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Analysis of T-Cell and B-Cell Responses to Recombinant *M. leprae* Antigens in Leprosy Patients and in Healthy Contacts: Significant T-Cell Responses to Antigens in *M. leprae* Nonresponders[1]

Jelle E. R. Thole, Anneke A. M. Janson, Alemayu Kifle, Rawleigh C. Howe, Kimberly McLean, Ayenew Nurilygn, Elaine Filley, Edward J. Shannon, G. Jaffero Bulla, Jo Hermans, Rene R. P. de Vries, Dominique Frommel, and Tobias F. Rinke de Wit[2]

It is believed that upon infection with *Mycobacterium leprae* the nature of the T-cell immune response in the exposed individual determines whether leprosy will develop or whether protection will be induced. Healthy exposed individuals and tuberculoid leprosy patients are both characterized by a strong T-cell response to *M. leprae* which limits infection in healthy individuals but which may be pathogenic to the host in tuberculoid patients. Lepromatous leprosy is typically characterized by a specific T-cell nonresponsiveness to *M. leprae* and is associated with dissemination of the leprosy bacillus throughout the host.

To identify the molecules of *M. leprae* involved in these immune responses, biochemical purification procedures and

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[2] J. E. R. Thole, Ph.D.; A. A. M. Janson; R. R. P. de Vries, M.D., Department of Immunohaematology and Bloodbank, University Hospital Leiden, P. O. Box 9600, 2300 RC Leiden, The Netherlands. A. Kifle; R. C. Howe, Ph.D.; K. McLean, Ph.D.; A. Nurilygn, B.Sc.; D. Frommel, M.D., Ph.D.; T. F. Rinke de Wit, Ph.D., Armauer Hansen Research Institute (AHRI) P. O. Box 1005, Addis Ababa, Ethiopia. E. J. Shannon, Ph.D., Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, P. O. Box 25072, Baton Rouge, Louisiana 70894, U.S.A. G. J. Bulla, Leprosy Control Division of ALERT (ALC), P. O. Box 165, Addis Ababa, Ethiopia. J. Herman, Ph.D., Department of Medical Statistics, University Hospital Leiden, P. O. Box 9600, 2300 RC Leiden, The Netherlands. E. Filley, B.Sc., Department of Medical Microbiology, University College London Medical School, Riding House Street, London W1P 7PN, U.K.

Reprint requests to J. E. R. Thole, Ph.D., Department of Medical Microbiology, St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG, U.K. FAX = 44-71-2626299.

TABLE 1. M. leprae *antigens used in this study.*

Name	Description	Reference
M. leprae	Sonicate of *M. leprae* (batch CD179) derived from armadillo tissue	
M. tuberculosis	Sonicate of *M. tuberculosis*	
hsp10	Recombinant *M. leprae* 10-kDa heat-shock antigen	15
hsp18	Recombinant *M. leprae* 18-kDa heat-shock antigen	5
hsp65	Recombinant *M. leprae* 65-kDa heat-shock antigen	14
L1	Recombinant fusion protein containing amino acids 295–408 of a 45-kDa *M. leprae*-specific antigen	24, 25
L2	Recombinant fusion protein of 154-kDa	25
LL2	Recombinant fusion protein containing C-terminal 188 amino acids of the secreted *M. leprae* 30–31-kDa antigen 85 complex component A	22
L7	Recombinant fusion protein containing amino acids 55–266 of the secreted *M. leprae* 30–31-kDa antigen 85 complex component B	25, 28
L8	Recombinant fusion protein containing amino acids 23–112 of a *M. leprae* 15-kDa antigen	25, 26
L14	Recombinant fusion protein containing amino acids 58–287 of the secreted 25.5-kDa (43L) antigen of *M. leprae*	25, 31
L43	Recombinant fusion protein of 140-kDa	25
L44	Recombinant fusion protein containing amino acids 265–327 of the secreted *M. leprae* 30–31-kDa antigen 85 complex component B	25, 28

screening of recombinant DNA expression libraries with monoclonal antibodies and patient sera have led to the identification of a large number of protein antigens (reviewed in [33]). Analyses of T-cell responses of subjects exposed to *M. leprae* thus far have indicated that heat-shock proteins (hsp) of 10 kDa, 18 kDa, 65 kDa and 70 kDa, as well as a 36-kDa protein and a family of 30/31-kDa secreted fibronectin-binding proteins are important T-cell antigens ([2, 6, 8, 10, 11, 15–18]). However, the contributions of the various antigen-specific T-cell responses to protection or pathogenesis of leprosy remain unclear.

The present study was initiated to examine the ability of T cells as well as B cells to respond to a panel of *M. leprae* protein antigens in patients and healthy controls in leprosy-endemic regions of Ethiopia. The results demonstrate that distinct differences do exist between the abilities of individual antigens to stimulate proliferation (and antibody production) in the subjects studied. However, these antigen-specific responses did not show a clear association with leprosy status. Thus, no evidence was found for a potential role of particular antigens in the induction of protection or in the pathogenesis of leprosy. Secondly, it was established that the specific nonresponsiveness to *M. leprae* sonicates in lepromatous (LL) leprosy patients is not reflected by specific nonresponsiveness to any of the *M. leprae* antigens tested.

MATERIALS AND METHODS

Patients and contacts. Forty-seven leprosy patients, classified according to Ridley-Jopling criteria, were included in this study. Thirty-five patients were recruited from Addis Ababa and the surrounding Ethiopian highlands by the ALERT Leprosy Control Unit. Twelve patients were recruited from the Rift Valley near Sashamene by the National Leprosy Control Programme which is funded by the German Leprosy Relief Association (GLRA). Patients were grouped as paucibacillary [borderline tuberculoid leprosy (BT) = 26; polar tuberculoid leprosy (TT) = 3] and as multibacillary [lepromatous leprosy (LL) = 18]. Forty-two of the patients studied were receiving multiple drug treatment (MDT); five BT patients (BT20, 38, 63, 66, 70) and one TT patient (TT53) were studied before treatment. Twenty-one healthy household contacts (HC) of leprosy patients living in a rehabilitation village near the ALERT hospital in Addis Ababa were studied: 18 contacts were Mitsuda positive, 1 was negative and 2 were not tested. The household contacts were not related to any of the patients involved in this study.

Antigens. The antigens used in this study are listed in Table 1. Purified recombinant proteins *M. leprae* hsp10 and hsp65 were

obtained from J. van Embden through the WHO/TDR/IMMLEP Special Programme. *M. leprae* sonicates were obtained from R. Rees; *M. tuberculosis* sonicates from A. Kolk; purified *M. leprae* hsp18 from J. Watson. *Escherichia coli* strains carrying pEX2 containing *M. leprae* DNA inserts derived from λgt11 recombinants L1, L2, LL2, L7, L8, L14, L43, and L44 ([21, 24]) were established as described ([28]). Semipurified crobeta-galactosidase fusion proteins were prepared from induced lysates as described ([27]). Fusion proteins were approximately 50% pure, as estimated by the protein profiles from SDS/PAGE gels stained with Coomassie brilliant blue.

Peripheral blood mononuclear cells (PBMC) and sera. PBMC were collected by venipuncture using heparinized vacutainer tubes (Becton, Dickinson, Mechelen, Belgium). PBMC were isolated by Ficoll-Paque (Pharmacia, Uppsala, Sweden) centrifugation and resuspended in culture medium containing RPMI-1640 medium (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) supplemented with penicillin (100 IU/mlIBCO, Gaithersburg, Maryland, U.S.A.) and glutamine (2 mM; ICN Flow, High Wycombe, U.K.). Serum was separated from whole blood and stored, without preservative, at −70°C until use.

Proliferation assay. Isolated PBMC (10^5 cells/well) were cultured in 96-well, round-bottom, microtiter plates (Costar Corporation, Cambridge, Massachusetts, U.S.A.) in culture medium containing 10% pooled human serum and antigen. Sonicates of *M. leprae* and *M. tuberculosis* were used at two different concentrations of 1 μg/ml and 10 μg/ml; purified recombinant *M. leprae* antigens at concentrations of 2 μg/ml and 20 μg/ml. Semipurified fusion proteins were used at dilutions of 1/100 and 1/5000. Control wells contained either phytohemagglutinin (PHA, 2 μg/ml; Wellcome Diagnostics, Dartford, U.K.), semipurified crobeta-galactosidase (nonfused) proteins, or culture medium alone. Cultures were set up in triplicate and incubated for 6 days at 37°C in a fully humidified atmosphere containing 5% CO_2. For the last 18 hr, the cultures were pulsed with 1.0 μCi of ^{3}H-thymidine (specific activity, 5.0 μCi/mmol; Amersham International, Amersham, U.K.) per well. The cells were then harvested onto glass-fiber filters with a semi-automatic sample harvester. ^{3}H-thymidine incorporation was assessed by liquid scintillation spectroscopy. The results of the responses to sonicates of *M. leprae, M. tuberculosis* and to the purified proteins hsp10, hsp18 and hsp65 are expressed as a stimulation index (SI) which is the ratio of ^{3}H-thymidine incorporation of antigen-stimulated cultures to that of the control cultures, containing neither antigen nor PHA. The results of the responses to the (semi)purified recombinant fusion proteins L1, L2, LL2, L7, L8, L14, L43 and L44 are expressed as SI, which is the ratio of ^{3}H-thymidine incorporation of antigen-stimulated cultures to that of cultures containing semipurified crobeta-galactosidase proteins as expressed by pEX2 vector. A SI of ≥3 was taken as a positive response. The average PHA responses were 26, 805 counts per minute (cpm) for the 21 HC, 25,031 cpm for the 29 BT/TT patients, and 19, 453 cpm for the 18 LL patients.

Immunoblotting and ELISA. Eight percent sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli ([9]). Either 200 ng purified hsp10 or 50 ng purified hsp65 were loaded per lane. Gels were blotted onto nitrocellulose membranes (BA-83; Schleicher & Schuell, Dassel, Germany) using a 0.2 amp current for 2 hr in blotting buffer according to Maniatis ([13]). The nitrocellulose membranes were blocked overnight with 50 mM Tris-HCl pH 8.0; 150 mM NaCl; 5% ovalbumin; 0.01% NaN3 at room temperature prior to incubation with antisera. Sera were tested at 1/100 (HC and BT/TT) or 1/500 (LL) dilutions.

ELISA was performed essentially as described previously ([12]). Sonicates of *M. leprae* and *M. tuberculosis* and hsp10 and hsp65 antigens were tested at 200 ng/well; and phenolic glycolipid-I (PGL-I) coupled to human serum albumin was tested at 50 ng/well and compared to uncoupled human serum albumin (50 ng/well). Sera were tested at 1/100 dilutions for antibodies reactive with hsp10 and hsp65, and at dilutions of 1/150, 1/400 and 1/600 for sonicates of *M. leprae* and *M. tuberculosis.* The serum samples were added to quadruplicate wells of the microtiter plate (Dynatech Immulon): two with antigen and two without antigen. The plates were incubated at room temper-

ature for 2 hr. Alkaline phosphatase conjugated F(ab')2 fragments of affinity isolated anti-human gamma or anti-human mu chain or anti-human gamma, alpha and mu chain (Sigma) were used as secondary antibodies. P-nitrophenylphosphate disodium (Sigma 104 phosphatase substrate tablets) was used as a substrate and the reaction was stopped by the addition of 4 N NaOH after 20 min. Absorbances were read at 405 nm using a Titertek Multiscan spectrophotometer (Flow Labs, Richmond, Virginia, U.S.A.), and mean absorbance values were calculated. The antibody reactivity to the coated antigen for each serum sample was calculated by subtracting the mean absorbance of duplicate samples in buffer-coated wells from the mean absorbance of duplicate antigen-coated wells. Delta absorbances of ≥ 0.20 were considered positive.

RESULTS

Proliferative T-cell responses to *M. leprae* antigens. Pilot experiments demonstrated that freshly isolated human peripheral mononuclear cells (PBMC) gave significantly higher T-cell proliferative responses than did frozen PBMC upon stimulation with *M. leprae* and control antigens (data not shown). Therefore, we performed our studies on fresh PBMC within 48 hr after collection of blood samples. Tables 2A-2C summarize the T-cell proliferative responses of the subjects studied, grouped according to their disease status (HC, BT/TT and LL). Three purified *M. leprae* antigens (hsp10, hsp18 and hsp65) and eight semipurified *M. leprae* proteins fused to *E. coli* crobeta-galactosidase were tested.

High frequencies of responses to *M. leprae* sonicates were found in HC (95%) and in BT/TT subjects (72%). Only 11% of the Ll patients studied responded to *M. leprae* sonicate, a finding in agreement with the previously reported *M. leprae*-specific nonresponsiveness at the lepromatous pole of the leprosy spectrum. In all three subject groups the frequency of responses to *M. tuberculosis* sonicates was high (90%).

A large variability in responses to the three purified and eight semipurified recombinant *M. leprae* proteins tested was found in the three subject groups. For example, BT patient 49 recognized 6 of 11 T-cell antigens versus BT patient 45 who recognized 0 of 11 T-cell antigens.

Among the purified *M. leprae* antigens, hsp65 was most frequently recognized in HC (33%), followed by hsp10 (19%) and hsp18 (10%). In patients the frequencies of proliferative T-cell responses to these three antigens were more similar (7%-10% in BT/TT patients; 11%-22% in LL patients). Among the semipurified *M. leprae* antigens the frequencies of T-cell responses were highest to antigens L14 (6%-19%), LL2 (14%-17%) and L7 (6%-14%), all of which are secreted. Frequencies of recognition to the other, nonsecreted antigens ranged from 0% to 11%. None of the antigens was shown to be exclusively recognized by any of the three subject groups, which would suggest that none of these antigens played a particular role in protection or in disease.

With respect to MDT treatment, no correlation could be demonstrated between duration of treatment and recognition profiles of any tested recombinant *M. leprae* antigen. No differences in T-cell responses to any *M. leprae* antigens could be detected between MDT untreated and treated individuals.

B-cell recognition of *M. leprae* antigens. To evaluate the antibody responses to purified hsp10 and hsp65 antigens, we employed ELISA and Western blotting techniques (Tables 3A–3C). All LL patients (100%) and the majority of BT/TT patients (86%) and HC (90%) reacted to *M. leprae* sonicates in ELISA. With respect to *M. tuberculosis,* serum antibodies were detected in 78% of LL patients, 41% of BT/TT patients and 60% of HC.

Combining the ELISA and Western blotting results, antibodies to *M. leprae* hsp10 and hsp65 appeared more frequently in the LL group than in the BT/TT and the HC groups. The fact that in the LL group antibodies to hsp65 were only detected by Western blotting and not by ELISA suggests that these antibodies are mainly directed to linear determinants. The existence of mainly linear determinants on hsp65 has been reported previously, and is thought to reflect its predominant existence as an unfolded molecule which is more amenable to proteolytic degradation ([34]). Comparisons of T-cell versus B-cell responses in the three groups of subjects studied did not reveal any

TABLES 2A–2C. *T-cell proliferative responses of Ethiopian HC (Table 2A), BT/TT (Table 2B) and LL (Table 2C) to purified recombinant* M. leprae *heat-shock proteins (hsp18, hsp19, and hsp65), semipurified recombinant* M. leprae *antigens (L1, L2, LL2, L7, L8, L14, L43 and L44) and (as controls) PHA, sonicates of* M. leprae *(M. lep) and* M. tuberculosis *(M. tub). Patient information is shown in terms of Mitsuda tests (Mit), years of multidrug treatment (MDT), the areas where the subjects live (H = highlands, L = lowlands) and IgM antibodies against PGL-I as tested by ELISA. T-cell responses are given as SI values, calculated as described in Materials and Methods. Of the various antigen concentrations tested, the highest SI values are shown. SI values of ≥3.0 are considered as positive responses; SI values of <3.0 are represented by −. % = Percent positivity in each subject group.*

TABLE 2A. *Healthy contacts.*

subject	Mit	area	PGL1	PHA	M.lep	M.tub	hsp10	hsp18	hsp65	L1	L2	LL2	L7	L8	L14	L43	L44
HC02	+	H	-	15.5	-	-	-	-	-	-	-	-	-	-	3.2	-	-
HC03	+	H	-	65.6	11.1	36.8	-	-	-	-	-	-	4.3	-	-	-	-
HC04	ND	H	-	123.6	6.5	23.8	-	-	-	-	-	-	-	-	-	-	-
HC05	+	H	-	18.4	11.2	12.1	-	-	-	-	-	-	-	-	-	-	-
HC10	-	H	-	40.9	20.4	37.2	6.1	5.2	9.4	-	-	-	-	-	-	-	-
HC11	+	H	-	14.7	10.9	24.2	-	-	3.6	-	-	-	-	-	-	-	-
HC12	+	H	-	46.6	4.3	15.3	-	-	-	-	-	-	-	-	-	-	-
HC13	-	H	-	37.2	8.2	37.4	-	-	3.2	-	-	3.8	-	-	-	-	-
HC14	+	H	-	29.0	3.1	19.4	-	-	-	-	-	-	-	-	-	-	-
HC15	+	H	-	10.9	3.8	25.2	-	-	-	-	-	-	-	-	-	-	-
HC16	+	H	-	16.0	10.7	27.7	-	3.3	-	-	-	-	-	-	-	-	-
HC17	+	H	-	46.6	25.0	28.4	-	-	-	-	-	-	-	-	-	-	-
HC21	+	H	-	1.7	21.2	26.2	-	-	5.8	3.7	5.9	7.2	-	-	7.8	-	-
HC23	+	H	-	13.1	4.4	7.5	-	-	-	-	-	-	-	-	-	-	-
HC24	+	H	-	2.3	27.2	22.2	10.1	-	-	-	-	-	4.3	-	-	-	-
HC25	+	H	-	5.6	9.0	34.4	-	-	-	5.1	-	-	-	-	3.7	3.4	-
HC40	+	H	-	16.7	17.6	51.1	3.9	-	7.2	-	-	3.2	4.0	-	-	-	-
HC41	ND	H	-	4.1	18.9	30.6	-	-	5.0	-	-	-	-	-	3.4	-	-
HC42	ND	H	-	9.6	51.9	72.2	5.2	-	12.8	-	-	-	-	-	-	-	-
HC43	+	H	-	3.0	20.4	50.9	-	-	-	-	-	-	-	-	-	-	-
HC44	+	H	-	6.8	13.1	18.7	-	-	-	-	-	-	-	-	-	-	-
%					95	95	19	10	33	10	5	14	14	0	19	5	0

TABLE 2B. *Tuberculoid leprosy patients.*

subject	MDT	area	PGL1	PHA	M.lep	M.tub	hsp10	hsp18	hsp65	L1	L2	LL2	L7	L8	L14	L43	L44
BT01	1	H	-	60.1	13.8	16.7	-	-	-	-	-	-	-	-	-	-	-
BT18	1.5	H	-	23.6	7.1	6.6	-	-	-	-	-	-	-	-	4.5	-	-
TT19	1	H	-	56.3	22.6	30.1	-	-	-	-	-	4.5	3.0	-	-	3.1	-
BT20	-	H	-	3.8	31.6	43.0	-	-	3.5	4.4	-	3.3	-	-	-	-	-
BT22	?	H	-	ND	18.3	24.8	-	3.0	-	-	-	-	-	-	3.0	-	-
BT38	-	L	-	14.1	3.5	7.7	-	-	-	-	-	-	-	-	-	-	-
BT39	0.5	L	-	15.7	4.9	37.2	-	-	-	-	-	3.6	-	-	-	-	-
BT45	0.4	L	-	66.3	8.7	9.2	-	-	-	-	-	-	-	-	-	-	-
BT46	1.5	L	-	210.4	-	-	-	-	-	-	-	-	-	-	-	-	-
BT47	0.2	L	+	120.7	-	18.9	-	-	-	-	-	-	-	-	-	-	-
BT48	0.2	L	-	132.5	7.7	11.8	-	-	-	-	-	4.1	-	-	-	-	-
BT49	0.2	L	-	29.8	10.0	8.7	-	-	4.0	3.1	3.8	-	-	3.6	3.9	-	4.6
BT50	2	L	-	63.0	13.3	44.2	-	5.7	5.7	-	-	-	-	-	-	-	-
BT51	1	L	-	23.6	-	7.7	-	-	-	-	-	-	-	-	-	-	-
BT52	1.5	L	-	62.3	-	3.1	-	-	-	-	-	-	-	-	3.2	-	3.8
TT53	-	L	-	30.9	3.9	28.3	-	-	-	-	-	-	-	-	-	-	-
BT54	0.2	L	-	114.1	9.5	3.7	-	-	-	-	-	-	-	-	-	-	-
BT55	0.2	L	-	27.3	-	5.9	-	-	-	-	-	-	-	-	-	-	-
BT56	1.5	L	-	70.3	-	71.4	-	-	-	-	3.1	-	-	-	-	-	-
BT62	0.2	H	-	36.4	8.4	5.3	-	-	-	-	-	-	-	-	-	-	-
BT63	-	H	-	44.1	6.6	9.8	3.4	-	-	-	-	-	-	-	-	-	-
TT65	0.1	H	-	10.1	3.4	6.0	-	-	-	-	-	-	-	-	-	-	-
BT66	-	L	-	31.6	3.2	41.8	-	-	-	-	-	-	-	-	-	-	-
BT67	0.5	L	-	109.6	31.3	18.1	-	-	-	-	-	-	-	-	-	-	-
BT68	0.5	L	-	361.7	4.2	44.7	3.3	4.2	-	-	-	-	-	-	-	-	-
BT69	0.5	L	-	145.0	9.4	13.8	-	-	-	-	-	-	-	-	-	-	3.5
BT70	-	L	-	103.1	-	-	-	-	-	-	-	-	5.6	-	-	-	-
BT71	0.5	L	-	60.2	-	42.6	-	-	-	3.3	-	-	-	-	-	-	-
BT72	0.5	L	-	29.8	23.5	46.3	-	-	-	-	-	-	9.6	-	-	-	-
%					72	93	7	10	10	10	7	14	10	3	14	3	10

TABLE 2C. *Lepromatous leprosy patients.*

subject	MDT	area	PGL1	PHA	M.lep	M.tub	hsp10	hsp18	hsp65	L1	L2	LL2	L7	L8	L14	L43	L44
LL06	8	H	+	76.9	-	22.3	-	-	-	-	-	-	-	-	-	-	-
LL07	3.2	H	-	60.9	-	34.3	-	-	-	-	-	-	-	-	-	-	-
LL08	6	H	-	72.4	-	5.3	-	-	-	-	-	-	-	-	-	-	-
LL09	4.5	H	-	74.5	-	38.0	-	-	-	-	-	3.4	-	3.2	-	-	-
LL26	0.8	H	+	14.9	-	27.7	-	-	-	-	-	-	3.4	-	-	-	3.2
LL27	1.5	H	+	20.9	-	37.6	6.0	3.8	3.3	-	-	-	-	-	-	-	-
LL28	5	H	+	11.5	-	-	-	-	-	-	-	-	-	-	-	-	-
LL29	7.5	H	-	15.6	-	18.8	-	-	-	-	-	-	-	-	-	-	-
LL30	3.5	H	-	26.9	-	10.1	3.0	-	-	-	-	-	-	-	-	-	-
LL31	0.3	H	+	5.1	-	29.1	-	-	-	-	-	-	-	-	-	-	-
LL32	6	H	+	6.6	-	7.0	-	-	-	-	-	-	-	-	-	-	-
LL33	8	H	-	8.3	7.0	56.6	6.5	4.7	7.2	-	-	-	-	-	-	-	-
LL34	4	H	+	8.2	3.8	5.8	3.3	-	3.6	-	-	3.0	-	-	3.3	-	-
LL35	1.5	H	+	14.5	-	11.2	-	-	-	-	-	-	-	-	-	-	-
LL37	2	L	-	50.5	-	43.3	-	-	-	-	-	-	-	-	-	-	-
LL57	1.8	H	+	97.3	-	-	-	-	-	-	-	-	-	-	-	-	-
LL58	4.5	H	+	29.2	-	3.7	-	-	-	-	-	3.2	-	6.6	-	-	-
LL60	21	H	-	62.5	-	39.8	-	-	-	-	-	-	-	-	-	-	-
n					11	89	22	11	17	0	0	17	6	11	6	0	6

TABLE 3A. *Healthy contacts.*

	ELISA				W.blot	
subject	M.lep	M.tub	hsp10	hsp65	hsp10	hsp65
HC02	0.49	0.32	-	-	-	-
HC03	0.39	0.28	-	-	-	-
HC04	0.29	0.26	-	-	-	-
HC05	0.22	-	-	-	-	-
HC10	0.27	-	-	-	-	++
HC11	0.25	-	-	-	-	-
HC12	0.68	0.50	-	-	-	-
HC13	0.45	0.37	-	-	-	+
HC14	-	-	-	-	-	-
HC15	0.54	0.21	-	-	-	-
HC16	0.40	0.28	-	-	-	-
HC17	0.24	-	-	-	-	-
HC21	0.33	0.28	-	0.28	-	-
HC23	ND	ND	-	-	ND	-
HC24	0.51	0.45	-	-	-	-
HC25	0.30	0.23	-	-	-	-
HC40	0.24	-	-	-	ND	-
HC41	0.32	0.23	-	-	-	-
HC42	-	-	-	-	-	-
HC43	0.30	0.27	-	-	-	-
HC44	0.23	-	-	-	-	+
%	90	60	0	5	0	14

TABLE 3B. *Tuberculoid leprosy patients.*

	ELISA				W.blot	
subject	M.lep	M.tub	hsp10	hsp65	hsp10	hsp65
BT01	0.21	0.25	-	-	+	-
BT18	0.41	-	-	-	-	-
TT19	0.38	0.32	-	-	-	-
BT20	0.32	0.24	-	-	-	+
BT22	0.28	0.21	-	-	-	-
BT38	0.33	-	-	-	-	-
BT39	0.23	-	-	-	-	-
BT45	-	-	-	-	-	-
BT46	0.51	0.29	-	-	-	-
BT47	0.62	0.37	-	-	-	-
BT48	0.25	-	-	-	-	-
BT49	-	-	-	-	-	-
BT50	0.38	0.21	-	-	-	-
BT51	0.30	-	-	-	-	-
BT52	0.23	-	-	-	-	-
TT53	0.39	0.22	-	-	-	-
BT54	0.25	-	-	-	-	-
BT55	0.24	0.21	-	-	-	-
BT56	0.25	-	-	-	-	-
BT62	0.31	0.21	-	-	-	-
BT63	-	-	-	-	-	-
TT65	-	-	-	-	-	-
BT66	0.41	0.33	-	-	-	-
BT67	0.21	-	-	-	-	-
BT68	0.32	-	-	-	-	-
BT69	0.32	0.28	-	-	-	-
BT70	0.25	-	-	0.21	-	+
BT71	0.24	-	-	-	-	-
BT72	0.20	-	-	-	-	-
%	86	41	0	3	3	7

TABLE 3C. *Lepromatous leprosy patients.*

	ELISA				W.blot	
subject	M.lep	M.tub	hsp10	hsp65	hsp10	hsp65
LL06	0.61	0.35	-	-	-	-
LL07	0.55	0.35	-	-	-	-
LL08	0.41	0.31	-	-	-	-
LL09	0.72	0.40	-	-	-	-
LL26	0.69	0.43	-	-	-	-
LL27	0.54	0.27	-	-	-	-
LL28	0.59	0.30	0.28	-	+	-
LL29	0.63	0.29	-	-	-	++
LL30	0.62	0.34	-	-	-	-
LL31	0.74	0.43	0.33	-	+	++
LL32	0.50	0.23	-	-	+	+
LL33	0.48	0.22	-	-	-	-
LL34	0.62	0.38	-	-	-	+
LL35	0.59	0.29	-	-	-	+
LL37	0.57	0.33	0.35	-	+	+
LL57	0.51	0.24	-	-	-	++
LL58	0.48	-	0.42	-	+	++
LL60	0.28	-	-	-	-	+
%	100	78	22	0	28	50

TABLES 3A–3C. *Antibody responses as measured by ELISA and Western blots of HC (Table 3A), BT/TT (Table 3B), and LL (Table 3C) to sonicates of* M. leprae *(M. lep),* M. tuberculosis *(M. tub) and purified* M. leprae *hsp10 and hsp65 proteins. Western blot information was interpreted by visual inspection; ELISA values are given in Δ absorbance values. Δ absorbances of <0.20, which are considered negative responses, are represented by −. ND = Not done.*

striking correlations (Tables 2A-2C versus 3A-3C).

DISCUSSION

In this study we have compared immune responses to 11 *M. leprae* antigens in 21 healthy household contacts and 47 leprosy patients from Ethiopia, a country where leprosy is endemic.

A major question we wanted to address by this study was whether there is a role for any particular *M. leprae* antigen in either immunopathology or protection as judged by T-cell proliferation. A role for secreted antigens in protective immunity has been proposed since these antigens are actively secreted by live bacteria and, thus, are available for immune recognition early in infection.

A limited amount of protection in an animal model for tuberculosis after immuni-

zation with secreted proteins was recently reported ([20]). In the present study, we found that the response to secreted antigens, i.e., 30/31-kDa and 25.5-kDa antigens, is not restricted to any particular patient group and, thus, is not predictive for protection or pathogenesis. Our study confirms earlier data ([3, 4, 11]) that secreted antigens frequently induce proliferative responses ([23, 31]). However, the responses toward heat-shock proteins (hsp) were found to be equally high, and this finding indicates that antigens from different compartments serve as frequent targets for proliferative T cells. Mycobacterial hsp have been implicated in immunopathological phenomena ([21]). Autologous hsp may form a target for T cells that were originally raised to structurally similar *M. leprae* hsp molecules. Proliferative T cells specific for mycobacterial hsp65 have been shown to induce autoimmune phenomena in a rat model ([30]), and it has been hypothesized that a similar reactivity may be involved in some of the immunopathological phenomena associated with leprosy ([11]). Although a considerable proportion of the subjects in our study recognized hsp65 and other hsp, no support for a particular role for hsp in the induction of immunopathology was found, and hsp were also frequently recognized by healthy individuals.

Thus, with regard to hsp and secreted antigens and the other antigens studied, we found an overall similarity in the antigenic repertoire recognized in healthy individuals and patients. Although a different picture may emerge when using different read-out systems, like cytotoxicity or cytokine production, this similarity in antigen recognition may indicate that the key to distinguishing protective from disease-associated immunity may lie in the exact nature of the T-cell response. It may be that the magnitude, quality and, perhaps, timing of the T-cell response rather than the recognized antigenic repertoire ultimately determines the outcome of an infection with *M. leprae.*

A potentially important finding in this study was that T-cell nonresponsiveness of 5 LL patients (LL09, 26, 27, 30, 58), 4 BT patients (BT52, 56, 70, 71) and 1 HC (HC02) to *M. leprae* sonicates is not reflected by nonresponsiveness to individual antigens of *M. leprae* (Tables 2B-2C). For example, the *M. leprae* nonresponder LL27 showed significant T-cell proliferation to all three purified *M. leprae* hsp and the nonresponders LL09, LL26, LL58 and BT52 responded to two semipurified crobeta-galactosidase *M. leprae* fusion proteins. The above finding confirms previous work for recombinant *M. leprae* antigens ([16, 18, 26]), and opens up possibilities to study the mechanisms underlying specific nonresponsiveness to whole *M. leprae.* The outcome of such studies may, ultimately, be helpful in the design of immunotherapy aimed at triggering antigen-specific immunity in nonresponsive leprosy patients.

SUMMARY

The recognition of a panel of recombinant *Mycobacterium leprae* antigens by T cells and B cells from 29 borderline tuberculoid/tuberculoid (BT/TT) and 18 lepromatous leprosy (LL) patients and from 21 healthy controls (HC) in leprosy-endemic regions of Ethiopia was examined. All 11 antigenic molecules tested (including *M. leprae* hsp10, hsp18, hsp65 and several novel *M. leprae* antigens) were shown to be recognized by T cells, but clear quantitative differences existed between reactivities induced by individual antigens. Similar quantitative differences were observed when antibody responses to hsp10 and hsp65 antigens were determined. No associations were found between the antigen-specific responses and the subject status of either BT/TT and LL patients or HC. Fifteen percent of the patients who were nonresponsive to sonicates of *M. leprae* showed significant T-cell responses to one or more individual *M. leprae* antigens. This indicates that *M. leprae* constituents other than the proteins tested are responsible for the *M. leprae*-specific nonresponsiveness in these patients, which may be exploited for the design of vaccines or immunotherapeutic modalities aimed at inducing *M. leprae*-specific immunity in nonresponders.

RESUMEN

Se examinó el reconocimiento de un panel de antígenos recombinantes de *Mycobacterium leprae* por las células T y B de 29 pacientes con lepra tuberculoide/tuberculoide subpolar (BT/TT), de 18 pacientes con lepra lepromatosa (LL), y de 21 controles sanos (HC)

de regiones con lepra endémica en Etiopia. Las 11 moléculas antigénicas probadas (incluyendo hsp10, hsp18, hsp65 y varios antígenos nuevos de *M. leprae*) fueron reconocidos por las células T, aunque existieron claras diferencias cuantitativas entre las reactividades inducidas por los antígenos individuales. También se observaron diferencias cuantitativas similares en el caso de las respuestas en anticuerpo con los antígenos hsp10 y hsp65. No se encontraron asociaciones entre las respuestas específicas para un antígeno y el estado BT/TT, LL, o HC de los individuos. Quince porciento de los pacientes que no respondieron a sonicados totales de *M. leprae* mostraron significantes respuestas celulares (Lc T) hacia uno o más antígenos individuales de *M. leprae*. Se concluye que no son las proteínas probadas en este estudio, sino otros constituyents de *M. leprae*, los responsables de la anergia específica que muestran los pacientes hacia el bacilo de la lepra. Esto podría ser explotado en el diseño de vacunas o de modalidades inmunoterapéuticas tendientes a inducir inmunidad específica hacia *M. leprae* en los individuos no respondedores.

RÉSUMÉ

La reconnaissance d'un panel d'antigènes recombinants de *Mycobacterium leprae* par des cellules T et B provenent de 29 patients lépreux borderline tuberculoides et tuberculoides (BT/TT), de 18 patients lépromateux (LL) et de 21 témoins en bonne santé de régions d'Ethiopie endémiques pour la lèpre a été examinée. On a montré que toutes les 11 molécules antigéniques testées (y compris hsp10, hsp18, hsp65 et plusieurs antigènes "nouveaux" de *M. leprae*) étaient reeonnues par les cellules T, mais que des différences quantitatives évidentes existaient dans la réactivité induite par les antigènes individuels. De semblables différences quantitatives ont été observées quand on a déterminé les réponses en anticorps aux antigènes hsp10 et hsp65. Aucune association n'a été trouvée entre les réponses spécifiques pour l'antigène et le statut individuel des personnes, que ce soient des patients BT/TT ou LL ou des individuels en bonne santé. Quinze pourcents des personnes qui ne répondaient pas aux sonicats de *M. leprae* montraient des réponses significatives de leurs cellules T à un ou plusieurs antigènes individuels de *M. leprae*. Ceci indique que des constituants de *M. leprae* autres que les protéines testées sont responsables de la non-réponse spécifique pour *M. leprae* chez ces patients, ce qui pourrait être exploité pour la conception de vaccins ou de moyens immunothérapeutiques ayant pour but d'induire une immunité spécifique pour *M. leprae* chez les non-répondants.

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