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LYMPH NODE CELL RESPONSIVENESS IN BALB/c MICE INFECTED WITH *LEISHMANIA MEXICANA*

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In the present study we measured the blastogenic response of lymph node cells from BALB/c mice infected with Leishmania mexicana *throughout the course of infection. Results showed that infected mice displayed normal blastogenic responses in the lymph nodes until twenty weeks of infection. Thereafter, there was a gradual suppression. Comparison of the immunoresponsiveness in the spleen and lymph nodes, revealed normal responses in the lymph nodes several weeks after suppression in the spleen had occurred. Suppression of blastogenic responses in the lymph nodes was related to an adherent macrophage-like cell which actively suppressed normal proliferative responses to mitogens.*

BALB/c mice infected with *Leishmania mexicana* develop a chronic infection accompanied by progressive loss of immunoresponsiveness (Pérez, Arredondo & Gonzáles, 1978; Arredondo & Pérez, 1979). The nature and mechanism of immunosuppression are poorly understood but there is evidence that non-specific suppressor cells appear in the spleen of infected mice (Arredondo & Pérez, 1979). Recently we have found that macrophage-like cells arise in the spleen that actively suppress the ability of normal syngeneic lymphocytes to undergo proliferation after stimulation with T and B cell mitogens (Pérez & Bolívar unpublished results).

In the present study we have evaluated the response of lymph node cells (LNC) throughout the infection. Results indicated that LNC did not become suppressed during the first twenty weeks of infection but loss of LNC responsiveness occurred in BALB/c mice with long-term infection by *L. mexicana*. Further, evidence was found that suppression is related to an adherent suppressor cell.

MATERIAL AND METHODS

Host and infection: inbred female BALB/c mice were used in all experiments. These were provided by the breeding unit of the Instituto Venezolano de Investigaciones Científicas. Mice ten week-old at the start of the experiment were inoculated into the right hind footpad with 10^4 amastigotes of the AZV isolate of *L. mexicana* (Pérez, Labrador & Torrealba, 1979). Amastigotes were obtained from infected hamsters as described (Pérez et al., 1978, 1979). Infected mice were examined weekly for lesions. These were measured with a "Schnelltäster" caliper (Kroplin, Germany) as previously reported (Pérez et al., 1979).

Cell cultures: microcultures of LNC and spleen cells from four control and four infected mice, were prepared as described before (Pérez et al., 1978; Arredondo & Pérez, 1979). Briefly, single cell suspensions were made by homogeneization of lymph nodes (periaortic and inguinal) or spleens in a glass Ten Brook tissue grinder with cold RPMI-1640 medium (Grand Island Biological Co., N.Y., GIBCO) supplemented with antibiotics. Cell debris and clumps were allowed to sediment for 10 min and the supernatants were centrifuged at 180 x g for 10 min. After one wash lymphoid cells were resuspended in cold RPMI-1640 supplemented with 2 mM glutamine (GIBCO), 5% foetal calf serum (FCS, GIBCO) and antibiotics hereafter referred to RPMI-FCS. Cell suspensions containing 5×10^5 viable lymphocytes were dispensed into each well of flat bottomed microtiter plates (Falcon, Plastic, Oxnard, Calif., Nº 3040). Concanavalin A (Con A, Sigma Chemicals, St. Louis, Mo.) and phytohemagglutinin P (PHA, Difco Laboratories, Detroit, Mich.) at the desired concentration were added to cultures with were then incubated in a humidified atmosphere of 5% CO_2 in air at 37ºC. Deoxyribonucleic acid synthesis was assayed by adding 1 μ Ci of tritiated thymidine (specific activity 5.0 Ci / mM, New England Nuclear Corp. Boston, Mass.) for the final 18 h of the 72 h incubation period. Lymphoid cell responses of infected animals were compared to those of control cells obtained from age-matched uninfected mice. Cells were collected by suction on glass-fiber filter using a multiple automated sample harvester (Mash II, Microbiological Associates, Md.). The radioactivity was measured in a liquid scintillation spectrometer (Packard, Tri-Carb, Mod. 3320, Packard Instruments Co., Inc, Downers 1 II). Results are expressed as the arithmetic mean c.p.m. of triplicate cultures or as the per-cent change (enhancement or suppression for control cultures).

Cell separation technique: to separate adherent cells 3 ml of 1×10^7 LNC suspended in RPMI-FCS were incubated in Falcon 1007 petri dishes for 2 h at 37ºC. Nonadherent cells were removed with a Pasteur pipette and the remaining adherent cells were extensively washed and removed after adding 2% cold EDTA (Sigma) in phosphate buffered saline and incubating the dishes for 15 min at 4ºC. Adherent cells were washed with cold RPMI-FCS and adjusted to the required concentration. Nonadherent cells contained 1%

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or fewer phagocytic cells as measured by the neutral red uptake (Arnaiz-Villena & Hay, 1975), and adherent cells contained more than 90% phagocytic cells.

RESULTS

Course of infection: this was determined in ten mice. All animals exhibited the characteristic progressive lesion which ulcerated at 10-12 weeks and steadily increased in size until mice became terminally ill. Metastatic lesions were frequently seen and after 18 weeks of infection visceralization occurred. A typical course of infection is shown in Figure 1 for reference purposes.

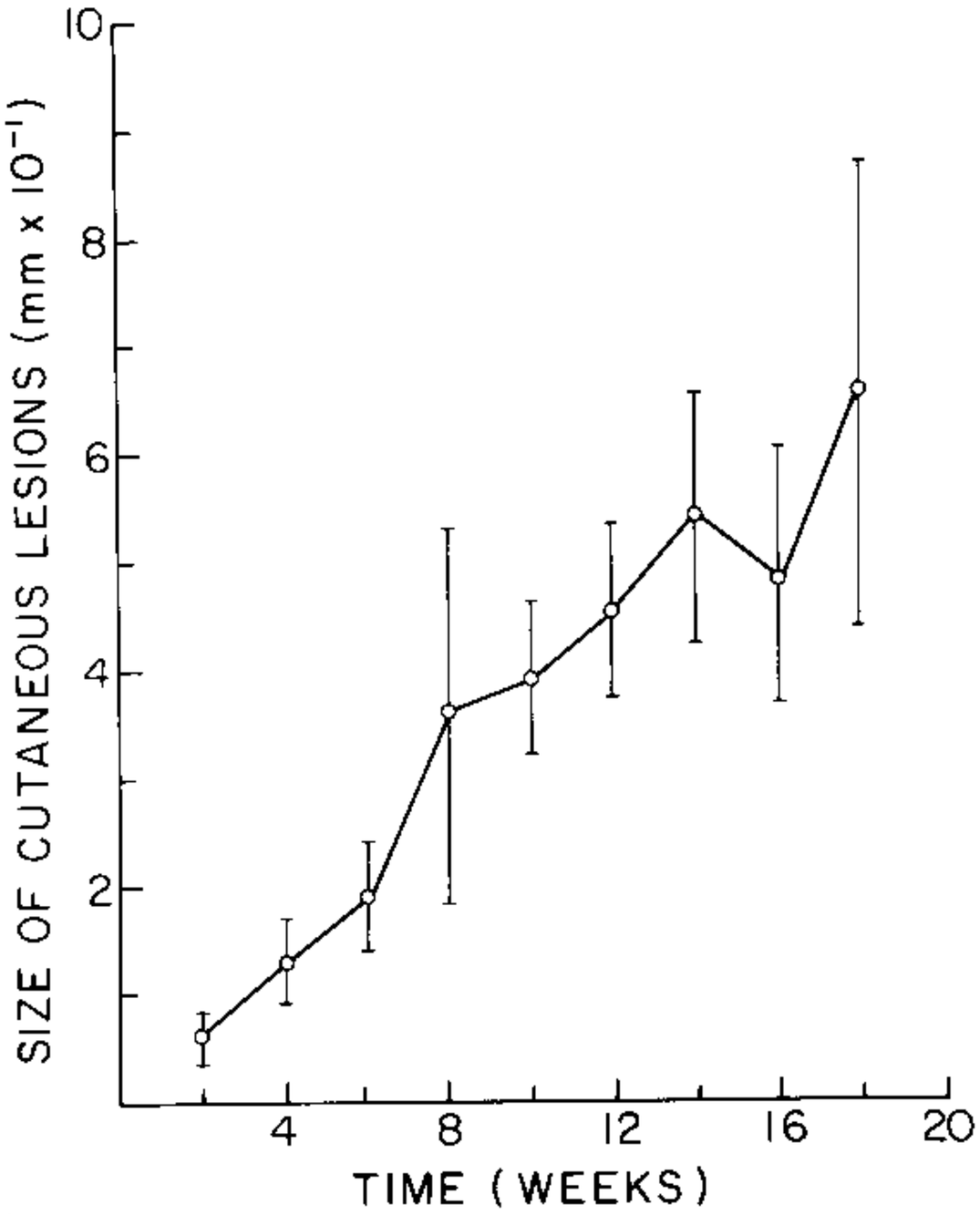

Fig. 1: course of cutaneous lesions in BALB/c mice infected with 10^4 amastigotes of *L. mexicana* (mean ± SD for ten mice).

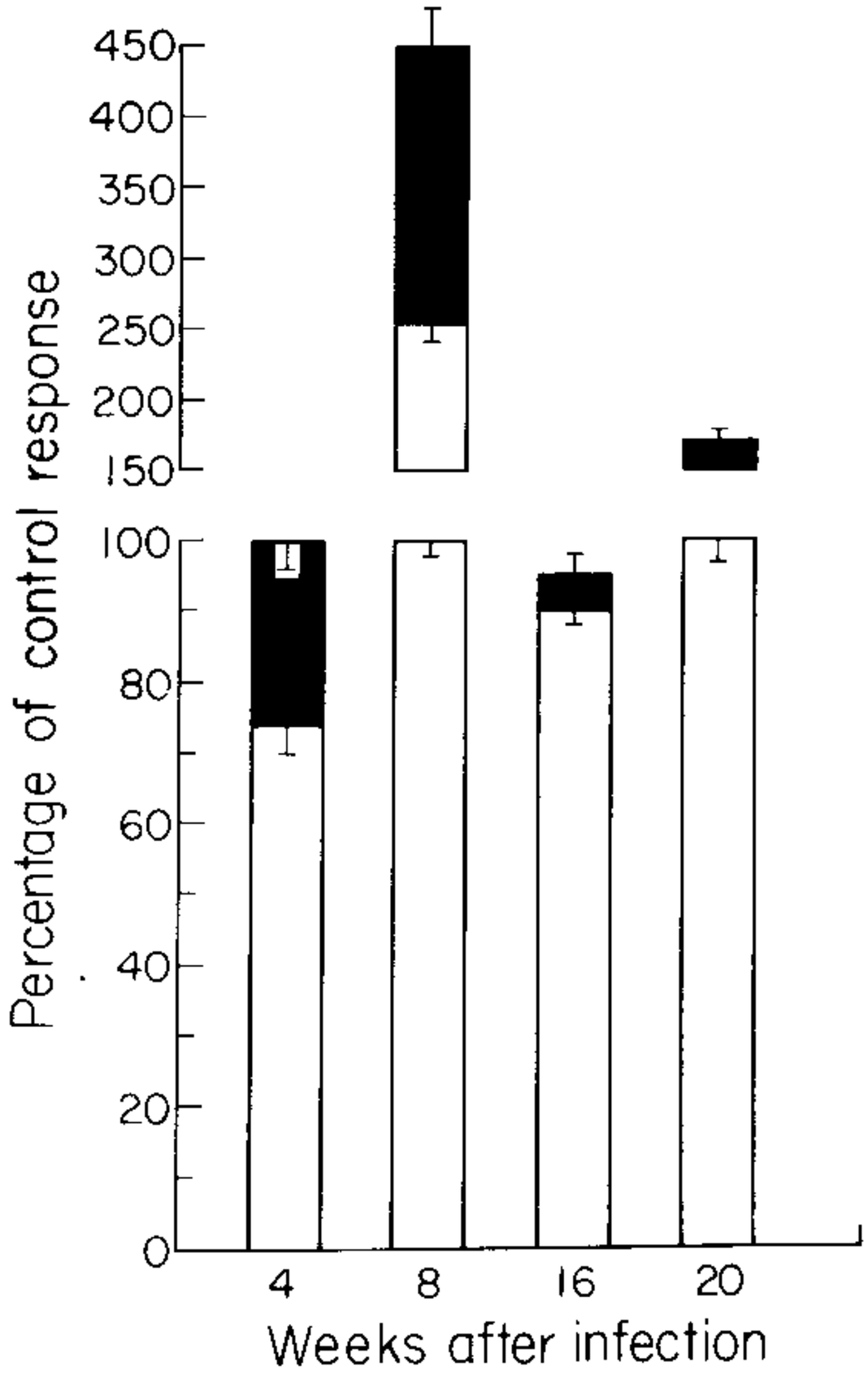

Fig. 2: lymph node cell responses of BALB/c mice infected with 10^4 amastigotes of *L. mexicana* to Con A. Results are expressed as percentage of responses of control lymph node cells for optimal (□, 1.25 μg/ml) and suboptimal (■, 0.62 μg/ml) doses of mitogen. Values indicate mean ± SD for three experiments.

TABLE I

The blastogenic response of spleen cells taken from BALB/c mice 16 week after the inoculation of 10^4 amastigotes of *L. mexicana*

Mitogens	Blastogenic response (Mean c.p.m. ± SD)	
	Control mice	Infected mice
Con A (μg/ml)		
1.25	128,287 ± 3,474	55,788 ± 4,246
0.62	103,873 ± 2,667	31,971 ± 1,867
PHA (μl/ml)		
5	45,568 ± 3,442	11,439 ± 1,007
1	34,512 ± 2,980	8,397 ± 1,001

Lymph node cell responsiveness in BALB/c mice infected with *L. mexicana*: we previously demonstrated that spleen cells from BALB/c mice infected with *L. mexicana* showed a marked depression of their blastogenic response to PHA and lipopolysaccharide (LPS) from eight weeks of infection onward and that nonspecific suppressor activity was evident at twelve weeks of infection (Arredondo & Pérez, 1979). It was of interest, therefore, to determine whether of not lymph nodes responsiveness was lost in BALB/c mice infected with *L. mexicana*. Fig. 2 shows that a four weeks of infection mice exhibited a slight depression of their blastogenic response to Con A (optimal dose) whereas at eight weeks responses to optimal and suboptimal doses of Con A were enhanced. At twenty weeks responses were either comparable (optimal dose) or stronger (suboptimal dose) than those of control cells. In contrast, at 16 weeks of infection spleen cells responses to Con A and PHA were markedly suppressed (Table I). Examination of the response of LNC taken at 24 and 28 weeks of infection revealed significantly reduced proliferative responses over a wide range of concentrations of Con A, Table II. To assertain whether suppressor cell activity was associated to the unresponsiveness acquired by LNC late during the infection, the responses of a mixture of 4×10^5 control LNC and 1×10^5 LNC taken at 28 weeks of infection to various doses of Con A were compared to those of 5×10^5 control LNC. Results, presented in Fig. 3, showed not only that LNC from long-term infected mice were intrinsically depressed but they were effective in suppressing the response of control cells to Con A. However, when infected LNC were depleted from the adherent cell population suppression was not longer manifested. These results suggested that loss of responsiveness in infected LNC was related to suppressor adherent cell effects. That was confirmed in a further experiment which showed that when 1×10^5 adherent LNC from long-term infected mice were added to cultures of 4×10^5 control LNC, responses to Con A were markedly suppressed as compared to those of control LNC cocultured with a similar number of control adherent LNC, Fig. 4.

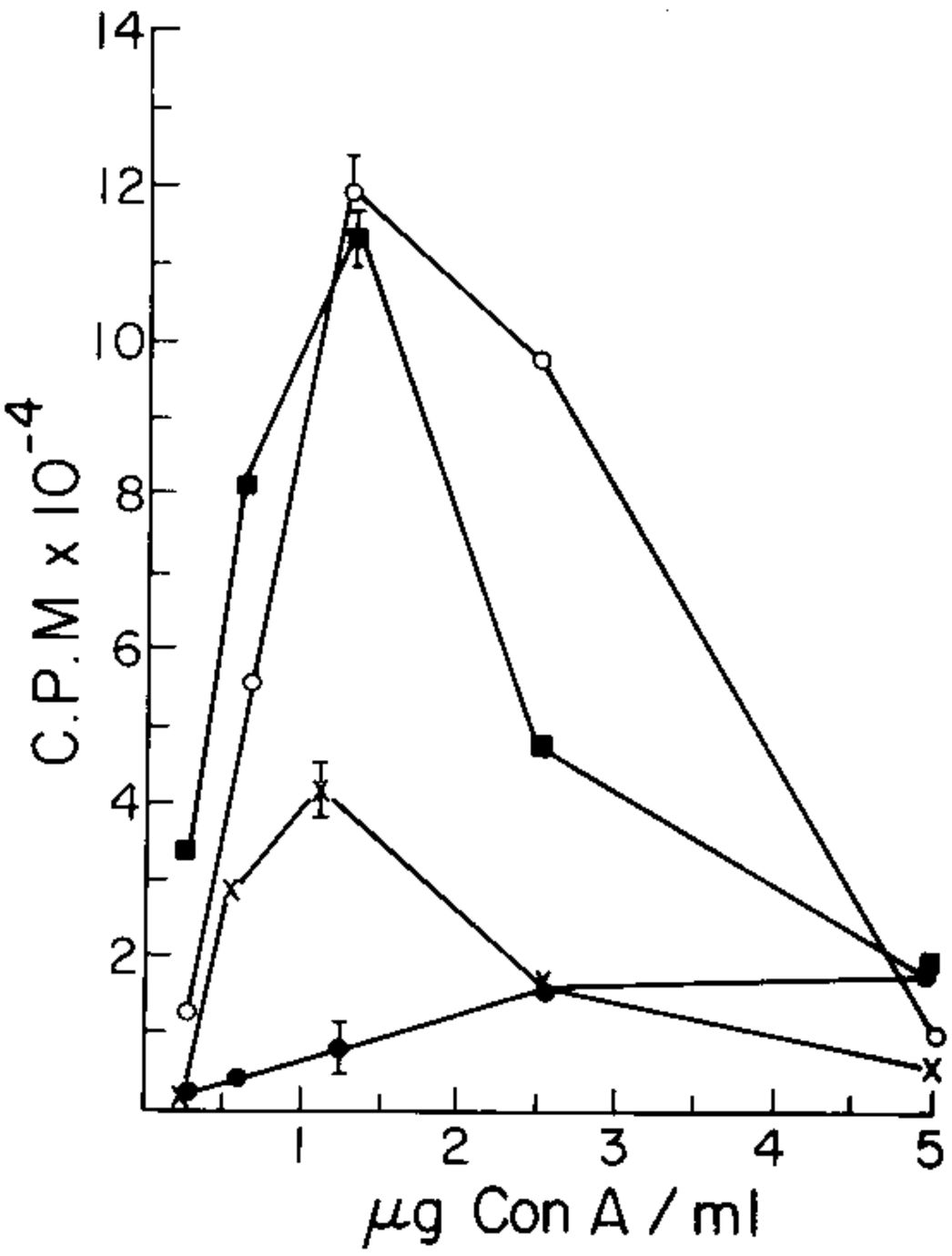

Fig. 3: effect of lymph node cells (LNC) taken at 28 weeks after the inoculation of 10^4 amastigotes of *L. mexicana* on the response of control LNC to Con A. Values correspond to 5×10^5 infected LNC (●—●), a mixture of 4×10^5 control LNC plus 1×10^5 infected LNC (x—x), a mixture of 4×10^5 control LNC plus 1×10^5 plastic non-adherent infected LNC (■—■) and 5×10^5 control LNC (○—○). Mean c.p.m. ± SD for two experiments. One SD shown.

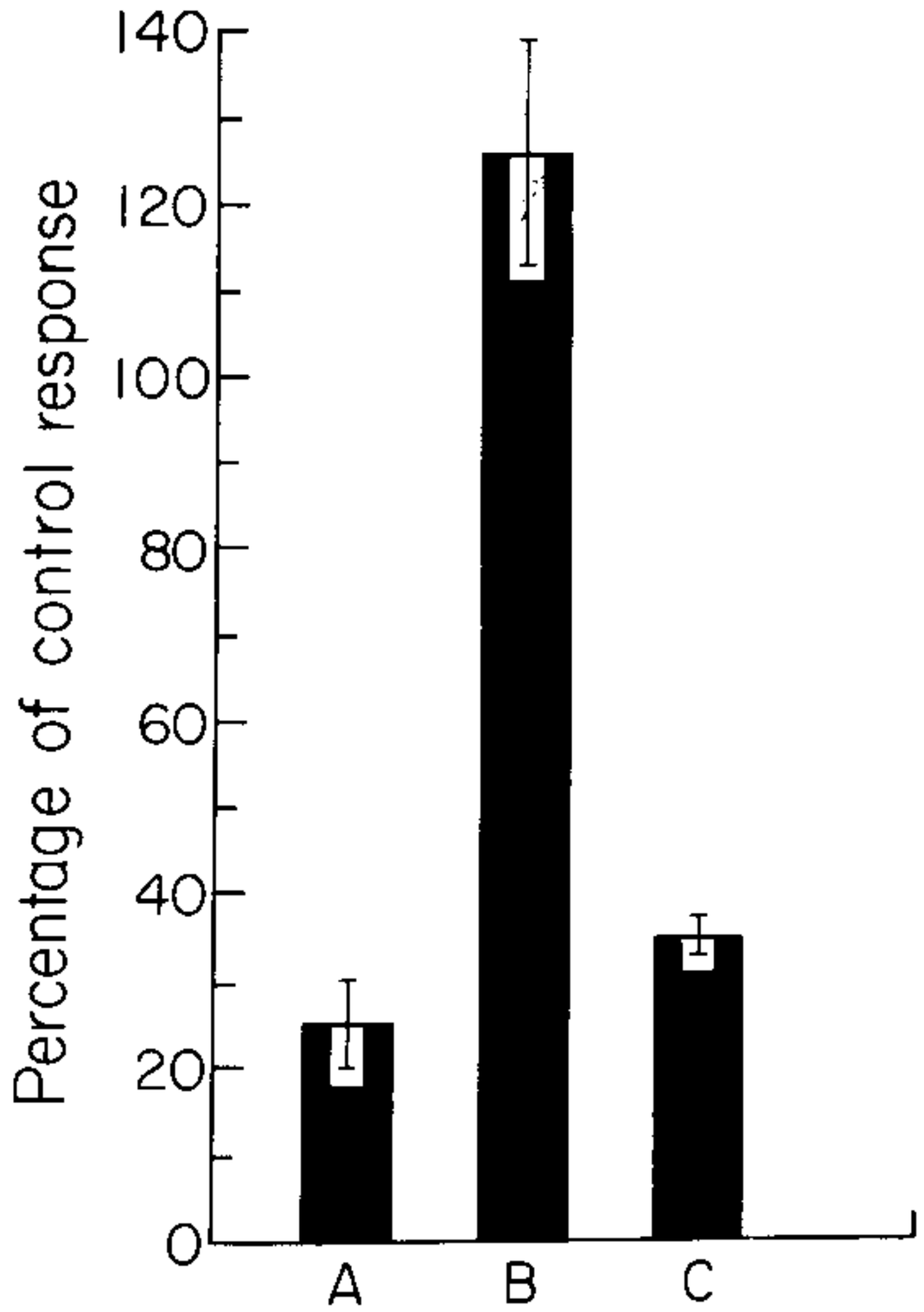

Fig. 4: effect of adherent cells obtained from the lymph nodes of BALB/c mice at 28 weeks after the inoculation of 10^4 amastigotes of *L. mexicana* on the response of control lymph node cells (LNC) to Con A. Values correspond 5×10^5 infected LNC (group A); 4×10^5 control LNC plus 1×10^5 control adherent LNC (group B) and 4×10^5 control LNC plus 1×10^5 infected adherent LNC (group C). Results are expressed as percentage of the response of 5×10^5 control LNC. Mean ± SD for two experiments.

DISCUSSION

BALB/c mice infected with *L. mexicana* show peculiar changes of their immunoresponsiveness. During an early phase of infection (4-8 weeks) mice are able to manifest delayed type hypersensitivity

TABLE II

The blastogenic response of lymph node cells of BALB/c mice with longterm infection by *L. mexicana*

Con A (μg/ml)	Blastogenic response (Mean c.p.m. ± SD)		
	Control mice	Infected mice	
		24 weeks	28 weeks
0.31	19,836 ± 1,636	11,185 ± 740	859 ± 155
0.62	76,147 ± 3,781	40,856 ± 4,027	3,382 ± 1,827
1.25	153,938 ± 7,791	68,499 ± 4,042	7,382 ± 2,421
2.50	173,080 ± 11,214	46,846 ± 1,561	16,148 ± 1,312
5.00	65,518 ± 247	4,500 ± 129	8,109 ± 300
10.00	1,471 ± 443	N.D	1,100 ± 783

N.D = Not done.

responses (DHR) to parasite antigens, enhanced IgM-PFC responses to sheep erythrocytes and increased spleen cell blastogenic responses to mitogens. However, from eight weeks of infection onward suppressed DHR to parasite antigens, accelerated growth of lesions and progressive suppression of the spleen blastogenic response to mitogens are observed (Arredondo & Pérez, 1979). A question of importance was whether or not immunosuppression occurred in lymph nodes. Data herein presented showed either normal or enhanced T cell responses in the lymph nodes during the first twenty weeks of infection. Hence, at a time when spleen cell responses were drastically suppressed those of lymph nodes were not significantly altered. Moreover, enhanced responses to suboptimal doses of Con A (8-20 weeks of infection) were noted. However, lymph nodes became suppressed after 23 weeks of infection. Dose curve analysis showed that diminished response to Con A occurred within a wide range of mitogen concentrations and it is thus not simply reflecting a shift in mitogen concentration inducing maximal stimulation. The reason for delayed occurrence of immunosuppression in lymph nodes is at present not understood but suppression was associated to the appearance of adherent suppressor cells. The latter is in keeping with other findings of our laboratory showing that the inability of spleen cells from BALB/c mice infected with *L. mexicana* to mount proliferative responses to T and B cell mitogens was attributable to a macrophage-like suppressor cell (Pérez & Bolívar unpublished results). Reduced proliferation to mitogens has been observed in cultures containing high number of macrophages (Keller, 1975). Therefore, it can be argued that the suppressed blastogenic response of lymph node cells from long-term infected mice is due to an increased number of macrophages in the lymph nodes. The latter possibility is not supported by the experiments showing that adherent cells from infected mice drastically suppressed the response of normal lymphoid cells to Con A when a similar number of control adherent cells did not significantly modify normal proliferative responses.

Suppressor macrophages have been found in several diseases (Cunningham & Kuhn, 1980; Klimpel & Henney, 1978; Sathish et al., 1983; Wadee, Sher & Rabson, 1980; Wellhausen & Mansfield, 1979; Wyler, Oppenheim & Koontz, 1979) and there is evidence that in BALB/c mice infected with *L. tropical* (Scott & Farrel, 1981) and in patients with diffuse cutaneous leishmaniasis (Petersen et al., 1982), immunosuppression was related to a macrophage-like suppressor cell. Experiments carried out to asess the effect of *Leishmania* parasites in the ability of macrophages from BALB/c mice to promote an immune response have shown that macrophages from infected mice pulsed *in vitro* with leishmanial antigen failed to sensitize syngeneic recipients for a DHR to parasite antigens (Handman, Ceredig & Mitchell, 1979). Likewise, skin-macrophages infected *in vitro* with *L. tropica* have a reduced capability to stimulate proliferation of specifically immune lymphocytes (Gorczynski & MacRae, 1982). Other studies indicated alterations in the pattern of high molecular weight components (Slutzky & Greenblatt, 1979), modifications of the pinocytic rate (Chang, 1980), reduced endocytic and digestive capacity (Kutish & Janovy, 1979; Rodríguez, Hernández & Merino, 1983) and decreased migration in response to chemotaxin (Bray et al., 1983) in macrophages either infected with *Leishmania spp* or treated with products derived from these parasites. Taken together, all these studies suggest that *Leishmania* parasites can provoke important functional alterations in the mononuclear phagocyte. It is noteworthy that products derived from *L. tropica* or *L. enriettii* have been shown to interfer with lymphocyte responses to PHA and PPD (Farah, Lazary & De Weck, 1976; Londner et al., 1983). Therefore, although macrophages are known to manifest suppressor activity via direct effects on regulatory T cells (Stobo, 1977) or through the production of soluble mediators (Allison, 1978), the possibility that parasites or their products are implicated in the immunosuppression exerted by macrophages from infected mice should also be explored. In this context evidence that parasite products can adversally affect immune responses (Albright & Albright, 1981) and that immunosuppressive activity could be released from macrophages following *in vitro* interaction with a trypanosome derived immunosuppressive fraction (Sacks et al., 1982) have been obtained. The possible mechanism by which macrophages from BALB/c mice with long-term infection by *L. mexicana* exert suppressor activity is the object of research in our laboratory.

RESUMO

No presente trabalho medimos a resposta blastogênica de células de nódulos linfáticos de camundongos BALB/c inoculados com *Leishmania mexicana*, no decurso da infecção. Os resultados mostraram que os animais infectados exibem respostas blastogênicas normais nos nódulos linfáticos até vinte semanas depois da infecção. Daí por diante houve uma supressão gradual. A comparação da capacidade de resposta imunitária no baço e nos nódulos linfáticos mostrou respostas normais nesses nódulos várias semanas depois da ocorrência da supressão no baço. A supressão das respostas blastogênicas nos nódulos linfáticos estava relacionada a uma célula aderente de tipo macrófago, que suprimia ativamente as respostas proliferativas normais aos agentes mitogênicos.

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