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Host-pathogen interaction— new *in vitro* drug test systems against *Mycobacterium leprae*— possibilities and limitations

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Introduction

The determination of the viability and drug sensitivity of *Mycobacterium leprae* in laboratory conditions has been a major problem, on account of the difficulty of growing this organism *in vitro*. To date the growth in the footpad of mice by the innoculated *M. leprae*, developed by Shepard[18] and later modified by Rees,[17] serves as the only recognized method to determine the viability of the organism, as well as evaluating the potency of antileprosy drugs. The major drawback of the mouse footpad test is that it is time-consuming and thus unsuitable for large numbers of tests or experiments involving several new compounds. However, several *in vitro* test systems to demonstrate viability and drug sensitivity of *M. leprae* have been described recently. They include the ^{3}H-thymidine incorporation method,[15,14] ATP quantitation method,[4] DOPA uptake system,[2] Fc receptor assay system.[3,6,12] Several other metabolic precursors have also been suggested as tools for determining viability of *M. leprae*. They are hypoxanthine, and amino acid;[8,9] labelled acetate.[19]

Recently Kvach & Veras[10] and Kvach *et al.*[11] have used a non-fluorescent fatty acid ester, Fluorescein-diacetate (FDA) and a nucleic acid stain, ethidium bromide (EB) for determining the viability of *M. leprae*. They showed that viable *M. leprae* convert FDA to fluorescent Fluorescein which accumulates within intact bacteria and make them fluoresce as green. The dead cells take up EB, due to defective membrane, and appear as orange red under the UV light.

In this presentation we would like to report several *in vitro* test systems that could identify viability of *M. leprae* and determine the drug sensitivity. The methods also enable screening new compounds for anti *M. leprae* activity. We would also like to show that these methods have excellent correlation with mouse footpad tests. In all these *in vitro* systems we have used *M. leprae* phagocytosed by macrophages and monitored various parameters to indicate viability or otherwise of the bacteria (Figure 1).

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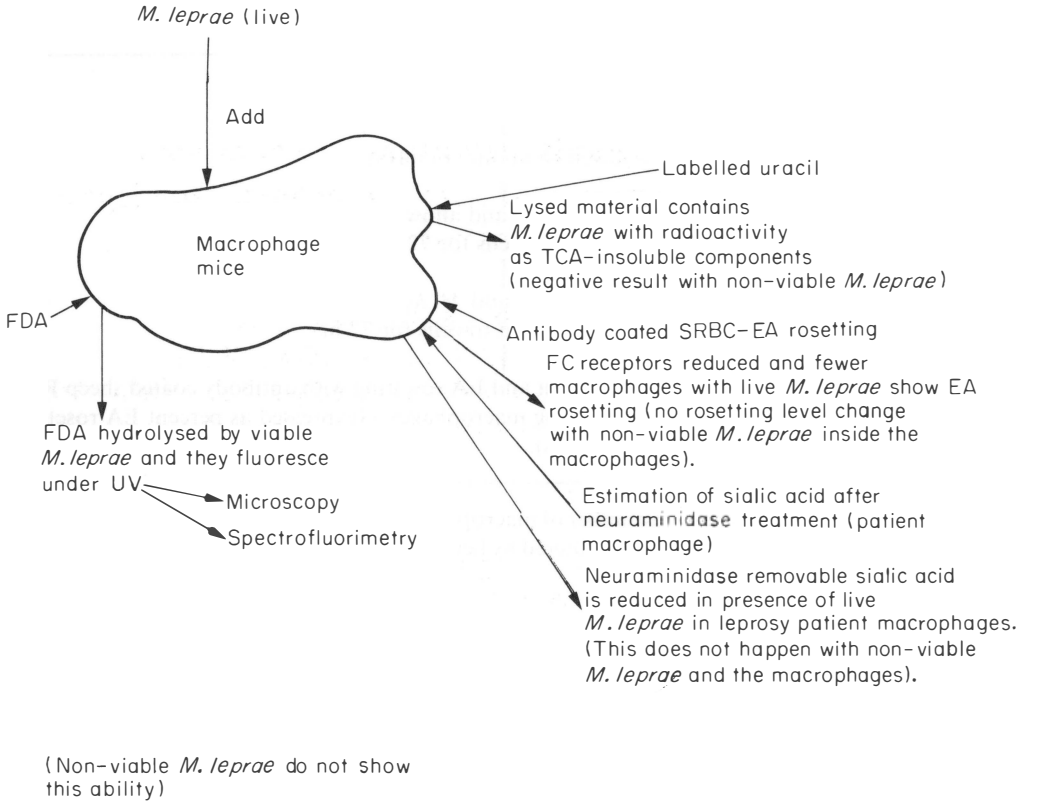

Figure 1.

Materials and methods

Mycobacterium leprae was obtained from infected armadillo tissues or human biopsies from untreated or partially treated lepromatous leprosy patients. Macrophages were from the peritoneal cavity of Swiss white mice. The preparations of both these components have been described in detail in our earlier publications.[6,12]

The Fc receptor assay system, using EA rosetting technique in relation to viability of *M. leprae* was described by Birdi *et al.*[3] using macrophages from humans. This was further adapted by using macrophages from peritoneal cavity of Swiss white mice.[12] The assay system is described in Table 1. Jagannathan and Mahadevan[6] had clearly demonstrated that dapsone, a drug used for *in vitro* assay systems, enters macrophages and the concentration inside the macrophages could be estimated in the solvent extracted sample by spectrofluorimetry.

Table 1. Fc receptor assay with macrophages *M. leprae* and drug.

Mouse macrophage (peritoneal) culture
↓
allowed to mature (3 days)
↓
DDS/RFP added and allowed to be exposed to cells for 72 hr
↓
Medium changed and *M. leprae* was added and exposed to cells for 72 hr
↓
Cells washed after removal of the supernatant and EA rosetting with antibody coated sheep RBC was done to determine Fc receptor expressing macrophages—(expressed as percent EA rosetting macrophages after scanning at least 200 cells).

Information background: The number of macrophages with Fc receptors is lowered by live *M. leprae* after their phagocytosis and it is unaltered by heat-killed *M. leprae* when compared to normal macrophages.[3]

Table 2. FDA assay with macrophages containing *M. leprae* with or without treatment with drug.

Mouse macrophages (peritoneal) (in 80% MEM + 20% human serum) allowed to mature 96 hr
↓
Add drugs
↓ 72 hr
Add *M. leprae* (armadillo/human 5×10^6/tube)
↓ 72 hr
Wash the cultures

- Used macrophages directly for FDA acid fast staining
- Lyse macrophages
  ↓ centrifuge
  separate the bacteria
  - FDA
  - Acid-fast staining
  - FDA
    ↓
    spectrofluorometry

FDA-EB assay system

This assay system was adopted using *M. leprae* phagocytosed by macrophages and was described earlier.[16] The basic steps in this assay system are given in Table 2. Further details on this method are under publication (Bhagaria and Mahadevan communicated).

Uracil uptake assay

The test system using the uptake of labelled uracil by *M. leprae* inside the macrophages has been described earlier (Vejare and Mahadevan, under publication). The broad details are presented in Table 3.

Table 3. Uracil incorporation test with *M. leprae* inside macrophages in presence or absence of drug.

Mouse peritoneal macrophages
↓
cultured for 3 days at 37°C (in 80% MEM + 20% human serum)
↓
infected with *M. leprae* (HK, live or RFP treated)
↓
24 hr later label with ^{3}H-uracil for 6 days
↓
After washing the cells, scrapped, count taken
↓
Lysed—freeze—thawed
↓
Centrifuged
↓
Bacteria counted and TCA insoluble radioactivity determined

Sialic acid assay system

The alteration in surface sialic acid due to viable bacteria inside the macrophages from patients has been reported.[1] The basic steps involved in the assay have already been published.[1]

EXPERIMENTAL RESULTS

Fc receptor assay system

That the *M. leprae* phagocytosed by macrophages are susceptible to drugs like dapsone and rifampicin is shown by the data presented in Tables 4 and 5 respectively. *M. leprae* in presence of drugs are unable to bring down the level of macrophages with Fc receptors but only live *M. leprae* are able to do so. The minimum inhibitory concentration effective in this assay system for dapsone is 0·028 μg/ml (28 ng/ml) and for rifampicin 0·11 μg/ml (114 ng/ml) (Tables 6 & 7).

Since the determination of EA rosetting is a subjective procedure, the technique of using ^{125}I labelled anti SRBC was adopted. The data presented in Tables 8 and 9 clearly show that this technique also shows the reduction in EA rosetting macrophages is quite clear in presence of live *M. leprae* and reversed in presence of anti-leprosy drug and *M. leprae*. In presence of live *M. leprae*, lesser amounts of labelled antibody bind to macrophages and this is reversed in presence of the drug with the *M. leprae*.

Lastly, using this assay system the viability of *M. leprae* exposed to dapsone and rifampicin in the macrophages, were determined in mouse footpad. It clearly showed that those *M. leprae* exposed to drugs and showing viability to lower the Fc receptors, also showed a poor growth in the mouse footpad. This indicated loss of viability by the *M. leprae* in presence of the drug inside the macrophages (Figures 2 and 3). The drug DDS showed that it acted clearly as bacteriostatic, since the *M. leprae* after removal from the drug-containing macrophage did show viability in mouse footpad. The rifampicin- (Figure 3) treated *M. leprae* showed loss of viability.

FDA-EB ASSAY SYSTEM

Data presented in Figure 4 show that in the presence of the anti-*M. leprae* drug dapsone the ability of *M. leprae* to break down FDA and express green fluorescence of the Fluorescein is drastically reduced. This could be due to the loss of viability in presence of the drug. A typical experimental result using this assay system with rifampicin is presented in Table 10.

Since determination of fluorescing *M. leprae* is a subjective experimental procedure, we have adopted estimation of fluorescence by viable *M. leprae* by

Table 4. Percent EA rosetting macrophages in presence of armadillo derived *M. leprae* with and without exposure to dapsone.

Expt. No.	Control (Møs only)	DDS only	Macrophages with: Live *M. leprae* only	DDS and *M. leprae*	Heat-killed *M. leprae* only
1	53	40	25	51	59
2	38	36	23	38	49
3	50	40	34	51	58
4	62	44	25	53	64
5	66	46	33	52	66
6	60	40	34	61	68
7	64	42	37	62	69
8	59	48	34	60	68
9	57	46	26	58	66
10	56	39	42	54	55
11	54	41	41	56	52
Mean ± SD	56 ± 2	42 ± 1	32 ± 2	54 ± 2	61 ± 2

Control, *M. leprae* added culture significant $p < 0{\cdot}05$; *M. leprae* only, drug + *M. leprae* significant $p < 0{\cdot}05$.

Table 5. Percent EA rosetting macrophages in presence of armadillo derived *M. leprae* with and without exposure to rifampicin

Expt. No.	Control (only Møs)	Rifampicin only	Macrophages with: Live *M. leprae* only	Live *M. leprae* and rifampicin	Heat-killed *M. leprae* only
	A	B	C	D	E
1	49	32	29	70	66
2	50	35	35	73	60
3	54	32	34	70	65
4	56	34	37	71	69
5	70	54	34	69	68
6	65	49	26	68	67
Mean ± SD	57 ± 4	39 ± 4	33 ± 2	70 ± 1	66 ± 2

P value A–C < 0·05 significant. *P* value D–C < 0·05 significant.

Table 6. Percent of macrophages exhibiting EA rosetting in presence of *M. leprae* and exposed to various concentrations of DDS.

DDS added per tube	Concentration of DDS (ng/ml medium)	Control macrophages only	Live *M. leprae* only	Macrophages containing: DDS only	DDS and live *M. leprae*	Heat-killed *M. leprae*
		A	B	C	D	E
10	14·2	49 ± 14	24 ± 6	30 ± 8	31 ± 9	55 ± 13
15	21·4	70 ± 7	46 ± 7	59 ± 3	59 ± 3	70 ± 0
*20	28·5	75 ± 5	48 ± 6	65 ± 5	76 ± 5	75 ± 5
50	71·4	72 ± 2	46 ± 8	60 ± 2	71 ± 2	72 ± 2
100	142·8	68 ± 3	42 ± 3	56 ± 9	70 ± 3	69 ± 5

Each value is an average of four experiments with each concentration of the drug added. A–B, $P < 0{\cdot}05$ significant; A–C, $P < 0{\cdot}05$ significant; A–D, $P < 0{\cdot}05$ significant up to 15 ng only; A–E, $P > 0{\cdot}05$ not significant; A–D, $P < 0{\cdot}05$ significant above 15 ng only.

* Minimum inhibitory concentration (MIC).

Table 7. Percentage of macrophages exhibiting EA rosetting in the presence of *M. leprae* and at various concentrations of rifampicin

REP conc. (ng) Per tube	(per ml) (ng)	Control Mθ only A	%EA rosetting (Mean ± S.D.) Mθ + *M. leprae* B	Mθ + RFP C	Mθ + RFP + *M. leprae* D	Mθ + *M. leprae* (H.K.) E
20	28·5	59 ± 3	31 ± 3	43 ± 7	49 ± 7	59 ± 3
50	71·4	65 ± 3	38 ± 4	51 ± 6	52 ± 5	66 ± 4
†80	114·2	65 ± 6	34 ± 3	51 ± 6	63 ± 4	64 ± 5
100	142·8	62 ± 4	36 ± 5	53 ± 4	62 ± 5	62 ± 1

* 3 experiments were carried out for each concentration and the mean ± SD is presented. 5×10^6/Leighton tube of *M. leprae* was used. Significance of difference (Student's *t* distribution test). *P* value is for data under each concentration of rifampicin used. A–B $P < 0{\cdot}05$; A–C $P < 0{\cdot}05$; A–D $P < 0{\cdot}05$ up to 50 ng only; A–E $P < 0{\cdot}05$; B–D $P > 0{\cdot}05$ up to 50 ng only.

† Minimum inhibitory concentration (MIC).

Table 8. Alteration in Fc receptor expressing macrophages in presence and absence of *M. leprae* as indicated by binding of ^{125}I labelled antibody coated SRBC.

Sample	Counts/400 s			
	1	2	3	AV ± SD
Control macrophages	772	438	423	544 ± 197
Macrophage + live *M. leprae*	188	142	142	157 ± 26
Macrophage + heat-killed *M. leprae*	692	535	310	512 ± 158
Macrophage + DDS (0·03 μg/ml)	266	230	190	228 ± 38
Macrophage + live *M. leprae* + DDS (0·03 μg/ml)	728	406	390	508 ± 149

The dose of *M. leprae*, 5×10^6/Leighton tube.

DDS dose—0·03 μg/ml—MIC level with Fc receptor system.

Table 9. Alteration in Fc receptor expressing macrophages in presence and absence of *M. leprae* as indicated by binding of ^{125}I labelled antibody coated SRBC.

Sample	Counts/400 S			
	1	2	3	AV ± SD
Control macrophage	595	540	434	523 ± 81
Macrophage + live *M. leprae*	214	274	158	215 ± 58
Macrophage + heat-killed *M. leprae*	518	613	410	534 ± 109
Macrophage + rifampicin (0·1 μg/ml)	372	378	236	328 ± 78
Macrophage + live *M. leprae* + rifampicin (0·1 μg/ml)	623	566	442	543 ± 92

The dose of *M. leprae*, 5×10^6/Leighton tube.

spectrofluorometry. Data presented in Table 11 show that with treatment of *M. leprae* inside the macrophages with rifampicin (1·14 μg/ml) the FDA degrading the fluorescing bacteria come down as indicated by reduction in the fluorescence level at an excitation wavelength of 485 nm and emission wavelength of 520 nm in the fluorimeter.

Lastly to show that the bacteria whose viability after rifampicin treatment was low, by FDA assay, were also tested in mouse footpad. The growth patterns of drug untreated *M. leprae* and drug treated *M. leprae* are presented in Figure 5.

Table 10. Effect of rifampicin on *M. leprae* inside the macrophages as indicated by their ability to break down FDA—an indication of viability.

	Phagocytosis (%)		No. of bacilli phagocytosed/ 100 macrophages		Viability (%)
	GFB	AFB	GFB	AFB	
Macrophages + live *M. leprae* only	70·5	92	423	2823	15
With 0·14 μg/ml rifampicin	4·5	90	18	2553	0·70
With 0·7 μg/ml rifampicin	3·2	85	8	2634	0·30

AFB, acid-fast bacteria; GFB, green fluorescing bacteria.

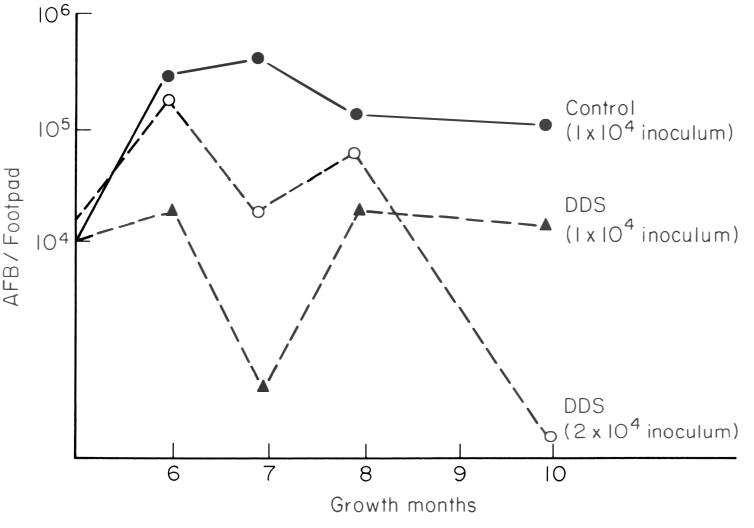

Figure 2. Loss of viability of *M. leprae* inside macrophages with dapsone (DDS) (*in vivo* assessment). Viability of Fc receptor: control, 80% rosetting macrophages; + *M. leprae*, 31% rosetting macrophages (loss of viability); + 0·03 μg DDS, 77% rosetting macrophages.

This clearly indicated that what appear as non-viable bacteria after RFP treatment by FDA test, also showed no viability in the mouse footpad.

URACIL UPTAKE SYSTEM

We had already demonstrated that uracil is taken up by free *M. leprae* and *M.*

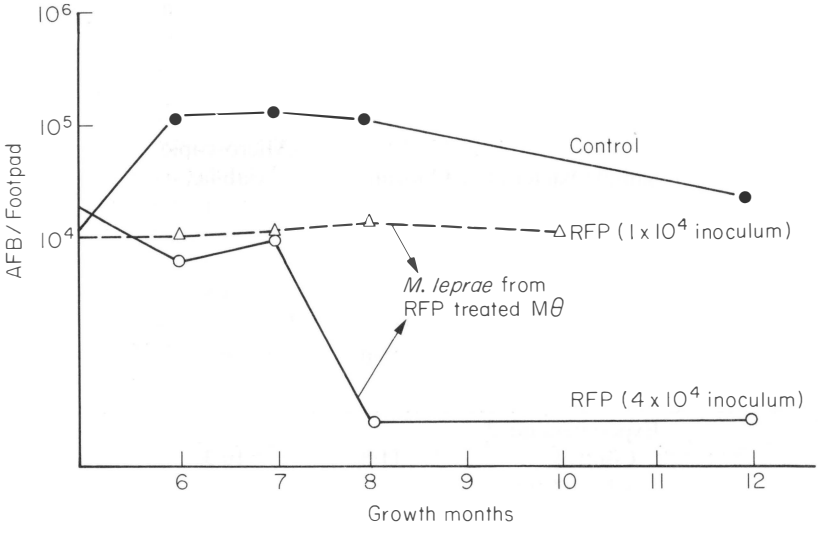

Figure 3. Loss of viability of *M. leprae* inside macrophages with rifampicin (*in vivo* assessment). Viability of Fc receptor: control, 50% rosetting macrophages; $+M.$ *leprae*, 31% rosetting macrophages; $+0{\cdot}1$ μg/ml RFP, 49% rosetting macrophages.

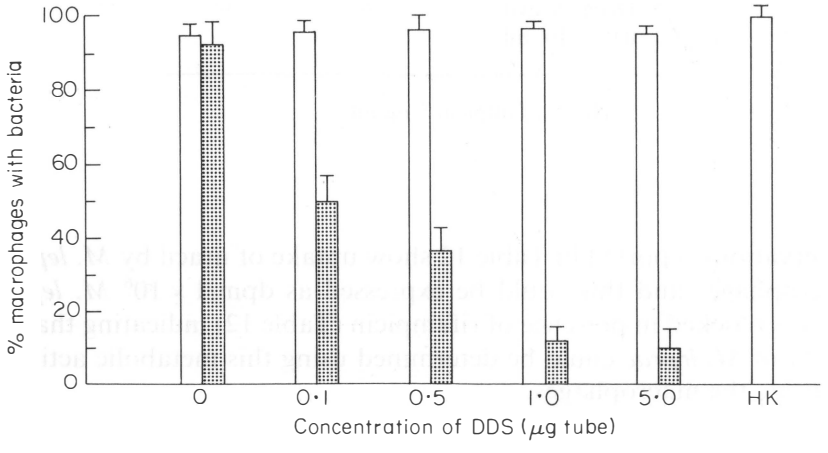

Figure 4. Effect of increasing levels of DDS on the percentage of macrophages with viable bacteria (green fluorescing) in relation to the total phagocytosis as indicated by the number of acid-fast bacteria (means $\pm$ SD of five experiments each with a count of 200 macrophages). Open columns, acid-fast bacteria; dotted columns, green fluorescing bacteria.

leprae inside the macrophages. We had also demonstrated that per unit amount of *M. leprae* more labelled uracil is taken up while the bacteria are inside the macrophages, as compared to the freely suspended bacteria (Vejare and Mahadevan, under publication).

Table 11. Loss of viability as indicated by reduced fluorescing bacteria determined by both microscopic count and spectrofluorimetry.

Sample/bacterial number	Fluorimetric reading	Microscopic viability (%)
Experiment no. 1		
Control $0{\cdot}3 \times 10^6$/ml	50·8	5·5
Drug treated $0{\cdot}3 \times 10^6$/ml	5·0	0
Experiment no. 2		
Control $0{\cdot}1 \times 10^6$/ml	11·8	10·3
Drug treated $0{\cdot}1 \times 10^6$/ml	2·5	0
Experiment no. 3		
Control $0{\cdot}05 \times 10^6$/ml	111·3	5·2
Drug treated $0{\cdot}05 \times 10^6$/ml	31·6	0

Drug, rifampicin 5 μg/ml.

Observations reported in Table 12 show uptake of uracil by *M. leprae* inside the macrophages and this could be expressed as dpm/1×10^6 *M. leprae*. This uptake was blocked in presence of rifampicin (Table 12) indicating that the drug sensitivity of *M. leprae* could be determined using this metabolic activity of *M. leprae* inside the macrophages.

SIALIC ACID ASSAY SYSTEM

We had reported that in the macrophages from bacillary negative lepromatous leprosy patients, live *M. leprae* induce a lowering of surface sialic acid.[1] This observation is also borne out by data presented in Table 13. This alteration of sialic acid level by *M. leprae* was shown to be both host and bacteria specific. Heat killed *M. leprae* were not able to do so. Thus, extrapolating this information we have also demonstrated that if viability of *M. leprae* is reduced by rifampicin (5 μg/ml) inside the macrophages, then they also lose the ability to reduce the surface sialic acid level of macrophages.

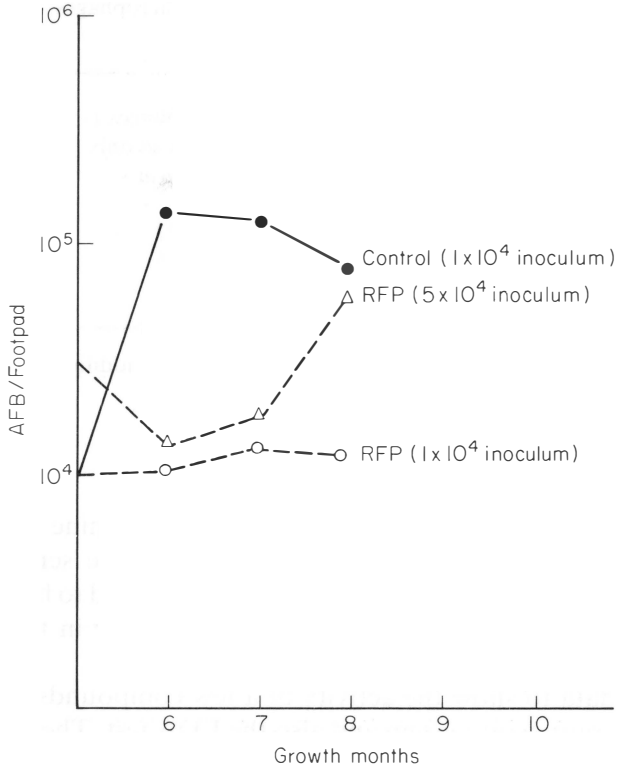

Figure 5. Loss of viability of *M. leprae* inside macrophages with rifampicin (RFP) (*in vivo* assessment). FDA viability: control, 30%; RFP, 1·14 μg/ml, 12·5%.

Table 12. Incorporation of ^{3}H-uracil by *M. leprae* (armadillo & human) inside macrophages (1×10^6) in presence of RFP.

	Ar_1	Ar_2	FMR 801*	FMR 805*	
Heat-killed *M. leprae*	1660	496	840	140	$p < 0{\cdot}01$ significant
Live *M. leprae* only	2950	1705	1140	994	$p < 0{\cdot}025$ significant
+ RFP (10 μg/ml) treated *M. leprae*	659	577	320	272	

* From patients.
Incorporation is expressed as dpm/1×10^6 *M. leprae*.

Table 13. Levels of removable sialic acid as n moles/10^6 macrophages in presence and absence of antileprosy drug, rifampicin.* (Macrophages from bacillary negative lepromatous leprosy patients.)

Expt. No.	Control macrophages	Macrophages + *M. leprae*	Macrophages + rifampicin only (5μg/ml)	Macrophages + rifampicin + *M. leprae*
1	25	10	22	25
2	28	8	25	26
3	30	12	28	30

* Rifampicin added to macrophages 48 hr before addition of *M. leprae*.

NEW COMPOUNDS—ANTI-M. LEPRAE ACTIVITY

If the above *in vitro* assay systems are useful to determine viability and drug sensitivity of *M. leprae* then new compounds could be screened for activity against *M. leprae* using these test systems. If they are found to be active against *M. leprae*, they should also show activity against *M. leprae* in the mouse footpad assay system.

We present data to show the activity of a few compounds against *M. leprae* using the Fc receptor assay system and also the FDA test. The compounds tested were Deoxyfructoserotonin, Brodimoprim (in combination with DDS), ciprofloxacin, methyoxy-2-Indole-derivative and Folate analogues, and K.130 in

Table 14. Percent EA rosetting of macrophages exposed to varying concentrations of deoxyfructoserotonin (DFS) in presence of *M. leprae*. (Percent EA rosetting (AV ± SD).)

DFS concentration (ng/ml)	Control macrophages only	Macrophages + live *M. leprae*	Macrophages + DFS	Macrophages + DFS *M. leprae*	Macrophages + heat-killed *M. leprae*
20	64 ± 7	37 ± 5	49 ± 3	41 ± 6	67 ± 6
50	57 ± 8	33 ± 4	45 ± 8	44 ± 8	58 ± 11
*70	62 ± 10	40 ± 14	46 ± 10	61 ± 10	63 ± 12
†80	62 ± 11	32 ± 16	51 ± 9	65 ± 16	62 ± 18
100	70 ± 18	35 ± 5	63 ± 20	78 ± 9	70 ± 11

* MIC of DFS.
† Concentration from which DFS could be immunomodulating.
Results are an average of 3 experiments.
Dose of *M. leprae*—5×10^6/Leighton tube.

Table 15. Percent EA rosetting by macrophages in presence of *M. leprae* and of varying concentrations of ciprofloxacin. (Percent EA rosetting Av ± SD).

Drug concentration (ng/ml)	Control macrophages only	Macrophages + live *M. leprae*	Macrophages + ciprofloxacin	Macrophages + ciprofloxacin + *M. leprae*	Macrophages + heat-killed *M. leprae*
20	61 ± 6	41 ± 8	46 ± 7	46 ± 3	65 ± 6
*50	61 ± 10	41 ± 16	47 ± 11	59 ± 7	61 ± 9
80	62 ± 9	40 ± 5	51 ± 8	65 ± 8	63 ± 14
100	63 ± 12	42 ± 9	56 ± 2	65 ± 6	59 ± 11

Results are an average of 3 expts at each concentration.
Dose of *M. leprae*—5×10^6/Leighton tube.
* MIC of ciprofloxacin—50 ng/ml.

combination with DDS (Tables 14, 15, 16, 17, 18). The data are presented and observations are mentioned in the legend under each table.

Among these compounds Deoxyfructoserotonin, Brodimoprim and K-130 (in combination with DDS) have been reported to be active on *M. leprae* in mouse footpad tests[13] (Gelber *et al.*, Dhople *et al.*, personal communications).

Discussion

The methods adopted for determining viability of *M. leprae* by us have been based on the behaviour of *M. leprae* and the host cell (macrophages) when they are together. None of the test systems depend on the necessity of multiplication of *M. leprae*. But they depend on the expression of the metabolic activity of *M. leprae* (FDA assay, uracil uptake) or changes introduced by the pathogen-host interaction in the host cell (Fc receptor changes, sialic acid change). It is clear from the data presented on the effect of drug on these parameters, that there is a definite MEC (minimum effective concentration) in all these cases. It was shown to be 0·028 μg/ml for DDS and 0·11 μg/ml rifampicin in Fc receptor assay system and 0·1 μg/ml. DDS and 0·17 μg/ml rifampicin in FDA assay system (Jagannathan & Mahadevan, 1986, Bhagaria & Mahadevan, under preparation). Thus it is clear that the phenomenon observed is basically an actual event which is controlled by the level of drug exposed to *M. leprae*.

Further it has been demonstrated when loss of viability of *M. leprae* was indicated in presence of the drug in the Fc receptor assay system (inability to reduce EA rosetting macrophages). Such *M. leprae* had also shown loss of

Table 16. Percentage of macrophages exhibiting Fc receptors in the presence of *M. leprae* (ML) with or without added dapsone (DDS) or brodimoprim (BDP) (means + SD from at least 3 experiments for each concentration).

Dosages of drugs added (μg/ml)		Control (macrophages only)	Treatment							Fractional inhibitory index
DDS	BDP		ML	BDP	DDS	DDS + BDP	DDS + ML	BDP + ML	BDP + DDS + ML	
0·022	—	70 ± 1	46 ± 7	—	55 ± 3	—	59 ± 3	—	—	—
0·028	—	75 ± 5	48 ± 6	—	65 ± 5	—	76 ± 5	—	—	—
—	5·7	66 ± 1	34 ± 2	45 ± 4	—	—	—	55 ± 1	—	—
—	7·1	70 ± 3	44 ± 3	55 ± 1	—	—	—	71 ± 5	—	—
0·014	2·8	69 ± 8	35 ± 2	56 ± 5	—	38 ± 2	—	60 ± 8	71 ± 11	0·9
0·007	1·4	78 ± 12	40 ± 7	58 ± 9	—	43 ± 4	—	50 ± 4	79 ± 12	0·45*
0·007	0·70	78 ± 3	42 ± 3	52 ± 1	—	64 ± 3	—	46 ± 3	78 ± 2	0·35*
0·007	0·35	74 ± 14	49 ± 4	50 ± 7	—	58 ± 7	—	49 ± 7	41 ± 8	—

* The most effective combinations of the two drugs.

Table 17. Percent EA rosetting of varying concentrations of K-119 (Indole-2-carboxylic acid) against *M. leprae*. (Percent EA rosetting (Av±SD).)

Drug concentration (ng/ml)	Control macrophages only	Macrophages + *M. leprae*	Macrophages + K-119	Macrophages + K-119 *M. leprae*	Macrophages + heat-killed *M. leprae*
20	65±9	36±8	46+8	49+14	65±9
50	59±8	32±9	36±4	38±7	58±6
*80	55±6	35±9	35±8	60±11	53±4
100	60±10	33±15	47±7	58±10	59±12

Results are an average of 3 experiments.
Dose of *M. leprae*—5×10^6/Leighton tube.
* MIC of K-119—80 ng/ml.

viability through their inability to hydrolyse FDA and consequent failure to exhibit green fluorescence. There is correlation between the two *in vitro* methods.

The fundamental correlation of loss of viability of *M. leprae* inside the macrophages in presence of drug and as determined by Fc receptor or FDA assay was the demonstration that such *M. leprae* showed no growth or at best poor growth in the mouse footpad. The *M. leprae* prepared in a similar way from macrophages without drug treatment showed good viability.

The routine procedure of using microscope and determining either fluorescence of the bacteria or rosetting macrophages was found to be a valid technique, since the observations obtained were correlated with spectrofluorimetric measurements or ^{125}I labelled antibody binding to Fc receptors in the respective tests mentioned above.

Thus it is very clear that we have demonstrated new *in vitro* assay systems capable of determining drug sensitivity and viability of *M. leprae* in less than 10–15 days. Consequently one should be able to determine the potentiality of anti-*M. leprae* nature of some new compounds. We have been able to do these kind of tests and have identified the following compounds as active against *M. leprae*: 1, Deoxyfructoserotonin; 2, Indole-2-methoxy compound; 3, Diflunisal (Merck, Sharp and Dohme); 4, Ciprofloxacin (Bayer); 5, Brodimoprim alone or in combination with DDS (Dr J K Seydel); and 6, K-130 (Dr J K Seydel).

Leprologists will accept efficacy of these drugs if they could be demonstrated to show activity against *M. leprae* infected in mice. The role of mouse metabolism preferentially inactivating the drug or poor pharmacokinetics in mice can lead to wrong data. But this is ignored.

Nevertheless if correlation comes, everybody is satisfied. Thus anti-*M. leprae* activity against *M. leprae* in mice has been shown for deoxyfructoserotonin,[13]

Table 18. Percent EA rosetting of macrophage with *M. leprae* exposed to varying concentrations of the drug K-130 along with DDS. (Percent EA rosetting (Av ± SD).)

Drug concentration		Control						
DDS (μg/ml)	K-130 (μg/ml)	(macrophages only)	Macrophages + *M. leprae*	Macrophages + K-130	Macrohages + K-130 + DDS	Macrophages + K-130 + *M. leprae*	Macrophages + K-130 + DDS + M. leprae	Macrophages + heat-killed *M. leprae*
0·007	0·01	65 ± 15	35 ± 7	—	42 ± 1	—	55 ± 14	64 ± 15
*0·007	0·02	80 ± 6	45 ± 11	—	61 ± 1	—	79 ± 6	78 ± 6
—	0·70	55 ± 8	31 ± 1	43 ± 15	—	39 ± 2	—	59 ± 7
†—	1·00	50 ± 1	31 ± 1	34 ± 3	—	50 ± 3	—	48 ± 2

* Amount of the drug K-130 for synergism with DDS—0·02 μg/ml.
† MIC of the drug K-130—1 μg/ml.
Dose of *M. leprae*—5×10^6/Leighton tube.
Data expressed as Av ± SD from a minimum number of 3 expts at each concentration.

Brodimoprim in combination with DDS (Gelber *et al.* personal communication), and K-130 (Dhople, personal communication).

We are now in the process of testing ciprofloxacin, diflunisal and Indole-2-methoxy compounds in the mouse footpad system in our laboratory.

It would be worthwhile to note, following the *in vitro* assay system in our laboratory, that DFS was identified as anti-*M. leprae*[2,7] and later confirmed in mouse footpad and is now in clinical trial in Bombay, India. This should be considered as a unique success in the drug development programme against leprosy. This success opens up vast potential for exploitation of the lead shown by the *in vitro* assay system developed in our laboratory.

The advantages of these *in vitro* assay systems are: (a) it is completed in less than 10–12 days; (b) *in vitro* MIC can be determined; (c) synergistic activity between two different drugs can also be established; and (d) static or cidal effect can be assessed. Among the drawbacks: (a) one needs at least 5–10 million *M. leprae* for each assay as compared to 1×10^4 in mouse footpad; and (b) as patients improve on drug therapy, viability goes down and thus to monitor the viability one has to use higher numbers of bacilli and this may lead to ambiguous information.

Nevertheless, we are in a position now to identify potential anti-*M. leprae* compounds much faster than we were 5 years ago. This is a definite advance in drug research in the area of leprosy control.

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Diflunisal and indole-2-carboxylic acid were identified as possible antileprotic agents and made available to us for testing as a result of an extensive study of tyrosinase inhibitors by our collaborators E G Beveridge, M Hooper and S K Yeap. *J Pharm Pharmac* 1985; **37**(Suppl.) 149P.

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