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A high incidence of viable *Mycobacterium leprae* in post-MDT recurrent lesions in tuberculoid leprosy patients

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Introduction

The differentiation between relapse and late reversal reaction in post-MDT recurrent lesions in paucibacillary cases of leprosy is difficult under field conditions.[1] Even histopathological studies are of limited use.[2] Therefore, a therapeutic course of corticosteroid is recommended[3] and is used in practice particularly in tuberculoid (paucibacillary) cases presenting with fresh activity in old and/or appearance of new lesions after release from treatment. This is based on the assumption that a delayed hypersensitivity type reaction to persisting dead bacterial products (antigen), causes late reversal reaction. The present study was carried out to determine whether viable bacteria are present in recurrent lesions of tuberculoid and borderline tuberculoid leprosy cases. The type and activity of the lesions are assessed histopathologically. The results obtained in a small group of 25 patients are presented in this paper.

Material and methods

Twenty-five skin lesional biopsies obtained from 25 cases of borderline tuberculoid (BT) leprosy referred to the Foundation between 1996 to 1998 and presenting with recurrent lesions 1–13 years after the release from treatment were studied histopathologically and also tested for the presence of viable bacteria, using the standard mouse foot pad method. Only confirmed cases of tuberculoid leprosy with reliable treatment and follow up details were included in this study. For each patient, a detailed history was taken, including pretreatment presenting symptoms, treatment details, reactions if any, followed by a clinical charting to record the old and the new skin lesions as well as nerve lesions. Skin smears and lepromin testing were also done. A deep incision skin lesional biopsy was obtained from an active lesion, using local anaesthesia.

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Part of each skin biopsy was fixed in formal Zenker and embedded in paraffin. Five micron thick sections stained with Trichrome modified Fite Farraco (TRIFF) were used for determining the classification (as per the Ridley–Jopling classification),[4] type and extent of cellular infiltrate and presence of acid fast bacilli (AFB) if any, in the section. Another part of the biopsy was homogenized within 24 h and bacterial load per gram weight was determined using the standard protocol (WHO/CDS/Lep/86.4.1987).[5] The weight of the tissue available for homogenization ranged between 0·1 and 0·3 g. The final volume of the suspension was maintained at 1 ml per 0·1 g of tissue weight. The homogenate thus obtained, regardless of presence or absence of any AFB, was injected into both the hind foot pads (0·03 ml/foot pad and inocula size not exceeding 1×10^4 *M. leprae*), of a minimum of 10 Swiss white mice in each case. Foot pad harvests were carried out at 6, 7 and/or 8 and 12 months post-inoculation using Shepard's method.[5] In short, a known volume of foot pad tissue suspension was spread over the spot slide, fixed and stained. AFB were counted in a minimum of 200 microscopic fields/sample. The lower limit of detectability by this countering method is 1×10^4 *M. leprae*/ml. A minimum of two counts per foot pad were obtained at 6, 7 and 8 months. All the remaining mice were harvested at the 12th month.

DEFINITION OF SIGNIFICANT GROWTH IN THE FOOT PADS OF NORMAL S/W MICE

One or more per foot pad counts showing $\geq 1 \times 10^5$ *M. leprae* in the harvests carried out at the 6th month or later (WHO/CDS/LEP/86.4,1987).[5]

RESULTS

Clinical findings

All 25 cases in this study were smear negative, lepromin positive and clinically presented with borderline tuberculoid (BT-TT) type of leprosy at the time of recurrence of lesions. All had presented with at least one impaired nerve (mostly sensory) and one or more skin lesions (maximum five). None of the cases had overt clinical signs of type 1 reaction. In most cases the recurrent skin lesion/s were mildly erythematous. Their pretreatment classification was also on the tuberculoid end of the spectrum, viz. TT-BT. The duration of treatment varied from 6 months to 3 years. All except five cases were treated with two drugs, viz. DDS 100 mg daily and RFP 600 mg monthly. In five cases, CLF was added later apparently for persisting neuritis. Fifteen (60%) had received a therapeutic course of corticosteroid during or after release from treatment (see Table 1).

HISTOPATHOLOGY

Thirteen biopsies showed features characteristic of active BT leprosy, viz. the presence of granulomatous infiltrate consisting of epithelioid cells and Langerhans type of giant cells surrounded by lymphocytes (see Figure 1a,b). Two biopsies had a few AFB (1+) in the TRIFF stained sections. Thus, both clinical and histopathological evidence was suggestive of BT reactivation/relapse. The remaining 12 lesions studied showed features of type I reaction, viz. increase in intra- and extracellular oedema, fibroblast proliferation, increased

vascularity along with epithelioid cell granuloma (see Figure 2a,b). A few AFB (1+) were seen in one of these lesions (see Table 1).

BACTERIAL LOAD AND THE VIABILITY TEST RESULTS (TABLE 1)

Of the 13 biopsies that had shown active BT pathology, only two cases were a few AFB were noted in the homogenate. The bacterial loads were 7 and 3×10^5/g wt. In the others no AFB were observed in 200 microscopic fields. Nevertheless, five of the inocula showed over 10-fold growth in the foot pad, (this includes one of the two cases that showed AFB in the section as well as homogenate) thus viable bacteria were detected in 5/13 relapsed BT cases (38·5%).

Of the 12 biopsies that had features of reversal reaction, one had AFB in the homogenate. Seven of the inocula showed significant growth in the foot pad; thus viable bacteria were detected in 7/12 reactional BT lesions (58·3%).

Although the slit skin smears were negative in all 25 patients, the homogenate showed AFB in three cases and viable bacteria were detected using the mouse foot pad technique in 12 lesions.

Discussion

The present study looked for viable mycobacteria in post-MDT recurrent lesions in borderline tuberculoid leprosy. Viable mycobacteria were detected in 48% of the recurrent lesions in this borderline tuberculoid group of patients, despite the lower limit of detection sensitivity being 1×10^4/ml in the system used. This implies that the occurrence of viable bacteria in tuberculoid lesions is fairly common. Interestingly the incidence of viable bacteria in lesions showing histopathological evidence of reaction was higher than that of the non-reactional lesions. Therefore, it can be deduced that these reversal reactional lesions are probably relapses presenting with reaction. In retrospect, the presence of a growing bacterial focus could be playing a crucial role in triggering the reaction. Fifteen of the cases in the present study, before the biopsy, had received a therapeutic course of steroid during and/or after release from treatment or on recurrence of lesions. It would be premature to comment on whether the use of corticosteroid had any role to play in promoting the survival of *M. leprae*, but results obtained in the present study suggest that a more systematic study should be undertaken. The WHO control studies report that in PB cases 32·9% of relapse occur during the first year, 34·1% of relapse during the second year and 16·5% of relapse during the third year. Thus the majority of relapse (83·5%) occurred during the first three years of release from treatment.[6] It is noteworthy that seven of the relapses in this series occurred 5–13 years after the release from treatment and five of them had viable mycobacteria in the foot pad testing. Persistence of solidly stained osmiophylic bacilli in the peripheral nerve were recorded through electron microscope, in 31% of BT cases long after stopping the treatment.[7] These findings suggest that even in PB patients, reactivation of the disease due to persisters occur as late as 5–13 years after the release from treatment.

In the present study, the mouse foot pad results were interpreted applying the criteria given by the WHO/CDS/LEP/86.4 1987 report. We have shown that, using foot pads of

Table 1. Summary of clinical and bacteriological findings in 25 recurrent BT lesions. RFT, release from treatment, MFP, mouse foot pad; PB-MDT, DDS + RFP; MB-MDT, DDS + RFP + CLF; (+S) patients who had received therapeutic course of corticosteroid

Case no.	Details of patients/ relatives	RFT duration in years	Histopathology	Bacterial load/g wt	Viability in MFP (count $\times 10^4$/foot pad)		
1	BK/67 F PB-MDT × 3 years	7	BT granuloma	0	6th 7th 12th	0 0 147, 33, 28, 1·3, 0, 0	0 0
2	JV/51 M PB-MDT × 2 years (+S)	2	BT granuloma (+RR)	0	6th 7th 12th	0 0 0/6	0 0
3	KBD/50 M PB-MDT × 3 years (+S)	2	BT granuloma	0	6th 7th 12th	0 0 0/4	0 0
4	SV/47 F PB-MDT × 1 year (+S)	2	BT granuloma	0	6th 7th 12th	0 0 0/5	0 0
5	HD/32 M PB-MDT × 2 years (+S)	9	BT granuloma (+RR)	0	6ht 7th 12th	0 0 135, 134, 133	0 0
6	KS/16 F PB-MDT × 1 year (+S)	2	BT granuloma (+RR)	0	6th 7th 12th	0 0 0/4	0 0
7	TS/41 F MB-MDT × 7 months (+S)	3·7	BT granuloma (+RR)	0	6th 7th 12th	0 27 0/4	0 0
8	AP/38 F PB-MDT × 3 years (+S)	3	BT granuloma (+RR)	0	6th 8th 12th	0 13 0/4	0 0
9	PS/25 M MB-MDT × 3 years (+S)	9	BT granuloma	0	6th 7th 12th	14 0 0/4	4·0 0
10	DH/35 M PB-MDT × 2 years (+S)	10	BT granuloma	0	6th 7th 12th	0 0 227, 18, 411, 197, 49, 12, 96, 0	0 0
11	ST/28 M MB-MDT × 2 years (+S)	3	BT granuloma	0	6th 7th 12th	0 0 0/5	0 0
12	PK/30 F PB-MDT × 1 year MB-MDT × 2 years (+S)	1	BT granuloma (+RR)	0	6th 7th 12th	0·7 0 0/4	0 0

normal Swiss White mice, it was possible to obtain a significant fold increase in 12/25 inocula that apparently contained less than 1×10^4 *M. leprae* per ml of tissue homogenate (0·1 g of tissue). It is important to note that in all except four cases, a significant fold increase in the foot pad was obtained only at the 12th month. A similar delay in growth in the foot pads of normal mice was recorded by us in two cases of secondary drug resistance.[8]

Table 1. Continued

Case no.	Details of patients/ relatives	RFT duration in years	Histopathology	Bacterial load/g wt	Viability in MFP (count×10^4/foot pad)		
13	SK/35 F PB-MDT × 6 months (+S)	1·9	BT granuloma	3 × 105	6th 7th 12th	0 0 0/5	0 0
14	GR/39 M PB-MDT × 6 months	2·5	BT granuloma (+RR)	7×10^5	6th 7th 12th	0 0 139, 65, 147, 111	0 0
15	PG/58 M PB-MDT × 1 year (+S)	2	BT granuloma (+RR)	0	6th 7th 12th	0 27·5 0/4	0 0
16	SM/28 M PB-MDT × 6 months	5	BT granuloma	0	6th 7th 12th	0 0 0/5	0 0
17	GK/14 F PB-MDT × 6 months	6	BT granuloma	0	6th 7th 12th	0 0 0/4	0 0
18	KV/48 M PB-MDT × 6 months	2	BT granuloma (+RR)	0	6th 7th 12th	0 0 0/6	0 0
19	UK/40 F PB-MDT × 10 months	1	BT granuloma	0	6th 7th 12th	0 0 0/6	0 0
20	GR/14 F PB-MDT × 6 months	3	BT granuloma	0	6th 7th 12th	0 0 0/5	0 0
21	YT/18 M PB-MDT × 6 months (+S)	2·3	BT granuloma (+RR)	0	6th 7th 12th	0 0 47, 0, 0, 0	0 0
22	IN/19 F PB-MDT × 18 months	2	BT granuloma	0	6th 7th 12th	0 0 1·4, 4, 8, 14, 1·4, 2·7, 0, 0·6	0 0
23	DB/15 M PB-MDT × 1 year	6	BT granuloma	7×10^5	6th 7th 12th	2·3 1 11, 1, 0, 0, 0	0 0
24	YP/21 M PB-MDT 6 months (+S)	2	BT granuloma (+RR)	0	6th 7th 12th	0 0 0/4	0 0
25	PK/32 M MB-MDT × 2 years	13	BT granuloma (+RR)	0	6th 7th 12th	0 0 4·8, 32·6, 115, 3, 3, 37, 66	0 0

In the present series, in four cases *M. leprae* fold increase was noted at one of the earlier intervals but not at the 12-month harvest. Known contributory factors to such inconsistent results are subminimal number of bacteria in the inocula, tendency of *M. leprae* to clump and loss of inocula due to dissemination.[9] Our findings reiterates the importance of carrying out regular foot pad harvests and extending at least up to the 12th month besides proving that the foot pads of the normal Swiss white mice could serve as a good, more importantly

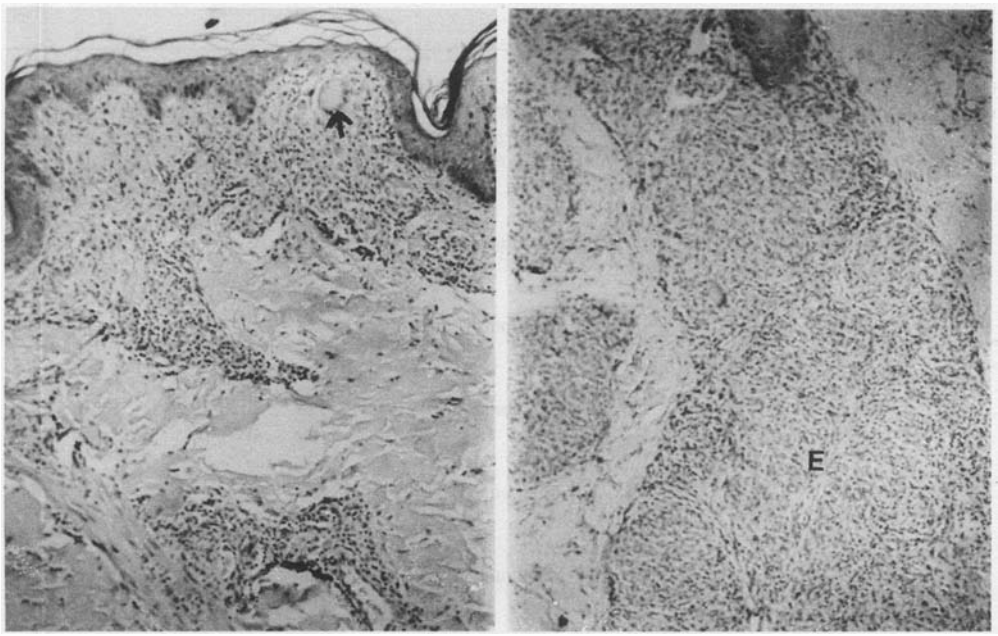

Figure 1. a Characteristic borderline tuberculoid type of infiltrate consisting of epithelioid cells, Langerhans giant cells (arrow) and lymphocyetes are seen in the superficial dermis in a relapsed BT patient (case 10). TRIFF stained section. Magnification × 150. **b** Similar large granulomatous infiltrate (I) is seen coursing along the hair folicle in the mid dermis, in the above lesion. Magnification × 150.

a specific and a time tested system that allows the expansion of an inocula containing less than 1×10^4 organisms. The time taken indeed is a major disadvantage. The quicker molecular based technique such as RNA based polymerase chain reaction detection system could be desirable under these circumstances provided a proper concordance is established.[10]

In summary, a total of 25 skin lesional biopsies obtained from 25 borderline tuberculoid cases of leprosy, presenting with recurrence of lesions 1–13 years after the release from MDT were studied using histopathology. They were also tested for the presence of viable *M. leprae* using the mouse foot pad method. Forty-eight percent (12/25) of the biopsies showed presence of viable *M. leprae* as determined by the significant growth ($>1 \times 10^5$/ foot pad) in the foot pads of normal Swiss white mice. The incidence of viable *M. leprae* in the lesions that showed histopathological evidence of reversal reaction (RR), viz. 7/12 (58%) was higher than the ones with no evidence of RR (5/13 = 38·5%). These findings have far reaching implications in the management of such cases.

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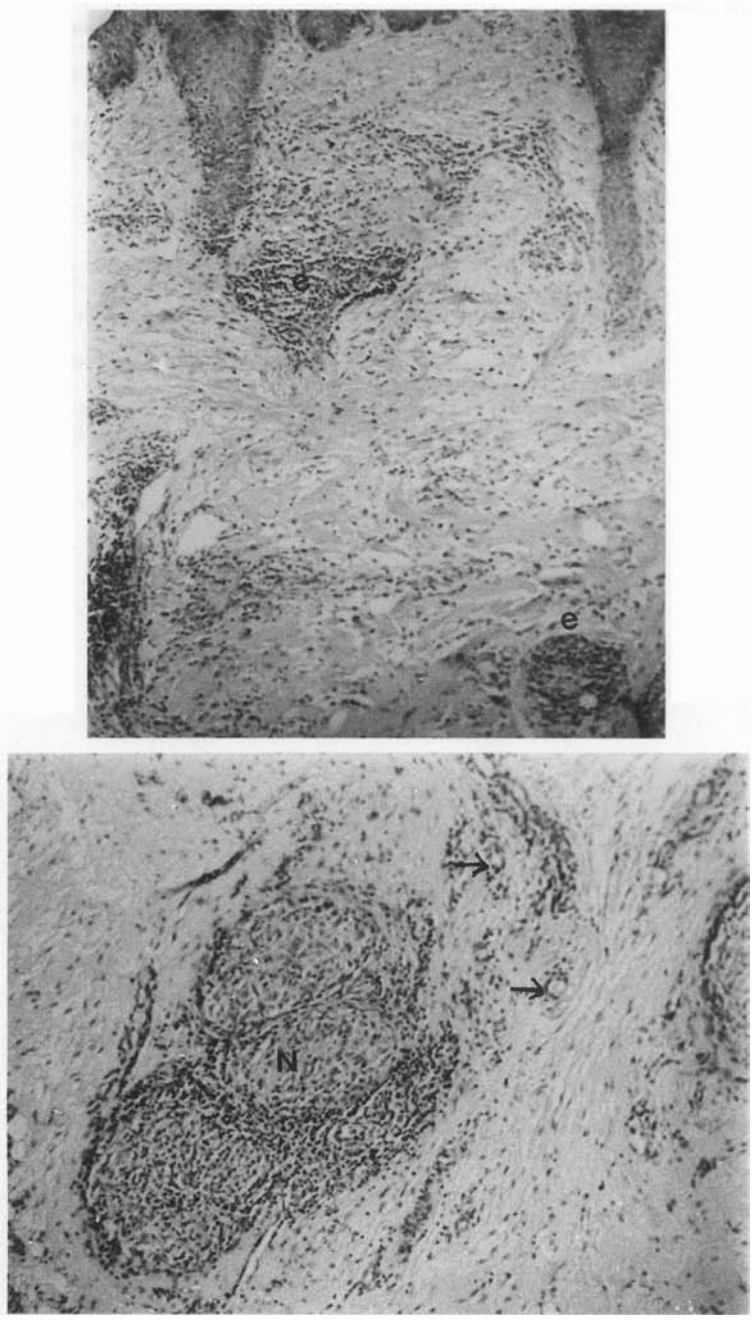

Figure 2. a A characteristic early type 1 reaction in a BT lesion (case 5). Note the presence of epithelioid cell foci (e) and proliferation of fibroblasts on an oedematous background in the superficial dermis. TRIFF stained section. Magnification × 150. **b** In the same lesion (**a**) infiltrated and oedematous dermal nerves (N) are seen in the deeper dermis. Note the proliferation of fibroblasts and blood vessels (arrow). Magnification × 150.

References

[1] Pannikar V, Jesudasan K, Vijayakumaran P, Christian M. Relapse or late reversal reaction. *Int J Lepr*, 1989; **57**: 526–528.

[2] Kesava Reddy P, Anil C. Relapse in leprosy after multidrug therapy and its differential diagnosis with reversal reaction. *Ind J Lepr*, 1991; **63**: 61–69.

[3] WHO. *A guide to elimination of leprosy as public health problem*, second edition. WHO/LEP/97.7, 1997.

[4] Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five group system. *Int J Lepr*, 1966; **34**: 255–273.

[5] WHO. *Laboratory techniques for leprosy*. WHO/CDS/LEP/86.4, 1987.

[6] WHO. The leprosy unit, division of control of tropical disease. In: *Risk of relapse in leprosy*. Document No. WHO/CTD/94.1 WHO, Geneva, 1994.

[7] Shetty VP, Suchitra K, Uplekar MW, Antia NH. Persistence of *Mycobacterium leprae* in the peripheral nerve as compared to the skin of multi drug treated leprosy patients. *Lepr Rev* 1992; **63**: 329–336.

[8] Shetty VP, Mistry NF, Uplekar MW *et al*. Dormancy, drug resistance or dependency, some thoughts to ponder. *Int J Lepr*, 1996; **64**: 455–456.

[9] Levy L, Moon N, Murray LP *et al*. Studies of the mouse foot pad technique for cultivation of *Mycobacterium leprae*. 1. Fate of inoculated organisms. *Int J Lepr*, 1974; **42**: 165–173.

[10] Gabrielle ME, van der Vliet, Sang-Nae Cho *et al*. Use of NASBA RNA amplification for detection of Mycobacterium leprae in skin biopsies from untreated and treated leprosy patients. *Int J Lepr*, 1996; **64**: 396–403.