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Identification of T-cell-activating Recombinant Antigens Shared Among Three Candidate Antileprosy Vaccines, Killed *M. leprae*, *M. bovis* BCG, and *Mycobacterium w*[1]

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Leprosy is a chronic mycobacterial disease caused by *Mycobacterium leprae*. Resistance against leprosy correlates with cell-mediated immune (CMI) responses to the infectious agent. Killed *M. leprae* purified from infected armadillo tissue are highly potent in inducing CMI responses in animal models ([14, 22]) and in human volunteers ([5, 6]). Vaccination with killed *M. leprae* protects mice from viable *M. leprae* challenge ([24]). These characteristics of killed *M. leprae* along with its nontoxic and nonpathogenic properties make it an ideal candidate for an antileprosy vaccine. However, since *M. leprae* has not been grown *in vitro,* infected armadillo tissue remains the major source of supply of the bacilli. In addition to high cost, this source may not provide enough bacteria required for vaccinating all of the people who are at risk of infection. Therefore, attempts are also being made to develop antileprosy vaccines based on cultivable mycobacteria, e.g., *M. bovis* BCG and *Mycobacterium w,* etc. Vaccination of animals with BCG ([24]) and *Mycobacterium w* ([20]) results in the induction of *M. leprae* skin-test reactivity. Immunization of mice with live BCG protects them from viable *M. leprae* challenge ([23]). Vaccination of *M. leprae* skin-test-negative lepromatous patients with *Mycobacterium w* converts about 60% of the patients into an *M. leprae* skin-test-positivity status and also results in clinical improvements ([2]). The above effects are said to be due to the sharing of those antigens of *M. leprae* by BCG and *Mycobacterium w* which are involved in CMI functions. However, the identity of such antigens is largely unknown.

In the present study, some of the antigens shared among *M. leprae,* BCG, and *Mycobacterium w* have been identified by testing the recombinant antigens of *M. leprae* and *M. tuberculosis* against human T-cell lines and clones raised from BCG and killed-*M. leprae*-vaccinated subjects.

MATERIALS AND METHODS

Antigens. Killed *M. leprae* were kindly supplied by Dr. R. J. W. Rees through the WHO/IMMLEP Bank. BCG was obtained from Serum Institute, Copenhagen, Denmark. Killed *Mycobacterium w* was a kind gift from Professor G. P. Talwar, National Institute of Immunology, New Delhi, India. These mycobacteria were used at concentrations optimal for T-cell proliferation, i.e., *M. leprae* at 5×10^7 bacilli/ml, BCG at 10 μg/ml (wet weight) and *Mycobacterium w* at 5×10^6 bacilli/ml.

The recombinant antigens of *M. leprae* identified by monoclonal antibodies which recognize *M. leprae* antigens of molecular weight 65 kilodalton (kDa), 36 kDa, 20 kDa, 18 kDa, and 12 kDa ([30]); the *M. leprae* 13B3 recombinant antigen identified from the recombinant *M. leprae* λgt11 library using human T-cell clones as probes (A. S. Mustafa, *et al.,* submitted for publication); and recombinant *M. tuberculosis* antigens identified by monoclonal antibodies which recognize *M. tuberculosis* antigens of molecular weight 65 kDa, 19 kDa and 14 kDa ([29]) were used in the present study. *Escherichia coli* lysates containing or lacking recombinant mycobacterial antigens were prepared according to the protocols described earlier

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([15, 21]). The lysates at a protein concentration of 50 μg/ml were used for T-cell proliferation assays.

T-cell clones. Forty-two *M. leprae*-induced, CD4+ T-cell clones were raised from the peripheral blood mononuclear cells (PBMC) of BCG-vaccinated and killed-*M. leprae*-immunized healthy volunteers ([15]). Eleven of these T-cell clones were *M. leprae* specific and the remaining crossreacted with other mycobacteria. Three of the *M. leprae*-specific and 12 crossreactive T-cell clones have been used in the present study.

T-cell lines. To establish T-cell lines from BCG-vaccinated and killed-*M. leprae*-immunized healthy subjects ([5]), 2×10^6 PBMC/ml complete medium (RPMI 1640 + 15% AB serum + 1% penicillin-streptomycin) were stimulated with optimal concentrations of *M. leprae*, BCG, and *Mycobacterium w* in the wells of 24-well Costar plates (Costar, Cambridge, Massachusetts, U.S.A.). The plates were incubated for 6 days at 37°C in an atmosphere of 5% CO_2 and 95% air. To expand the antigen-reactive cells, starting from day 6 recombinant interleukin-2 (IL-2) (a gift from Cetus Corporation, Emoryville, California, U.S.A.) was added to the cultures at 100 U/ml twice a week. After 4–5 weeks, the T-cell lines were tested for antigen responsiveness in proliferative assays.

Proliferative assay. Autologous adherent cells from 1×10^5 irradiated (2500 R) PBMC added to the wells of 96-well Costar plates served as antigen-presenting cells. Ten thousand cells of *M. leprae*-induced, CD4+ human T-cell clones or 1×10^5 cells of T-cell lines raised against *M. leprae*, BCG, and *Mycobacterium w* were added to the wells. Experimental cultures were stimulated with antigens in triplicates. The control cultures did not have antigen. To assess the response against recombinant antigens, the experimental cultures were stimulated with *E. coli* lysates containing recombinant antigens, and the cultures with *E. coli* lysates lacking recombinant antigens were taken as controls. The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. On day 3, 0.045 MBq ^{3}H-thymidine (specific activity 185×10^3 MBq/mmole) was added to each well. The plates were further incubated for 4 hr at 37°C. The cultures were harvested, and ^{3}H-thymidine incorporation was assessed by standard procedures ([16]). Median values of counts per minute (cpm) from triplicate have been used to express the results. The proliferative response to a given antigen was considered positive when a T-cell clone or a T-cell line showed $\Delta\text{cpm} \geq 1000$ and $T/C > 2$, and such values in the tables are underlined, where Δcpm = (Median cpm in experimental) − (Median cpm in control cultures) and

$$T/C = \frac{\text{Median cpm in experimental cultures}}{\text{Median cpm in control cultures}}.$$

Cytotoxicity assay. The neutral red uptake assay as described earlier ([17, 19]) was used to assess the cytotoxic potential of T-cell clones against antigen-pulsed adherent cells. In brief, autologous adherent cells from 1×10^6 irradiated PBMC in the individual wells of 24-well Costar plates pulsed with *M. leprae*, BCG, and *Mycobacterium w* served as targets. Control wells did not have antigen. The effector cells were *M. leprae*-induced CD4+ human T-cell clones added at 1×10^5 cells/well. The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. On day 6, nonadherent cells were washed off, and the remaining macrophages differentiated from adherent monocytes were allowed to take up the neutral red. The dye taken up by macrophages was released in a solution of 0.05 M acetic acid in 50% ethanol and was quantitated by measuring OD540 on a spectrophotometer. The results are expressed in terms of percentage cytotoxicity which is defined as: % cytotocixity =

$$\frac{\text{OD540 control} - \text{OD540 experimental}}{\text{OD540 control}} \times 100$$

where OD540 control = OD540 of cultures with adherent cells + antigen, OD540 experimental = OD540 of cultures with adherent cells + T-cell clone + antigen.

RESULTS

Responses of *M. leprae*-induced T-cell clones. Fifteen *M. leprae*-induced CD4 +

TABLE 1. *Proliferation of* M. leprae*-induced, CD4+ T-cell clones to* M. leprae, *BCG, and* Mycobacterium w.

T-cell clones[a]	Proliferation (cpm × 10^{-3}) in response to			
	No antigen	*M. leprae*	BCG	*Mycobacterium w*
	M. leprae-specific			
ATT 6/10F[b]	0.1	13.3[c]	0.1	1.4
NHH 1/E7	0.4	11.7	0.1	0.2
NHH 5/ID	0.1	1.2	0.1	0.1
	Crossreactive			
JM A7[b]	0.3	14.7	28.1	3.8
JM 2C6	0.2	10.9	12.9	0.1
JM 3C7	0.2	11.4	11.6	6.9
ATT 4/1F	0.1	3.4	43.2	1.6
ATT 5/2B	0.1	54.2	41.8	27.4
ATT 5/6B	0.1	3.3	17.8	1.1
ATT 4/4C	0.1	39.8	49.4	0.2
ATT 2/3D[b]	0.1	35.2	50.7	0.1
ATT 1/6B	0.1	20.8	25.7	0.1
ATT 6/9B	0.1	64.1	69.4	0.2
AF 6/C8	0.1	2.0	34.4	3.3
AF 5/C10	0.1	1.8	1.8	0.5
Positive/ tested		15/15	12/15	7/15

[a] ATT, NHH, JM and AF are the subjects whose PBMC were used to establish the T-cell clones.

[b] The T-cell clones ATT 6/10F, JM A7, and ATT 2/3D, proliferated in response to the 18-kDa recombinant antigen of *M. leprae* ([15]), 65-kDa recombinant antigens of *M. leprae* and *M. tuberculosis* ([19]), and 13B3 antigen of *M. leprae* (A. S. Mustafa, *et al.*, paper submitted for publication), respectively.

[c] A clone showing Δ cpm ≥ 1000 and T/C > 2 in response to a given antigen was considered positive. Such values are underlined.

T-cell clones from four BCG-vaccinated, killed-*M. leprae*-immunized subjects were tested for their proliferative responses to *M. leprae*, BCG, and *Mycobacterium w*. Three of these T-cell clones were *M. leprae* specific and others in addition showed reactivity to other mycobacteria (data not shown and [15]). None of the *M. leprae*-specific T-cell clones responded to BCG, but the clone ATT 6/10F which recognizes *M. leprae* 18-kDa protein ([15]) showed a weak response to *Mycobacterium w* (Table 1). All of the cross-reactive T-cell clones responded to *M. leprae* and BCG; only five of these clones showed response to *Mycobacterium w*, including the T-cell clone JM A7 (Table 1) which recognizes *M. leprae* and *M. tuberculosis* 65-kDa antigen ([19]). The 13B3 *M. leprae* antigen-reactive T-cell clone ATT 2/3D (A. S. Mustafa, *et al.*, submitted for publication) proliferated to *M. leprae* and BCG but not to *Mycobacterium w* (Table 1).

TABLE 2. *Cytotoxicity of* M. leprae*-induced CD4+ T-cell clones against adherent cells pulsed with* M. leprae, *BCG, and* Mycobacterium w.

T-cell clones	% cytotoxicity of adherent cells pulsed with		
	M. leprae	BCG	*Mycobacterium w*
ATT 6/10F	96	3	87
JM A7	76	100	60
JM 3C7	60	98	88
ATT 4/1F	87	100	92
ATT 6/9B	98	100	−13

Five *M. leprae*-induced T-cell clones were tested for cytotoxicity against *M. leprae*-, BCG-, and *Mycobacterium w*-pulsed monocytes. A correlation was found between proliferative and cytotoxic activities of these clones. The T-cell clones A7, 3C7, and 4/1F, which proliferated to *M. leprae*, BCG, and *Mycobacterium w*, were also cytotoxic for monocytes pulsed with them (Table 2). The T-cell clone 6/10F did not proliferate to BCG and did not kill BCG-pulsed monocytes (Table 2). The T-cell clone 6/9B did not proliferate to *Mycobacterium w* (Table 1) and could not kill *Mycobacterium w*-pulsed monocytes (Table 2).

Response of *M. leprae*-, BCG-, and *Mycobacterium w*-induced T-cell lines. *M. leprae*-, BCG-, and *Mycobacterium w*-induced T-cell lines were raised from 7, 8 and 5 BCG-vaccinated and killed-*M. leprae*-immunized subjects, respectively. All of the seven T-cell lines raised against *M. leprae* responded to *M. leprae* and *Mycobacterium w*, and six of these T-cell lines responded to BCG (Table 3). All of the five T-cell lines raised against *Mycobacterium w* responded to *M. leprae*, BCG, and *Mycobacterium w* (Table 4). Although all of the BCG-induced T-cell lines recognized BCG in proliferative assays, only three and two of these T-cell lines responded to *M. leprae* and *Mycobacterium w*, respectively (Table 5).

TABLE 3. *Proliferation of* M. leprae*-induced T-cell lines to* M. leprae, *BCG, and* Mycobacterium w.

T-cell lines	Proliferation (cpm × 10^{-3}) in response to			
	No antigen	*M. leprae*	BCG	*Mycobacterium w*
AF ML	1.2	7.5[a]	21.0	5.6
WR ML	0.7	2.0	6.3	1.9
JM ML	0.4	8.0	48.5	2.4
HK ML	35.6	162.4	256.1	145.2
PO ML	77.2	146.4	134.6	176.4
ATT ML	0.6	41.9	119.5	49.7
NHH ML	11.4	69.4	66.7	30.1
Positive/ tested		7/7	6/7	7/7

[a] A T-cell line showing Δ cpm ≥ 1000 and T/C > 2 in response to a given antigen was considered positive. Such values are underlined.

To identify mycobacterial antigens activating the T cells, the cell lines were tested against the recombinant antigens of *M. leprae* and *M. tuberculosis*. The *Mycobacterium w*-induced T-cell line from donor AF (AF *M. w*) and all three cell lines from donors JM and NHH responded to the 65-kDa recombinant antigen (Table 6). The T-cell lines from donor NHH were most interesting with respect to their response to recombinant antigens. BCG- and *Mycobacterium w*-induced cell lines from NHH proliferated to 19-kDa *M. tuberculosis* antigen,

TABLE 4. *Proliferation of* Mycobacterium w*-induced T-cell lines to* M. leprae, *BCG, and* Mycobacterium w.

T-cell lines	Proliferation (cpm × 10^{-3}) in response to			
	No antigen	*M. leprae*	BCG	*Mycobacterium w*
AF MW	1.0	14.1[a]	29.9	6.9
JM MW	0.9	2.8	49.7	4.5
HK MW	8.0	103.5	196.8	106.3
PO MW	21.6	96.3	126.4	142.0
NHH MW	10.3	36.6	55.4	39.7
Positive/ tested		5/5	5/5	5/5

[a] A T-cell line showing Δ cpm ≥ 1000 and T/C > 2 in response to a given antigen was considered positive. Such values are underlined.

TABLE 5. *Proliferation of BCG-induced T-cell lines to* M. leprae, *BCG, and* Mycobacterium w.

T-cell lines	Proliferation (cpm × 10^{-3}) in response to			
	No antigen	*M. leprae*	BCG	*Mycobacterium w*
AF BCG	4.0	8.5[a]	18.8	6.2
WR BCG	0.6	1.1	8.0	0.8
JM BCG	1.8	17.1	54.7	6.1
TO BCG	1.7	1.6	4.0	1.8
HK BCG	34.1	56.7	122.1	51.8
PO BCG	50.5	57.0	109.1	89.8
ER BCG	5.0	5.7	15.1	6.6
NHH BCG	1.6	34.2	145.0	13.1
Positive/ tested		3/8	8/8	2/8

[a] A T-cell line showing Δ cpm ≥ 1000 and T/C > 2 in response to a given antigen was considered positive. Such values are underlined.

M. leprae- and *Mycobacterium w*-induced cell lines responded to 18-kDa *M. leprae* antigen, BCG-induced cell line also responded to 14-kDa *M. tuberculosis* antigen, and *Mycobacterium w*-induced cell line responded to 28-kDa *M. leprae* antigen (Table 6). The T-cell lines from the remaining subjects did not proliferate to any of the recombinant antigens tested (data not shown).

From the reactivity pattern of the T-cell clone 2/3D, which responded to recombinant antigen 13B3, it was shown that this antigen is shared between *M. leprae* and BCG (Table 1). However, only the BCG- but not *M. leprae*-induced NHH T-cell line responded to 13B3 antigen (Table 6). The reason could be that the majority of the T cells in the *M. leprae*-induced NHH T-cell line recognized antigens other than 13B3, e.g., 65-kDa and 18-kDa antigens, and the 13B3 reactive T cells, although present, were too few in number to yield a positive response. If this were the case, it should be possible to enrich these T cells by culturing the cell line in the presence of the respective antigen and thereby gain a response to it. To test this possibility, the *M. leprae*-induced NHH T-cell line (NHH-ML) was cultured with *E. coli* lysates containing 13B3 antigen as well as with 65-kDa *M. tuberculosis* antigen and 18-kDa *M. leprae* an-

TABLE 6. *Proliferation of T-cell lines to the recombinant antigens of* M. leprae *and* M. tuberculosis.

E. coli lysates containing recombinant antigens of	Proliferation (cpm × 10^{-3}) of T-cell lines								
	AF ML	AF BCG	AF MW	JM ML	JM BCG	JM MW	NHH ML	NHH BCG	NHH MW
M. tuberculosis									
65-kDa[a]	3.7	21.6	56.2[b]	14.2	9.2	2.5	29.5	11.2	7.7
19-kDa	7.3	6.3	3.1	0.6	0.3	0.2	5.8	4.8	7.8
14-kDa	7.7	13.6	4.6	0.9	NT[c]	0.3	6.2	4.6	0.6
M. leprae									
36-kDa	8.9	10.4	5.7	0.6	NT	0.6	6.8	0.6	1.0
28-kDa	8.1	8.9	3.7	0.4	NT	0.7	9.7	0.6	2.7
18-kDa	13.1	10.2	4.6	0.7	0.3	0.4	46.3	0.2	1.8
12-kDa	11.4	7.5	4.9	0.8	NT	0.4	5.3	0.3	0.5
13B3	6.7	17.4	4.3	0.7	0.4	0.4	3.7	5.6	0.8
Control *E. coli* lysate	8.5	14.0	3.2	0.4	0.3	0.4	6.4	0.2	0.8

[a] The 65-kDa antigens of *M. leprae* and *M. tuberculosis* have greater than 95% amino acid sequence homology ([26]) and in general have common antibody and T-cell-reactive epitopes ([1, 12, 19]). Therefore, to determine the reactivity against 65-kDa antigen, the T-cell lines were tested against recombinant 65-kDa antigen of *M. tuberculosis* only.

[b] The cpm values showing significant proliferation (cpm ≥ 1000 and T/C > 2) in response to *E. coli* lysates containing recombinant antigens as compared to control *E. coli* lysates are underlined.

[c] NT = Not tested.

tigen containing lysates as positive controls, and with 19-kDa *M. tuberculosis* antigen containing lysate and *E. coli* lysate lacking recombinant antigen as negative controls. The NHH-ML T-cell line after 2 weeks of culture with *E. coli* lysates containing or lacking recombinant antigens maintained its responsiveness to *M. leprae*, BCG, and *Mycobacterium w* (Table 7). Similarly, the cell line cultured with 65-kDa *M. tuberculosis* antigen or 18-kDa *M. leprae* antigen maintained the responsiveness to 65-kDa *M. tuberculosis* and 18-kDa *M. leprae* antigens, respectively. The T-cell line cultured with 13B3 antigen gained the responsiveness to 13B3, but the cell line cultured with 19-kDa *M. tuberculosis* antigen failed to proliferate in response to the homologous antigen (Table 7).

DISCUSSION

The present study was undertaken with the aim to identify T-cell-activating antigens shared by *M. leprae*, BCG, and *Mycobacterium w*. Such crossreactive antigens may be responsible for the induction of *M. leprae*-reactive CMI after BCG immunization ([23]) or *Mycobacterium w* vaccination ([2, 20]). The positive response of some of the *M. leprae*-induced T-cell clones to BCG and *Mycobacterium w* establishes the antigenic crossreactivity for a single specificity. The proliferative and cytotoxicity assays used in the present study to demonstrate antigenic crossreactivity may have biological relevance. Mycobacterial antigen-induced proliferation of human T-cell clones parallels with the production of important lymphokines like IL-2, γ-interferon and granulocyte-macrophage colony stimulating factors ([18]). All of these lymphokines can directly or indirectly have a role in the regulation of CMI responses and in the destruction of pathogens in the host monocytes/macrophages. However, those monocytes/macrophages which somehow have become incompetent to kill ingested bacilli may serve as fertile soil for unabated bacterial growth. Their killing by cytotoxic T cells will facilitate the release of bacteria from the protected intracellular sites to be taken up by fresh and competent monocytes. The inhibitory effect of lymphokine-producing cytotoxic T-cell clones on the *in vitro* growth of mycobacteria inside macrophages has been demonstrated by Kaufmann, *et al.* ([9]).

TABLE 7. *Proliferative response of NHH-ML T-cell line after 2 weeks of culture with recombinant antigens.*

T-cell line cultured with *E. coli* lysates containing	Proliferative (cpm × 10^{-3}) response to				
	M. leprae	BCG	*Mycobacterium w*	*E. coli* lysate lacking recombinant antigen	*E. coli* lysate containing homologous recombinant antigen[a]
No recombinant antigen	20.6[b]	53.3	12.8	2.1	NA
M. tuberculosis					
65-kDa antigen	42.4	83.0	14.4	2.8	34.9
19-kDa antigen	31.3	45.6	22.6	3.0	1.8
M. leprae					
18-kDa antigen	31.0	15.1	15.7	3.0	49.0
13B3 antigen	18.1	69.0	20.3	6.0	30.8

[a] Homologous recombinant antigens are the antigens used to culture the T-cell line NHH-ML for the 2-week period.

[b] The cpm values showing significant proliferation in response to the antigens are underlined.

T-cell clones are excellent tools to study antigenic crossreactivity for a single specificity. However, in an individual, the T cells activated in response to a complex organism like mycobacteria will respond to antigens of varied specificity ([18, 21]). The *in vitro*-raised T-cell clones which are a selected population may not represent the complete repertoire of activated T cells. Therefore, in addition to T-cell clones, the crossreactivity of *in vitro*-established T-cell lines against *M. leprae*, BCG, and *Mycobacterium w* was also determined. As compared to T-cell clones, these T-cell lines are easy to raise and maintain. Like T-cell clones, the T-cell lines have also given evidence of antigenic crossreactivity among *M. leprae*, BCG, and *Mycobacterium w*. Although all except one of *M. leprae*- and *Mycobacterium w*-induced T-cell lines responded to BCG and *Mycobacterium w*, only 3 of 8 and 2 of 8 BCG-induced T-cell lines responded to *M. leprae* and *Mycobacterium w*, respectively. This may mean that in the subjects tested, the dominant T-cell-activating antigens of *M. leprae* and *Mycobacterium w* are crossreactive with BCG, but the dominant T-cell-activating antigens of BCG may not crossreact with *M. leprae* and *Mycobacterium w*. Such a conclusion is supported from our earlier studies where >70% of the *M. leprae*-reactive T-cell clones from five killed-*M. leprae*-vaccinated subjects responded to BCG ([15]) but <10% of BCG-reactive T-cell clones from nine BCG-vaccinated subjects responded to *M. leprae* ([18]).

The T-cell lines were also used to identify T-cell-activating recombinant antigens present in *E. coli* lysates. The antigens previously identified by T-cell clones, i.e., 65-kDa antigens of *M. leprae* and *M. tuberculosis* ([19, 21]), 18-kDa *M. leprae* antigen ([15]), 19-kDa *M. tuberculosis* antigen ([21]), 14-kDa *M. tuberculosis* antigen ([13]), and 13B3 *M. leprae* antigen (A. S. Mustafa, *et al.*, submitted for publication) were also identified by T-cell lines. Additional reactivity was found with one of the *Mycobacterium w*-induced T-cell lines responding to the *M. leprae* 28-kDa antigen, for which T-cell reactivity has not yet been reported. However, the T-cell lines are a mixture of different reactivities. The bulk of their response may be directed toward a few dominant antigens. The T cells capable of responding to other antigens may be present but be so few in number that, unless enriched for these reactivities, they may not be detected by the assay system used. This seems to be the reason for the nonresponsiveness of the *M. leprae*-induced NHH T-cell line to 13B3 antigen in Table 6. The response was specifically restored after the cell line was cultured to enrich for 13B3

antigen-reactive T cells. This enrichment technique may allow the identification of other recombinant antigens with less-dominant T-cell-activating epitopes, which will be missed due to the low frequency of responding T cells in either the T-cell cloning procedures or in the assays with nonenriched T-cell lines.

The results of testing *M. leprae*-induced T-cell clones, and *M. leprae*-, BCG-, and *Mycobacterium w*-induced T-cell lines with recombinant antigens show that the 65-kDa antigen is shared among the three mycobacteria. Amino acid sequence analysis of 65-kDa antigens from *M. leprae*, BCG, and *M. tuberculosis* indicates >95% homology ([26]). Most of the antibody and T-cell epitopes present on this protein crossreact with other mycobacteria ([1, 12, 19]). However, it may also have species-specific antibody and T-cell epitopes ([1, 21]). Seven of the 20 *in vitro*-established T-cell lines from vaccinated volunteers (Table 6) and 20% of the *M. tuberculosis*-reactive T cells from immunized mice ([10]) responded to the 65-kDa antigen. Immunization of experimental animals with whole bacilli induced CMI reactivity to the 65-kDa antigen and vice versa ([7, 9, 27]). These studies indicate that the 65-kDa protein is a dominant T-cell antigen of mycobacteria.

M. leprae and *M. tuberculosis* possess another immunologically important protein of almost identical molecular weight, i.e., the 18-kDa and 19-kDa proteins. But, unlike the 65-kDa proteins, the 18-kDa protein of *M. leprae* and the 19-kDa protein of *M. tuberculosis* are completely different from each other. The DNA encoding the two proteins does not hybridize ([8]), and the antibodies or the T-cell clones responding to the respective proteins do not crossreact ([8, 15, 21]). The results presented in this paper suggest that 18-kDa *M. leprae* protein is shared by *Mycobacterium w* and that the 19-kDa *M. tuberculosis* protein is present in BCG and *Mycobacterium w*.

The mouse monoclonal antibodies and the polyclonal sera raised against *M. leprae* and *M. tuberculosis* antigens react to a limited number of proteins when tested either against their lysates ([3, 4]) or their recombinant proteins ([8, 25, 28–30]). However, the majority of the T-cell clones and T-cell lines in our hands do not respond to these recombinant proteins ([15, 19, 21] and this report). The reason may be that λgt11 recombinants usually produce only a portion of the protein of interest in the form of β-galactosidase fusion protein. The amino terminal part which is missing in the recombinant proteins may contain most of the T-cell epitopes. In this connection, Klatser, *et al.* ([11]) have reported that of the five T-cell clones which responded to 36-kDa *M. leprae* native protein, only one T-cell clone responded to recombinant 36-kDa protein. In addition, some T-cell epitopes may be present on proteins that cannot be identified by antibody probes. The use of T-cell clones as primary probes to identify such antigens resulted in the identification of a recombinant antigen from the *M. leprae* λgt11 library tentatively termed as 13B3 antigen (A. S. Mustafa, *et al.*, submitted for publication). The gene for this antigen as analyzed by restriction enzyme analysis is different from the genes of the other known recombinant antigens of mycobacteria. The availability of complete recombinant proteins identified by antibody or T-cell probes may help to elucidate further the dominant, species-specific and crossreactive antigens of mycobacteria. This will be important for the development of a new generation of recombinant vaccines, specific diagnostic tools, and for the selection of the most suitable cultivable mycobacterium as a vaccine candidate against leprosy.

SUMMARY

Antigenic crossreactivity among three candidate antileprosy vaccines, killed *Mycobacterium leprae,* BCG, and *Mycobacterium w,* was studied using T-cell lines and clones raised from BCG- and killed-*M. leprae*-vaccinated subjects. To identify the crossreactive antigens, the T-cell lines and clones were tested against *Escherichia coli* lysates containing 65-, 36-, 28-, 18-, and 14-kilodalton (kDa) and 13B3 *M. leprae* antigens and 65-, 19-, and 12-kDa *M. tuberculosis* antigens. The short-term T-cell lines, which compared to T-cell clones are easy to raise and maintain, were equally effective in identifying the T-cell-activating recombinant antigens. The reactivity pattern of the T-cell lines and the clones suggested that 65-kDa *M. leprae* and *M. tuberculosis* an-

tigens are present in *M. leprae*, BCG, and *Mycobacterium w*; 18-kDa *M. leprae* antigen is shared between *M. leprae* and *Mycobacterium w*, 13B3 *M. leprae* antigen is possessed by *M. leprae* and BCG. These and other unidentified T-cell-activating antigens shared among candidate leprosy vaccines may be the basis for induction of *in vivo* sensitization to *M. leprae* antigens after vaccination with BCG or *Mycobacterium w*.

RESUMEN

Se estudió la reactividad cruzada entre 3 vacunas antileprosas potenciales (*Mycobacterium leprae* muerto, BCG, y *Mycobacterium w*) usando líneas y clonas de células T obtenidas de sujetos vacunados con BCG y con *M. leprae* muerto. Para identificar los antígenos de reacción cruzada, las líneas y clonas de células T, se probaron contra lisados de *Escherichia coli* conteniendo los antígenos de 65-, 36-, 28-, 18-, y 14- kilodaltones (kDa) del *M. leprae*, contra el antígeno 13B3 de *M. leprae*, y contra los antígenos 65-, 19-, y 12-kDa del *M. tuberculosis*. Las líneas de células T, las cuales son más fáciles de generar y mantener que las clonas de células T, fueron igualmente efectivas en la identificación de los antígenos recombinantes activadores de las células T. Los patrones de reactividad de las líneas de células T y de las clonas, sugirieron que los antígenos de 65-kDa de *M. leprae* y *M. tuberculosis* están presentes en *M. leprae*, BCG y *Mycobacterium w*; que el antígeno de 18-kDa de *M. leprae* es compartido por *M. leprae* y *Mycobacterium w*, y que el antígeno 13B3 de *M. leprae* está presente en *M. leprae* y en BCG. Estos y otros antígenos no identificados, activadores de las células T y compartidos por algunas vacunas con potencialidad anti-leprosa, pueden ser la base para la inducción de la sensibilización *in vivo* contra los antígenos del *M. leprae* cuando se vacuna con BCG o con *Mycobacterium w*.

RÉSUMÉ

On a étudié la réactivité croisée antigénique entre trois préparations envisagées comme vaccins éventuels contre la lèpre, à savoir *Mycobacterium leprae* tué, le BCG, et *Mycobacterium w*. Cette étude a été menée sur des lignées de cellules-T et de clones développées à partir d'individus vaccinés par le BCG et par *M. leprae* tués. En vue de mettre en évidence la réactivité croisée des antigènes, les lignées de cellules-T et les clones ont été testées contre des lysats d'*Escherichia coli* contenant les antigénes de 65-, 36-, 28-, 18-, et 14-kilodaltons (kDa), ainsi que des antigénes 13B3 de *M. leprae* et des antigénes 65-kDa, 19-kDa et 12-kDa de *M. tuberculosis*. Les lignées de cellules-T à courte vie, plus faciles à developper et à maintenir que les clones de cellules-T sont tout aussi efficaces pour reconnaître les antigènes recombinants qui activent les cellules-T.

Le profil de réaction des lignées de cellules-T et des clones suggèrent que les antigènes 65-kDa de *M. leprae* et de *M. tuberculosis* sont présents chez *M. leprae*, dans le BCG, et chez *Mycobacterium w*; l'antigène 18-kDa de *M. leprae* existe chez *M. leprae* et chez *Mycobacterium w*, l'antigène 13B3 de *M. leprae* est présent chez *M. leprae* et dans le BCG. Ces antigènes, de même que d'autres antigènes non identifiés qui activent les cellules-T, et que l'on retrouve dans toutes les préparations actuellement envisagées comme pouvant servir de vaccin contre la lèpre, peuvent fournir la base pour induire une sensibilisation *in vivo* aux antigènes de *M. leprae* après vaccination par le BCG ou par le *Mycobacterium w*.

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