Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. V, 105-109, 1992

IMMUNOPATHOLOGY OF AMERICAN CUTANEOUS LEISHMANIASIS

CLAUDE PIRMEZ

Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

American mucocutaneous leishmaniasis is a granulomatous disease clinically characterized by ulcerated skin lesions that can regress spontaneously. A small percentage of the affected individuals can however develop a severe destruction of the nasal, oral, pharyngeal and/or laryngeal mucous membranes many years after the healing of the primary lesion. The human immune response to the infection and the possible mechanisms underlying the pathogenesis of the disease, determining either the self-healing or the development of chronic and destructive mucosal lesions, are discussed.

Key words: mucocutaneous leishmaniasis – immunopathology – T-cells – cytokines

American cutaneous leishmaniasis (ACL) is a disease of skin and mucous membranes caused by many different species of *Leishmania*, a protozoon that invades and grows within macrophages. *Leishmania (Viannia) braziliensis* is the most common species causing human disease in Brazil. The mildest clinical form of the disease is localized cutaneous leishmaniasis (LCL), which presents as a cutaneous ulcer at the site of the sandfly bite. The lesion is often self-limiting, and heals after a period of several months. The principal characteristic that distinguishes *L. braziliensis* from other species is its propensity to manifest a metastatic spread to the nasal, pharyngeal and oral mucosae resulting in the complete destruction of these tissues and associated cartilage (mucocutaneous leishmaniasis – MCL). This manifestation, commonly called espundia, is a relentlessly progressive process that can be interrupted only by specific therapy (Marsden & Nonata, 1975; Marsden et al., 1984). Morphologically, both LCL and MCL lesions are depicted by an intense mononuclear inflammatory infiltrate and a remarkable scarcity of parasites.

Experimental infections of resistant and susceptible mice strains with different species of *Leishmania* have shown that the resolution of the infection relates with a specific T-cell response (Handman et al., 1979; Mitchell et al., 1980, 1981). Clinical observations in human infection have shown that spontaneous healing of cutaneous lesions is associated with the development of cell-mediated immune response against *L. braziliensis* antigens (Castes et al., 1983; Saravia et al., 1989). Interestingly, lymphocytes from MCL patients show a higher *in vitro* and *in vivo* reactivity than those from LCL patients. This has led to speculation that the chronic destructive nature of the MCL lesions is either due to a defective immune response restricted to the site of the lesion (Carvalho et al., 1985) or that lesions result from a hyperreactive immune response (Castes et al., 1984; Coutinho et al., 1987).

Studies on the properties and functional responsiveness of T lymphocytes from *L. braziliensis* human lesions have shown that i) CD4+ T-memory cells are the predominant phenotype in both LCL and MCL lesions as well as in Montenegro reactions, ii) cells containing IFN-γ mRNA were present in similar frequencies in LCL, MCL and Montenegro biopsy specimens and, iii) T-cells derived from both LCL and MCL lesions responded equally well to *L. braziliensis in vitro* (Conceição-Silva et al., 1990; Pirmez et al., 1990). These data indicate that components of a delayed-type hypersensitivity (DTH)-like response are present in MCL lesions and are neither increased nor diminished in comparison to LCL lesions. Despite the clinical and experimental evidence of a good correlation between DTH reactivity and resistance to the infection, it is likely that the DTH reaction, present either in LCL or MCL lesions, might also result in the concomitant tissue injury. This hypothesis is further supported by experimental data show-

ing that the enhancement of the DTH reaction by cyclophosphamide or pertussigen in immunized BALB/c mice results in exacerbation of the disease (Dhaliwal & Liew, 1987).

Studies on genetically resistant and susceptible mice strains suggest that the inability to control the infection is due to the expansion of a T helper 2 (T_H2) subset that produces IL-4, IL-5 and IL-10 but not IFN-γ, whereas effective immunity is accompanied by expansion of a IFN-γ producing subpopulation (T_H1 type) (Locksley et al., 1987; Scott, 1989; Locksley & Scott, 1991). If this idea holds true in human leishmaniasis, T_H1 cells should be present in LCL form where spontaneous healing is the rule, whereas in the MCL form T-cells should fall into the T_H2 category. The two distinct patterns of cytokine production were analyzed in MCL and LCL lesions by means of polymerase chain reaction (PCR) on lesion-derived mRNA. Lymphokine mRNAs resembling murine T_H1 (e.g., IL-2, IFN-γ and lymphotoxin) were present in LCL, MCL, and Montenegro reactions. In contrast, the TH2 profile (e.g., IL-4, IL-5, and IL-10) were most evidently expressed in MCL lesions. Cytokine mRNAs predominantly produced by macrophages such as IL-β, granulocyte colony-stimulating factor (GM-CSF), IL-6, and tumor necrosis factor (TNF-α) were equally expressed in both LCL, MCL and Montenegro specimens (Pirmez et al., 1993). These results suggest that LCL lesions appear to typify the type 1 response, characteristic of limited and/or self-healing lesions, whereas the type 2 response is likely to predominate in the progressive and destructive MCL lesions. The type 1 patterns was also found in the Montenegro reaction, a typical DTH response.

IFN-γ is a T_H1 product well known to enhance production of reactive Oxygen and Nitrogen intermediates (Nathan et al., 1983; Green et al., 1990) and to facilitate the intracellular killing of *Leishmania* (Nacy et al., 1981; Murray et al., 1983). Progression of the disease in BALC/c mice infected with *L. major* is associated with an inability of the animals to produce IFN-γ (Sadick et al., 1986). On the other hand, IL-4 induces B cells to switch to the production of IgG1, increases the expression of class II antigens, and activates macrophages. T cells that produce IL-4 or IFN-γ act as reciprocal regulatory agents in the determination of Ig isotype responses. IFN-γ inhibits the action of IL-4 on resting B cells, including induction of class II MHC molecule expression and costimulation of proliferation (Snapper & Paul, 1987). The cross-regulatory effects of IL-4 and IFN-γ in the host response to infection have been well demonstrated in the murine leishmaniasis model. In *L. major* infections, healing in C57/BL mice is accompanied by an increase in IFN-γ production by *Leishmania*-antigen-specific cells and in the susceptible BALB/c mice, there is an increase in IL-4 production (Heinzel et al., 1989).

Many cytokines other than IFN-γ and IL-4 seem to influence the outcome of infection such as GM-CSF, IL-3, IL-10 or TNF-α. For instance, TNF-α strongly activates murine peritoneal macrophages for killing of *L. major* amastigotes, an effect that is enhanced in the presence of low amounts of IFN-γ (Liew et al., 1990) and treatment *in vivo* with anti-TNF-α aggravates the course of the disease (Titus et al., 1989). GM-CSF has a detrimental effect in *L. major* infected mice (Greil et al., 1988), and there is a direct correlation between susceptibility and production of high levels of IL-3 (Lelchuk et al., 1988). Data of a number of studies show however conflicting results. While it has been demonstrated that the IFN-γ-mediated activation of murine peritoneal macrophages to kill *L. major* promastigotes can be antagonized by IL-3 or IL-4 (Liew et al., 1989), others had shown that IL-4 cooperates with IFN-γ for the resistance to *L. major* (Bogdan et al., 1991). Resolution of the lesions has been shown by *in vivo* treatment with either IL-4 (Carter et al., 1989) or anti-IL-4 (Sadick et al., 1990). Furthermore, Titus et al. (1991) have shown that BALB/c mice parasite-specific T cells that are clearly of the T_H1 type can exacerbate cutaneous leishmaniasis, despite the fact that the cells secrete large amounts of IFN-γ when they are activated with parasite antigens. Although many of the discrepancies can be explained by differences in the experimental designs, depending upon the source of the macrophage, the parasite form used, and the timing of addition of the cytokines, one can speculate that a T_H1 subset might not be protective when it does not produce the additional cytokine that optimally activate macrophages to destroy the parasite. Mixture of cells types is therefore likely to result in complex functions, and the balance among cytokines might be crucial in determining the outcome of the infection. The presence of IL-2, IFN-γ, and lymphotoxin detected either in LCL or MCL lesions is likely to contribute to

the healing of the localized cutaneous lesions, but IL-4 might play a key role in the pathogenesis of MCL lesions, perhaps by partially inhibiting the effect of IFN-γ on activating macrophages, resulting in the chronicity and tissue injury which characterize this form of leishmaniasis.

The pathogenesis of ACL is likely to be dependent on a complex array of factors. Different organisms as well as epidemiologic variables may be important in determining differences of the disease expression in different geographic regions. In contrast to predominance of CD4+ cells in LCL lesions in Brazil, CD8+ cells predominate in LCL lesions in Venezuela (Modlin et al., 1985). Nevertheless, the presence of high percentages of a T-cytotoxic phenotype in both LCL and MCL lesions is of particular interest since the resistance to *L. donovani* or *L. major* seems to require the activation not only of CD4+ cells, but also CD8+ cells (Stern et al., 1988; Farrell et al., 1989). γδ T-cells also merits some attention since they are present in LCL but not MCL lesions and may contribute to granuloma formation (Modlin et al., 1989). These γδ T-cells release a factor or factor that synergizes with GM-CSF to induce macrophage adhesion, aggregation and proliferation, are clonally selected by specific antigen, and undergo an oligoclonal expansion in distinct tissue microenvironments (Uyemura et al., 1992). The delayed-type hypersensitivity reaction is another component likely to participate in the pathogenesis of the lesions. In this context, it is interesting to note that T_H1 cells mediate a DTH reaction when injected with antigen into the footpads of mice with features more similar to the Jones-Mote than to the tuberculin-type DTH reaction (Cher & Mosmann, 1987), suggesting that this latter reaction is more likely to result from a combination of T_H1 and T_H2 cells (Mosmann & Moore, 1991). The demonstration of the simultaneous presence of T_H1 and T_H2 subsets in *L. major* infected mice (Lohoff et al., 1989) argues for a regulated balance between these subsets. On the other hand, it has been demonstrated that antigen-presenting cells (APC) can regulate the differentiation of T_H cells, since different APC can preferentially present different classes of antigen (Gajewski et al., 1991). It is noteworthy that Langerhans cells, present in high numbers in LCL lesions, are virtually absent in MCL lesions (Pirmez & Modlin, unpublished observation; Martínez-Arends et al., 1991). Langerhans cells are APC of the epidermis, and can present antigen to both T_H1 and T_H2 cells, but when UV irradiated present to only T_H2 cells (Simon et al., 1990). It is thus possible that the dominant APC in MCL lesions preferentially presents antigen to type 2 lymphokine producing cells. This is supported by experiments showing that T-cells in lymphoid organs draining non-mucosal tissue sites produce IL-2 while those draining mucosal sites produce IL-4 (Daynes et al., 1990). Another hypothesis that should be considered is the MHC restriction element which may select particular functional T-cell populations. Murray et al. (1989) have shown that CD4+ T cells activated in the primary *in vivo* response to antigen produce distinct patterns of cytokines depending upon the MHC class II haplotype of the responding mice.

Definition of the mechanisms influencing the cytokine regulation in ACL is likely to permit more comprehensive view of the pathogenesis of the disease. Further investigation of T-cytotoxic cells and TCR-γδ cells, as well as comparison of the antigen repertoire of T-cells in lesions might be critical in understanding and differentiating the clinical presentation and immune response to infection caused by *L. braziliensis*.

REFERENCES

BOGDAN, C.; STENGER, S.; RÖLLINGHOFF, M. & SOLBACH, W., 1991. Cytokine interactions in experimental cutaneous leishmaniasis. IL-4 synergizes with interferon-γ to activate murine macrophages for killing *Leishmania major* amastigotes. *Eur. J. Immunol., 21:* 327-333.

CARTER, K. C.; GALLAGHER, G.; BAILLIE, A. J. & ALEXANDER, J., 1989. The induction of protective immunity to *Leishmania major* in the BALB/c mouse by interleukin-4 treatment. *Eur. J. Immunol., 19:* 779-782.

CARVALHO, E. M.; JOHNSON, W. D.; BARRETO, E.; MARSDEN, P. D.; COSTA, J. L. M.; REED, S. & ROCHA, H., 1985. Cell mediated immunity in American cutaneous and mucosal leishmaniasis. *J. Immunol., 135:* 4144-4148.

CASTES, M.; AGNELLI, A. & RONDON, A. J., 1984. Mechanisms associated with immunoregulation in human American cutaneous leishmaniasis. *Clin. Exp. Immunol., 57:* 279-286.

CASTES, M.; AGNELLI, A.; VERDE, O. & RONDON, A. J., 1983. Characterization of the cellular immune response in American cutaneous leishmaniasis. *Clin. Immunol. Immunopathol., 27:* 176-186.

CHER, D. J. & MOSMANN, T. R., 1987. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by Th1 clones. *J. Immunol., 138:* 3688-3694.

CONCEIÇÃO-SILVA, F.; DÓREA, R. C. C.; PIRMEZ,

C.; SCHUBACH, A. & COUTINHO, S. G., 1990. Quantitative study of *Leishmania braziliensis braziliensis* reactive T cells in peripheral blood and in the lesions of patients with American mucocutaneous leishmaniasis. *Clin. Exp. Immunol., 79:* 221-226.

COUTINHO, S. G.; PIRMEZ, C.; MENDONÇA, S. C.; CONCEIÇÃO-SILVA, F. & DÓREA, R. C. C., 1987. Pathogenesis and immunopathology of leishmaniasis. *Mem. Inst. Oswaldo Cruz, 82:* 214-218.

DAYNES, R. A.; ARANEO, B. A.; DOWELL, T. A.; HUANG, K. & DUDLEY, D., 1990. Regulation of murine lymphokine production *in vivo*. III. The lymphoid tissue microenvironment exerts regulatory influences over T helper cell function. *J. Exp. Med., 171:* 979-996.

DHALIWAL, J. S. & LIEW, F. Y., 1987. Induction of delayed-type hypersensitivity to *Leishmania major* and the concomitant acceleration of disease development in progressive murine cutaneous leishmaniasis. *Infect. Immun., 55:* 645-651.

FARRELL, J. P.; MÜLLER, I. & LOUIS, J. A., 1989. A role for Lyt-2+ T cells in resistance to cutaneous leishmaniasis in immunized mice. *J. Immunol., 142:* 2052-2056.

GAJEWSKI, T. F.; PINNAS, M.; WONG, T. & FITCH, F. W., 1991. Murine TH2 and TH2 clones proliferate optimally in response to distinct antigen-presenting cell populations. *J. Immunol., 146:* 1750-1758.

GREEM, S. J.; CRAWFORD, R. M.; HOCKMEYER, J. T.; MELTZER, M. S. & NACY, C. A., 1990. *Leishmania major* amastigotes initiate the L-arginine-dependent killing mechanism in IFN-γ-stimulated macrophages by induction of tumor necrosis factor-α. *J. Immunol., 145:* 4290-4297.

GREIL, J.; BODENDORFER, B.; RÖLLINGHOFF, M. & SOLBACH, W., 1988. Application of recombinant granulocyte-macrophage colony-stimulating factor has a detrimental effect in experimental murine leishmaniasis. *Eur. J. Immunol., 18:* 1527-1533.

HANDMANN, E.; CEREDIG, R. & MITCHELL, G. F., 1979. Murine cutaneous leishmaniasis: disease patterns in intact and nude mice of various genotypes and examination of some differences between normal and infected macrophages. *Aust. J. Exp. Biol. Med. Sci., 57:* 9-30.

HEINZEL, F. P.; SADICK, M. D.; HOLADAY, B. J.; COFFMAN, R. L. & LOCKSLEY, R. M., 1989. Reciprocal expression of interferon-γ or interleukin-4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J. Exp. Med., 169:* 59-72.

LIEW, F. Y.; MILLOTT, S.; LI, Y.; LELCHUCK, R.; CHAN, W. L. & ZILTENER, H., 1989. Macrophage activation by interferon-γ from host-protective T cells is inhibited by interleukin (IL)-3 and IL-4 produced by disease promoting T cells in leishmaniasis. *Eur. J. Immunol., 19:* 1227-1232.

LIEW, F. Y.; PARKINSON, C.; MILLOTT, S.; SEVERN, A. & CARRIER, M., 1990. Tumor necrosis factor (TNFα) in leishmaniasis. I. TNFα mediates host protection against cutaneous leishmaniasis. *Immunol., 69:* 570-573.

LELCHUK, R.; GRAVELEY, R. & LIEW, F. Y., 1988. Susceptibility to murine cutaneous leishmaniasis correlates with the capacity to generate interleukin-3 in response to *Leishmania* antigen *in vitro*. *Cell. Immunol., 111:* 66-76.

LOCKSLEY, R. M.; HEINZEL, F. P.; SADICK, M. D.; HOLADAY, B. J. & GARDNER, K. D., 1987. Murine cutaneous leishmaniasis: susceptibility correlates with differential expansion of helper T-cell subsets. *Ann. Inst. Pasteur/Immunol., 138:* 744-749.

LOCKSLEY, R. M. & SCOTT, P., 1991. Helper T-cell subsets in mouse leishmaniasis: induction, expansion and effector function. *Immunol. Today, 12:* A58-61.

LOHOFF, M.; SOMMER, F.; SOLBACH, W. & RÖLLINGHOFF, M., 1989. Coexistence of antigen-specific Th1 and Th2 cells in genetically susceptible BALB/c mice infected with *Leishmania major*. *Immunol., 179:* 412-421.

MARSDEN, P. D.; LLANOS-CUENTAS, A.; LAGO, E. L.; CUBA, C. A. C.; BARRETO, A. C.; COSTA, J. M. & JONES, T. C., 1984. Human mucocutaneous leishmaniasis in Três Braços, Bahia, Brazil. An area of *Leishmania braziliensis braziliensis* transmission. III. Mucosal disease: presentation and initial evolution. Rev. Soc. Bras. Med. Trop., 17: 179-186.

MARSDEN, P. D. & NONATA, R. R., 1975. Mucocutaneous leishmaniasis. A review of clinical aspects. *Rev. Soc. Bras. Med. Trop., 9:* 309-326.

MARTÍNEZ-ARENDS, A.; TAPIA, F. J.; CÁCERES-DITTMAR, G.; MOSCA, W.; VALECILLOS, L. & CONVIT, J., 1991. Immunocytochemical characterization of immune cells in lesions of American cutaneous leishmaniasis using novel T cell markers. *Acta Trop., 49:* 271-280.

MITCHELL, G. F.; CURTIS, J. M.; HANDMAN, E. & McKENZIE, I. F., 1980. Cutaneous leishmaniasis in mice: disease patterns in reconstituted nude mice of several genotypes infected with *Leishmania tropica*. *Aust. J. Exp. Biol. Med. Sci., 58:* 521-532.

MITCHELL, G. F.; CURTIS, J. M.; SCOLLAY, R. G. & HANDMAN, E., 1981. Resistance and abrogation of resistance to cutaneous leishmaniasis in reconstituted BALB/c nude mice. *Aust. J. Exp. Biol. Med. Sci., 59:* 539-554.

MODLIN, R. L.; PIRMEZ, C.; HOFMAN, F.; TORIGIAN, V.; UYEMURA, K.; REA, T. H.; BLOOM, B. R. & BRENNER, M. B., 1989. Lymphocytes bearing antigen-specific γδ T-cell receptors accumulate in human infectious disease lesions. *Nature, 339:* 544-548.

MODLIN, R. L.; TAPIA, F. J.; BLOOM, B. R.; GALLINOTO, M. E.; CASTES, M.; RONDON, A. J.; REA, T. H. & CONVIT, J., 1985. *In situ* characterization of the cellular immune response in American cutaneous leishmaniasis. *Clin. Exp. Immunol., 60:* 241-248.

MOSMANN, T. R. & MOORE, K. W., 1991. The role of IL-10 in crossregulation of Th1 and Th2 responses. *Immunol. Today, 12:* A49-A53.

MURRAY, H. W.; RUBIN, B. Y. & ROTHERMEL, C. P., 1983. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon is the activating lymphokine. *J. Clin. Invest., 72:* 1506.

MURRAY, J. S.; MADRI, J.; TITE, J.; CARDING, S. R. & BOTTOMLY, K., 1989. MHC control of CD4+ T

cell subset activation. *J. Exp. Med., 170:* 2135-2140.

NACY, C. A.; MELTZER, M. S.; LEONARD, E. J. & WYLER, D. J., 1981. Intracellular replication and lymphokine-induced destruction of *Leishmania tropica* in C3H/HeN mouse macrophages. *J. Immunol., 127:* 2381-2386.

NATHAN, C. F.; MURRAY, H. W.; WIEBE, M. E. & RUBIN, B. Y., 1983. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med., 158:* 670-689.

PIRMEZ, C.; COOPER, C.; PAES-OLIVEIRA, M.; SCHUBACH, A.; TORIGIAN, V. K. & MODLIN, R. L., 1990. Immunologic responsiveness in American cutaneous leishmaniasis lesions. *J. Immunol., 145:* 3100-3104.

PIRMEZ, C.; YAMAMURA, M.; UYEMURA, K.; PAES-OLIVEIRA, M.; CONCEIÇÃO-SILVA, F. & MODLIN, R. L., 1993. Citokine patterns in American cutaneous leishmaniasis. *J. Clin. Invest., 91:* 1390-1395.

SADICK, M. D.; HEINZEL, F. P.; HOLADAY, B. J.; PU, R. T.; DAWKINS, R. & LOCKSLEY, R. M., 1990. Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody. Evidence for a T cell-dependent, interferon-γ-independent mechanism. *J. Exp. Med., 171:* 115-1127.

SADICK, M. D.; LOCKSLEY, R. M.; TUBBS, C. & RAFF, H. U., 1986. Murine cutaneous leishmaniasis: resistance correlates with the capacity to generate interferon-γ in response to *Leishmania* antigens *in vitro*. *J. Immunol., 136:* 655-661.

SARAVIA, N. G.; VALDERRAMA, L.; LABRADA, M.; HOLGUIN, A. F.; NAVAS, C.; PALMA, G. & WEIGLE, K. A., 1989. The relationship of *Leishmania braziliensis* subspecies and immune response to disease expression in New World leishmaniasis. *J. Infect. Dis., 159:* 725-735.

SCOTT, P., 1989. The role of TH1 and TH2 cells in experimental cutaneous leishmanisis. *Exp. Parasitol., 68:* 369-372.

SIMON, J. C.; CRUZ, P. D.; BERGSTRESSER, P. R. & TIGELAAR, R. E., 1990. Low dose ultraviolet B-irradiated Langerhans cells preferentially activate CD4+ cells of the T helper 2 subset. *J. Immunol., 145:* 2087-2091.

SNAPPER, C. M. & PAUL, W. E., 1987. Interferon-γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science, 236:* 944-947.

STERN, J. J.; OCA, M. J.; RUBIN, B. Y.; ANDERSON, S. L. & MURRAY, H. W., 1988. Role of L3T4+ and Lyt-2+ cells in experimental visceral leishmaniasis. *J. Immunol., 140:* 3971-3977.

TITUS, R. G.; SHERRY, B. & CERAMI, A., 1989. Tumor necrosis factor plays a protective role in experimental murine cutaneous leishmaniasis. *J. Exp. Med., 170:* 2097-2104.

TITUS, R. G.; MÜLLER, I.; KIMSEY, P.; CERNY, A.; BEHIN, R.; ZINKERNAGEL, R. M. & LOUIS, J. A., 1991. Exacerbation of experimental murine cutaneous leishmaniasis with CD4+ *Leishmania major*-specific T cell lines or clones which secrete interferon-γ and mediate parasite-specific delayed-type hypersensitivity. *Eur. J. Immunol., 21:* 559-567.

UYEMURA, K.; KLOTZ, J.; PIRMEZ, C.; OHMEN, J.; WANG, X. H.; HO, C.; HOFFMAN, W. L. & MODLIN, R. L., 1992. Microanatomic clonality of γ/δ T cells in human leishmaniasis lesions. *J. Immunol., 148:* 1205-1211.