Short Report

Cellular immune responses to *Leishmania braziliensis* in patients with AIDS-associated American cutaneous leishmaniasis

Alda Maria Da-Cruz[1], Marise Mattos[2], Manoel P. Oliveira-Neto[2], Ziadir Coutinho[2,3], Elizabeth S. Machado[4] and Sergio G. Coutinho[1]

[1]Laboratório de Imunidade Celular e Humoral em Protozooses, Departamento de Protozoologia/Imunologia, Instituto Oswaldo Cruz-FIOCRUZ, RJ, Brazil; [2]Centro de Pesquisas Hospital Evandro Chagas, FIOCRUZ, RJ, Brazil; [3]Hospital Universitário Gaffrée-Guinle, UNIRIO, RJ, Brazil; [4]Hospital dos Servidores do Estado, SUS-Ministério da Saúde, RJ, Brazil

Keywords: cutaneous leishmaniasis, *Leishmania braziliensis*, AIDS, cellular immunity, T-cell proliferation, T-cell phenotypes, IFN-γ production, disease course, Brazil

American cutaneous leishmaniasis (ACL), caused by *Leishmania* parasites, constitutes a major health problem in Central and South America. Cell-mediated immune responses (CMI) have a strong influence on the immunopathological mechanisms involved in the course of leishmaniasis but also are necessary for recovery.

HIV infection produces a complex pathogenic process that affects the T-cell compartment required for production of an effective immune response. In this regard, reports concerning AIDS-associated ACL have described unexpected clinical pictures of leishmaniasis probably related to the inability of the T-cell-mediated immune response to control the spread of the parasite (ALVAR *et al.*, 1997). Despite an increasing number of relapsing or atypical cases of leishmaniasis in HIV-positive patients there are still few reports addressing the influence of the immunological abnormalities produced by HIV on the *Leishmania*-specific CMI and consequently on the outcome of leishmaniasis (DA-CRUZ *et al.*, 1999; NIGRO *et al.*, 1999).

In this report from Brazil, 4 previously reported clinical cases of AIDS-associated ACL caused by *Leishmania braziliensis* were grouped to evaluate a possible relationship between the outcome of the disease and the development of CMI in terms of T-cell phenotypes and interferon-gamma (IFN-γ) production. Patients no. 1 (MACHADO *et al.*, 1992), no. 2 (NOGUEIRA-CASTAÑON *et al.*, 1996) and no. 3 (MATTOS *et al.*, 1998) displayed disseminated cutaneous lesions. Patient no. 4 (DA-CRUZ *et al.*, 1999) presented mucosal lesions.

The patients were initially treated with conventional antimonial therapy (meglumine antimonate; Glucantime®, Rhodia). When therapeutic failure occurred patients were treated with second-line drugs such as amphotericin B (Fungizone®, Squibb) (patients no. 3 and no. 4) or combined immunochemotherapy (ICT: antimonial associated with immunotherapy with whole promastigote antigens) (patient no. 4). ICT has already been used as an alternative therapy with favourable results (GENARO *et al.*, 1996).

T-cell responses elicited *in vitro* by *L. braziliensis* (Lb) antigen stimulation were assessed as described elsewhere (COUTINHO *et al.*, 1996). In brief, peripheral blood mononuclear cells (PBMC) were cultured *in vitro* in the presence of the equivalent of 10^6 sonicated Lb promastigotes (MHOM/BR75/M2903) or 4 μg of concanavalin A (Con A, Sigma Chemical Co., USA). Stimulation indexes (SI, mean counts in wells containing antigen or mitogen divided by the mean counts in non-stimulated wells) equal to or higher than 2·5 were considered positive. The levels of IFN-γ in the culture supernatants were determined by enzyme-linked immunosorbent assay (Quantikine™ Human IFN-γ Immunoassay, R&D Systems, MN, USA).

In parallel, leishmanial antigen-reactive T cells, obtained after stimulation of PBMC *in vitro*, were separated by centrifugation over a discontinuous Percoll gradient (Sigma). For phenotypic analysis, PBMC or Lb-reactive T cells were incubated with monoclonal antibodies for $CD2^+$ (T11-RD1), anti-$CD4^+$ (T4-FITC), and anti-$CD8^+$ (T8-RD1, Coulter Corporation, FL, USA), prior to analysis by flow cytometry (EPICS 751, Coulter).

The immunological studies were performed during active disease or after the remission of lesions. Patients no. 1 and no. 2, despite very low lymphocyte proliferative response (LPR) stimulation indexes, produced high levels of IFN-γ in the cell cultures, and their lesions remitted with therapy. Patient no. 3 initially achieved remission of his lesions with antimonial therapy. At that time he presented a positive LPR to Lb (SI 4·7), but low IFN-γ production (56 pg/mL) in the culture supernatants. When reactivation occurred, LPR and IFN-γ production became negligible (Table) and no improvement of lesions was achieved.

Patient no. 4 showed very low T-cell stimulation indexes (SI 1·4) and IFN-γ production (44 pg/mL) before the ICT (Table). However, positive LPR to Lb antigens were observed after the 1st (SI 4·7) and 2nd (SI 3·1) course of ICT. In these culture supernatants, the levels of IFN-γ were 1240 pg/mL and 1107 pg/mL, respectively. However, both LPR and IFN-γ showed a striking decrease after the 3rd course of the ICT (Table). Thereafter, the SI returned to the low levels found before therapy (SI 0·96). In this patient, the healing of lesions and development of an Lb-reactive T-cell response point to the possibility that ICT induced a *Leishmania*-specific immune response leading to cure (DA-CRUZ *et al.*, 1999). The beneficial T-cell responses induced after the 1st and 2nd course of ICT apparently were sufficient to induce the remission of the disease. We may speculate that homing of Lb-reactive IFN-γ producing T cells to the inflammatory site could explain the observed low T-cell proliferation and lack of IFN-γ production after the 3rd course of ICT. Despite the absence of LPR and IFN-γ production observed after the 3rd course of ICT, the lesions remained healed until the death of the patient 4 months later.

Taken together, those results show that despite a poor LPR induced by leishmanial antigens, a favourable clinical response to therapy preferentially occurred when IFN-γ was clearly detected in cultures stimulated with parasite antigens. The use of exogenous IFN-γ plus antimonial therapy for treatment of leishmaniasis also provided good results in patients suffering from visceral leishmaniasis and AIDS (DE GORGOLAS *et al.*, 1993). Moreover, the levels of IFN-γ production observed in patients no. 1, 2 and 4 (during ICT) were comparable to those detected in immunocompetent ACL patients (COUTINHO *et al.*, 1996). The role of IFN-γ could be activation of local macrophages leading to reduction of the parasite burden.

The majority of T lymphocytes that proliferated under stimulation *in vitro* with leishmanial antigen had a $CD8^+$ phenotype. The percentages of $CD8^+$ reactive cells in patients no. 2 and 3 were 34·2% and 45·7%, respectively. Patient no. 4 showed positive LPR only after the first course of ICT and the majority of Lb-reactive proliferat-

Address for correspondence: Sergio G. Coutinho, Laboratório de Imunidade Celular e Humoral, Departamento de Imunologia, PCC 5° andar, Instituto Oswaldo Cruz - FIOCRUZ, Av. Brasil, 4365, CEP 21045-900, Rio de Janeiro, Brazil; phone +55 21 598 4318/+55 21 598 4461, fax +55 21 590 3545, e-mail coutinho@gene.dbbm.fiocruz.br

Table. Cellular immune responses to *Leishmania braziliensis* in four patients with AIDS-associated American cutaneous leishmaniasis

Case no.	Clinical status of leishmaniasis lesions	Montenegro skin test (mm³)	Peripheral blood T-cell counts (/mm³) (before stimulation)		T-cell responses after stimulation *in vitro*					
					Lymphoproliferative responses (SI)		*Leishmania*-reactive T-cell phenotypes (%)		IFN-γ production (pg/mL)	
			$CD4^+$	$CD8^+$	Con A	Lb	$CD4^+$	$CD8^+$	Bg	Lb
1	Active[a]	3	139	485	2·0	1·2	ND	ND	39	3939
2	Active[a]	5	143	296	2·9	1·2	0·7	34·2	0	1083
3	Active[a]	15	ND	ND	74·3	4·7	7·5	45·7	0	56
	Reactivation	NR	23	292	1·5	1·2	ND	ND	0	0
4[b]	Active[a]	NR	64	204	0·8	1·4	ND	ND	0	44
	After 1st course ICT	ND	70	273	2·4	4·7	2·2	33·0	44	1240
	After 2nd course ICT	ND	19	155	2·4	3·1	2·2	34·7	0	1107
	End of ICT	ND	29·8	296	0·8	0·9	ND	ND	0	0

SI, stimulation index; Con A, concanavalin A; Lb, *Leishmania braziliensis*; Bg, background; ND, not determined; NR, not reactive.

[a]Tendency to remission of lesions.

[b]Case no. 4 was treated with three 10-day courses of combined immunochemotherapy (ICT) (*Leishmanin* plus an antimonial).

ing lymphocytes were T cells (47·5%), most of them showing $CD8^+$ phenotype (33%). Similar results were seen when the phenotypic analyses were performed in cultures after the 2nd course of ICT (DA-CRUZ *et al.*, 1999). In all cases, the percentages of $CD4^+$ antigen-reactive T cells were very low (Table). No phenotypic analysis was performed in patients no. 1 and 4 before therapy because very few antigen-responder cells were found in the cultures.

Our findings indicate that antigen-responding $CD8^+$ cells, even when $CD4^+$ cells were practically absent, were able to mediate mechanisms associated with healing of leishmanial lesions.

These results suggest that even in advanced stages of AIDS, leishmaniasis patients can retain a beneficial immunological response able to control *Leishmania* infection. These results provide further evidence that the healing process seems to depend not only on the anti-leishmanial therapy, but also on the induction of an appropriate *Leishmania*-specific T-cell response characterized by IFN-γ production where the majority of Lb-reactive T cells had a $CD8^+$ phenotype (COUTINHO *et al.*, 1996; DA-CRUZ *et al.*, 1999; KEMP *et al.*, 1999).

Although not many cases of ACL–HIV infection have been described, the current expansion of AIDS cases and the spreading of ACL around large cities increase the probability of this co-infection and the occurrence of severe cases of leishmaniasis (ALVAR *et al.*, 1997). Thus, the understanding of the immunopathological mechanisms involved may help decisions on therapeutic and prophylactic approaches leading to a better clinical follow-up for *Leishmania*–HIV co-infected patients.

Acknowledgements

We are grateful to Dr Alvaro Bertho and to Ms Marta Santiago for flow cytometry analysis, to Dr M. Nogueira-Castañon for contribution and to Ms R. Pellegrino and Ms A. Oliveira for the excellent secretarial assistance. The authors dedicate this paper in honour of the Oswaldo Cruz Institute, on the occasion of the centenary of its foundation, 25 May 1900. This work was supported by grants from Economic European Community, CAPES and CNPq.

References

Alvar, J., Cañavate, C., Gutiérrez-Solar, B., Jiménez, M., Laguna, F., López-Vélez, R., Molina, R. & Moreno, J. (1997). *Leishmania* and human immunodeficiency virus coinfection: the first 10 years. *Clinical Microbiology Reviews*, **10**, 298–319.

Coutinho, S. G., Oliveira, M. P., Da-Cruz, A. M., De Luca, P. M., Mendonça, S. C. F., Bertho, A. L., Soong, L. & McMahon-Pratt, D. (1996). T-cell responsiveness of American cutaneous leishmaniasis patients to purified *Leishmania pifanoi* amastigote antigens and *Leishmania braziliensis* promastigote antigens: immunologic patterns associated with cure. *Experimental Parasitology*, **84**, 144–155.

Da-Cruz, A. M., Filgueiras, D. V., Coutinho, Z., Mayrink, W., Grimaldi, G. Jr, De Luca, P. M., Mendonça, S. C. F. & Coutinho, S. G. (1999). Atypical mucocutaneous leishmaniasis caused by *Leishmania braziliensis* in an acquired immunodeficiency syndrome patient: T-cell responses and remission of lesions associated with antigen immunotherapy. *Memórias do Instituto Oswaldo Cruz*, **94**, 537–542.

De Gorgolas, M., Castrillo, J. M. & Fernandez Guerrero, M. L. (1993). Visceral leishmaniasis in patients with AIDS: report of three cases treated with pentavalent antimony and interferon-gamma. *Clinical Infectious Diseases*, **17**, 56–58.

Genaro, O., Toledo, V. P. C. P., Costa, C. A., Hermeto, M. V., Afonso, L. C. C. & Mayrink, W. (1996). Vaccine for prophylaxis and immunotherapy, Brazil. *Clinics in Dermatology*, **14**, 503–512.

Kemp, K., Theander, T. G., Hviid, L., Garfar, A., Kharazmi, A. & Kemp, M. (1999). Interferon-γ and tumour necrosis factor-α producing cells in humans who are immune to cutaneous leishmaniasis. *Scandinavian Journal of Immunology*, **49**, 655–659.

Machado, E. S., Braga, M. P., Da-Cruz, A. M., Coutinho, S. G., Vieira, A. R. M., Rutowitsch, M. S., Cuzzi-Maya, T., Grimaldi, G. Jr & Menezes, J. A. (1992). Disseminated American muco-cutaneous leishmaniasis caused by *Leishma-*

nia braziliensis braziliensis in a patient with AIDS: a case report. *Memórias do Instituto Oswaldo Cruz*, **87**, 487–492.
Mattos, M., Caiza, A., Fernandes, O., Gonçalves, A. J. S., Pirmez, C., Souza, C. S. F. & Oliveira-Neto, M. P. (1998). American cutaneous leishmaniasis associated with HIV infection: report of four cases. *Journal of the European Academy of Dermatology and Venereology*, **10**, 218–225.
Nigro, L., Cacopardo, B., Preiser, W., Braner, J., Cinatl, J., Palermo, F., Russo, R., Doerr, H. W. & Nunnari, A. (1999). *In vitro* production of type 1 and type 2 cytokines by peripheral blood mononuclear cells from subjects coinfected with human immunodeficiency virus and *Leishmania infantum*. *American Journal of Tropical Medicine and Hygiene*, **60**, 142–145.
Nogueira-Castañon, M. C. M., Pereira, C. A. C. & Furtado, T. (1996). Unusual association of American cutaneous leishmaniasis and acquired immunodeficiency syndrome. *International Journal of Dermatology*, **35**, 295–297.

Received 1 December 1999; revised 18 April 2000; accepted 18 April 2000

Books Received*

An Update on Zoonoses. *OIE Scientific and Technical Review*, **19** (1), 1–336. P.-P. Pastoret (Co-ordinator). Paris: Office International des Epizooties, 2000 (April). 336pp. Price Euro 40/FF 270/US$ 45 (air mail postage is included for all countries). ISBN 92-9044-512-2. ISSN 0253-1933. [Obtainable from OIE, 12 rue de Prony, 75017 Paris, France; phone +33 (0)1 44151888, fax +33 (0)1 42670987, e-mail pub.sales@oie.int]

The papers are printed in English (mainly), French or Spanish but have summaries in all 3 languages. Topics covered include the biological conditions conducive to the transmission of pathogens from animals to humans, infections due to hantavirus, bunyavirus, filovirus, monkeypoxvirus, the Hendra and Nipah viruses, bat lyssavirus, influenza A virus, and Borna virus, new variant Creutzfeldt–Jakob disease, Lyme disease, cat-scratch fever, and West Nile encephalitis, as well as more general papers on zoonoses. The papers contain Tables and Figures (some in colour) as appropriate and Reference lists.

Meeting the Nutritional Needs of Infants During Emergencies: Recent Experiences and Dilemmas. Report of an International Workshop, Institute of Child Health, London, November 1999. M. McGrath, A. Seal, A. Taylor & L. Gostelow (researchers and compilers). London: Save the Children, 2000. 72pp.

The first part of the report contains abstracts of the presentations together with summaries of the key concerns and of the Working Group action points. The second (major) part of the report documents the research conducted in Macedonia, which provided a substantial part of the information base from which action points were developed.

SEAMIC Health Statistics 1999. Tokyo: International Medical Foundation of Japan, 2000. 278pp. Price not stated. ISBN 4-930783-83-6. SEAMIC Publication No. 83.

Part I presents comparative statistics (in the form of Tables and Graphs) from Brunei, Indonesia, Japan, Malaysia, Philippines, Singapore, Thailand, and Viet Nam for selected health and related topics. Part II provides the background information on how these statistics have been collected, processed and produced for each of these countries. The contents of this book are also accessible through the Internet (http://www.seamic-imfj.or.jp).

WHO Recommended Surveillance Standards (second edition—October 1999). Geneva: World Health Organization, 1999. ii+158pp. WHO/CDS/CSR/ISR/99.2. Distribution general. Available in English.

The document, produced jointly by technical clusters of WHO, as well as by UNAIDS, brings together WHO recommended standards for the surveillance of communicable disease. First produced in November 1997, this manual has been completely revised and is intended to be updated on a regular basis. The diseases or syndromes are listed alphabetically, and for each there is a description of the rationale for surveillance, case definition, types of surveillance, minimum data elements, data analyses, principal uses of data for decision making, and the relevant WHO contact details.

Measles Eradication Field Guide. Technical Paper No. 41. Prepared by the Special Program for Vaccines and Immunization. Washington, D.C.: Pan American Health Organization, 1999. vi+70pp. ISBN 92-75-13041-8.

Canine Leishmaniasis: an Update. Proceedings of the International Canine Leishmaniasis Forum, Barcelona, Spain—1999. R. Killick-Kendrick (editor). Wiesbaden, Hoechst Roussel Vet, 2000. 104pp.

This soft-backed volume, with coloured illustrations, contains full papers complete with Tables, Figures and References.

Leishmania/HIV Co-infection in South-Western Europe 1990–98. Retrospective Analysis of 965 Cases. Geneva: World Health Organization (Department of Communicable Disease Surveillance and Response), 2000. vi+12pp. WHO/LEISH/2000/42. Distribution general. Available in English.

War and Public Health. Handbook on War and Public Health. P. Perrin. Geneva: International Committee of the Red Cross, 1996. xxiii+446pp. Price not known. ISBN 2-88145-077-6.

This paper-backed book [received in the Editorial Office this year] contains chapters on planning, food and nutrition, water and environmental health, communicable diseases, medical and surgical care, epidemiology, the health-care system, disasters and development, protecting the victims of armed conflicts, and humanitarian ethics.

*Inclusion of titles in this list does not necessarily imply recommendation by the Society, Editorial Board, or Editor.