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Nonspecific Immunodepression and Protective Immunity in Mice Infected with *Leishmania mexicana*

HILDA PÉREZ,[1]* MARISOL POCINO,[2] AND INÉS MALAVÉ[2]

Centro de Microbiologia[1] and Centro de Medicina Experimental,[2] Instituto Venezolano de Investigaciones Científicas, Caracas 1010A, Venezuela

C57BL/6 mice infected with *Leishmania mexicana* showed depression of the in vitro immunoglobulin M-plaque-forming cell response to sheep erythrocytes. Immunodepression was present 3 weeks after inoculation and was maximal at 11 weeks. Thereafter, there was a gradual return to normal immunoresponsiveness correlated with the resolution of lesions. At the time of maximal immunodepression, spleen cells from infected mice diminished the plaque-forming cell response to sheep erythrocytes of normal spleen cells. On the other hand, specific responses, as exemplified by protective immunity to a challenge infection and delayed hypersensitivity responses to parasite antigens, were apparently unaffected. These responses were both present in mice bearing primary lesions and were maximal in recovered mice. The significance of these findings is discussed in relation to a current hypothesis on parasite-induced immunodepression.

Parasitic infections have been associated with important changes in the host immune response to various antigens (23, 25). Studies have usually been concerned with modifications in the nonspecific immune responses accompanying fatal infections, but there is less information on the alterations of the host immune response occurring during the course of self-limiting infections.

Inoculation of C57BL/6 mice with 10^3 amastigotes of *Leishmania mexicana* results in the development of lesions which ulcerate at 6 weeks and show maximal sizes at 10 weeks, when they are covered with a crust. Thereafter, healing starts, and by week 20, lesions have resolved. Recovered mice show a strong delayed hypersensitivity response (DHR) to leishmanial antigen and protective immunity to a challenge infection with *L. mexicana* (15-17).

In the present work, we have investigated the development of DHR to leishmanial antigen and protective immunity to a challenge infection with *L. mexicana*. In parallel, we have studied the antibody response to sheep erythrocytes (SRBC) throughout the course of a primary infection with *L. mexicana* in C57BL/6 mice.

MATERIALS AND METHODS

Hosts. Female C57BL/6 mice (8 weeks old) and outbred male hamsters (12 weeks old) were used.

Parasites and infection. The strain AZV of *L. mexicana* used in the present study was isolated in 1972 from a human with American cutaneous leishmaniasis and has since been maintained in vivo in outbred hamsters and in vitro in lactoalbumin hydrolysate medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 5% fetal calf serum (FCS) (GIBCO).

The procedures used for infection of animals and assessment of the course of the infection have been described previously (15-18).

Development of protective immunity. Protective immunity to a challenge infection with *L. mexicana* was assayed as described elsewhere (16). Groups of seven mice were challenged with 10^3 amastigotes at regular intervals after a primary infection. Animals received the challenge parasite inoculum into the previously uninfected foot. The size of lesions in reinfected mice was measured with Schnelltester calipers (H. C. Kroplin, Germany) and compared with those in age-matched primarily infected controls.

DHR to leishmanial antigen. The DHR was estimated by the footpad swelling after subcutaneous injection of 50 μg of leishmanial antigen (17) into the uninfected hind footpad. DHR to *L. mexicana* peaks between 24 and 48 h after antigen elicitation. Infected and control mice (four in each group) were injected with antigen, and skin reactions were measured with Schnelltester calipers at 24 h and compared with the zero time reading (15-18). Each animal was skin tested on one occasion only.

In vitro immunization with SRBC. Spleen cells were immunized in vitro with SRBC by the method described by Mishell and Dutton (14). Pooled cells from at least five animals per group were used in each experiment. Cells were cultured in modified Eagle medium (GIBCO) supplemented with 5% FCS (Rehatuin and Reheis Chemical Corp., Phoenix, Ariz.), 0.1 mM glutamine (GIBCO), and 2-mercaptoethanol (BDH Ltd., Poole, England) at a final concentration of 5×10^{-5} M. Volumes (1 ml) containing 1×10^7 to 2×10^7 spleen cells were placed in Falcon 1008 dishes (Falcon Plastics, Oxnard, Calif.) and stimulated with 10^7 SRBC. Cultures were incubated at 37°C in an

atmosphere of 10% CO_2–7% O_2–83% N and rocked at 7 cycles per min on a rocking platform (Bellco Glass, Inc., Vineland, N.J.). Each culture was fed daily with 50 μl of nutritional cocktail (14) and 50 μl of FCS. After 5 days of incubation, cultures were harvested, and the number of immunoglobulin M (IgM) antibody-forming cells was determined by a modification of the hemolytic plaque-forming cell (PFC) assay (12). Results were expressed either as the arithmetic mean ± standard error of the mean of the number of PFC per 10^6 recovered cells, calculated from the results of quadruplicate cultures, or as a percentage of the responses of control spleen cells.

RESULTS

Development of protective immunity in mice infected with *L. mexicana*. It was previously shown that C57BL/6 mice which recover from a primary infection with *L. mexicana* are resistant to a challenge infection with this parasite (16). To study the development of protective immunity, mice were primarily infected with 10^3 amastigotes of *L. mexicana* and challenged at 5, 10, 15, and 20 weeks of infection with 10^3 amastigotes in the uninfected footpad. The size of lesions at week 10 after challenge was compared with that of primarily infected controls inoculated with the same number and batch of parasites (16). A significant resistance to a challenge infection was present as early as 5 weeks after primary infection, and by week 15, mice were highly resistant to a challenge infection (Fig. 1).

Development of DHR to parasite antigens. Mice recovered from a primary infection with *L. mexicana* show a strong DHR to leishmanial antigen (15–17). Examination of this response at regular intervals after primary infection revealed that mice infected with 10^3 amastigotes of *L. mexicana* showed little or no detectable DHR during the first 2 weeks of infection. The DHR was first detected at week 3, and thereafter, the intensity of this response increased until a plateau was reached at about week 10. DHRs to leishmanial antigen persisted in recovered mice (Fig. 2).

In vitro IgM-PFC responses to SRBC in mice infected with *L. mexicana*. The in vitro IgM-PFC response to SRBC was studied at various times after inoculation with 10^3 amastigotes of *L. mexicana*. Immunodepression was present at week 3 of infection, and it was maximal at week 11, when the lesion was at its peak. Thereafter, the in vitro IgM-PFC response of infected mice gradually returned to normal levels in parallel with the resolution of lesions. Recovered mice examined 21 weeks after infection showed normal IgM-PFC responses to SRBC (Fig. 3). A replicate experiment reproduced these results, with the exception that immunodepression appeared later and was not detected until week 5 of infection.

To ascertain whether suppressor cells were involved in the nonspecific immunodepression observed in mice infected with *L. mexicana*, spleen cells from infected mice were mixed with spleen cells from normal mice and cocultured in vitro with SRBC. Addition of 10^7 spleen cells from 6- and 9-week-infected mice to 10^7 normal

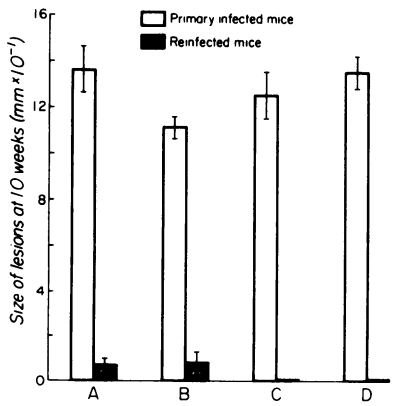

FIG. 1. *The response to a challenge infection (as measured by lesion size) 10 weeks after challenge with 10^3 amastigotes. Open bars indicate the size of lesions (mean ± standard deviation) in primarily infected mice; closed bars indicate the size of lesions in mice challenged during weeks 5 (A), 10 (B), 15 (C), and 20 (D) after primary infection. Nonmeasurable lesions were detectable on challenge at week 15. Experimental groups consisted of seven mice each.*

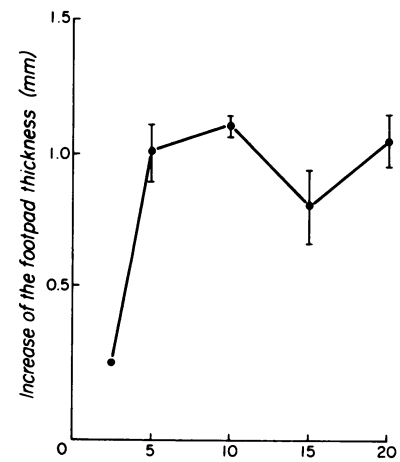

FIG. 2. *Footpad DHR (mean ± standard error) of C57BL/6 mice infected with 10^3 amastigotes of L. mexicana. Groups of four mice were skin tested with 50 μg of leishmanial antigen, and the footpad swelling reactions were measured 24 h later.*

spleen cells resulted in PFC responses lower than those observed in control cultures of 2×10^7 normal cells. Furthermore, these responses were lower than the expected PFC response calculated from the added responses of 10^7 normal cells and 10^7 infected cells. Spleen cells taken from mice recovering from the infection (17 to 21 weeks) did not inhibit the PFC response of normal cells (Table 1).

DISCUSSION

These studies showed that mice with a primary infection of *L. mexicana* exhibited substantial resistance to a challenge infection before primary lesions had healed, which is in accordance with previous findings in CBA mice infected with *Leishmania tropica* and guinea pigs infected with *Leishmania enriettii* (20, 22). This suggests that protective immunity to a challenge infection is already present before the immune response becomes sufficient to provoke healing of primary lesions, which is compatible with the hypothesis that in leishmaniasis the mechanisms of healing and protective immunity are separate processes (3).

High levels of DHR to parasite antigens and protective immunity to a challenge infection were both present early in the course (5 weeks) of primary infection with *L. mexicana*. A possible participation of the DHR in immunity to *Leishmania* spp. lacks sufficient experimental support, but in guinea pigs, inoculation of *L. enriettii* at the site of a DHR to *Mycobacterium bovis*, BCG, or dinitrochlorobenzene drastically inhibits the growth of lesions (2). DHRs are characterized by a large infiltration of lymphocytes and macrophages, and recent findings (8, 9, 13) suggest that activated macrophages participate in the destruction of *Leishmania* parasites. It may be possible, therefore, that the DHR provoked the recruitment and activation of macrophages at the site of reinoculation, leading to elimination of parasites.

Immunodepression is a prominent feature of parasitic infections and has been frequently associated with failure to control the infection (25). In these studies, mice showing progressive primary lesions exhibited depression of the primary antibody response to SRBC concomitantly with protective immunity to a homologous challenge and high levels of DHR to parasite antigens. Nonspecific immunodepression was a transient event, and the primary antibody response to

TABLE 1. *Effects of spleen cells from mice infected with L. mexicana on the in vitro IgM-PFC response of normal spleen cells to SRBC*

Weeks after infection	No. of spleen cells in culture: Control	No. of spleen cells in culture: Infected	No. of PFC/10^6 recovered cells[a]	% Suppression[b]
6	10^7	0	1,056 ± 59	
	0	10^7	311 ± 54	
	2×10^7	0	831 ± 184	
	10^7	10^7	524 ± 77	62 (47)
9	10^7	0	327 ± 106	
	0	10^7	211 ± 53	
	2×10^7	0	616 ± 72	
	10^7	10^7	223 ± 53	59 (62)
13	10^7	0	1,835 ± 154	
	0	10^7	415 ± 8	
	2×10^7	0	ND[c]	
	10^7	10^7	1,742 ± 184	23
17	10^7	0	646 ± 100	
	0	10^7	525 ± 38	
	2×10^7	0	617 ± 49	
	10^7	10^7	912 ± 115	22 (NS[d])
21	10^7	0	395 ± 83	
	0	10^7	321 ± 17	
	2×10^7	0	617 ± 49	
	10^7	10^7	567 ± 47	20 (8)

[a] Mean ± standard error.

[b] Percent suppression of 10^7 infected and 10^7 normal spleen cells was calculated on the basis of the expected response of 10^7 normal plus 10^7 infected cells or the PFC response of 2×10^7 normal spleen cells (shown within parentheses).

[c] ND, Not done.

[d] NS, Not suppressed.

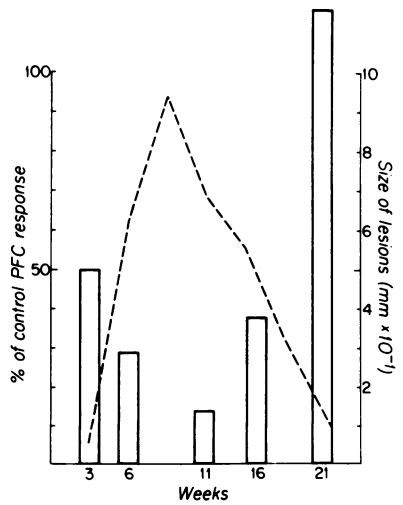

FIG. 3. *Effect of L. mexicana infection on the in vitro antibody response to SRBC of spleen cells from infected mice. Results are expressed as the percentage of responses of 10^6 control spleen cells. The dashed curve represents the course of lesions in mice primarily infected with 10^3 amastigotes.*

SRBC was restored as mice recovered from infection. Thus, in the self-limiting infection of C57BL/6 mice with *L. mexicana*, no direct correlation was found between the nonspecific depression of the antibody response of spleen cells and the host capability to develop a successful response to the parasite. The present study did not exclude the possibility that the immunosuppressive effects of *L. mexicana* were mainly restricted to the spleen. Mice infected with *Plasmodium berghei* subsp. *yoelii* exhibited a depressed splenic antibody response to SRBC, but the infection had little effect on antibody formation in lymph nodes (26).

Specific antibodies able to agglutinate *Leishmania* parasites are found in the sera of infected mice at the critical time of immunodepression (18). This paradoxical situation, where the presence of antiparasitic antibodies is accompanied by suppression of the antibody response to other antigens, has also been described in mice infected with the BUT64 strain of *Trypanosoma brucei* (10), but the immune mechanism involved is poorly understood. Some parasitic infections seem to mimic the effect of B-cell mitogens (25a), but we found no evidence of polyclonal activation in C57BL/6 mice infected with *L. mexicana* (data not shown). Thus, depression of the primary response to SRBC in the spleens of mice infected with *Leishmania* spp. is probably associated with suppression rather than direct B-cell unresponsiveness.

Nonspecific T-cell suppressor activity has been described in mice infected with *T. brucei* (5, 11). In our experimental model, mice exhibited an intensive DHR to leishmanial antigen and protective immunity to *L. mexicana* in coincidence with nonspecific immunodepression. Thus, if the depression of the primary antibody response to SRBC is determined by activation of nonspecific suppressor cells, these cells do not interfere with the specific immune response to the parasite.

Leishmania spp. infection may induce alterations in the physiological status of macrophages (19), which could influence regulation of the immune response. The in vitro function of macrophages can be modulated from a nonspecific helper role to a nonspecific suppressor role by the parasite load (27). It could be speculated that during the course of infection with *L. mexicana*, overloading of macrophages with *L. mexicana* antigens interfered with the macrophage's ability to process newly introduced antigens. Alternatively, soluble products released by infected macrophages could inhibit the function of other cells participating in the antibody response.

Products released by the parasites may constitute additional in vivo modulating factors of the immune response, and recent studies indicate that subcellular components of trypanosomes may induce nonspecific suppression of the immune response of uninfected mice (4). *Leishmania* spp.-excreted factors have been found in cultures of promastigotes and macrophages infected with *L. tropica* and *L. donovani* (6, 24), but there are possible effects of parasite-derived products in the physiology of macrophages or other cells participating in the antibody response.

The depression of the primary PFC response to SRBC in mice infected with *L. mexicana* can also be interpretated in terms of antigenic competition between *L. mexicana* and SRBC antigens. Initial stimulation of the immune system by parasite antigens may stimulate suppressor cells or inhibitors which regulate the immune response to a second antigen (7, 21). As the mice recovered from infection and the antigenic parasite load was reduced, they recovered their capability to respond to other antigens.

In comparing these studies with those previously reported (1, 15), it appears that, in mice infected with *L. mexicana*, the fate of nonspecific immunodepression is largely determined by the host's capability to control the infection and could hence be at least partially related to parasitic load. Thus, in highly susceptible BALB/c mice, a chronic infection is accompanied by a persistent suppression of the antibody response to SRBC (1), whereas in self-healing C57BL/6 mice, the depression of the antibody response to SRBC is only a transient event. It remains to be shown whether the same or different mechanisms are responsible for the nonspecific immunodepression developed by susceptible and resistant mice infected with *L. mexicana*.

LITERATURE CITED

1. **Arredondo, B., and H. Pérez.** 1979. Alterations of the immune response associated with chronic experimental leishmaniasis. Infect. Immun. **25**:16–22.
2. **Behin, R., J. Mauel, and D. S. Rowe.** 1978. Mechanism of protective immunity in experimental cutaneous leishmaniasis of the guinea pig. III. Inhibition of leishmanial lesion in the guinea-pig by delayed hypersensitivity reactions to unrelated antigens. Clin. Exp. Immunol. **29**:320–325.
3. **Bryceson, A. D. M.** 1975. Mechanism of disease in leishmaniasis, p. 85–100. *In* M. Taylor and R. Miller (ed.), Pathogenic processes in parasitic infections. Symposia of the British Society for Parasitology. Blackwell Scientific Publications, Oxford.
4. **Clayton, C. E., D. L. Sacks, B. M. Ogilvie, and B. A. Askonas.** 1979. Membrane fractions of trypanosomes mimic the immunosuppressive mitogenic effects of living parasites in the host. Parasite Immunol. **1**:241–250.
5. **Eardley, D. D., and A. N. Jayawardena.** 1977. Suppressor cells in mice infected with *Trypanosoma brucei*. J. Immunol. **119**:1029–1033.
6. **El-On, J., L. F. Schnur, and C. L. Greenblatt.** 1979.

Leishmania donovani: physicochemical, immunological and biological characterization of excreted factor from promastigotes. Exp. Parasitol. **47**:254–269.

7. **Gershon, R. K., and K. Kondo.** 1971. Antigenic competition between heterologous erythrocytes. I. Thymic dependency. J. Immunol. **106**:1524–1531.
8. **Handman, E., and A. W. Burgess.** 1979. Stimulation by granulocyte-macrophage-colony stimulating factor of *Leishmania tropica* killing by macrophages. J. Immunol. **122**:1134–1137.
9. **Handman, E., and D. T. Spira.** 1977. Growth of *Leishmania* amastigotes in macrophages from normal and immune mice. Z. Parasitenkd. **53**:75–81.
10. **Hudson, K. M., and R. J. Terry.** 1979. Immunosuppression and the course of infection of a chronic *Trypanosoma brucei* infection in mice. Parasite Immunol. **1**: 317–326.
11. **Jayawardena, A. N., and B. H. Waksman.** 1977. Suppressor cells in experimental trypanosomiasis. Nature (London) **265**:539–541.
12. **Jerne, N. K., C. Henry, A. A. Nordin, H. Fuji, A. M. C. Koros, and I. Lefkovits.** 1974. Plaque-forming cells: methodology and theory. Transplant. Rev. **18**:130–191.
13. **Mauel, J., Y. Buchmüller, and R. Behin.** 1978. Studies on the mechanism of macrophage activation. I. Destruction of intracellular *Leishmania* in macrophages activated by cocultivation with stimulated lymphocytes. J. Exp. Med. **148**:393–407.
14. **Mishell, R. I., and R. W. Dutton.** 1967. Immunization of dissociated spleen cells cultured from normal mice. J. Exp. Med. **126**:423–442.
15. **Pérez, H., B. Arredondo, and M. Gonzalez.** 1978. Comparative study of American cutaneous leishmaniasis and diffuse cutaneous leishmaniasis in two strains of inbred mice. Infect. Immun. **22**:301–307.
16. **Pérez, H., B. Arredondo, and R. Machado.** 1979. *Leishmania mexicana* and *Leishmania tropica*: cross immunity in C57BL/6 mice. Exp. Parasitol. **47**:9–16.
17. **Pérez, H., F. Labrador, and J. W. Torrealba.** 1979. Variations in the response of five strains of mice to *Leishmania mexicana*. Int. J. Parasitol. **9**:27–32.
18. **Pérez, H., I. Malavé, and B. Arredondo.** 1979. The effect of protein calorie malnutrition on the course of *Leishmania mexicana* infection in C57BL/6 mice. Clin. Exp. Immunol. **38**:453–460.
19. **Poulter, L. W.** 1976. Changes in macrophages status *in vivo* during infection with *Leishmania enriettii*. Cell Immunol. **27**:17–25.
20. **Poulter, L. W.** 1979. The kinetics and quality of acquired resistance in self-healing and metastatic leishmaniasis. Clin. Exp. Immunol. **36**:30–37.
21. **Press, H. F., and D. Eidinger.** 1974. Antigenic competition: a review of non-specific antigen induced suppression. Adv. Immunol. **18**:133–168.
22. **Preston, P. M., and D. C. Dumonde.** 1976. Experimental cutaneous leishmaniasis. V. Protective immunity in subclinical and self-healing infection in the mouse. Clin. Exp. Immunol. **23**:126–138.
23. **Schwab, J.** 1975. Suppression of the immune response by microorganisms. Bacteriol. Rev. **39**:121–143.
24. **Slutzy, G. M., and C. L. Greenblatt.** 1979. Analysis by SDS-polyacrylamide gel electrophoresis of an immunologically active factor of *Leishmania tropica* from growth media, promastigotes, and infected macrophages. Biochem. Med. **21**:70–77.
25. **Terry, R. J.** 1977. Immunodepression in parasitic infections. INSERM **72**:161–178.

25a. **Terry, R. J., K. M. Hudson, and M. Faghihi-Shirazi.** 1980. Polyclonal activation by parasites, p. 259–272. *In* H. Van Den Bossche (ed.), The host-invader interplay. Third International Symposium on the Biochemistry of Parasites and Host-Parasite Relationships. Elsevier/North Holland Biomedical Press, Amsterdam.

26. **Weidanz, W. P., and R. G. Rank.** 1975. Regional Immunosuppression induced by *Plasmodium berghei yoelii*. Infect. Immun. **11**:211–212.
27. **Wyler, D. J., J. J. Oppenheim, and L. C. Koontz.** 1979. Influence of malaria infection on the elaboration of soluble mediators by adherent mononuclear cells. Infect. Immun. **24**:151–159.