Lepr Rev (1988) **59,** 195–199

A comparative evaluation of serological assays for lepromatous leprosy

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Accepted for publication 15 April 1988

Summary A comparative antibody analysis of sera from 26 patients with lepromatous leprosy showed consistently high titres to the phenolic glycolipid I disaccharide and to the ML04 epitope of the 35 kD protein antigen of *Mycobacterium leprae.* Antibody titres of these two specificities were positively correlated ($p < 0{\cdot}01$) and both declined after chemotherapy, although this trend was apparent earlier after the onset of therapy for the anti-35kD antibody response. Two healthy subjects (out of 18 tested) from the leprosy endemic area had pronounced anti-PGL-1 but no demonstrable anti-35kD antigen activities. In contrast with the above results, antibody levels to lipoarabinomannan were much lower and with great individual variation between the LL patients. Finally, antibody levels to the *M. leprae*-specific IIIE9 epitope (peptide 422–436) of the 65kD protein antigen were not demonstrable in the majority of LL patients.

Introduction

Serological studies in leprosy have been pursued for the past 80 years[1] with interest in diagnosis, classification within the spectrum of disease, monitoring of chemotherapy, prognosis of subclinical infection[2] and response to vaccination.[3] Recent progress in the molecular definition of *Mycobacterium leprae*-specific, antigenic determinants provided a new impetus to these studies. The phenolic glycolipid I (PGL-1) and its terminal disaccharide have been used in a solid-phase binding enzyme-linked assay,[4–6] whereas a competition test has been applied using monoclonal antibodies (MAB) to the 35 kD antigen and other protein or polysaccharide antigens,[7–12]

These assays confirmed results from previous studies which showed that serum antibody levels are profoundly increased in multibacillary (lepromatous) but not in paucibacillary (tuberculoid) leprosy. The relative immunodominance of the respective antigens has not as yet been fully evaluated. The purpose of this paper has been to compare antibody levels to distinct epitopes in individual patients with lepromatous leprosy.

0305-7518/88/059195+05 $01.00

Materials and Methods

SERA

Thirty patients, attending the clinic of the JALMA Institute for Leprosy (Agra, India) were classified as lepromatous leprosy (LL) according to the Ridley–Jopling scale. The 18 control sera were from healthy subjects living in the Agra region. Sera were collected from patients before and at various times after chemotherapy which was given in accordance with WHO recommendations.[13]

ANTI 35 KD PROTEIN, ML04-COMPETITION ASSAY

Microtitre plates (Immulon, M129B Dynatech) were coated with 50 μg/ml (25 μl/well) *M. leprae* soluble extract (MLSE) for 20 hr at 4°C. After washing 3 times with phosphate buffered saline (PBS) the plates were blocked with 3% bovine serum albumin (BSA) in PBS for 1 hr at 20°C and serum samples, serially diluted (100–51200) in 3% BSA–0·05% Tween −20 were added to wells. After 30 min incubation at 37°C in a humidified chamber, peroxide-labelled ML04 MAB (25 μl/well) in BSA–Tween was added and incubated for 1 hr at 37°C. Plates were washed and incubated with 3,3′,5′5′ tetramethyl benzidine (TMB)-H_2O_2 substrate (100 μl/well, of a fresh solution containing 50 μg/ml TMB and 0·006% H_2O_2 in 0·08 M Na Citrate pH 5·0) for 30 min at 20°C. The reaction was stopped with 50 μl/well of 0·5 M H_2S0_4 and the optical density read at 450 nm in a titertek multiscan reader. Antibody titres are expressed as reciprocal serum dilutions giving 50% inhibition of ML04 binding to MLSE coated wells (ID_{50}).

ANTI-LIPOARABINOMANNAN (LAM) ML34-COMPETITION ASSAY

This test was performed with MAB ML34 which binds to an epitope expressed on (LAM).[15,16] The competition assay was done essentially as described in the previous paragraph, but using ^{125}I-labelled ML34 as in the original technique.[17]

PHENOLIC GLYCOLIPID BINDING ASSAY

Microtitre plates were coated with 5 μg/ml phenolic glycolipid I disaccharide (PGDS) (3,6-Dimethyl-β-D-Glucosyl (1–4) 2,3-Dimethyl-α-L-Rhamnosyl) conjugated to BSA (from R Gigg) in PBS (50 μg/well) for 20 hr at 4°C, washed with PBS and blocked with 3% BSA for 1 hr at 37°C in a humid chamber. Serial doubling dilutions (100–51200) of test sera in 1% BSA Tween (50 μl/well) were added to PGDS-coated or uncoated plates and incubated for 2 hr at 37°C. Plates were washed with Tween/PBS, then incubated with 50 μl/well of 1:1000 diluted peroxidase coupled goat anti-human IgM (Sigma Chem. Co. UK) for 1 hr at 37°C. After washes with Tween/PBS, substrate was added and the reaction read as described above. Relative binding values were calculated over the range of serum dilutions with OD values corrected for binding to the uncoated plate, using a positive reference serum as 100%. Antibody titres were expressed as reciprocal serum dilutions giving 15% binding (ABT_{15}).

ANTI-65kD ANTIGEN TESTS

Microtitre plates were coated overnight at 4°C with 50 μl/well of the *M. leprae*-specific IIIE9 peptide[18] dissolved in 0·1 M Na bicarbonate at 0·1 μg/ml concentration. Plates were washed with PBS and blocked for 1 hr at room temperature with BSA/Tween. After incubation with sera for 2 hr and washing with PBS, plates were incubated for 1 hr with 1/1000 dilution of peroxidase conjugated rabbit anti-human IgG (Sigma Chemical Co) in BSA/Tween, washed, and developed as described above. The IIIE9 MAB was used as the positive control.

Additional tests were performed with radiolabelled IVD8 antibody[12] using the competition assay as described before.[17]

STATISTICAL ANALYSIS

Correlations betweeen antibody titres of the various specificities and for antibody titres related to years of chemotherapy were analysed using correlation coefficients.

Table 1. Antibody levels in patients with lepromatous leprosy

Sample no.	Years of therapy:	BI*	Antibodies† to: 35kD ID_{50}	PGDS ABT_{15}	LAM ID_{50}
Patients					
1	0	3·7	8440	4380	71
2	0	4	6860	> 5120	87
3	0	5	8440	1698	31
4	0	4	7350	3308	14
5	0	4	8440	15219	577
6	0	5	2110	1537	172
7	0	4	690	12775	69
8	0	NA	8440	6182	268
9	0	4·5	6400	5385	102
10	0	NA	1710	4217	291
11	3	0	1260	< 100	13
12	3	0	5200	221	68
13	3	2	320	1131	219
14	3	NA	1130	3591	> 625
15	3	3	350	1440	68
16	4	3	12800	16781	125
17	5	5	3200	18243	268
18	6	0	1130	2588	115
19	7	0	800	282	25
20	8	0	120	2983	87
21	10	1	920	572	74
22	10	0	< 100	976	20
23	12	0	300	146	20
24	12	0	< 100	428	21
25	12	0	400	100	17
26	15	NA	< 100	5761	16
Controls					
1–16			< 100	< 100	10–50
17			< 100	1479	26
18			< 100	1130	25

* Bacteriological index. NA, not available.

† The values represent reciprocal dilutions (titres) of test sera giving 50% inhibition (ID_{50}) of binding of monoclonal antibody ML04 (35kD protein) and ML34 (LAM) or giving 15% binding to PGDS coated micotitre plates.

Results and Discussion

Sera from untreated patients showed consistently high antibody levels in the PGDS binding and the anti-35kD (ML04) competition assays (Table 1). The performance of the ML04 competition test using peroxidase-labelled ML04 monoclonal antibody showed considerably higher serum titres than in previous assays which employed a radiolabelled ML04.[7,8]

Unlike the uniformly raised anti-PGDS and anti-35kD protein antibodies, we observed considerable variation between individual LL patients in their anti-LAM antibody response, represented by the ML34-competition test. The individual variations could not be related to the bacteriological index. These results related only to one epitope (ML34) of LAM and the immunogenicity of epitopes detected by other MABs (ML02)[15] (L9)[10] was not evaluated.

In view of the cross-reactivity of the ML34 epitope of LAM with several mycobacteria,[16] it is not surprising to find antibody levels in healthy controls which consequently limits the usefulness for diagnosis of leprosy. High titres of antibodies against phenolic glycolipid I were found in 2 out of 18 control sera which were negative in the ML04 competition test. This result is in agreement with previous reports of 'false' positive reactions to PGL-1 in 5% of control sera.[5,12]

Correlation between these tests was examined with the sera from patients prior to therapy and those with up to 5 years of therapy. PGDS *vs* ML34 and PGDS *vs* ML04 showed positive correlation at the $p < 0{\cdot}02$ and $p < 0{\cdot}01$ levels respectively whilst no significant correlation was found between ML04 *vs* ML34.

All three tests showed a statistically significant correlation between decrease of titres and length of chemotherapy. The ML04-competition test showed by far the best correlation ($p < 0{\cdot}001$) and may therefore be the most suitable for monitoring the response of patients to chemotherapy.

Marginal levels of binding activity to the IIIE9 peptide of the 65KD protein were demonstrable in sera of 2 patients only. Competition experiments using radiolabelled IVD8 antibody, whose epitope is also contained on the IIIE9 peptide, showed similarly marginal activity in sera with very high anti-35kD and PGDS antibody levels (results not shown). Therefore, our results suggest that the *M. leprae* specific epitope of the 65kD antigen is poorly immunogenic in patients with lepromatous leprosy.

In conclusion, we found that essentially all patients with LL produce high levels of antibodies towards PGL-1 and 35kD protein antigen. This strong immunodominance and the species-specificity of both epitopes is particularly favourable for further serodiagnostic and seroepidemiological evaluation. This is apparent in view of the fact that sera from LL patients do not contain significant antibody titres to two other *M. leprae* specific epitopes defined by the ML06/ML10 MABs on the 12kD antigen[7] and by the IIIE9/IVD8 MABs on the 65kD antigen. Of the other MAB-defined *M. leprae* specific epitopes, it appears that only half of the LL patients react with the 18kD antigen[10] whilst the response to the 28kD antigen[19] has not yet been evaluated. Technically, the availability of chemically synthesized PGDS is favourable for assay standardization although variable levels of non-specific IgM binding to the solid phase interfere with the analysis of sera at low dilutions. On the other hand, the competition assay is less prone to non-specific factors and therefore possibly more reliable to detect low antibody levels.

Acknowledgments

We thank V Aber for statistical evaluation, R Gigg for the supply of PGL-disaccharide, P Kim and R Young for the IIIE9 peptide, T Buchanan for the IVD8 monoclonal antibody, J Duncan for peroxidase conjugation of antibodies and D Young for advice.

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LEPROSY CONTROL AND FIELD WORK

Prometheus–PHC (Primary Health Care)

Dr Manuel Quimper, Instituto de Medicina Tropical, 'Alexander von Homboldt', A.P. 5045, Lima, Peru, South America, has developed a project for the use of microcomputers to improve primary health care services in Peru. The descriptive leaflet summarizing this interesting initiative includes the following:

Prometheus–PHC: A computerized, integrated health information system:

Designed to improve the quality of primary health care services . . . and other medical services in Peru and other countries.

Information technology which works first at the community level . . . where the real needs of the people are and secondly at the level of direct supervision.

Computer-based expert assistance for health promotors . . . and an automated system for planning and supervision at health centres and area hospitals.

Appropriate high level technology: Powerful microcomputers which work with batteries, have no moving parts and are recharged by inexpensive solar panels (less than $20 each) made in Peru.

An 'intelligent' system: an exact, continuously up-dated community census, which monitors and records all health services activities, automatically schedules future primary health care visits, monitors the growth and development of all community children, records all immunization and precisely targets future needs, automatically manages inventories of medicines and supplies, and provides expert assistance for the diagnosis and treatment of: diarrhoea, respiratory infections, malnutrition, high risk pregnancy, fever, trauma, emergency. Rapid error-free transmission of information between computers of all types at different levels of the Health System.

Avoiding loss of data through redundant storage of information.

The Prometheus–PHC prototype being used, at this moment in the central jungle region of Peru:

In 10 health posts along the Rio Pichis, the Prometheus–PHC pilot project has shown that:

Prometheus–PHC is reliable, well received, and can be easily used by local health promotors in their native communities.

Local promotors can be trained in the use of the system in less than 1 week even those who have never seen a typewriter before.

Dr Quimper has supplied the following additional technical information:

The equipment about which you asked me in your letter of 29 March is a real Microcomputer, an item which belongs to the so called 'Lap top personal computers' with a RAM (Random Access Memory) of 275 Kbytes, powered by a rechargeable 9 Volt battery.

The computer, a Hewlett–Packard 110 model, had no mobile parts and was shown to be a robust, and reliable piece of equipment (at least for the first 2 years).

We developed in the country the solar energy unit for the recharging of the batteries, using discarded (broken) materials brought from a factory in the USA, and manufactured the panels with our own craftsmen. They cost less than US $5 each. The software was designed by us and the programming was in Basic language.

At the beginning of the project we ran into some difficulties because of the small storing capacity of the batteries. However, introducing a 12 Volt standard acid battery (car type), as an additional accumulator, we improved the capacity of power storing and went on without any further problems.

At the end of the project, some of the computers presented problems related to the lifespan of the internal batteries, which diminished the amount of charge they could hold. Again, that was solved by replacing the batteries with new ones, a task performed locally, though it required a special type of screwdriver.

(The approach, on the evidence so far, seems of potential value for the collection of data in the field and it is apparently workable by local health staff. As many people in South America and elsewhere, are already familiar with keyboards, visual display units and computers, this initiative warrants further attention. *Editor.*)

United Nations Association International Service; Brazil

We are indebted to Jane Carter, General Secretary of UNAIS, 3 Whitehall Court, London SW1A 2EL, for keeping us informed about a series of volunteers from the UK who have been recruited to work in the Manaus area of Brazil, under the supervision of Professor Sinesio Talhari. Qualified nurses are being sent out to help supervise the multiple drug therapy programme in the Amazonas; they do this from small health centres or clinics in 4 designated centres of population, at varying distances from the capital of Manaus. Experience has already shown that this improves training of local staff and adequate follow-up of patients. This project, operating under difficult conditions, well away from the more developed centres in Brazil, is supported by CAFOD and LEPRA and is likely to be on-going over the next 5 years at least.