Immunological Reactions to Mycobacterial Proteins in the Spectrum of Leprosy

TO THE EDITOR:

Leprosy, still a public health probelm in many parts of the world, is a chronic mycobacterial disease produced by an intracellular parasite, *Mycobacterium leprae*, which multiplies mainly within tissue macrophages and Schwann cells in peripheral nerves. A good immunological response toward *M. leprae* depends on the development of a T-cell-mediated immune response.

It has not been established whether individual proteins are sufficient for the development of protective immunity in mycobacterial infections. Also, the availability of purified and well-charaterized antigens is a prerequisite for studying the role of individual molecules in the pathogenesis of the disease.

We have previously studied the effect of soluble *M. leprae* extract on the proliferative response of peripheral blood lymphocytes from leprosy patients, their family members and other contacts. In tuberculoid patients and in contacts we detected differential reactivity to the various proteins isolated ([9]).

Using molecular biology techniques and monoclonal antibodies, it has been possible to obtain individual proteins from mycobacterial antigens ([1, 2, 6, 8, 15]), which has been very helpful for understanding the biology of this microorganism and its relationship with the host.

In this study, we have compared cellular and humoral reactivity toward various whole, fractionated and recombinant mycobacterial antigens from *M. bovis* and *M. leprae* in patients from the outpatient clinic of the Instituto de Biomedicina, Caracas, Venezuela. These patients were clinically and histologically diagnosed according to Ridley and Jopling's classification ([11]). The group included 20 lepromatous (LL) and borderline lepromatous (BL) patients and 20 tuberculoid (TT) patients, as well as a group of 10 family and nonfamily Mitsuda-positive contacts. All LL and BL (85% males, 15% females) and TT (40% males, 60% females) patients were adults.

The antigens used for this study were: bacilli from experimentally infected armadillo lesions purified using Draper's method, BP ([4]); soluble *M. leprae* extract

TABLE 1. *Percentages of proliferation to complex extracts and bacillary antigens.*[a]

Patients	No.	BCG[b]	MlSA[c]	PPD[d]	BP[e]
Multibacillary (LL and BL)	20	30	10	50	10
Paucibacillary (TT)	20	70	80	80	25
Controls (contacts)	10	90	90	90	40

[a] Stimulation index >2 positive.
[b] *M. bovis* BCG.
[c] *M. leprae* soluble antigens.
[d] *M. tuberculosis* purified protein derivative.
[e] *M. leprae* purified from infected armadillos.

from the same type of bacilli, ruptured in a French pressure cell ([3]); *M. bovis* BCG (Connaught Laboratories, Willowdale, Ontario, Canada); *M. tuberculosis* purified protein derivative (PPD) (Statens Seruminstitutet, Copenhagen, Denmark); and recombinant antigens 70 kDa from *M. tuberculosis*, 65 kDa from *M. bovis*, and 36 kDa, 28 kDa, 18 kDa, and 10 kDa from *M. leprae*. These recombinant antigens were obtained from the Recombinant Protein Bank of the World Health Organization. All soluble antigens were used at a 20-μg/ml final concentration in proliferation assays. Phytohemagglutinin (PHA) (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) was used as the positive control at a 25-μg/ml final concentration.

Lymphocyte transformation assay. Mononuclear cells from 20 ml of peripheral blood were obtained through a density gradient, diluted, and placed in flat-bottom, 96-well plates (Falcon 3072; Falcon Plastics, Oxnard, California, U.S.A.) at 2×10^5 lymphocytes per well. Cell proliferation was measured as previously reported ([9]). Results were reported as the percentage positivity, and an index higher than 2 was considered positive. The various percentage positivities in the spectrum, with both recombinant proteins and complex antigens are shown in Tables 1 and 2.

Multibacillary patients (18/20) did not show *in vitro* cellular reactivity to the recombinant antigens used, with a mean stimulation index of 0.98 ± 0.30, similar behavior to that shown with soluble extract (MlSA); only two showed cellular reactivity with *M. tuberculosis* 70 kDa and *M. bovis* 65 kDa, with a stimulation index of 2.26 ± 0.67 and 2.24 ± 0.85, respectively. These patients presented clinical spontaneous reversion. Paucibacillary patients showed reactivity with all of the recombinant proteins, more intense with *M. leprae* 18-kDa, *M. tuberculosis* 70-kDa, and *M. leprae* 10-kDa proteins. Healthy family or nonfamily contacts reacted toward all of the antigens used. The highest values were obtained with *M. tuberculosis* 70-kDa, *M. bovis* 65-kDa, and *M. leprae* 10-kDa proteins.

Studies done in Nepali leprosy patients and healthy controls showed strong correlation between BCG and *M. leprae* antigens. Patients and controls did not respond toward complex preparations nor toward recombinant antigens ([12]).

We had similar results in lepromatous patients, who lack T-cell responses, toward MlSA and recombinant antigens. T cells of tuberculoid patients and positive contacts recognized BCG/PPD in 70%/80% and 90%/90% of cases, respectively. Positive contacts recognized recombinant 70-kDa and 65-kDa proteins; in tuberculoid patients there were deficiencies in the cell-mediated response, it being much greater with the 70-kDa protein (Table 2).

TABLE 2. *Percentages of proliferation to mycobacterial antigens.*[a]

		M. tuberculosis	*M. bovis*	*M. leprae*			
Patients	No.	70	65	36	28	18	10
Multibacillary (LL and BL)	20	5	5	20	5	10	0
Paucibacillary (TT)	20	30	10	20	15	35	20
Contacts	10	50	40	30	25	30	40

[a] Stimulation index >2 positive.

TABLE 3. *ELISA, serum pools with mycobacterial recombinant antigens.*

Patients	Recombinant proteins						Soluble extract *M. leprae*
	70	65	36	28	18	10	
Multibacillary	0.453[a]	0	0.221	0.154	0.317	1.399	1.718[c]
(LL and BL)	0.641[b]	0.107	0.736	0	0.866	1.264	2.310[d]
Paucibacillary	ND[e]	0.030	ND	0.185	ND	ND	0.442
(TT)	ND	0.045	ND	0	ND	ND	1.107
Contacts	0.140	0	ND	0.076	0.220	0	0.234
	0.291	0	ND	0.222	0.451	0	0.590

[a] OD values protein concentration 5 μg/ml.
[b] OD values protein concentration 20 μg/ml.
[c] 2 μg/ml.
[d] 10 μg/ml.
[e] ND = Not done.

Work done in an endemic region of Ethiopia with 24 tuberculoid and 18 lepromatous patients and 21 healthy contacts using 11 antigenic molecules including *M. leprae* heat-shock protein (hsp) 10 and hsp18 and hsp65 did not show any association between the antigen-specific cellular response and the status of the various patients and controls studied ([13]).

Concerning cellular immunity, it is evident that the complete *M. leprae* protein extract is more immuogenic than individual proteins; if we study the percentage of positivity in the lymphocyte transformation tests of paucibacillary patients and family or nonfamily contacts, it is clear that several proteins are undoubtedly involved in the immune response. The stimulation index of T lymphocytes from lepromatous patients toward MlSA varied between 0.55, and 1.52; in tuberculoid patients the index varied between 1.72 and 10.02; in contacts, between 2.21 and 39.82.

ELISA study. IgG antibodies directed toward recombinant proteins were determined in an enzymatic assay ([10]). The values of circulating lgG antibodies directed toward complete extracts (cytosol and cell wall) and recombinants from *M. leprae* and *M. tuberculosis* were expressed in optical density (OD). Microtiter plates were sensitized with 5 μg/ml and 20 μg/ml recombinant antigens and 2 μg/ml and 10 μg/ml soluble *M. leprae* extract, 50 μl per well; one well was left with no antigen and two wells with antigen for each serum pool (LL and BL, T, and contacts) ([14]). Preliminary results using serum pools in the leprosy spectrum are shown in Table 3.

IgG antibodies responded in greater proportion to complex MlSA and *M. leprae* 10-kDa protein, and in decreasing order to *M. tuberculosis* 70, *M. leprae* 18, and *M. leprae* 36 kDa. With *M. bovis* 65 kDa it was not possible to obtain good detection in the groups studied. Other workers have found that in the majority of leprosy patients and their contacts, lgG reacted to both of the 70-kDa proteins from *M. tuberculosis* and *M. bovis* (BCG) ([7]). Quantitative differences found in this study using a pool of sera from patients and contacts are not clear. At present, we are developing sequential studies before, during and after multidrug therapy in individual patient's sera.

—Elsa Maria Rada, M.Sc.
Nacarid Aranzazu, M.D.
Jacinto Convit, M.D.

Instituto de Biomedicina
Laboratorio de Leprología
Apartado 4043
Caracas 1010A, Venezuela

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REFERENCES

1. BOOTH, R. J., HARRIS, D. P., LOVE, J. M. and WATSON, J. D. Antigenic properties of *Mycobacterium leprae*, complete sequence of the gene for the 18 kDa protein. J. Immunol. **140** (1988) 597–601.

2. BUCHANAN, T. M., NOMAGUCHI, H., ANDERSON, D. C., YOUNG, R. A., GILLIS, T. P., BRITTON, W. J., IVANJI, J., KOLK, A. H., CLOSS, O., BLOOM, B. R. and MEHRA, V. Characterization of antibody-reactive epitopes on the 65-kilodalton protein of *Mycobacterium leprae*. Infect. Immun. **55** (1987) 1000–1003.
3. CONVIT, J., ARANZAZU, N., ULRICH, M., PINARDI, M. E., REYES, O. and ALVARADO, J. Immunotherapy with a mixture of *Mycobacterium leprae* and BCG in different forms of leprosy and Mitsuda-negative contacts. Int. J. Lepr. **50** (1982) 415–424.
4. DRAPER, P. Protocol 1/79: Purification of *M. leprae*. Report of the enlarged Steering Committee for Research on the Immunology of Leprosy (IMMLEP) Meeting of 7–8 February 1979. Geneva: World Health Organization, 1979, Annex 1, p. 4.
5. KAPLAN, G. and COHN, Z. A. The immunobiology of leprosy. Int. Rev. Exp. Pathol. **28** (1986) 45–78.
6. KLATSER, P. R., DE WITT, M. Y. and KOLK, A. H. J. An ELISA-inhibition test using monoclonal antibody for the serology of leprosy. Clin. Exp. Immunol. **62** (1985) 468–473.
7. LAUNOIS, P., NIANG, M. N., DROWART, A., VAN VOOREN, J. P., SARTHOU, J. L., LALU, T., MILLAN, J. and HUYGEN, K. IgG response to purified 65- and 70-kDa mycobacterial heat shock proteins and to antigen 85 in leprosy. Int. J. Lepr. **62** (1994) 48–54.
8. MEHRA, V., BLOOM, B. R., BAJARDI, A. C., GRISSO, C. L., SIELING, P. A., ALLAND, D., CONVIT, J., FAN, X.-D., HUNTER, S. W., BRENNAN, P. J., REA, T. H. and MODLIN, R. L. A major T cell antigen of *Mycobacterium leprae* is a 10 kD heat-shock cognate protein. J. Exp. Med. **175** (1992) 275–284.
9. RADA, E., SANTAELLA, C., ARANZAZU, N. and CONVIT, J. Preliminary study of cellular immunity to *Mycobacterium leprae* protein in contacts and leprosy patients. Int. J. Lepr. **60** (1992) 189–194.
10. RADA, E., ULRICH, M., ARANZAZU, N., SANTAELLA, C., GALLINOTO, M. E., CENTENO, M., RODRIGUEZ, V. and CONVIT, J. A longitudinal study of immunologic reactivity in leprosy patients treated with immunotherapy. Int. J. Lepr. **62** (1994) 552–558.
11. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity; a five-group system. Int. J. Lepr. **34** (1966) 255–273.
12. ROCHE, P. W., THEUVENET, W. J. and BRITTON, W. Cellular immune responses to mycobacterial heat shock proteins in Nepali leprosy patients and controls. Int. J. Lepr. **60** (1991) 36–43.
13. THOLE, J. E. R., JANSON, A. A. M., KIFLE, A., HOWE, R. C., MCLEAN, K., NURILYGN, A., FILLEY, E., SHANNON, E. J., BULLA, G. J., HERMANS, J., DE VRIES, R. R. P., FROMMEL, D. and RINKE DE WIT, T. Analysis of T-cell and B-cell responses to recombinant *M. leprae* antigens in leprosy patients and healthy contacts, significant T-cell responses to antigens in *M. leprae* nonresponders. Int. J. Lepr. **63** (1995) 369–380.
14. ULRICH, M., SMITH, P., SAMPSON, C., ZUÑIGA, M., CENTENO, M., GARCÍA, V., MANRIQUE, X., SALGADO, A. and CONVIT, J. IgM antibodies to native phenolic glycolipid-I in contacts of leprosy patients in Venezuela; epidemiological observations and a prospective study of the risk of leprosy. Int. J. Lepr. **59** (1991) 405–415.
15. YOUNG, D. B., KAUFMANN, S. H. E., HERMANS, P. W. M. and THOLE, J. E. R. Mycobacterial protein antigens. Mol. Microbiol. **6** (1992) 133–145.