animals harbored approximately 3×10^6 parasites (Figure 4). Thus, it appears that animals resolving infection with this parasite strain will express resistance against a more virulent strain of L. donovani.

Studies with an isolate from a U.S. soldier in Panama (L. chagasi;

WR317) were prompted by the fact that this organism came from a cutaneous lesion. A demotropic strain of L. chagasi would have obvious implications in terms of vaccination against visceral leishmaniasis. In our hands, however, this organism behaves exactly like other L. chagasi strains we have studied. We did not observe any evidence of cutaneous lesions in either mice or hamsters follow intradermal inoculation of this parasite. Following intravenous inoculation into BALB/c mice, hepatic parasite burdens increased by 32-fold from 1 to 21 days which is within the range of the Santana strain of L. chagasi. Mice inoculated intradermally with this strain develop no demonstrable resistance to an intravenous challenge infection with L. chagasi (Santana) which is similar to results we have previously obtained showing that mice inoculated intradermally with the 2S strain of L. donovani develop little immunity to intravasular challenge infections. In hamsters, however, dermal parasite inoculation with WR317 induce resistance to an IC challenge with the Santana strain (Figure 5), but this resistance was no greater than that seen with other visceral isolates.

One additional observation on strain behavior in animals deserves note. As part of an attempt to identify visceral strains of Leishmania which can produce consistent visceral infections in hamsters following ID inoculation, a large group of animals was inoculated with the Santana strain of L. chagasi. None of these animals developed microscopically patent infections. However, we were able to culture promastigotes from the spleens of these hamsters as long as 7 months following inoculation. The implications of these persistent low level infections are yet to be determined.

Immunity to L. donovani in the Golden Hamster Much of our effort concentrated on the study of immunological responses in hamsters infected with the 2S (Sudan) strain L. donovani. Briefly, we utilized three model systems:

1) IC infections - Inoculation of 1-10 10^6 amastigotes intracardially results in a progressive visceral disease in which parasites multiply unchecked in spleen and liver tissue, and death ultimately results from a fulminating infection.

2) ID infections - Inoculation of 1-10 x 10^6 amastigotes intradermally into hamsters results in transient dermal lesions which usually resolve within 6-8 weeks. These animals display significant acquired resistance to re-infection.

3) ID - IC Challenge infections - Inoculation of 1-10x10^6 amastigotes ID followed several weeks later by an IC challenge with similar numbers of organisms results in visceral infections in which splenic and hepatic parasite burdens are significantly lower than those seen in primary IC infections. Acquired resistance is not absolute, however, since parasite numbers eventually increase and animals ultimately succumb to infection.

Spleen cells from these infected animals were tested in in vitro lymphocyte proliferative assays for response to mitogen and antigen (sonicated 2S promastigotes). The number of parasites was determined from Giemsa stained impression smears. Intradermal inoculation did not

result in visceral accumulation of parasites as seen from impression smears of spleen and liver. However, small number of parasites were detectable in smears of the infected footpads as well the draining lymph nodes. Cultures of spleen and liver were very rarely found positive for promastigotes. Spleen cells from such ID inoculated hamsters respond to both Con A and leishmanial antigen, and thus provide a baseline for measuring optimum response to both stimulants. In constrast IC inoculation results in a high parasite load in the spleen and the liver by 4-6 weeks of infection and death between 6-8 weeks. Spleen cells from these hamsters respond to leishmania antigen and Con A at two weeks after infection. However, as the infection progressed, pronounced suppression of both responses was observed in the IC group (Figure 6&7). Unresponsiveness to antigen was more acute and occurred faster. By 4 weeks post-infection, antigen response in the IC group was less than 10 percent of that observed in the ID group. The latter showed a progressively increasing response during the experimental period of 6 weeks. Suppression of the response to the T-cell mitogen, Con A, was more gradual and characterized the chronic phase. Six weeks after infection, the response to antigen and mitogen was 2% and 11% of the ID response respectively (Figures 6&7). To test if the presence of antigen would further enhance the suppression of the mitogen response, spleen cells from hamsters infected 5 weeks previously, were incubated either with antigen, Con A alone, or with antigen and Con A together. The results showed that the depressed response to mitogen of IC spleen cells was not exacerbated by the presence of leishmania antigen (data not shown).

To test for the presence of suppressor cells in IC infected hamsters, $2.5x10^5$ spleen cells from ID infected hamsters were cultured in the presence of $2.5x10^5$ spleen cells from IC infected (6weeks) animals.

Co-culture of spleen cells from IC and ID infected hamsters at 1:1 ratio showed marked suppression of the ID response to antigen (90%) and to Con A (60%) as can be seen in Figure 8. Spleen cells from hamsters that had been inoculated IC with normal hamster tissue, used as controls, did not induce any suppression when co-cultured with cells from ID inoculated group. To test the course of development of these suppressor cells appear, spleen cells at 2, 4, and 6 weeks post-infection were assayed for suppressive activity against ID spleen cells. Spleen cells from hamsters inoculated IC with hamster tissue preparation were also added to the control culture. Absence of lymphocyte proliferative response in spleen cells of IC infected hamsters coincides with the development of these suppressor cells. They are functionally absent at 2 weeks after infection, when IC spleen cells are responsive to antigen, but present at 4 and 6 weeks of infection.

Antigen and mitogen-induced proliferative response of cells from tissues other than the spleen were also assayed to determine if the suppression observed in IC infected animals is generalized. Peripheral blood lymphocytes (PBL) from these IC infected hamsters showed very poor response to antigen, although response to Con A was not significantly affected. The corresponding cells from ID infected hamsters responded normally to both antigen and Con A (Figures 9 & 10). Proliferative response of lymph node cells to antigen and mitogen was significantly depressed in animals infected intracardially.

To test if these suppressor cells, as seen in the spleen, were also present in other tissues where the parasite load is minimal, an equal number of lymph node cells, PBL, or peritoneal cells, either resident or antigen induced 3-4 days previously, were cultured with spleen or lymph node cells from ID infected animals. As it can be seen in Figures

11 & 12, these cells failed to suppress the ID cell response to antigen and mitogen, despite the fact that these cells, themselves, were unresponsive.

To identify the suppressor cell population in IC infected animals, spleen cells were fractionated using nylon wool, Sephadex G-10, and plastic adherence. Removal of adherent cells and replacement with mitomycin c treated normal spleen cells ($1.25\text{-}2.5\text{x}10^5$) significantly enhanced the response to both antigen and mitogen. Addition of normal spleen cells to the non-fractionated population did not enhance the response. Similar removal of plastic adherent cells from peripheral blood lymphocytes, however, failed to reconstitute responsiveness to leishmania antigen. Since this observation suggested that infected adherent cells may be responsible for the lack of response, unfractionated, plastic adherent or non-adherent fractions from IC spleen were mitomycin c treated and added to an equal number of ID spleen cells. Addition of plastic non-adherent fraction from infected spleen, unlike the unfractionated or the adherent fraction, did not suppress the antigen or mitogen response in an <u>in vitro</u> proliferation assay (Figures 13&14). The adherent fraction, however was suppressive, even at a lower ratio, while simiarly obtained adherent cells from normal hamsters did not have any effect when added at 1:1 ratio, the highest concentration tested. Further fractionation of these non-adherent population by a second round of adherence on anti-hamster IgG coated plates to remove B-cells did not significantly enhance the proliferative response to mitogen. Cell fractionation, however, was sometimes complicated by high background counts (especially true of cells fractionated by nylon wool and Sephadex G-10) in co-cultured cells containing a non-adherent fraction from IC spleens added to total spleen cells from ID infected ham-

sters. This is possibly due to stimulation of ID cells by parasite antigen (live amastigotes) present in the infected cell (IC) preparation, whose effect became more pronouced on removal of the adherent population. Very rarely was this high background count observed when one of the cell population is the unfractionated IC spleen cell. The suppressive adherent cells were found to be (90%) non-specific esterase positive after two rounds of adherence and phagocytosed latex beads indicating that they are macrophages (Table 1).

Since macrophages are known to suppress lymphocyte proliferation via production of metabolites like prostaglandins and hydrogen peroxide, a role for these metabolites in depressed hamster responses was tested. Neither indomethacin, (5ug/ml) nor catalase (200ug/ml), alone or in combination, added at the beginning of the assay could reverse the suppression in lymphocyte proliferation of spleen cells from IC infected hamsters (Figure 15). This indomethacin insensitive suppression by adherent cells is suggestive of the presence of suppressive factors other than prostaglandins. Despite this, cell-free supernates from 48 hour culture of IC spleen cells were found to contain prostaglandins, in radioimmunoassay (Table 2). The concentration, as to be expected, was higher when only adherent cells were cultured. These adherent cell supernates, high in prostaglandins, were also suppressive when added to cell cutures at 50% concentration.

Cell free supernates from adherent spleen cells have also been tested for inhibitory action in lymphocyte proliferative assays. Spleen cells ($10x10^6$/ml) were adhered to plastic for two hours, non-adherent cells washed off and adherent cells incubated with fresh media for 24, 48, and 72 hours. Preliminary results showed that 72 hours but not 24 and 48 hour supernates were inhibitory. Control supernates from ID

infected hamster cells were not inhibitory. Since this method encountered variability, various concentrations ($1x10^5$-$5x10^6$/ml) of adherent cells, pooled from several ID or IC infected or normal hamsters, were cultured for 5 days, the supernates collected, dialyzed in phospate buffered saline for 24 hours and added to ID spleen cell cultures at 50% concentration. The results showed that supernates form $1x10^6$/ml or more cells were inhibitory to lymphocyte blastogenesis. Dialysis of these supernates (8,000 cut off) in PBS for 24 hours did not remove the suppressive activity. Concentration of the supernates using amicon filters (10,000) showed that the factor has a molecular weight greater than 10,000.

Spleen cells ($4x10^6$/ml) from normal, ID or IC infected hamsters were stimualted with 5ug/ml Con A for 48 hours and the supernates were filtered and serial dilutions tested for their ability to support the growth of an IL-2 dependent cell line (CTLL); growth was determined by the incorporation of ^3H thymidine. Spleen cells from IC infected hamsters, unlike ID and normal hamsters, have marked impairment in the production of IL-2. In addition, these cells also supress IL-2 production by normal or ID spleen cells when cultured together at 1:1 ratio. Removal of plastic adherent cells improves the ability of cells from infected hamsters to produce IL-2 and alleviates the suppressive effect exerted by infected cells on IL-2 production by normal or ID spleen cells. To test if this deficient IL-2 production is responsible for the depressed blastogenic response persistently observed in infected spleen cells, cultures were supplied with exogenous IL-2. However, addition of IL-2 containing spleen cells supernatant, or 10 U/ml recombinant IL-2 , with or without indomethacin, added to unfractionated, infected spleen cells at the beginning of a blastogenesis assay, failed to reconstitute

the response. Addition of higher concentration of IL-2, (100U, 250U/ml), in general gave a higher background count and showed some enhanced proliferation over cells cultured alone but the net antigen response was still 12 to 23 percent of ID spleen and no net increase in mitogen response was seen. Similarly, addition of 250U or 500U/ml recombinant IL-1 did not restore the proliferative response.

To test a role for humoral factors in immunosuppression, the inhibitory activity of serum from chronically ihfected hamster (5 weeks) was tested in lymphocyte transformation assay using normal hamster serum as a control. Sera were pooled from several infected animal and assayed using spleen cells from ID infected animals. Since circulating immune complexes are known to occur in sera of L. donovani infected animals as well humans, serum samples were also centrifuged in a high speed airfuge, at 30 psi, for 30 minutes and added to cells cultures. In general addition of serum (1:40 final dilution), from IC infected hamsters markedly suppressed antigen-induced proliferative responses compared to serum from normal or ID infected hamsters (Figure 16). Centrifugation of the sera at high speed for possible removal of antigen-antibody complexes did not remove the suppressive activity.

Hamsters were skin-tested by inoculation of 50 ul of leishmanin in one footpad and phenol saline in the other. Increase in footpad thickness was measured at 24 and 48 hours. ID inoculated hamsters showed increased footpad thickness in response to antigen starting 2 weeks after infection. No skin test responsiveness was observed in IC inoculated hamsters. Figure 17 shows the differential skin test response 15 days after infection.

To see if this skin test unresponsiveness was due to suppression, antigen-responsive cells from spleen and lymph nodes of ID inoculated

hamsters ($3x10^6$) were injected with antigen ($10x10^6$ formalin-fixed promastigotes), locally into the footpads of normal or hamsters infected IC 4 weeks previously. The co-lateral pad received antigen alone. Infected hamsters failed to respond in a local skin test reaction while normal hamsters did respond with positive DTH (Figure 18).

In another assay, the ability of cells from IC infected hamsters to transfer DTH to normal hamsters was tested. Whole or plastic non-adherent fraction of spleen cells from IC infected hamsters (DTH^-) were injected with antigen to footpads of normal hamsters. Figure 19 shows that only the non-adherent fraction of infected spleen cells elicited a positive skin test reaction in normal recipients showing adherent cells, which suppress in vitro responses, also inhibit DTH skin reaction in vivo.

Human visceral leishmaniasis is characterized by a lack of demonstratable cell mediated immunity during active infection since delayed hypersensitivity responses are negative until successful drug cure. In addition, peripheral blood lymphocytes fail to respond to leishmanial antigens in proliferation assays. The golden hamsters infected with L. donovani appears to be an excellent model for both the clinical and immunological aspects of human Kala-ozar. The results presented here show that cells from hamsters with visceral infections fail to respond to parasite antigens. However, it is possible to demonstrate antigen-reactive cells in the spleen of these hamsters following removal of adherent suppressor cells. These adherent cells, which are probably macrophages, mediate suppression through the production of prostaglandins and other higher molecular weight factors which have not yet been defined. There is no evidence for active suppression by T lymphocytes in these animals, but serum factors can be shown to suppress in

vitro responses to parasite antigens. It is of special interest that splenic lymphocytes can mediate delayed hypersensitivity when removed from the suppressive environment of the infected host. It is also of interest that no peripheral responses to antigen could be demonstrated during infection, despite attempts to remove potential suppressor cells. It is possible that antigen-reactive cells fail to circulate and are trapped in visceral organs where parasite (antigen) densities are high.

FIGURE 1

Courses of Infection of WR503 in Hamsters following IC Inoculation of Promastigotes

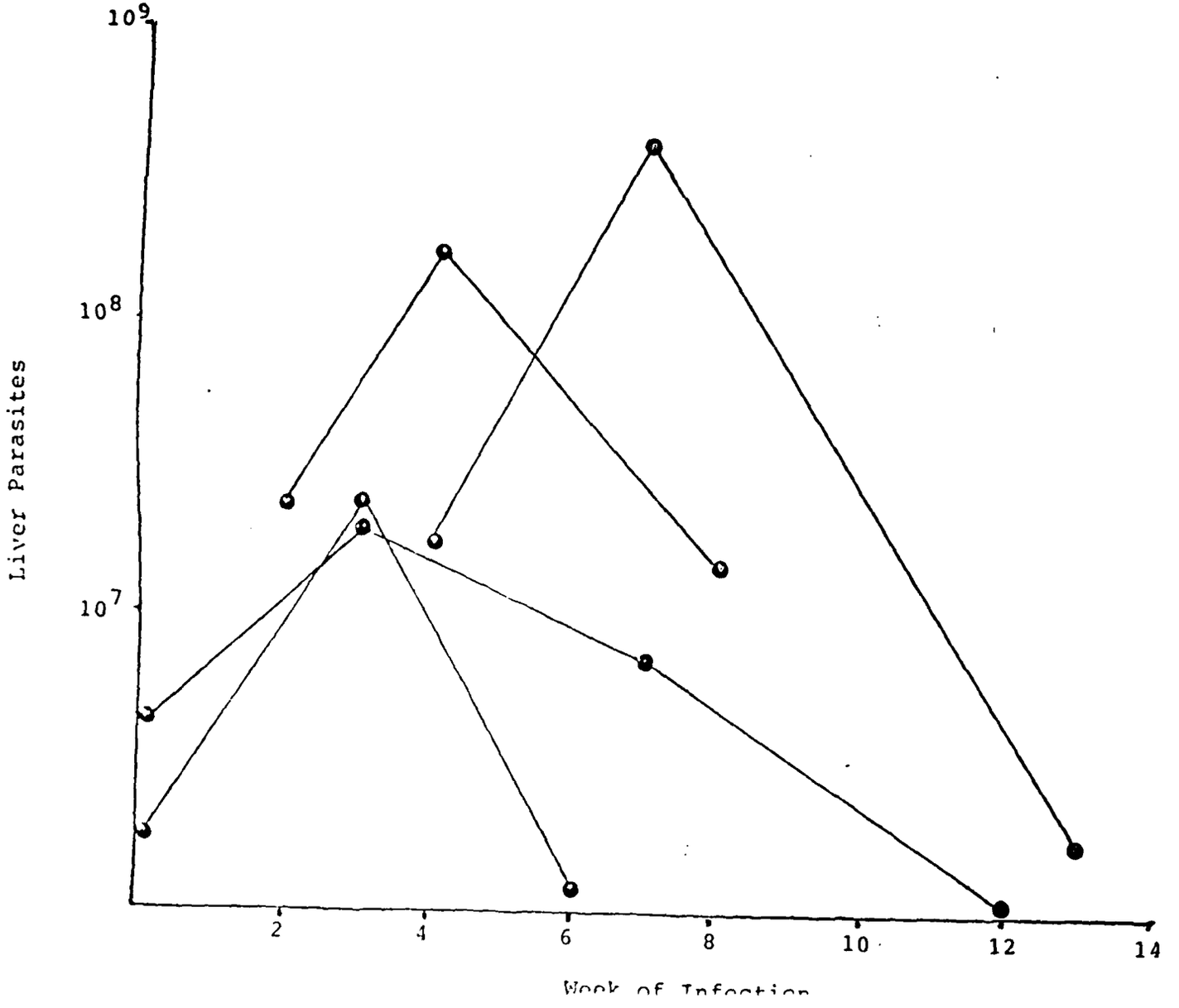


FIGURE 2

Effect of Cortisone (2.5 mg/100g) Treatment
on Parasite Burdens in Hamsters
Infected with WR503

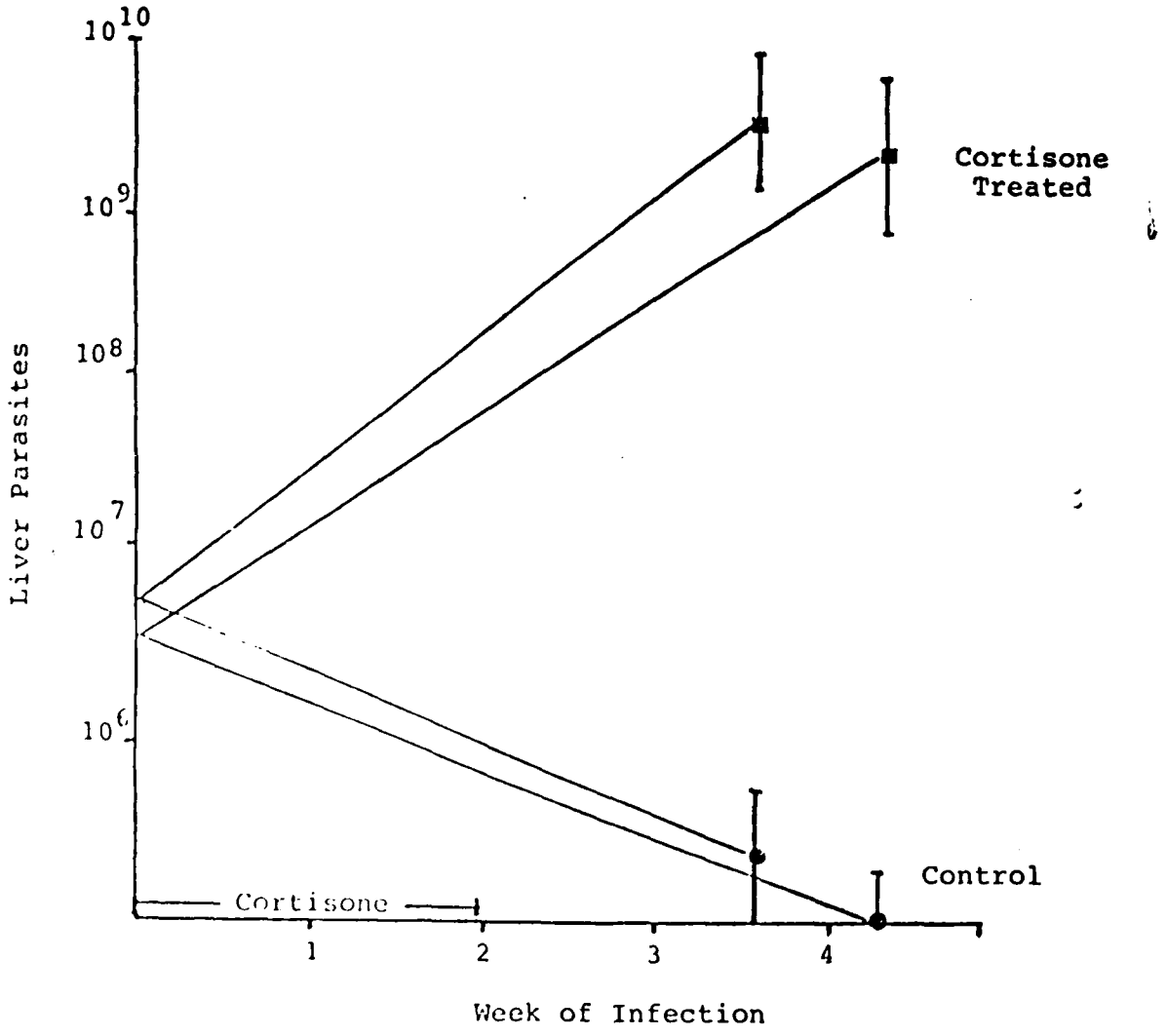


FIGURE 3

Challenge Infection with L. donovani (2S) in Control versus WR503 "Healed" Hamsters

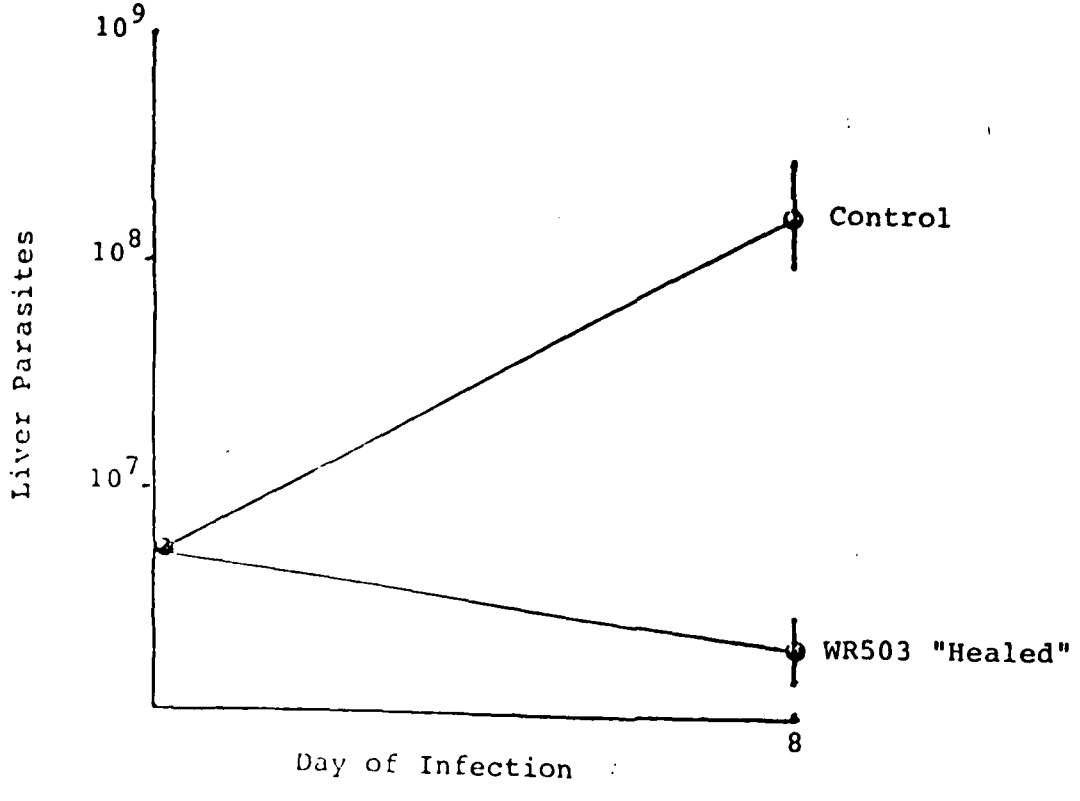


FIGURE 4

Course of Infection of L. donovani (2S) versus WR503 in BALB/c Mice

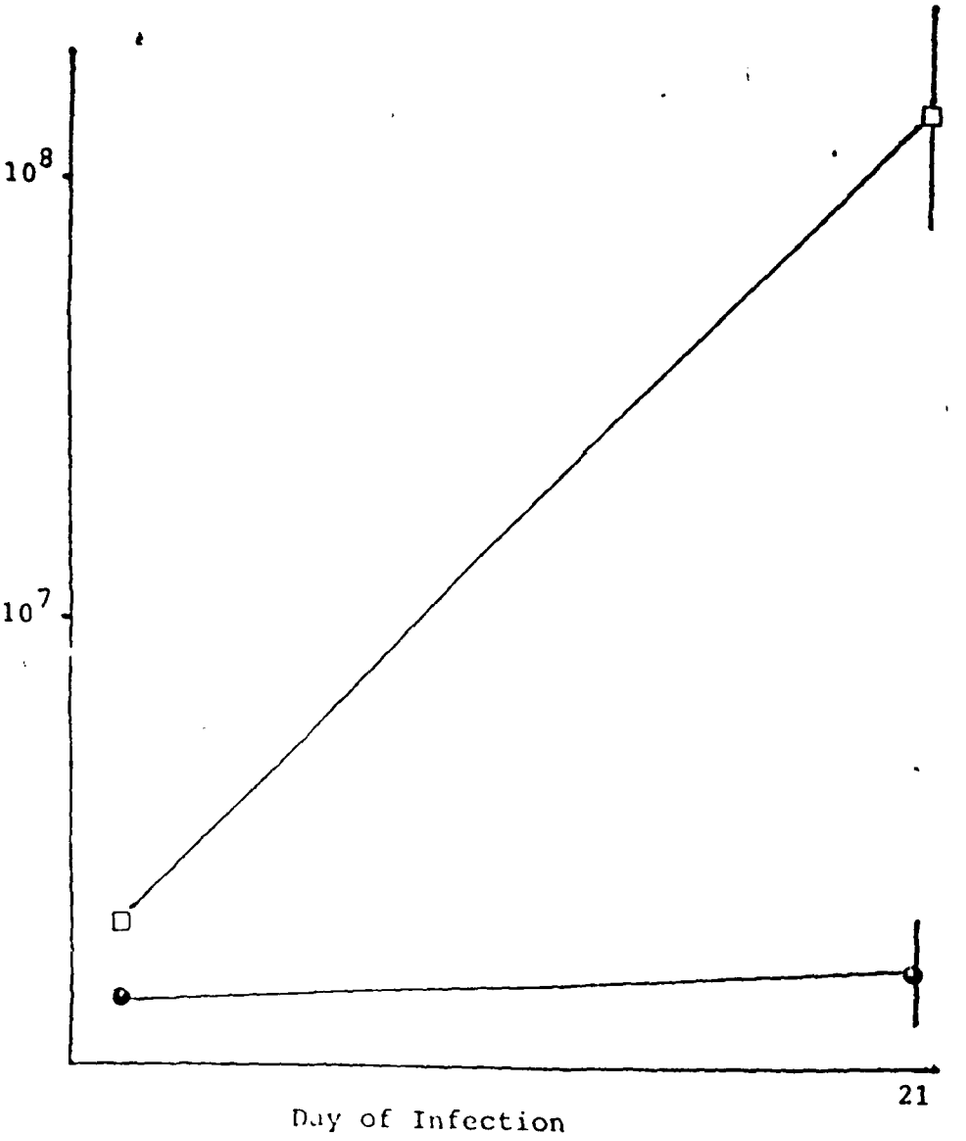
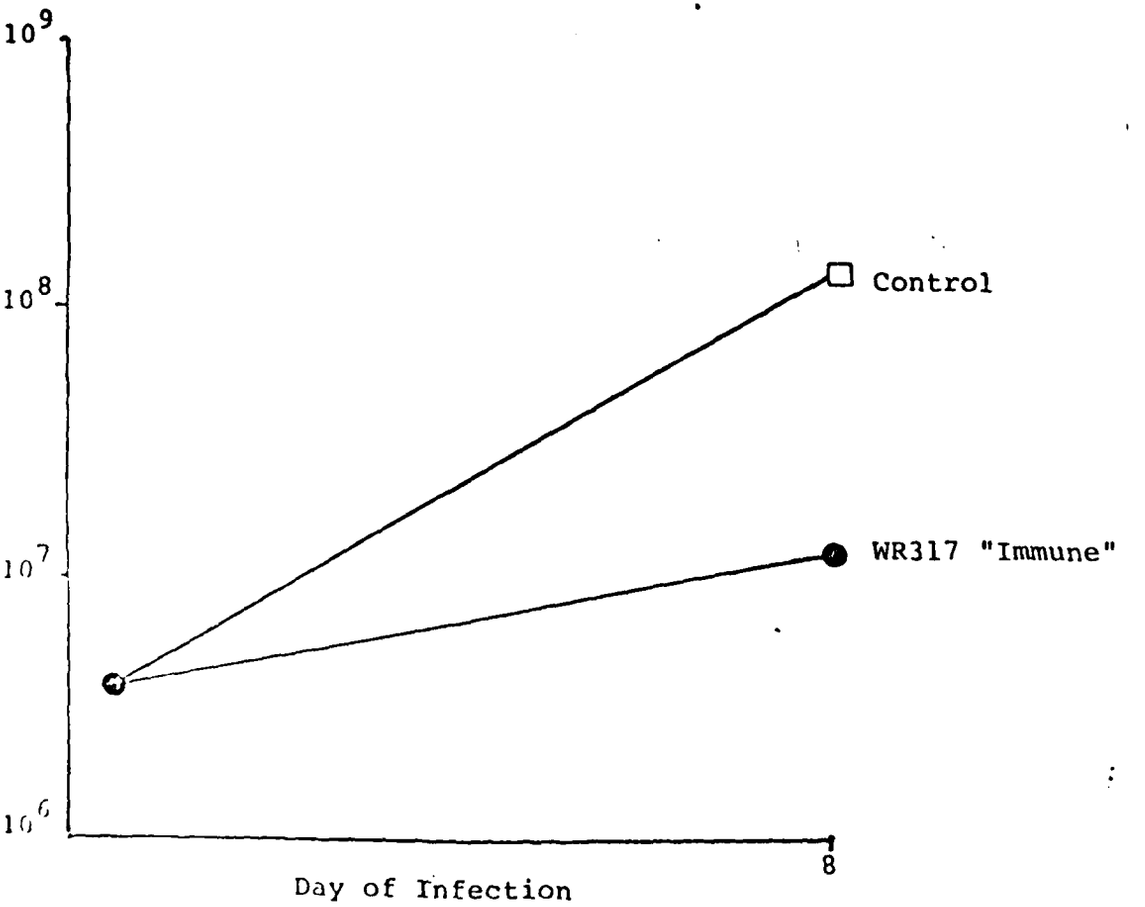


FIGURE 5

L. chagasi (Santana) Challenge Infections in Control Hamsters versus Hamsters Inoculated Intradermally with WR317



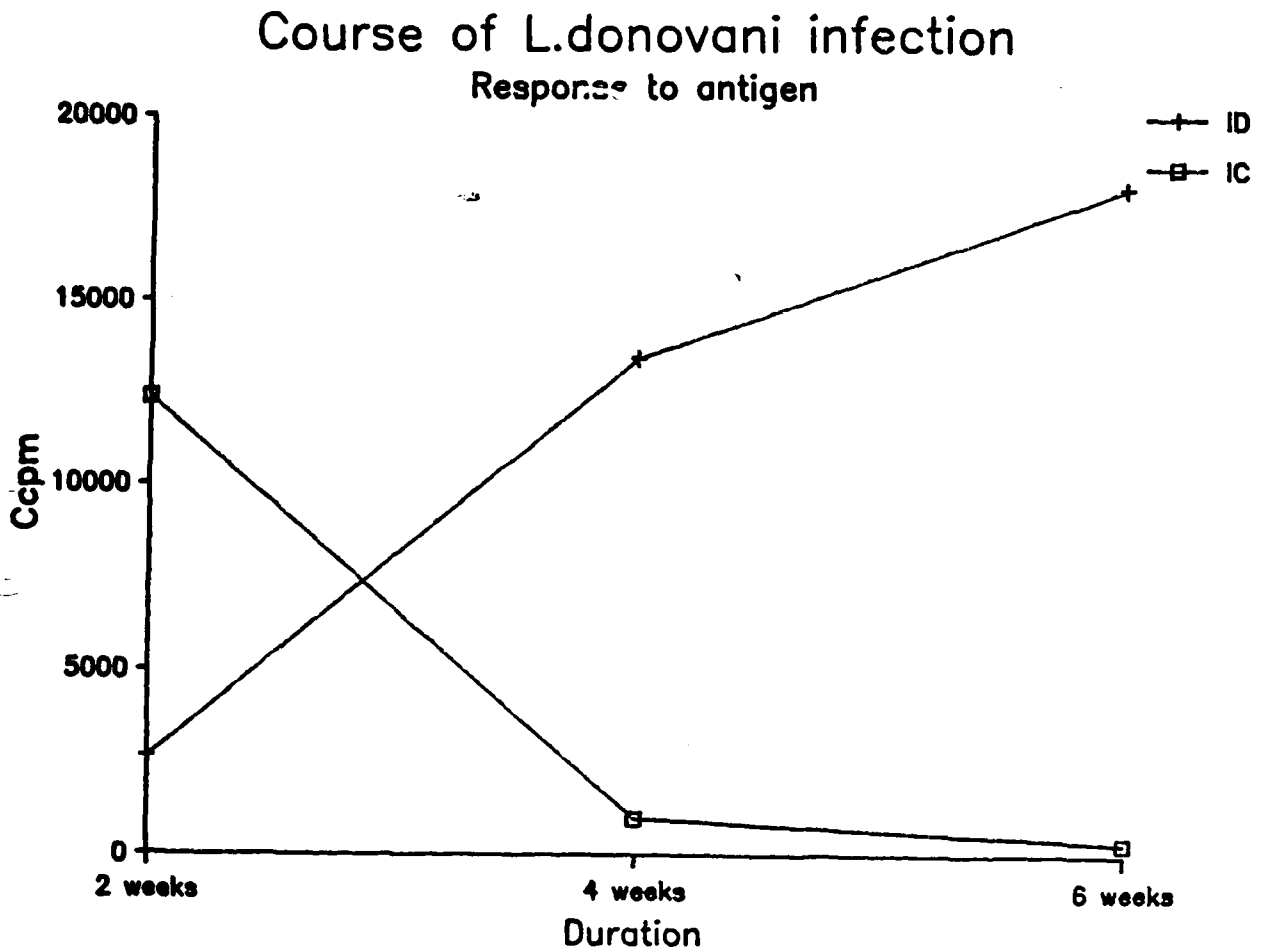


Figure 6. Antigen-induced proliferative response of spleen cells from hamsters infected either intracardially (IC) or intradermally (ID) with L. donovani. Ccpm = mean counts per minute of stimulated cultures- mean counts per minute of unstimulated cultures.

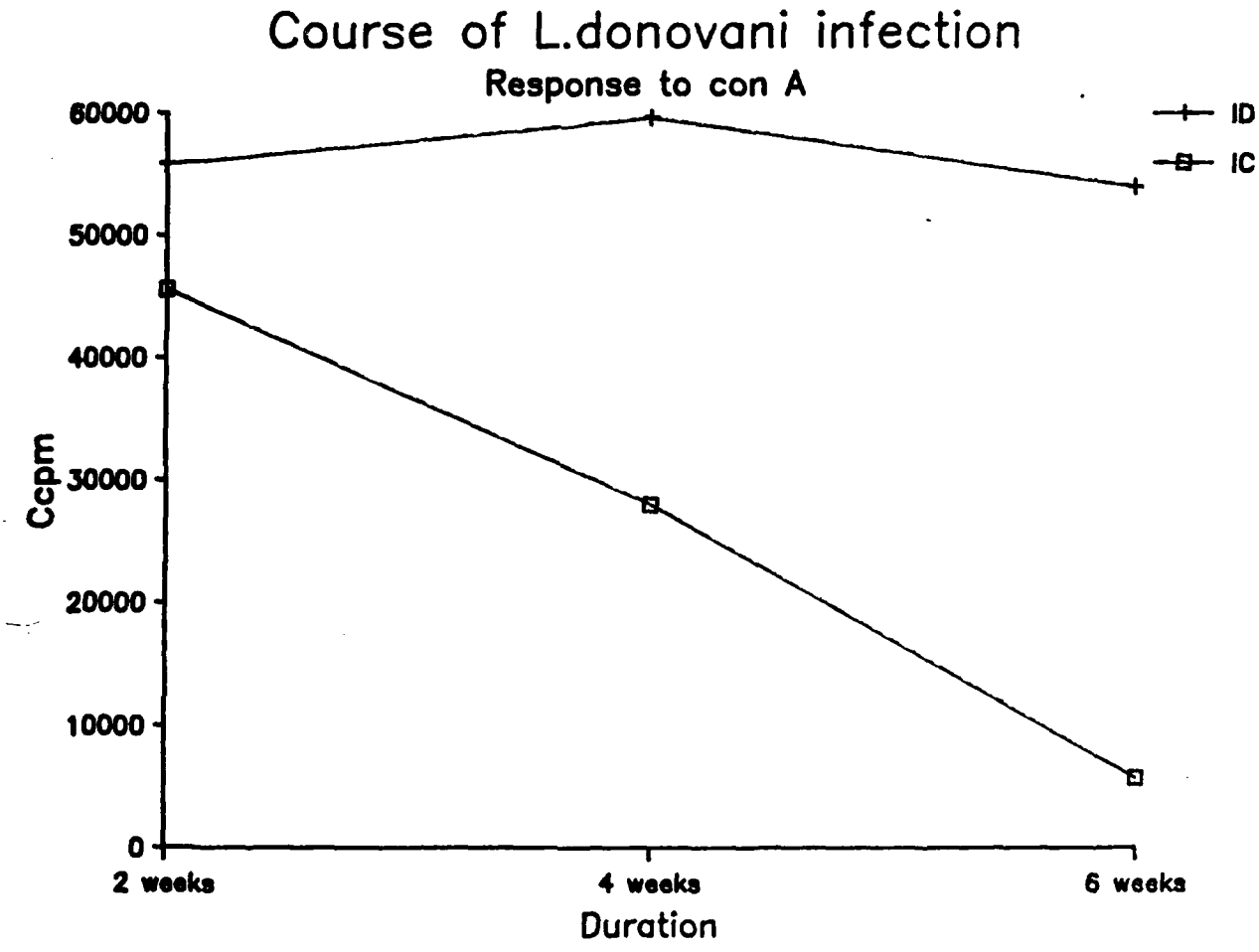


Figure 7. Proliferative response to Con A of spleen cells from hamsters infected intradermally (ID) or intracardially (IC).

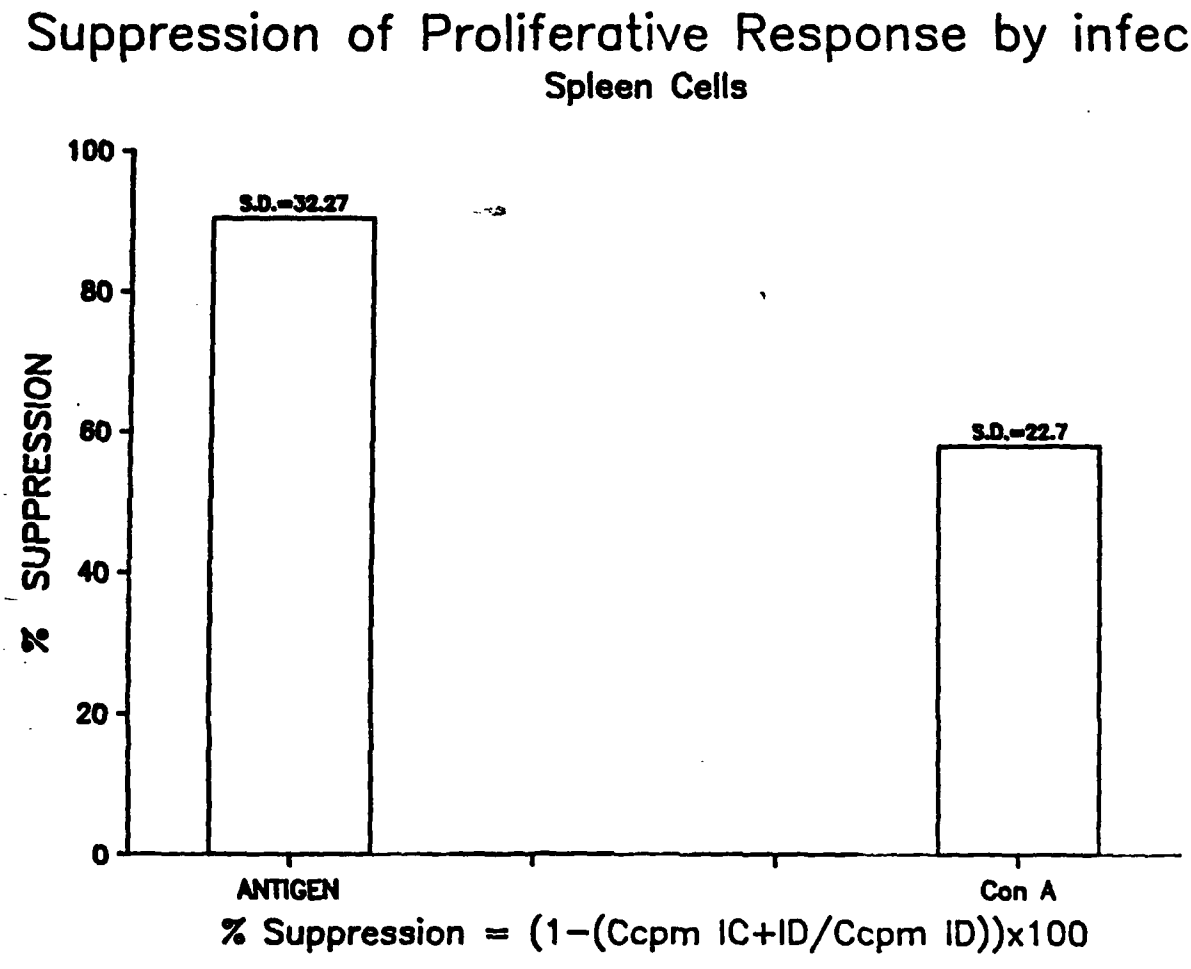


Figure 8. Spleen cells ($2.5x10^5$) from hamsters infected (IC) 6 weeks previously were cultured with an equal number of spleen cells from ID infected hamsters. Result based on the mean of 18 animals from 4 experiments.

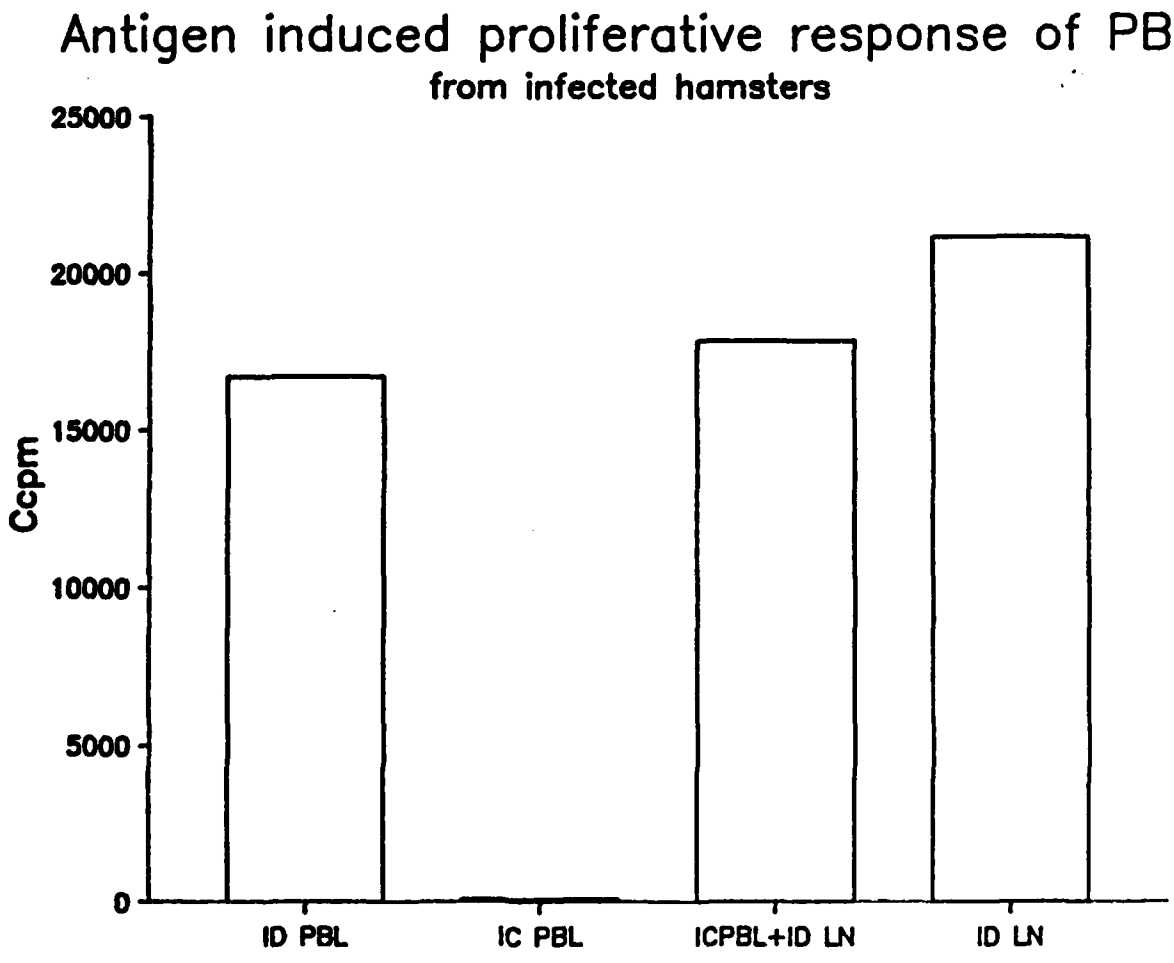


Figure 9. Peripheral blood lymphocytes from ID or IC infected hamsters were stimulated with Leishmania antigen; cells from IC infected hamsters were pooled and added to ID lymph node cells to test for suppressive activity.

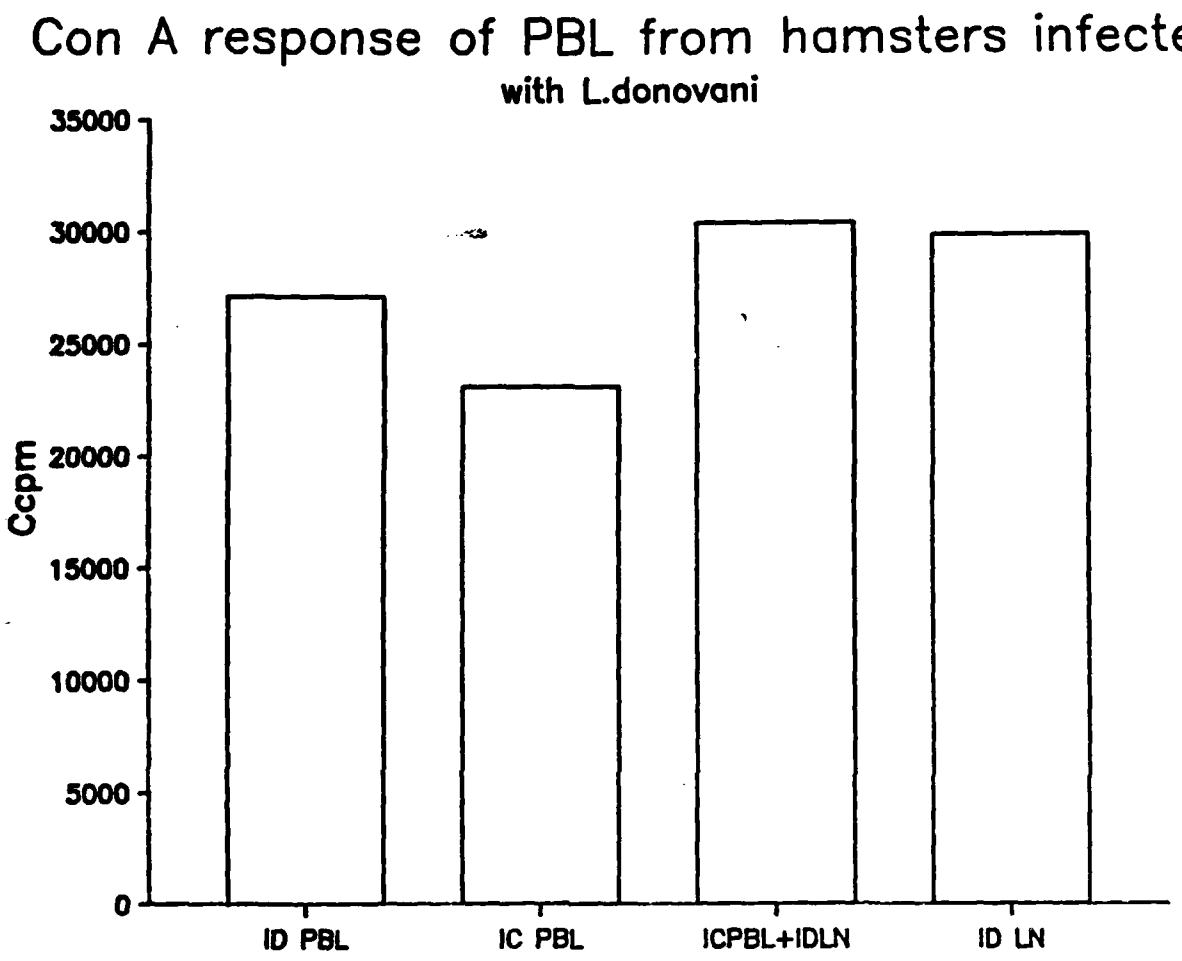


Figure 10. ID PBL, IC PBL = Peripheral blood lymphocytes from hamsters infected intradermally or intracardially with L. donovani
ID LN = lymph node cells from ID inoculated hamsters.

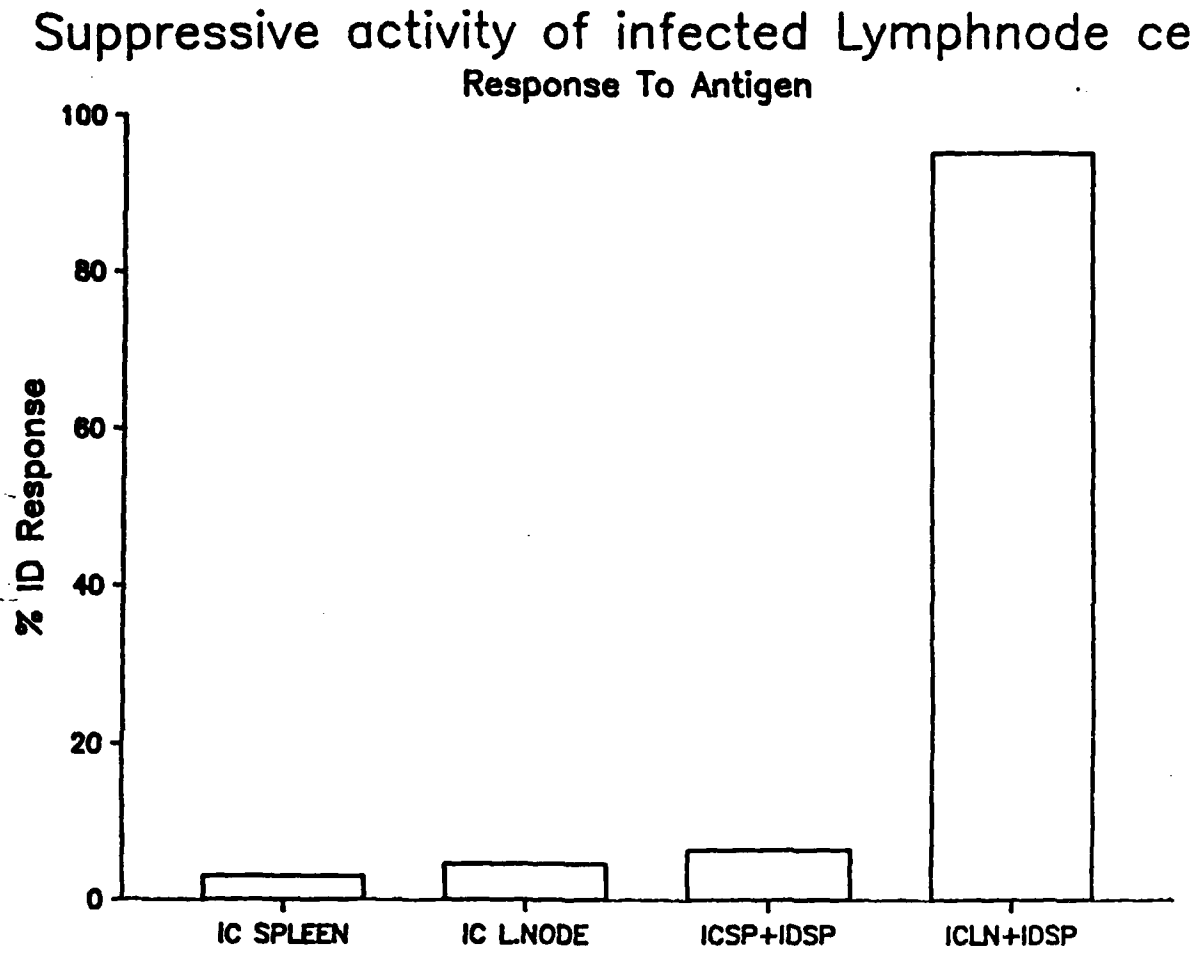


Figure 11. Spleen (IC SP) or lymph node (IC LN) cells from IC infected hamsters were co-cultured with spleen cells from ID infected (ID SP) hamsters to test for suppressive activity.

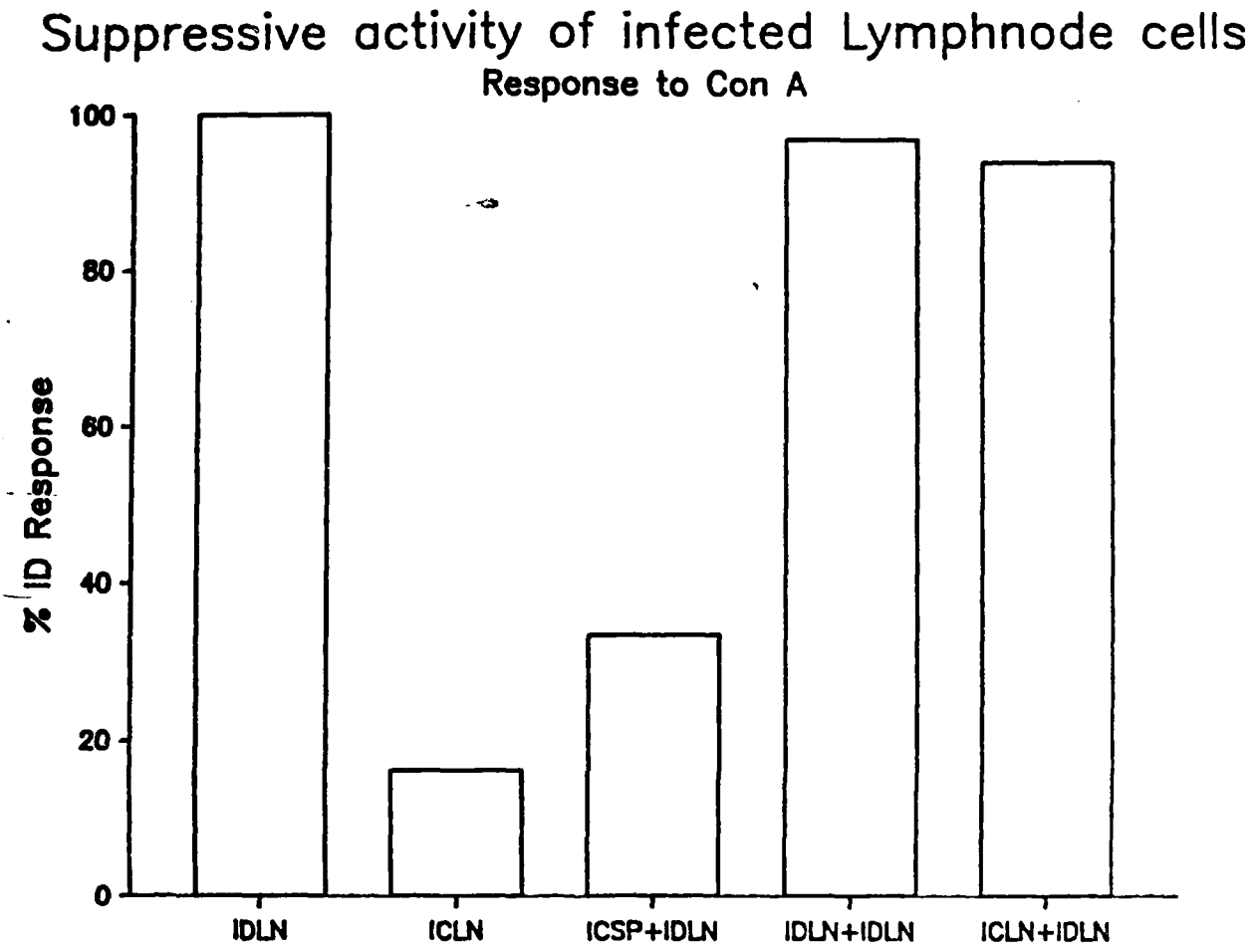


Figure 12. ID LN, IC LN = Lymph node cells from hamsters infected with L. donovani ID or IC.

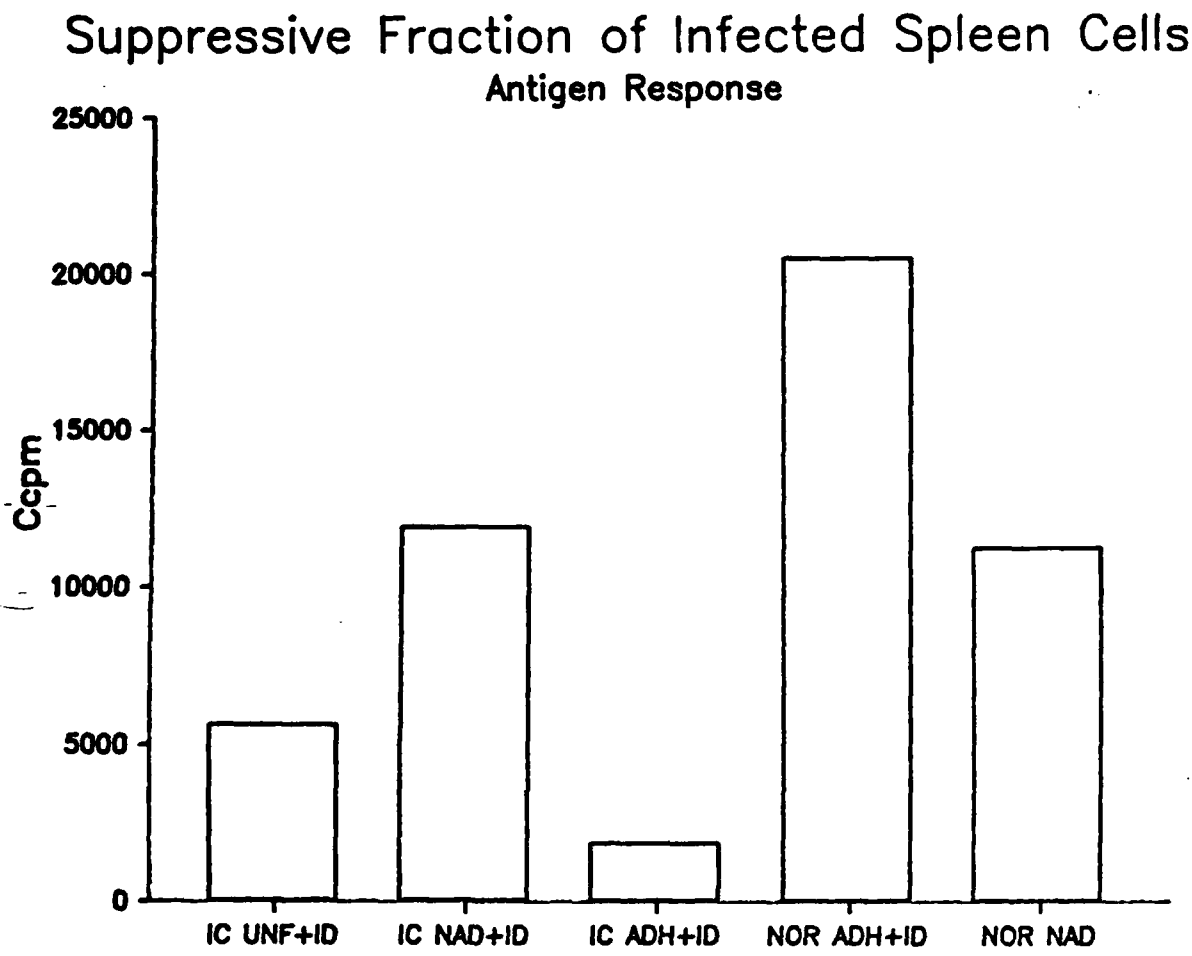


Figure 13. Infected spleen cells either unfractionated (ICUNF), plastic non-adherent (ICNAD), or plastic adherent (ICADH) were co-cultured with an equal number of spleen cells from ID inoculated hamsters. Plastic adherent (NOR ADH) and non-adherent (NOR NAD) from uninfected hamsters were used for controls.

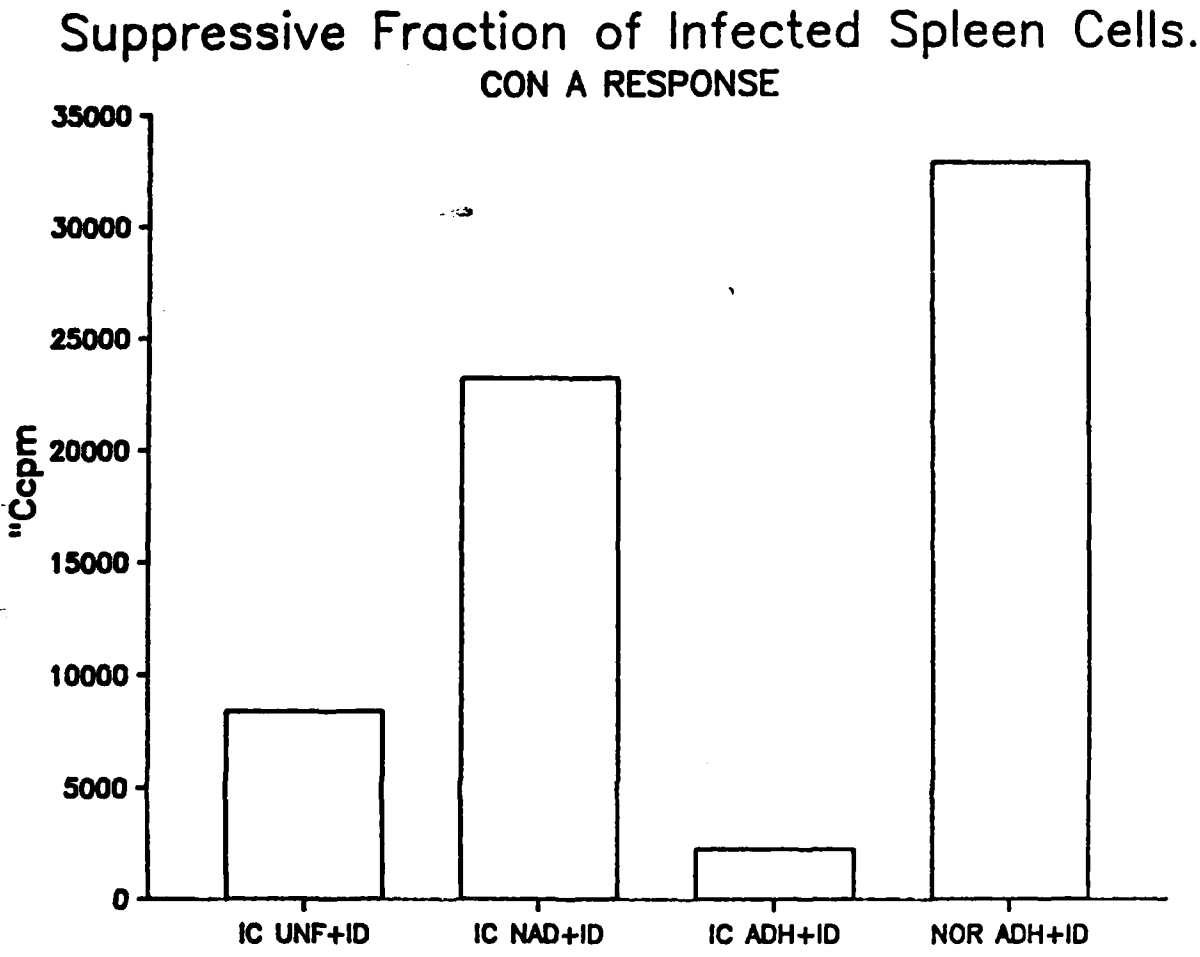


Figure 14. Con A - induced proliferative response of ID spleen cells in the presence of unfractionated infected (ICUNF), plastic adherent (IC ADH), infected non-adherent (IC NAD) or adherent spleen cells from normal hamsters (NOR ADH).

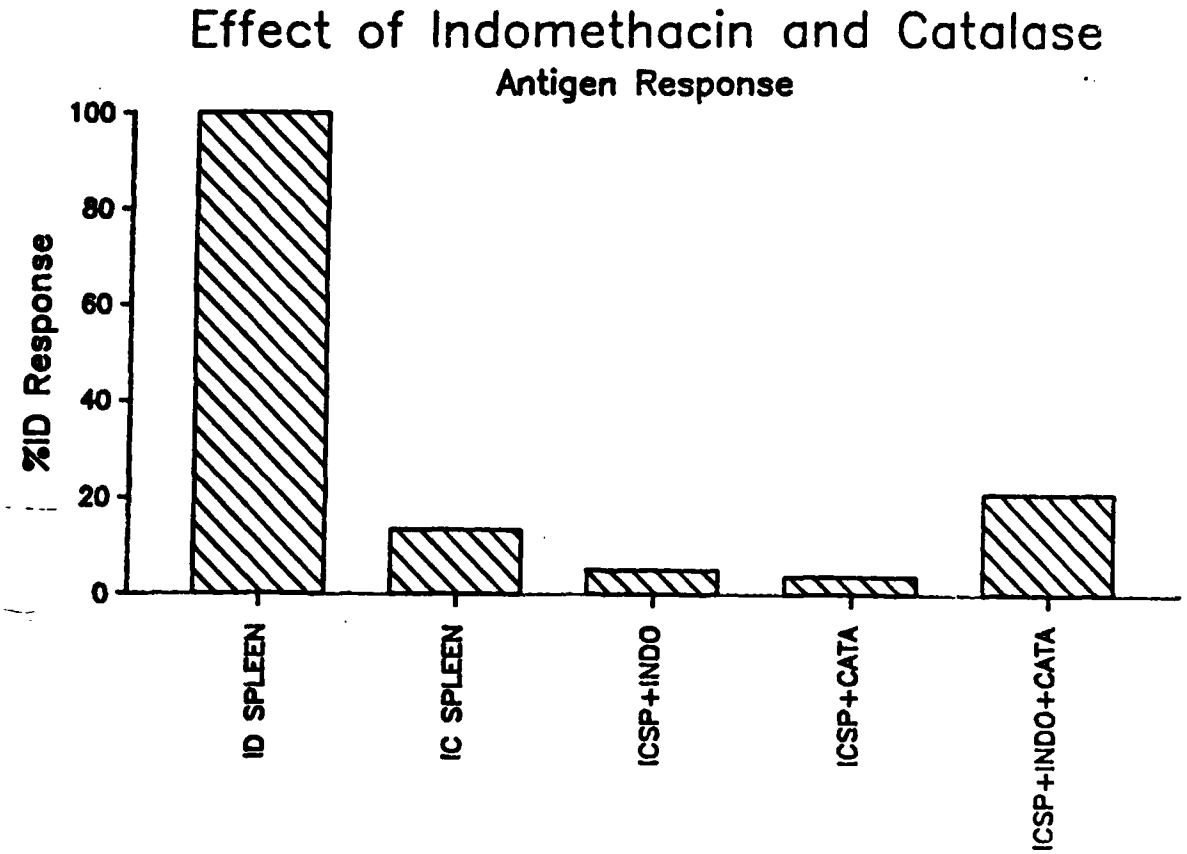


Figure 15. Indomethacin (5 ug/ml), catalase (200 ug/ml), either alone or in combination, were added to cultures of infected spleen cells at the beginning of lymphocyte proliferation assays.

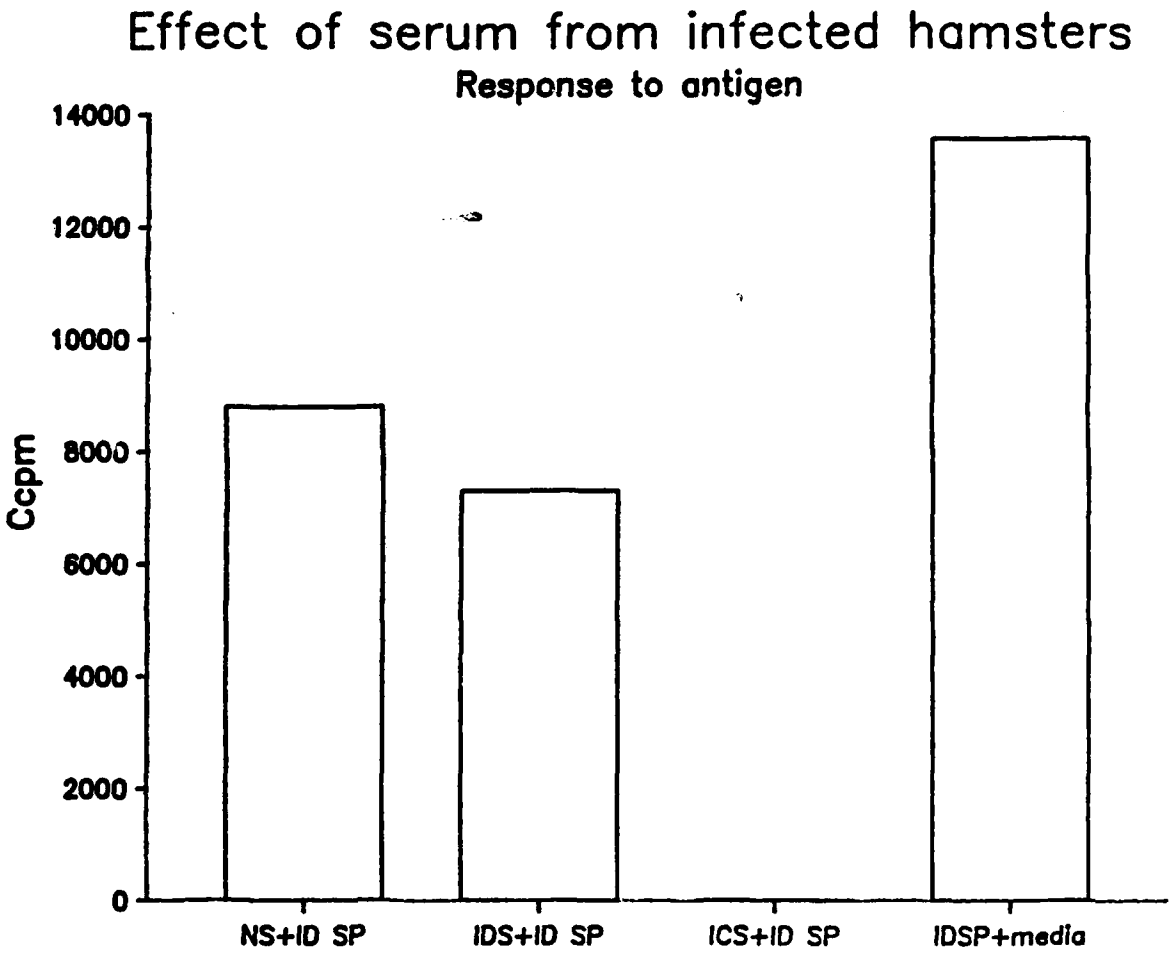


Figure 16. Pooled sera from normal hamsters (NS), or hamsters infected either IC (ICS) or ID (IDS) 5 weeks previously were tested for effect on antigen-induced proliferative response of ID spleen cells (IDSP).

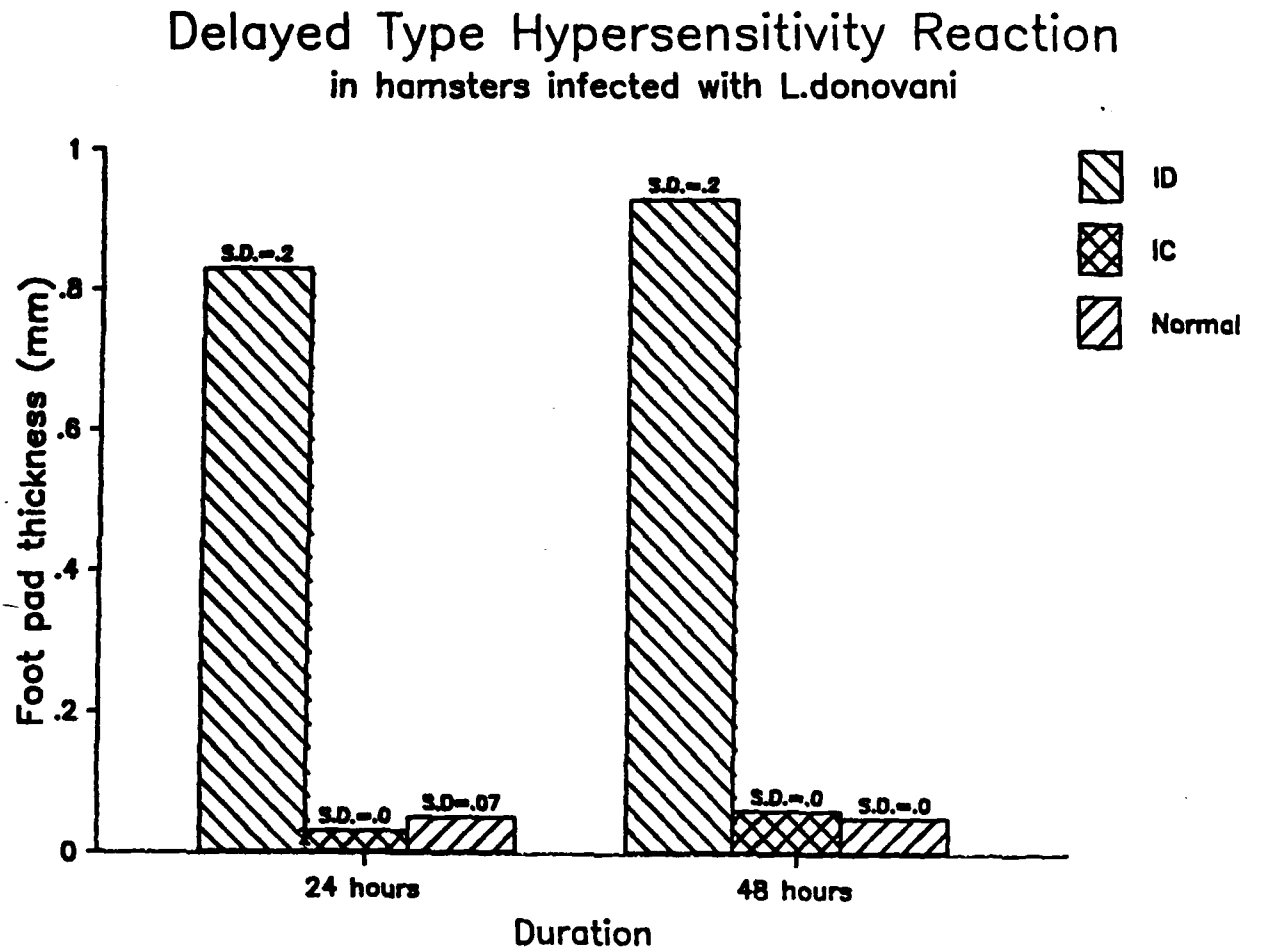


Figure 17. Normal hamsters or hamsters infected with L. donovani amastigotes intradermally (ID) or intracardially (IC) 2 weeks previously were skin tested with 50 ul leishmanin in one of the foot pads; the other pad received phenol saline alone. The difference in foot pad thickness is presented.

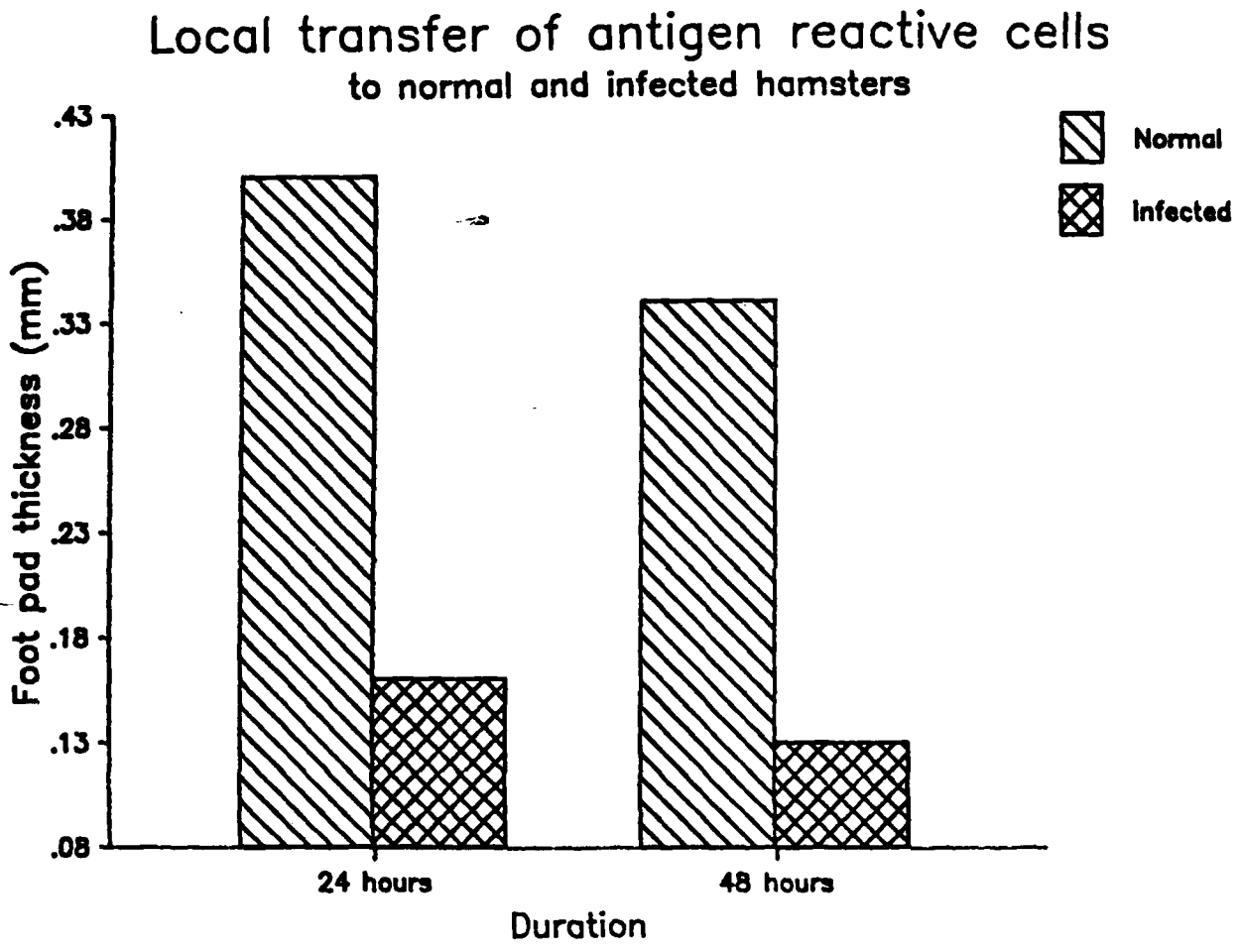


Figure 18. Spleen cells from ID infected hamsters ($3x10^6$) were injected locally with antigen ($10x10^6$ formalin fixed promastigotes) to one of the hind footpads of normal hamsters or hamsters infected with L. donovani (IC) 4 weeks previously. The co-lateral pad received antigen alone and the difference in foot pad thickness is presented.

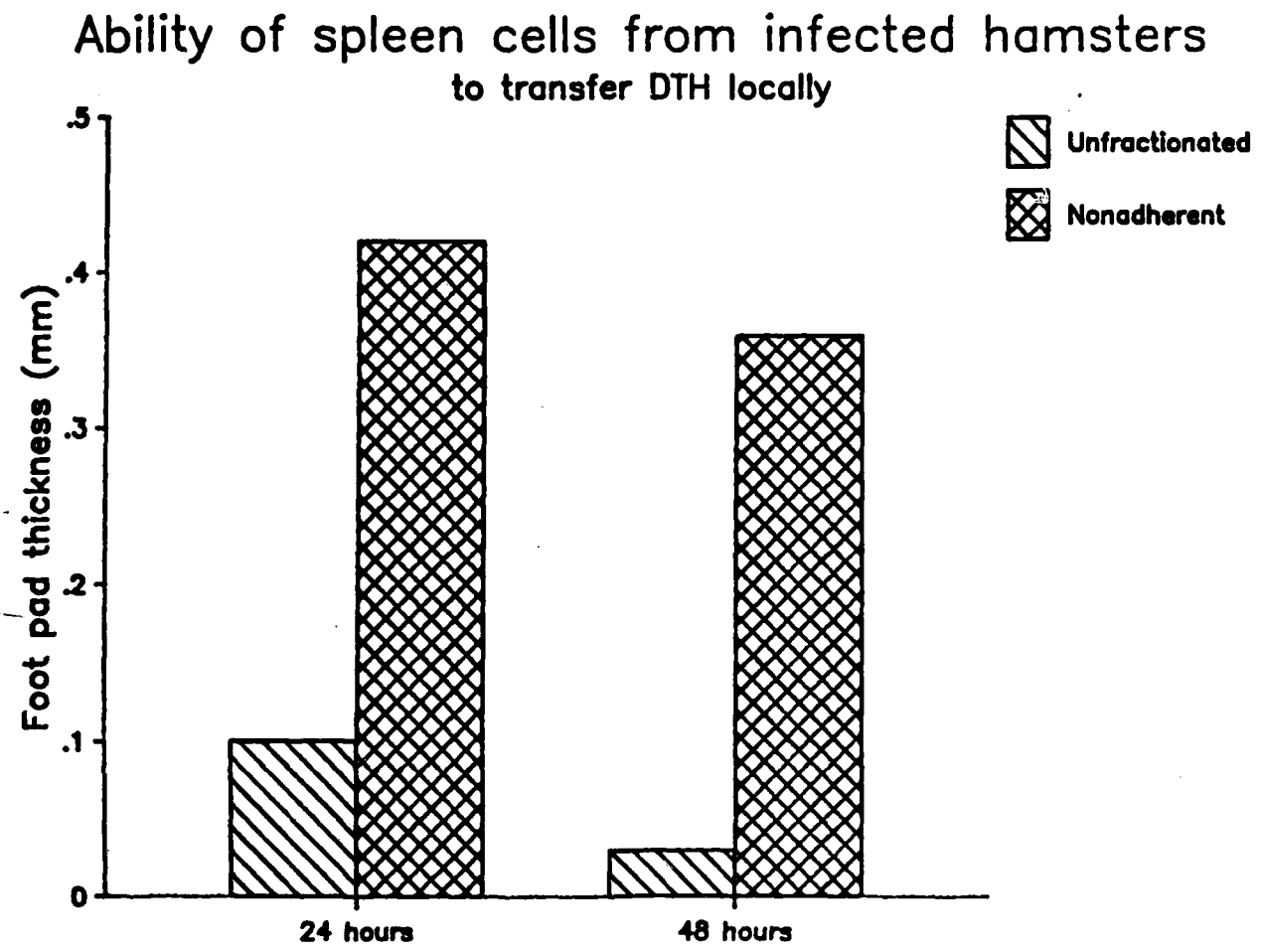


Figure 19. Unfractioned or plastic non-adherent fractions of spleen cells from infected hamsters (IC) were inoculated with antigen ($15x10^6$ formalin fixed promastigotes) into one of the hind foot pads of normal hamsters. The contralateral pad received only antigen and the difference in foot pad swelling at the indicated times is presented.

Table 1

Properties of adherent cells from spleens of hamsters infected with *L. donovani*.

	Percentage
Esterase staining	90.6% ± 4.5
Phagocytosis of latex beads	60.0% ± 4.2

Phagocytosis (normal peritoneal cells) 72%

N.B. a number speading, macrophage-like cells did not take up latex beads.

Table 2.

Production of prostaglandins by spleen cells from L. donovani infected hamsters.

Cell source	No. of cells/ml	Pg/ml ± S.D.
Normal spleen	3.5×10^6	3240 ± 1886
ID spleen	2.5×10^6	881 ± 470
IC spleen	2.5×10^6	19470 ± 6595

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