INFECTION AND IMMUNITY, Dec. 1987, p. 3142–3148
0019-9567/87/123142-07$02.00/0

Vol. 55, No. 12

The Cellular Immune Response to a Purified Antigen from *Leishmania mexicana* subsp. *amazonensis* Enhances the Size of the Leishmanial Lesion on Susceptible Mice

MAURICIO MARTINS RODRIGUES,[1,2] LÚCIA MENDONÇA-PREVIATO,[3] ROSANE CHARLAB,[1,4] AND MARCELLO A. BARCINSKI[1]*

Carlos Chagas Filho Institute of Biophysics[1] and Institute of Microbiology of the Federal University of Rio de Janeiro,[3] Rio de Janeiro, 21941, Discipline of Parasitology, Escola Paulista de Medicina, São Paulo, 04023,[2] and Federal Rural University of Rio de Janeiro, Rio de Janeiro,[4] Brazil

Received 16 June 1987/Accepted 2 September 1987

Immunization of BALB/c mice with gp10/20, a glycoconjugate purified from *Leishmania mexicana* subsp. *amazonensis*, induced a delayed-type hypersensitivity response to the antigen, and a significant increase was elicited in the size of the lesion induced by a subcutaneous infection with this parasite. The increase in the lesion size was observed when mice were immunized by the subcutaneous and the intraperitoneal routes. The subcutaneous immunization with gp10/20 was unable to reverse the prophylactic effect of an intravenous injection of irradiated promastigotes. An L3T4$^+$ T-cell line specific for gp10/20 was able to transfer this lesion-enhancing effect and specific delayed-type hypersensitivity reactivity to normal syngeneic recipients. The same T-cell line was a good producer of a hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor.

Cutaneous leishmaniasis is a granulomatous disease induced by some species of the genus *Leishmania* and characterized by a single cutaneous lesion that resolves spontaneously or with the aid of pentavalent antimonial therapy. In a very few patients, the disease can develop into a diffuse, usually fatal form characterized by dissemination of parasite-rich nodes. The full spectrum of the disease can be reproduced in different inbred strains of mice according to their genetic constitution (11, 27).

When *Leishmania major* was the etiological agent, T lymphocytes were shown to play a major role in the resistance of the "self-healing" strains of mice (18, 28) and in the progression of the lesion in the highly susceptible BALB/c mice (18, 22, 24, 31, 32). All of these observations support the notion that L3T4$^+$ T lymphocytes are critically involved in the pathogenesis of experimental cutaneous leishmaniasis induced by *L. major*. Less is known about the murine model of cutaneous leishmaniasis induced by *L. mexicana* subsp. *amazonensis* although there is evidence for similarities among some of the genetic (27), immunological (4), and pathological (3) findings within the different models. However, the description of an inbred strain of mice (CBA/Ca) which is self-healing with respect to *L. major* infection and highly susceptible to *L. mexicana* subsp. *mexicana* (3) points to the existence of differences in the genetic control of the fate of the disease induced by those two species of the genus *Leishmania*.

Recently, extensive experimental work has been done to characterize biologically active molecules purified from different leishmanial species. Such molecules are potentially useful tools for analyzing mechanisms of parasite-host cell interactions (6, 30) as well as for studies on the taxonomy of *Leishmania* spp. (13, 14) and on the prophylaxis of leishmanial infection (9).

The present work is an extension of our previous characterization of the cellular immune response to chemically defined glycoconjugates purified from *L. mexicana* subsp. *amazonensis* (29). In this paper we analyze the effect of the cellular immune response to one of those fractions (gp10/20) on the development of cutaneous leishmaniasis in BALB/c mice. We find that the induction of an immune state, either by intraperitoneal (i.p.) or subcutaneous (s.c.) injection of this glycoprotein or by the adoptive transfer of an antigen-specific L3T4$^+$ T-cell line, significantly enhances the primary lesion size in the infected mice. We also observe that s.c. immunization with gp10/20 is unable to reverse the prophylactic immunization induced by the intravenous (i.v.) injection of irradiated promastigotes.

MATERIALS AND METHODS

Animals. Male and female BALB/c, C3H/HeJ, C57 BL10(B10), C57B110.A(B10.A), and (BALB/c × B10)F_1 mice, 6 to 16 weeks old, raised in our own facilities, were used throughout this study.

Parasites. *L. mexicana* subsp. *amazonensis* H-21 was provided by the Wellcome Parasitology Unit, Instituto Evandro Chagas, Belém, Brazil. Promastigotes were grown for 7 days at 28°C in screw-cap tubes containing brain heart infusion broth (37 g/liter), folic acid (0.02 g/liter), and hemin (0.01 g/liter dissolved in 2 M NaOH). Cells were harvested by centrifugation and washed once in Hanks balanced salt solution (HBSS) before use. Amastigotes were obtained from infected BALB/c mice. Lesions were aseptically dissected, washed in sterile HBSS, finely teased, and ground in a glass homogenizer containing cold HBSS. The suspension was then filtered through gauze and centrifuged at 200 × *g* to remove large debris; the amastigotes from the supernatant were washed at least once in HBSS. The parasites were counted in a Neubauer chamber and adjusted to the desired concentration.

Purification of gp10/20. Frozen cells were rapidly thawed and extracted as described previously (19). The aqueous extract was fractionated by gel filtration chromatography on a Bio-Gel P 10 column (120 by 2 cm) (Bio-Rad Laboratories,

* Corresponding author.

Richmond, Calif.). The material emerging in the void volume was designated gp10/20. The antigen dose is given in terms of mass of dry weight.

Conventional antigens. Purified Protein Derivative (Connaught Medical Research Laboratory, Ontario, Canada) and keyhole limpet hemocyanin (KLH; Calbiochem-Behring Corp., La Jolla, Calif.) were diluted in HBSS and sterilely filtered before use.

Immunization. Mice were immunized according to three different protocols. The s.c. immunization was performed by injecting one or both hind footpads (0.05 to 0.1 ml per mouse). The following antigenic fractions were used: (i) gp10/20 (50 to 250 μg per animal); (ii) KLH (50 to 250 μg per animal). Each antigenic fraction was emulsified with an equal volume of complete Freund adjuvant (CFA; 0.4 mg of *Mycobacterium tuberculosis* H37Ra per ml; Difco Laboratories, Detroit, Mich.). In some experiments, incomplete Freund adjuvant (IFA; Difco) was used. The i.p. immunization was performed with 1 mg of gp10/20 mixed with 300 μg of *Corynebacterium parvum* and injected in a final volume of 0.2 ml. The i.v. prophylactic immunization was performed as described previously (16). Stationary promastigotes, at a concentration of 2×10^8 parasites per ml in HBSS, were irradiated with a total dose of 135,000 rads from a Co source before being injected (0.1 ml per mouse) into the tail vein.

Infection. Amastigotes and promastigotes were obtained as described above, and the concentration was adjusted to 1×10^7 to 2×10^7/ml. Mice were inoculated s.c. with 0.1 ml in one of the hind footpads. Infection was quantified by measuring the increase in thickness of the footpad with engineering calipers. The size of the lesion in the course of infection was calculated as the difference in thickness of the footpad before and after infection.

Measurement of delayed-type hypersensitivity (DTH) response. Immunized or adoptively transferred mice were injected in one of the hind footpads with 0.1 ml of the purified antigen or with amastigotes. The degree of swelling was expressed as the difference in millimeters between the thickness measured before and 24 or 48 h after the eliciting injection.

Cell culture. Complete medium for cell culture consisted of RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2-mercaptoethanol (5×10^{-5} M; Eastman Kodak Co., Rochester, N.Y.), L-glutamine (2 mM; Sigma Chemical Co., St. Louis, Mo.), gentamicin (10 μg/ml; Schering S.A., Rio de Janeiro, Brazil), and fetal calf serum (5 to 10%) (GIBCO) or normal inactivated human serum (5%). Cultures were grown at 37°C in a humid environment containing 5% CO_2. For the generation of short-term T-cell lines, the draining lymph nodes were collected 2 weeks after s.c. immunization in CFA. Single-cell suspensions of whole lymph node cells were prepared, and lymph node lymphocytes were obtained by eluting lymph node cells through nylon wool columns (15). Normal syngeneic splenocytes were pulsed with the appropriate antigen (50 to 100 μg/ml) and irradiated (3,000 rads) before being used as stimulator cells. Lymph node lymphocytes (5×10^6 per well) were cultured in complete medium containing antigen-pulsed stimulator cells (10^6 per well) in a final volume of 2 ml in a 24-well plate (Linbro Inc., Handen, Conn.). After 4 to 7 days in culture, blast cells were isolated on a continuous Ficoll-Paque gradient (Pharmacia Fine Chemicals, Uppsala, Sweden), washed in HBSS, and adjusted to the proper concentration for use in vitro and in vivo. The short-term T-cell lines (2×10^5 to 2.5×10^5 per well) were cultivated with irradiated spleen cells (2×10^5 per well) in a 96-well round-bottom microtiter plate (Linbro) containing the indicated antigen concentration. In some experiments, monoclonal antibodies (MAbs) against T-cell surface antigens L3T4 (GK 1.5) and Lyt-2 were added to the cultures. At 18 to 20 h before harvesting, each well received 1 μCi of tritiated thymidine (*methyl*-[^{3}H]TdR; specific activity, 6.7 Ci/mM; New England Nuclear Corp., Boston, Mass.). After 72 h, cultures were harvested onto glass fiber filters with the aid of a semiautomatic harvesting device. The amount of isotope incorporated was measured in a liquid scintillation counter. Results are expressed as mean ± standard deviation of triplicate cultures.

Colony stimulating activity of T-cell line supernatants. Supernatants of short-term anti-gp10/20 T-cell lines were obtained as follows. Specific T cells (5×10^6 per well) were cultured with irradiated syngeneic spleen cells pulsed with gp10/20 (10^6 per well) at a final volume of 2 ml in 24-well plates (Linbro). After 5 days, the cell culture supernatant was collected, filtered through membrane filters (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.), and frozen until use. Colony-stimulating activity of the collected supernatants was measured as described previously (10). Bone marrow cells were collected from normal BALB/c mice. A single-cell suspension was made in complete medium. The cells (75×10^3 per well) were cultured with indicated concentrations of the supernatant in 96-well, flat-bottom microtiter plates (Linbro) for 5 days. During the last 8 to 20 h before harvesting, each well received 2 μCi of *methyl*-[^{3}H]TdR. Cultures were harvested, and the amount of incorporated isotope was estimated as described above. To analyze the specificity of the colony-stimulating activity present in the supernatants, bone marrow cells were cultivated in semisolid medium as described previously (5) and the cells in the generated colonies were identified morphologically. The sources of the positive and negative control supernatants were, respectively, a lung-conditioned medium rich in granulocyte-macrophage colony-stimulating factor GM-CSF and GM-CSF-poor T-cell clone medium (SN-29), a gift of Peter Krammer (16).

Adoptive transfer of specific short-term T-cell lines. T-cell lines were adjusted to 10^8 cells per ml, and 0.1 ml of this suspension was injected into the tail vein of normal BALB/c mice 48 to 72 h before any assay was performed in the recipient animals.

RESULTS

Increase in the lesion size and generation of specific DTH response by immunization with purified gp10/20. To investigate the effect of the immune response to the purified glycoconjugate on the development of the experimental infection, BALB/c mice were immunized i.p. and s.c. with gp10/20 and then infected with *L. mexicana* subsp. *amazonensis* promastigotes. Both immunization protocols elicited the same type of response. The primary lesion increased regardless of whether mice received i.p. 1 mg of gp10/20 with *C. parvum* 2 weeks before the infection (Fig. 1A) or 0.25 mg via the s.c. route with CFA as adjuvant (Fig. 1B). The quantitation differences between the results of the two immunization schedules can probably be attributed to the different antigen doses used. Neither KLH (0.25 mg per animal) in CFA nor CFA alone had any effect on the size of the primary lesion (data not shown). gp10/20 is clearly immunogenic, since a DTH-type response was elicited when 10^5 amastigotes were injected in the footpad (Table 1).

To better characterize the interaction between the immune response to gp10/20 and the course of the leishmanial infec-

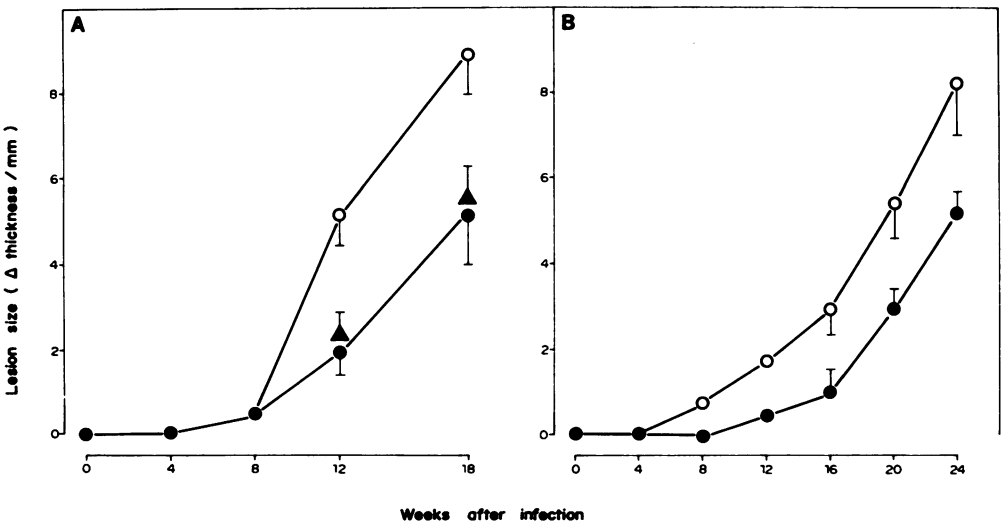

FIG. 1. Enhancement of the size of the primary lesion in infected BALB/c mice by immunization with gp10/20. (A) Groups of five mice were immunized i.p. with saline (●), with *C. parvum* alone (▲), or with *C. parvum* plus 1 mg of gp10/20 (○). (B) Groups of five mice were immunized s.c. with 0.25 mg of gp10/20 (○) or 0.25 mg of KLH (●) in CFA. Two weeks later, each mouse was inoculated with 10^6 promastigotes s.c. in one of the hind footpads. Symbols represent means ± standard deviations.

tion, we attempted to reverse the i.v. prophylactic immunization obtained using irradiated promastigotes (19) by s.c. immunization with gp10/20. Two immunizing protocols were used. The first group received three doses, at 1-week intervals, or 0.1 mg of the antigen emulsified in IFA. The second group received a single dose of 0.25 mg of gp10/20 in CFA. One week later, both groups received one i.v. injection of 2×10^7 irradiated promastigotes and were infected after a 7-day interval with 10^6 culture forms. The results were negative throughout the entire follow-up of the lesion. Table 2 shows the result of this experience at late points of the course of the lesion (16 and 20 weeks postinfection). At those time points, the enhancing effect of the s.c. injection of gp10/20 is already very clear (Fig. 1). So, the injection of gp10/20 in a total dose that significantly increased the size of the lesion was unable to reverse the prophylactic effect of injection of irradiated promastigotes.

In vitro characterization of anti-gp10/20 T-cell lines. To define the characteristics of the cell mediating the deleterious effect of immunization with gp10/20, antigen-specific T-cell lines were analyzed in vitro. The cells that were recovered after stimulation of immune lymph node lymphocytes for 4 to 7 days by syngeneic, irradiated spleen cells pulsed with the antigen were mainly $L3T4^+$ as identified by an antibody-dependent cytotoxicity assay using the anti-L3T4 MAb (data not shown). Functional evidence for the cell surface specificity is presented in Fig. 2. Only the MAb anti-L3T4 could specifically inhibit the in vitro proliferative response of the T-cell line to gp10/20. This MAb is not toxic for cultured cells, and the MAb anti-Lyt-2 antigen in the concentration used could inhibit the proliferative response of spleen cells to concanavalin A (data not shown). The recognition of gp10/20 by the short-term T-cell line was dependent on the antigen-presenting cell and was H-2 restricted (results not shown). The in vitro restimulation of the anti-gp10/20 T-cell lines was also obtained when living promastigotes were used as the antigen source (Table 3). As few as 5×10^3 culture forms lead to an intense in vitro proliferative response, indicating that the gp10/20 epitope present in the intact parasite is very efficiently presented to T cells by antigen-presenting cells.

Specific enhancement of the primary lesion in infected BALB/c mice is induced by anti-gp10/20 $L3T4^+$ T lymphocytes that mediate DTH response. After the in vitro characterization of the $L3T4^+$ anti-gp10/20-specific short-term T-cell line, we attempted to investigate whether these cells could adoptively transfer the detrimental effect of the immunization with gp10/20. Normal BALB/c mice received 10^7 T cells i.v. 48 to 72 h before being infected with 10^6 amastigotes.

TABLE 1. DTH response of BALB/c mice immunized with gp10/20 to viable amastigotes[a] of *L. mexicana* subsp. *amazonensis*

Immunogen[b]	Footpad thickness (mm)[c] at:	
	24 h	48 h
CFA (control)	0.70 ± 0.75	0.18 ± 0.40
CFA + gp10/20	3.60 ± 1.30[d]	2.57 ± 1.13[e]

[a] 10^5 viable forms per footpad.
[b] Mice immunized in the footpad with 100 μg per mouse, 14 days before injection eliciting DTH.
[c] Results are means ± standard deviations of experiments in seven mice per group.
[d] $P = 0.005$ with respect to control at 24 h.
[e] $P = 0.003$ with respect to control at 48 h.

TABLE 2. Inability of s.c. injection of gp10/20 to reverse a prophylactic i.v. immunization with irradiated promastigotes

Preimmunization	i.v. immunization (no. of irradiated promastigotes) (10^7)	Wk post-infection	Lesion size (mm, mean ± SD)
IFA	0	20	4.7 ± 1.2
IFA	2	20	0.44 ± 0.08
IFA + gp10/20[a]	2	20	0.65 ± 0.65
CFA[b]	0	16	4.35 ± 1.2
CFA	2	16	0.88 ± 0.8
CFA + gp10/20[c]	2	16	1.02 ± 0.14

[a] Three weekly injections of 0.1 mg of gp10/20 (seven mice per group).
[b] $P = 0.003$.
[c] Single injection of 0.25 mg of gp10/20 (five mice per group).

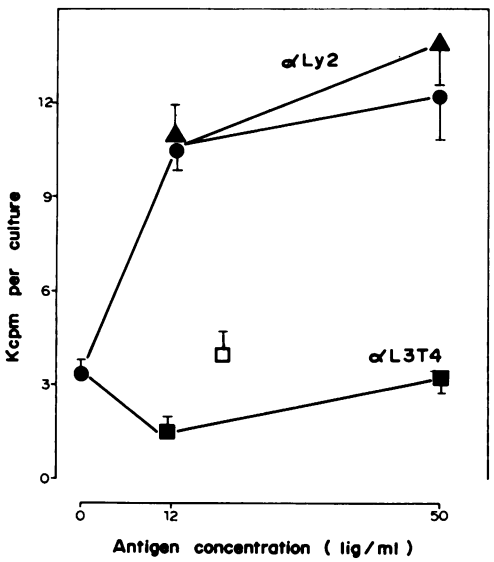

FIG. 2. L3T4 phenotype of the proliferating cells of an anti-gp10/20 T-cell line generated from lymph node cells of BALB/c mice immunized with gp10/20 in CFA. The T-cell line was restimulated in vitro with syngeneic irradiated spleen cells plus gp10/20 (closed symbols) in the presence of MAb anti-L3T4 (■), anti-Lyt-2 (▲), or with no MAb added (●). □, Restimulation with syngeneic irradiated spleen cells plus Purified Protein Derivative.

There was a specific enhancement of the size of the primary lesion in recipients of the anti-gp10/20 T-cell line (Fig. 3). The transference of an anti-KLH had no effect on the course of the disease. The anti-gp10/20 T-cell line that could adoptively transfer enhancement of the primary lesion size could also transfer the DTH reaction specific for anti-gp10/20 DTH (Table 4).

TABLE 3. In vitro restimulation of T-cell line[a] anti-gp10/20 with living promastigotes of *L. mexicana* subsp. *amazonensis*

No. of APC[b] in culture	Antigen in culture medium (dose)	[^{3}H]TdR incorporated (cpm)
25,000	None	7,439 ± 110
	gp10/20 (50 μg/ml)	47,945 ± 2,361
	Promastigotes (5 × 10^3)	30,279 ± 535
	Promastigotes (25 × 10^3)	37,086 ± 721
50,000	None	9,834 ± 310
	gp10/20 (50 μg/ml)	43,458 ± 2,391
	Promastigotes (5 × 10^3)	36,961 ± 1,677
	Promastigotes (25 × 10^3)	53,385 ± 729

[a] The anti-gp10/20 T-cell line was generated from BALB/c mice lymph node lymphocytes; 2.5 × 10^5 lymphocytes were restimulated in vitro for 72 h. Results are means ± standard deviations of triplicate cultures.
[b] APC, Normal syngeneic adherent peritoneal cells.

Production of bone marrow blast cell growth factors by an anti-gp10/20 T-cell line. In BALB/c mice, the correlation between progression of the disease induced by *L. major*, the presence of immature monocytes and granulocytes in the spleen and the production of colony-stimulating factors by adherent spleen cells of infected mice has been pointed out (21). Although the cause-effect relationship was not determined, there is a possibility that the bone marrow liberation of these immature cells, which is induced by hematopoietic growth factors, is important in the natural history of the disease. Since the adoptive transference of anti-gp10/20 T-cell lines had a marked effect on the progress of the leishmanial lesion in BALB/c mice, we investigated the generation of hematopoietic growth factors by these cells. The supernatant of restimulated T-cell lines (generated as

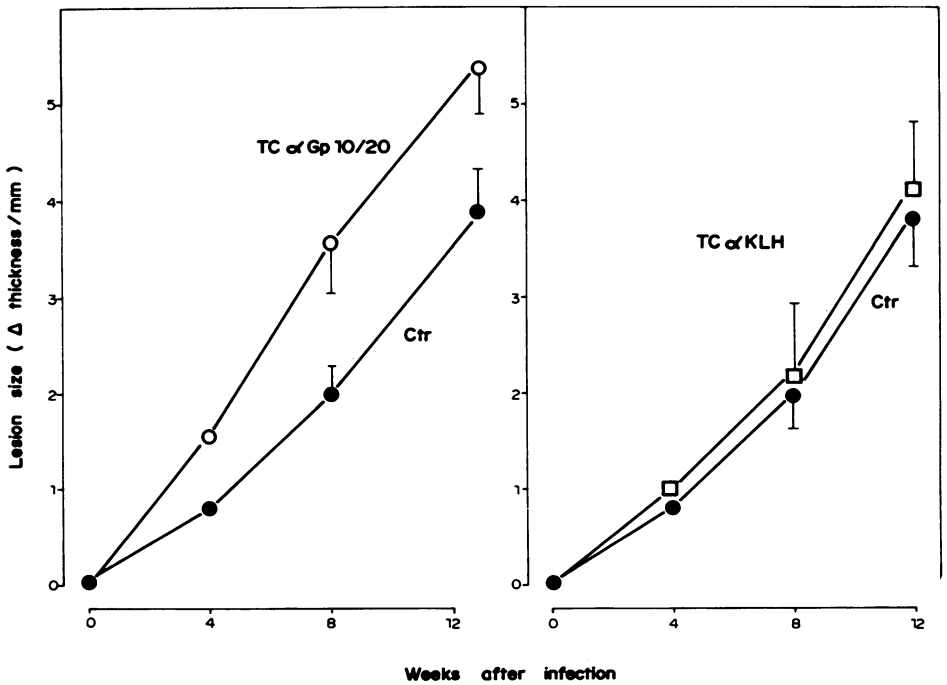

FIG. 3. Enhancement of the primary lesion size by adoptive transfer of anti-gp10/20 T-cell line. Five mice per group were injected i.v. with 10^7 anti-gp10/20-specific (○) or anti-KLH-specific (□) T cells. Control mice (●) received no cells. Two days later, mice were infected in one of the hind footpads.

TABLE 4. Adoptive transfer of DTH response by a T-cell line[a] anti-gp10/20

T-cell line specificity	Antigen (50 μg)	Footpad thickness (mm, mean ± SD)	
		24 h	48 h
gp10/20	gp10/20	6.00 ± 0.90	3.00 ± 0.80
	KLH	0.50 ± 0.06	0.20 ± 0.05
KLH	gp10/20	0.20 ± 0.05	0.50 ± 0.06
	KLH	5.20 ± 0.10	3.80 ± 0.50

[a] 10^7 T cells were injected i.v. into four normal BALB/c mice 48 to 72 h before injection of the indicated antigens into the hind footpad.

described in Materials and Methods) had an intense mitogenic effect on bone marrow blast cells (Fig. 4). T-cell lines secrete larger amounts when restimulated by using gp10/20-pulsed spleen cells as adherent peritoneal cells (Fig. 4A). A T-cell clone supernatant (SN-29) low in GM-CSF had no detectable stimulating activity when compared with T-cell line supernatant or with the positive control, GM-CSF-rich lung medium (Fig. 4B).

DISCUSSION

The results reported here show for the first time that gp10/20, a chemically defined glycoprotein extracted from promastigotes of *Leishmania mexicana* subsp. *amazonensis* (29), can enhance the size of the lesion induced by this parasite in susceptible BALB/c mice. This enhancing effect can be observed when either the i.p. or the s.c. routes of injection are used. L3T4$^+$ T cells specific for this glycoconjugate can mediate this effect, since exacerbation of a cutaneous lesion is observed upon transference of such cells to naive syngeneic recipients. These same cells can transfer an antigen-specific DTH reaction. Exacerbation of the cutaneous leishmanial lesion induced by *L. major* by adoptive transfer of antigen-specific T cells and with s.c. injections of leishmanial antigens have been previously described (32). The *L. major*-specific T cells described by those authors were also able to transfer DTH reactions. In the present situation we show a similar phenomenon occurring with the lesion induced by *Leishmania mexicana* subsp. *amazonensis* in BALB/c mice. More importantly, we extend those findings by showing that the recently described antigen gp10/20 is a fraction capable of inducing all of these phenomena described above. Indeed, both s.c. and i.p. injections of this glycoconjugate lead to a significant exacerbation of the cutaneous lesion induced by the subsequent injection of *L. mexicana* subsp. *amazonensis*. The s.c. injection of leishmanial antigens obtained by freezing and thawing promastigotes of *L. major* has been shown to exacerbate the leishmanial lesion (19). However, the i.p. injection of extract from *L. major*-infected macrophages, or a glycolipid purified from promastigotes of *L. major* using exactly the same protocol as in the present study, with *C. parvum* as an adjuvant, can induce protection against a subsequent infection with *L. major* (9). Also, use of either s.c. or i.p. routes of immunization leads to completely different results when irradiated promastigotes are used as antigens; immunization via the i.p. route induces protection (although not so efficiently as the i.v. route [12]), whereas via the s.c. route the prophylactic effect of an i.v. immunization with this same antigen can be completely reversed (19). In this context, the unexpected effect of the i.p. injection of gp10/20 is very interesting. On one hand, it may merely reflect use of a leishmanial species different from the one used in the observations already published. Indeed, caution must be applied when extrapolating results obtained with *L. major* to parasites of the *L. mexicana* complex, since it has been shown

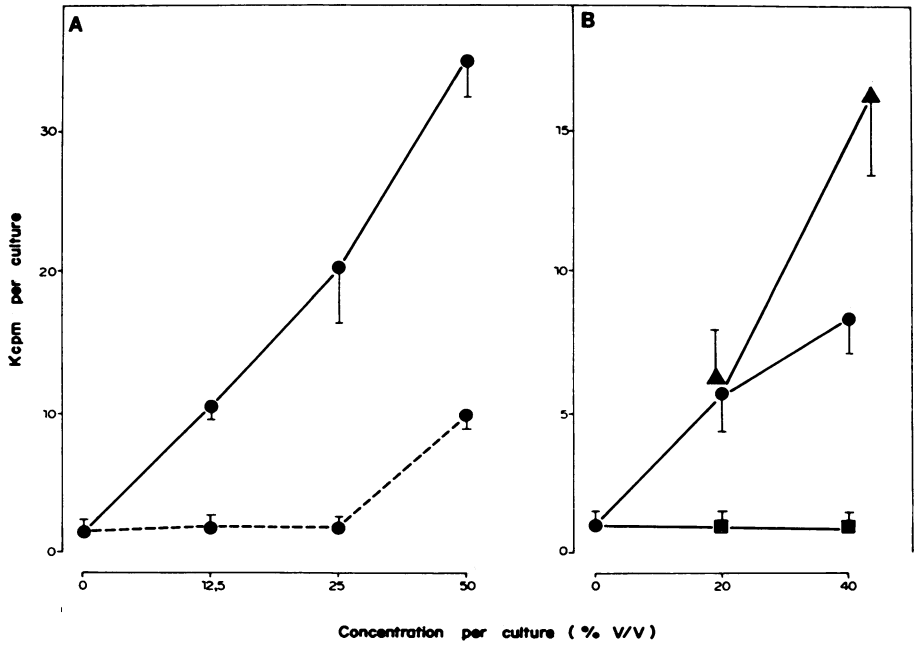

FIG. 4. Presence of bone marrow blast cell growth factors in the supernatant of anti-gp10/20 T-cell lines. The supernatants were generated as described in Materials and Methods. Bone marrow cells from normal BALB/c mice were cultured for 5 days with the indicated concentrations of supernatants of a T-cell line restimulated by irradiated spleen cells in the presence (●—●) or absence (●--●) of gp10/20 (A) and of the T-cell supernatant restimulated with gp10/20, a positive control supernatant rich in GM-CSF (lung-conditioned medium) (▲—▲) and a negative control supernatant low in GM-CSF (SM-29) (■—■) (B).

that some immunoregulatory phenomena are completely different when different *Leishmania* species are involved (2). On the other hand, the effect of gp10/20 indicates that not only the immunizing route but also the molecular form and the epitopes present in the immunogen are important variables in defining the fate of the infection, as recently proposed by Mitchell and Handman (25). It would be of interest to assess the effect of the i.v. inoculation of gp10/20, since no information is available concerning this route of immunization with this glycoconjugate.

It has been clearly shown in other systems that different epitopes in the same molecule can be presented to immune T cells and that they can define the type of effector mechanism to be triggered (1). More recently, Mitchell and Handman (26) have shown that a host-protective glycolipid of *L. major* contains an immunogeneic carbohydrate component that induces cutaneous lesion enhancement in infected mice. No data are available up to the moment to suggest similarities between this carbohydrate antigen and gp10/20. The possibility of clearly defining the epitopes recognized by the T cells that mediate lesion enhancement represents an advantage of using defined antigens for this type of study. The fact that the s.c. injection of gp10/20 fails to reverse the prophylactic effect of i.v. immunization with irradiated promastigotes might also be due to the use of different species or to differences in the molecular form of the antigen. However, one cannot forget the possibility that by using a higher dose of gp10/20 and a different preimmunizing schedule, a reversal of the protective effect might be observed. Indeed, Liew et al. (19) clearly showed that four weekly s.c. injections of irradiated promastigotes were consistently more efficient in reversing the protective effect than preimmunizing schedules using less frequent injections.

To characterize the T cells capable of mediating the lesion-enhancing effect of gp10/20, we phenotyped T-cell lines enriched for anti-gp10/20 cells and analyzed the effect on their proliferative capacity of anti-L3T4 and anti-Lyt-2 MAbs. The results (Fig. 2) define these cells as $L3T4^+$, $Lyt\text{-}2^-$ T cells. These gp10/20-specific, short-term T-cell lines are capable of transferring the lesion-enhancing effect to susceptible mice after a single i.v. injection (Fig. 3) and can also transfer the DTH reaction to gp10/20 (Table 3). The fact that the enhancing effect of the transferred cells is less pronounced than the enhancement obtained by active immunization may reflect the fact that the transferred cells were lines and not clones, and thus not all of them were necessarily anti-gp10/20.

The results cited above are in accordance with the ones obtained using *L. major*-specific T cells (32), and they add to evidence that is accumulating for an inverse correlation between DTH reactivity and protection against a cutaneous leishmanial infection (17). Experiments using T-cell clones specific for gp10/20 would be valuable to determine whether the epitope recognized by the cells that transfer DTH reactivity is the same as that mediating lesion enhancement. Indeed two opposing albeit not mutually exclusive views are now emerging to explain the role of $L3T4^+$ T cells in the susceptibility of BALB/c mice to the leishmanial infection. The first one proposes the existence of a T cell that can suppress the ability of effector T cells to activate macrophages for killing intracellular leishmanial forms and control the disease progression (7, 8, 18). In the second view, it is proposed that the T cells of infected BALB/c mice are very efficient in recruiting immature macrophages to the site of the lesion. These macrophages, by being less efficient in their leishmanicidal activity, become safe targets for the multiplication of the parasite (23). Very recent work by a Swiss group (20) elegantly reinforces this view. Those authors show that the transference of antigen-specific T cells to heavily irradiated recipient mice enhances a lesion only if syngeneic bone marrow cells depleted of mature T cells are concomitantly transferred. Within this perspective, we show (Fig. 4) that the same T-cell lines that can induce lesion size enhancement can also secrete large amounts of GM-CSF upon in vitro stimulation. This last result is consistent with the safe-target hypothesis and accounts for the findings obtained with the transference of ova-specific T cells (32). More indirect evidence for the role of GM-CSF in lesion enhancement is worth pursuing. We are working on this line at the moment.

ACKNOWLEDGMENTS

This work was supported by grants from the Brazilian National Research Council, Financing Agency for Studies and Projects, Federal University of Rio de Janeiro (CEPG), and UNDP-World Bank-World Health Organization Special Program for Research and Training in Tropical Disease.

We thank George A. Dos Reis for helpful discussions and Sandra B. da Rocha for typing the manuscript.

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