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Humoral immune responses to *M. leprae* in human volunteers vaccinated with killed, armadillo-derived *M. leprae*

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Summary
In a trial of a leprosy vaccine consisting of killed, armadillo-derived *M. leprae,* 4 groups of healthy human volunteers residing in a leprosy non-endemic country were given 1.5 x 10^7, 5 x 10^7, 1.5 x 10^8 and 5 x 10^8 bacilli intradermally. The antibody responses to *M. leprae,* in these volunteers, were measured before and up to 1 year after vaccination using two assays : a serum antibody competition (SAC) test and an enzyme linked immunosorbent assay (ELISA). Both assays revealed similar antibody profiles in the vaccinees. The 2 groups which received the lower doses of vaccine were observed to have very low levels of antibody before and up to 1 year after vaccination. The 2 groups which received the higher doses of vaccine showed low antibody titres before and up to 3 months after vaccination, with a discernible rise in antibody titres 6 months after vaccination which increased even further 1 year after vaccination.

Introduction

A candidate vaccine against leprosy, consisting of killed, armadillo-derived *Mycobacterium leprae,* is currently undergoing trial in human volunteers (1). In an early phase of this trial, 4 groups of healthy human volunteers, residing in a leprosy non-endemic country, were given 1.5 x 10^7, 5 x 10^7, 1.5 x 10^8, and 5 x 10^8 killed, armadillo-derived *M. leprae* intradermally (2). The vaccine was found to induce a significant increase in the skin test response to an *M. leprae* soluble antigen (MLSA) preparation in the groups which received the three highest doses of vaccine (2). A second assay, the *in vitro* lymphocyte transformation test (LTT) was also used to assess the ability of this vaccine to sensitize human volunteers. It was shown that the vaccine induced a significant increase in the proliferative responses of the peripheral blood mononuclear cells (PBMC) taken from the two groups which received the two highest doses of vaccine (3), to MLSA. In this study we report on the humoral immune response of the vaccinated subjects.

Materials and methods

SUBJECTS AND STUDY DESIGN

The subjects and study design have been described in detail previously (2). Briefly, four groups of individuals aged between 23 and 28 years were given 1.5 x 10^7, 5 x 10^7, 1.5 x 10^8, and 5 x 10^8 heat killed, armadillo-derived *M. leprae* intradermally. These volunteers were bled and then skin tested with PPD and MLSA, before and at various time intervals after vaccination. The plasma and mononuclear cells were separated from peripheral blood by density centrifugation on a Ficoll/metrizoate gradient (Lymphoprep, Nyegaard & Co., Oslo, Norway) and stored at -70°C and in liquid nitrogen, respectively, until tested. An unvaccinated control group was also included in the study.

ASSAYS FOR *M. LEPRAE* ANTIBODIES

Serum antibody competition test

The serum antibody competition (SAC) test has been described in detail previously (4). Briefly, 96 well plates were coated with sonicated armadillo-derived *M. leprae*. Antibody was measured by competitive binding to these antigen coated wells, between ^{125}I labelled monoclonal antibody (MLO4) and test sera from vaccinated subjects. The results are expressed in terms of ID_{50} values which represent the serum dilution achieving 50 % inhibition of ^{125}I-MLO4 binding to the antigen coated wells.

Enzyme-linked immunosorbent assay (ELISA)

Polystyrene NUNC-Immunoplates were coated with *M. leprae* sonicate. Additional sites were blocked with phosphate buffered saline (PBS, pH7.4) containing 0.1 % Tween 20 + 0.5 % BSA for 1hr at 37°C and washed x3 with PBS/Tween. Sera diluted 1:50 in PBS/Tween were applied in the wells and incubated for 1hr at 37°C. Between each of the following steps, the plates were washed x3 with PBS/Tween. Peroxidase conjugated anti-human IgG or anti-human IgM (DAKO Immunoglobulins, Copenhagen, Denmark) diluted 1:1000 in PBS/Tween were incubated with the plates for 1hr at 37°C. ABTS substrate (Boehringer Mannheim) was used, and the optical density was measured at 405 nm when the highest values in control wells containing pooled lepromatous serum (PLS) 1:100 were between 1.000 and 1.200. Antibody activity in the sera tested is expressed in per cent of the antibody activity in the pooled lepromatous serum (PLS) used for reference.

Results

Tables 1a-1d show the antibody responses to *M. leprae* in the vaccinated groups as measured by the SAC test. In the unvaccinated control group receiving skin tests only, antibodies could not be detected. The two groups vaccinated with 1.5 x 10^7(Table 1a) and 5 x 10^7 (Table 1b) bacilli, showed low antibody titres (<1), before as well as after vaccination. The two groups, which were vaccinated with 1.5 x 10^8 (Table 1c) and 5 x 10^8 (Table 1d) bacilli, show a rise in antibody titre 6 months after vaccination. This increase in antibody titre is statistically significant, ($Pr>0.001$) one year after vaccination in the group which received 5 x 10^8 bacilli.

Table 1a. Antibody responses in human volunteers before and after vaccination with 1.5 x 10^7 killed, armadillo-derived M. leprae

Subjects	Time				
	Before vaccination	After vaccination			
		1 month	3 months	6 months	12 months
KK	nd	<1[A]	<1	nd[B]	<1
SN	nd	nd	<1	nd	nd
JS	nd	<1	<1	nd	<1
KH	nd	<1	<1	nd	<1
THB	nd	<1	<1	nd	<1
MH	nd	nd	nd	nd	<1

[A] Antibody titre: See Materials and methods
[B] Not done

Table 1b. Antibody responses in human volunteers before and after vaccination with 5 x 10^7 killed, armadillo-derived M. leprae

Subjects	Time				
	Before vaccination	After vaccination			
		1 month	3 months	6 months	12 months
SE	<1[A]	<1	<1	nd[B]	<1
TV	<1	<1	<1	nd	<1
GJ	<1	<1	<1	nd	<1
EN	<1	<1	<1	nd	<1
MO	<1	<1	<1	nd	<1
PS	<1	<1	<1	nd	<1
ALH	<1	3.1	<1	nd	nd
JGH	<1	<1	<1	<1	<1
FH	<1	<1	<1	nd	<1

[A] Antibody titre: See Materials and methods
[B] Not done

Table 1c. Antibody responses in human volunteers before and after vaccination with 1.5 x 10^8 killed, armadillo-derived M. leprae

Subjects	Time				
	Before vaccination	After vaccination			
		1 month	3 months	6 months	12 months
AKA	<1[A]	<1	<1	<1	nd[B]
LH	<1	<1	<1	<1	<1
WR	<1	<1	<1	nd	nd
TR	1.7	<1	27	nd	26
KR	<1	<1	<1	3.3	<1
JM	<1	<1	nd	3.1	1.9

[A] Antibody titre: See materials and methods
[B] Not done

Table 1d. Antibody responses in human volunteers before and after vaccination with 5 x 10^8 killed, armadillo-derived M. leprae

Subjects	Time				
	Before vaccination	After vaccination			
		1 month	3 months	6 months	12 months
AF	<1[A]	<1	<1	8.4	14
NHH	<1	<1	<1	5.2	10
KS	<1	<1	<1	<1	10
ATT	<1	<1	<1	42	13
HK	<1	nd[B]	<1	7.5	12.5
ER	<1	<1	nd	nd	42

[A] Antibody titre. See materials and methods
[B] Not done

Figures 1a-1e show the antibody responses in the control and vaccinated groups as measured by an enzyme-linked immunosorbent assay. This assay measured the IgG and the IgM antibody responses. As far as IgG antibody is concerned, the pattern of responses exhibited by all the groups is similar to that observed with the SAC test. The control group (Fig. 1a) and the two groups which received the lower doses of vaccine, 1.5 x 10^7 (Fig. 1b) and 5 x 10^7 (Fig. 1c) show low IgG antibody titres before vaccination and throughout the duration of the study. The two groups which received the higher doses of vaccine, 1.5 x 10^8 (Fig. 1d) and 5 x 10^8 (Fig. 1e), showed a rise in the IgG antibody titre six months after vaccination. The differences for these groups in IgG antibody titres, between prevaccination and 6 months post vaccination ($0.05>Pr>0.02$ and $0.01>Pr>0.001$, respectively) and between pre-vaccination and one year post vaccination ($0.01>Pr>0.001$ and $0.01>Pr>0.001$, respectively) are statistically significant. While the IgG anti-*M. leprae* antibody activity showed a distinct pat-

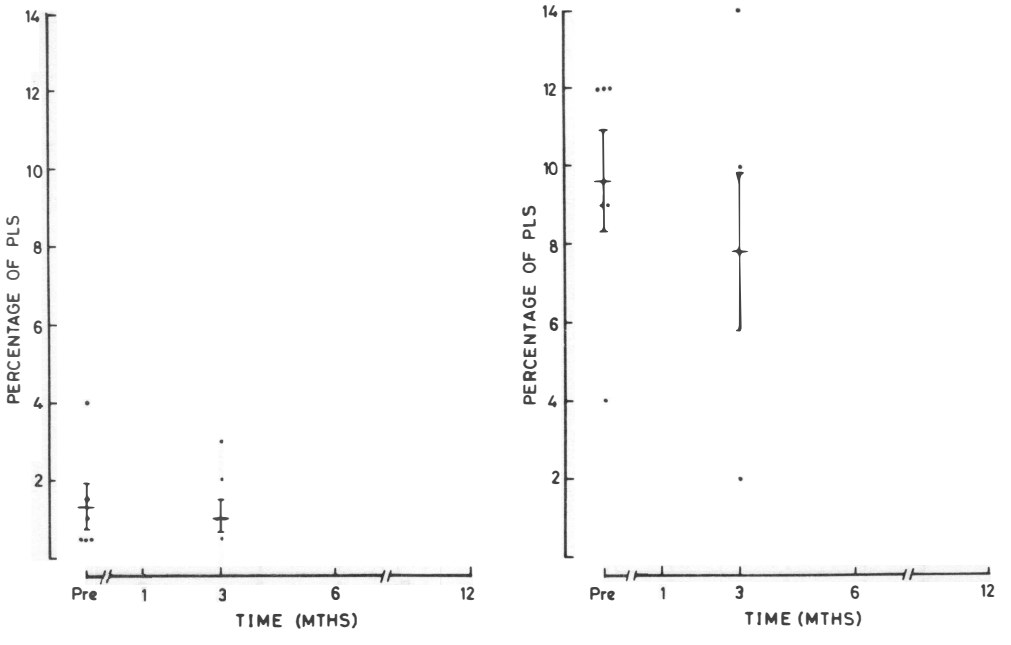

Figure 1 a.
Kinetics of the antibody responses to M. leprae *in unvaccinated volunteers. The left hand side of the figure shows the IgG responses while the right hand side shows the IgM responses. The results are expressed as the mean response of the group and the vertical bars represent the SEM.*

tern with a significant increase in the two groups which received the highest doses of vaccine, the IgM antibody levels showed less distinct changes. The IgM antibody levels were higher than the IgG levels in the control group and in the prevaccination samples.

Discussion

The purpose of the study reported here and previously (2,3) was to obtain as much information as possible on the performance of heat-killed, armadillo-derived *M. leprae* vaccine in normal, healthy individuals residing in a non-endemic country. This would be crucial before starting a trial in an endemic country which would, because of the nature of the disease, take a long time (>10 years) to complete. As protective immunity in leprosy is cell mediated, it was important to establish that the vaccine was able to induce cell mediated immune responses at doses that do not lead to unacceptable side effects in vaccinated individuals. This has been established both by the *in vivo* skin tests (2) and the *in vitro* LTT (3). Secondly, it was important to determine whether the vaccine induced humoral immune responses in these same individuals.

The vaccine does induce a humoral response at the 2 highest doses i.e. 1.5×10^8 and 5×10^8 bacilli. The kinetics of the humoral and CMI responses differ, in that while DTH and LTT responses to MLSA had risen to significant levels 3 months after vaccination with killed

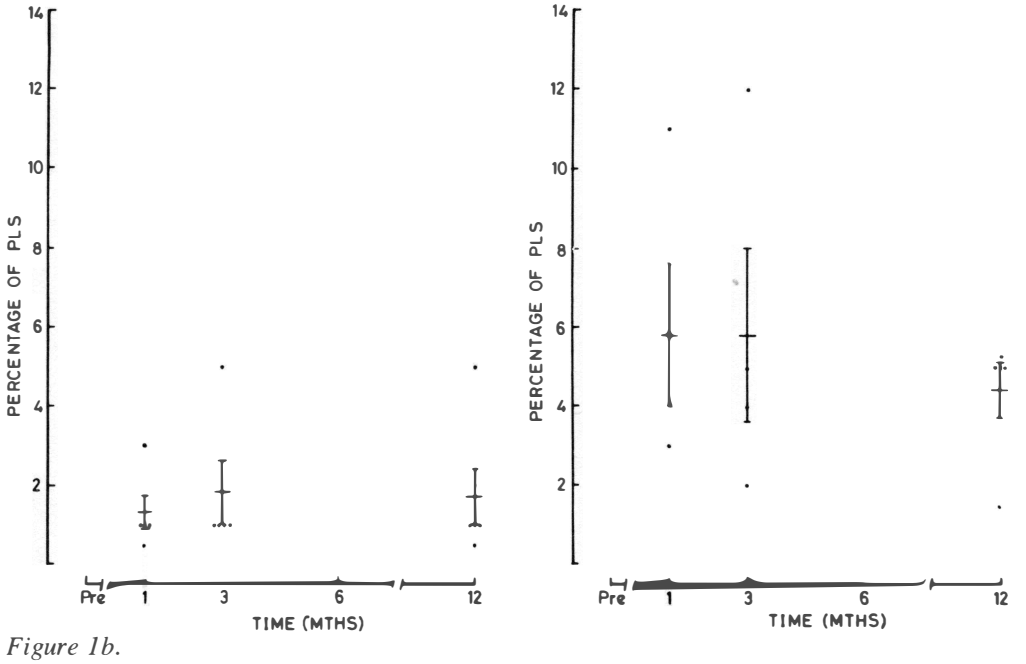

Figure 1b.
Kinetics of the antibody responses to M. leprae *in volunteers vaccinated with 1.5 x 10^7 killed* M. leprae. *The left hand side of the figure depicts the IgG responses while the right hand side depicts the IgM responses. The results are expressed as the mean response of the group and the vertical bars represent the SEM.*

M. leprae (2), antibody titres took a longer time to show an appreciable rise. It is only at 6 months after vaccination that an increase in antibody titres was discernible in these two groups.

Two assays, the SAC test and ELISA, were used to measure antibody titres in these vaccinated individuals. The antibody profiles revealed by both these assays were very similar. The SAC test uses the monoclonal antibody MLO4, which recognizes a 35 kD protein antigen on *M. leprae* (5). The higher titres revealed by this assay in the groups which received 1.5 x 10^8 and 5 x 10^8 bacilli must be indicative of their having recognized this antigen. As mentioned earlier, this finding is important in the search for the protective antigens on *M. leprae*. T cell clones from these vaccinated individuals have also been raised with this goal in mind (6). The ELISA measures cross-reacting antibodies as well. This may explain the high IgM titres in the control group and in the two groups which received the lower doses of vaccine, measured by ELISA.

In conclusion, the results presented here extend the results reported from the same group of healthy volunteers vaccinated with killed *M. leprae*. It appears, that the killed, *M. leprae* vaccine does induce a humoral response in the individuals which received the 2 highest doses of vaccine. An analysis of these antibodies may help in the ultimate identification of protective antigens.

Acknowledgements
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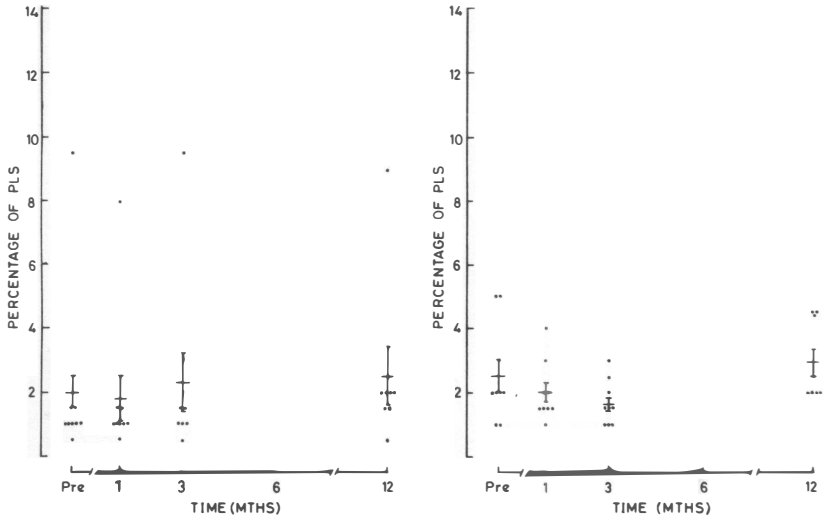

Figure 1c.
Kinetics of the antibody responses to M. leprae *in volunteers vaccinated with 5 x 10^7 killed* M. leprae. *The left hand side of the figure depicts the IgG responses while the right hand side depicts the IgM responses. the results are expressed as the mean response of the group and the vertical bars represent the SEM.*

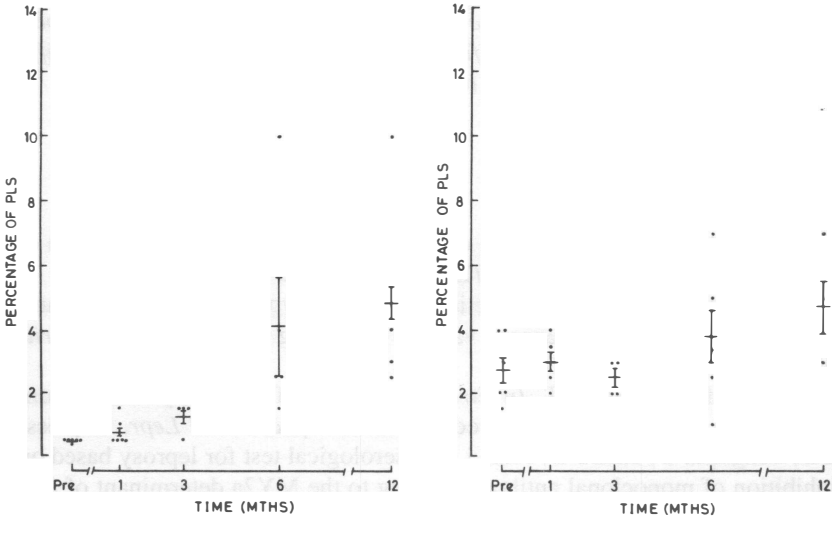

Figure 1d.
Kinetics of the antibody responses to M. leprae *in volunteers vaccinated with 1.5 x 10^8 killed* M. leprae. *The left hand side of the figure depicts the IgG responses while the right hand side depicts the IgM responses. The results are expressed as the mean response of the groups and the vertical bars represent the SEM. The difference between pre and 6 months post-vaccination IgG antibody responses was significant (0.05>Pr>0.02) as was the difference between pre and 1 year post-vaccination (0.01>Pr>0.001).*

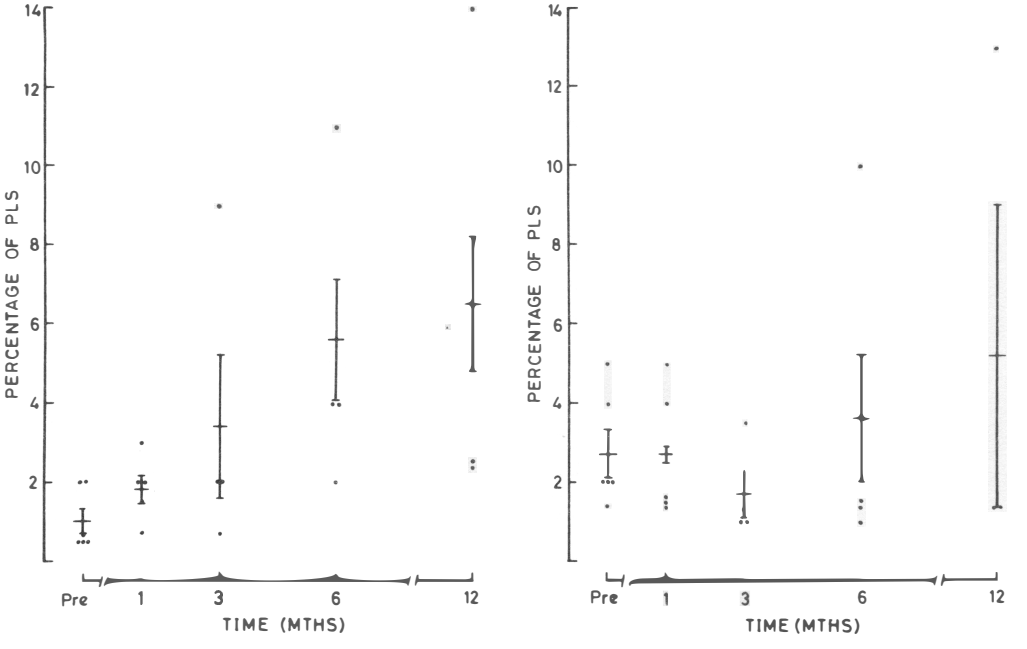

Figure 1e.
Kinetics of the antibody responses to M. leprae *in volunteers vaccinated with 5 x 10^8 killed* M. leprae. *The left hand side of the figure depicts the IgG responses while the right hand side depicts the IgM responses. The results are expressed as the mean response of the group and the vertical bars represent the SEM. The difference between pre and 6 months post vaccination IgG antibody responses was significant (0.01>Pr>0.001) as was the difference between pre and 1 year post vaccination (0.01>Pr>0.001).*

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