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SPECIAL ARTICLE

Hypothesis: Erythema nodosum leprosum is precipitated by an imbalance of T lymphocytes

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Summary Erythema nodosum leprosum (ENL) has so far been taken as an immune complex mediated disease. Failure to demonstrate these complexes in or around blood vessels showing perivasculitis in a substantial number of patients with ENL has never been clearly explained. It is proposed that initiation of ENL is mediated by an imbalance of T lymphocytes, especially suppressor T cells, leading to modulation of polymorphonuclear leukocyte function.

Erythema nodosum leprosum (ENL) occurs as a complication mainly in patients with lepromatous leprosy and few with borderline lepromatous leprosy. Clinically it is characterized by crops of tender erythematous skin nodules most commonly on the face, forearms and thighs lasting only a few days. Sometimes it can also affect nerve trunks causing them to be swollen, tender and painful. Nerve abscesses have been reported to occur during ENL attacks. Other manifestations include uveitis, orchitis, lymphadenitis, arthritis and glomerulonephritis. Histologically, the hallmark of the lesions is perivasculitis, initially the infiltrating cells are polymorphonuclear leukocytes (PMN) replaced later by mononuclear cells.[1–3] This clinico-pathological picture is usually seen when most of the bacilli in the tissues are granulated or fragmented and presumably non-viable. These histological findings in conjunction with the demonstration of immunoglobulin, complement and mycobacterial antigen deposits around blood vessels in some ENL lesions, as well as circulating immune complexes (ICs) in lepromatous leprosy, have been the basis of the concept that ENL is an immune complex mediated disease, akin to a type III Arthus reaction.[4–6] Demonstration of ICs in or around blood vessels is not universally found in ENL lesions,[4,5,7] various figures being reported. Although these discrepancies might depend on the

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timing of the biopsy relative to the onset of the ENL,[8,9] the numbers of ENL lesions without demonstrable ICs around blood vessels might indicate another pathogenic mechanism. Lucio phenomenon is thought to have pathogenetic mechanism similar to ENL, and thus occurs in the lepromatous end of the spectrum.[10] It has, however, been reported recently that 3 patients with tuberculoid leprosy had developed Lucio phenomenon.[11] This would argue strongly against a primary role for the immune complexes theory. Antibodies to *Mycobacterium leprae* have been found in the aqueous humour of leprosy patients and the evidence suggests that they are locally produced. The high level of these antibodies does not seem to be related to the classification of the patient, but rather to the presence of enlarged corneal nerves.[12] Uveitis as a complication of leprosy, however, occurs only during ENL. Similarly, *in vitro* experiments with skin tissues from leprosy patients show that antibodies reacting with *M. leprae*, together with complement components, can be synthesized locally in these patients.[13,14] Intradermal injection of *M. leprae* into lepromatous patients does not, however, elicit a classical Arthus type of reaction.[15] Lepromatous leprosy has some immunological features similar to diffuse cutaneous leishmaniasis, amongst which is specific anergy to the causative agent in the presence of circulating antibodies to their respective antigens. However, ENL-like lesions do not develop in leishmaniasis.

Arthus reaction is defined as a localized acute necrotizing vasculitis. PMN and complement components have been shown to be essential for its pathogenesis. In experimental animals an Arthus reaction develops when IgG antibody and antigen combine at a vessel wall after either is initially presented at an extravascular site. Neutrophils are thought to be recruited by the action of IgG alone or by leuko-attractants released after it has reacted with complement components. Neutrophil attraction and vasculitis is, however, not an exclusive property of Arthus reaction as it has been shown to occur in experimental allergic encephalomyelitis (EAE) produced in rhesus monkeys[16] and other animals.[17] EAE is induced in animals by injection of a low molecular weight myelin basic protein (MBP) from the central nervous system. On histological examination of the brain, typical delayed type hypersensitivity reaction is found and this disease can be transferred by living, washed lymphocytes but not with serum. MBP can, however, induce EAE lesions accompanied by intense PMN infiltration and fibrinous exudates even though anti-MBP antibody neither causes *in vivo* PNS or *in vitro* CNS demyelination.[18,19] Further, PPD, which classically induces delayed-type hypersensitivity, can elicit an early and predominantly PMN leukocyte infiltration in some individuals. The recruitment of PMN in acute vasculitis, therefore, need not be exclusively due to antibody-mediated reactions, but may on certain occasions be due to delayed-type-hypersensitivity reactions. Recently, it has been shown that cyclophosphamide given in small doses can enhance Arthus reaction in guinea-pigs.[20] Since the action of cyclophosphamide in enhancing DNCB

sensitization,[21] Jones-Mote reaction and tuberculin response is thought to be on rapidly dividing suppressor T cells,[20] it may be implied that Arthus reaction is also under the influence of T suppressor cells, whereby a decrease in the suppressor cells enhances Arthus reaction.

Human lymphocytes are a heterogenous population of cells, broadly subdivided into T lymphocytes (T cells) and B lymphocytes (B cells). Within each group there are further sub-classes reflecting different physiological functions. Suppressor T cells (Ts) are a distinct population whose role in pathogenesis of human diseases is just beginning to come to light.[22–26] A decrease in the T cells function leads to a concomitant increase of helper or effector functions which might be deleterious to the patient.[22,24,26–31] T cells mediating cell-mediated immunity produce a number of soluble products termed lymphokines. These lymphokines have a variety of biological functions, including increased vascular permeability to circulating proteins and polymorphonuclear and mononuclear cell accumulation. They have also been implicated in pathogenesis of tissue injury, for example by promotion of platelet aggregation and production of thrombin-like materials which lead to vascular occlusion secondary to thrombosis. Human PMN leukocytes, on the other hand, have a high content of neutral proteases within their granules which are released upon phagocytosis. These enzymes can cause tissue damage and stimulate lymphocyte proliferation as well as producing inflammatory response histologically indistinguishable from delayed hypersensitivity, in the absence of added antigen.[32] These substances, therefore, apart from causing local tissue damage, may act on resident mononuclear cells causing further release of lymphokines which in turn increase lysosomal enzyme and protease secretions by resident cells thus aggravating injury to blood vessels.

The cause of anergy in lepromatous leprosy is poorly understood, but recently the suggestion that suppressor cells are actively involved has been put forward. A decrease of this function during the disease process is expected to disturb this delicate balance and might be reflected in certain functional tests. It has recently been shown that whereas contact sensitivity to dinitrochlorobenzene (DNCB) is depressed in lepromatous leprosy, it is on the other hand not impaired or greatly attenuated during ENL,[33] indicating a depression of suppression. In addition, using monoclonal antibodies to different subsets of T cells, it has been shown that there is a depression of suppressor cells during ENL with a concomitant increase *in vitro* phytohaemagglutinin (PHA) response, although *in vivo* responses to *M. leprae* were not affected.[34] Factors known to precipitate ENL are many and varied. They seem to share very little in common in terms of immune complexes. Further, despite circulating anti-*M. leprae* antibodies, not all LL patients develop ENL as a complication. Most of the factors associated with the precipitation of ENL can, however, be taken as causing a disturbance in the T-cell balance, favouring a drop of the suppressor cells. Thus, a strongly positive Mantoux test which can precipitate

ENL can be thought of as a decrease of suppression. In some LL patients ENL occurred within 48 h of smallpox vaccination and increased in intensity up to 8–10 days.[35] This can also be explained by a disturbance of the T cell subpopulations starting with an increase in suppressor cells, as is common with viral infections, and then followed by a decrease of this population which results in ENL precipitation. Since it is live viruses which have been implicated in the induction of suppressor cells, it is important to note that intradermal inoculation with heat-inactivated virus did not precipitate or exacerbate ENL.[35] Furthermore, it has been reported that in children with erythema nodosum, together with pulmonary tuberculosis, conversion to Mantoux positivity occurred at the time of appearance of the erythema nodosum. This can now be thought of as loss of cell-mediated suppression. Based on these observations, therefore, it is proposed that ENL is initiated by a decrease, absolute or relative, of suppressor T cells. This hypothesis can explain clinical observations that are difficult to explain using the immune complex theory.[4,5] For example, large amounts of bacillary antigen and anti-*M. leprae* antibodies are always present in LL patients but not all LL patients develop ENL. Further, diverse factors known to precipitate ENL can be brought together by this hypothesis. It has been known for a long time that although ENL can occur in untreated patients, it is more frequent in patients receiving effective anti-leprosy chemotherapy. This has been thought to be due to rapid killing of *M. leprae* with release of antigens. Mice infected with large doses of BCG, develop a systemic disease with appearance of anergy to P.P.D. This anergy is thought to be mediated by suppressor T cells. Recently, it has been shown that early specific chemotherapy in these animals leads to a dramatic decrease of these suppressor cells, probably by reducing the numbers of viable organisms. By delaying chemotherapy, the established suppressor T cells were still able to suppress P.P.D. responses *in vitro* even when the numbers of viable bacilli had been tremendously reduced.[36] In all these animals, intradermal responses to P.P.D. were persistently negative. This would be an interesting model to study ENL in the sense that chemotherapy, although unable to completely abolish the suppression, can induce an imbalance between the helper and suppressor functions, and this may be enough to initiate ENL. It is therefore felt that the increased incidence of ENL in treated patients is not only due to antigenic release but rather due to the disturbance in T-lymphocyte subpopulations and the capacity of the individual patient to manifest such change. This can explain why some patients, despite effective chemotherapy never develop ENL. The clinical picture that can vary from acute to chronic ENL can also be taken as a function of the time-interval between these T-cell imbalances. Patients in whom decreases in suppression occur frequently and at short time-intervals will be expected to develop chronic ENL. The perpetuation phase of ENL will depend on many factors amongst which are the local presence of *M. leprae* or its antigens in tissues and antibodies towards these antigens.

The distinction of the 2 phases of ENL, namely the initiation and perpetuation phases, may be of relevance in the design and trial of drugs. Drugs that are able to prevent the depression of suppressor cells will be expected to prevent ENL attacks from occurring. Cyclosporin-A, a non-toxic immunosuppressant,[37–39] has been shown to increase the proportion of suppressor T lymphocytes with a concomitant clinical improvement in patients with primary biliary cirrhosis.[26] Due to nephrotoxicity, however, long-term use could not be assessed. A drug with similar effects, but non-toxic, would then be ideal for a clinical trial in diseases thought to have T-cell imbalance, like multiple sclerosis,[22,23] primary biliary cirrhosis[26,30,31] and now ENL. Thalidomide, a very effective agent in ENL, has not been reported to be able to prevent ENL from occurring, lending further support to the 2 phases of ENL Syndrome. Thalidomide has also been reported to have beneficial effects in other diseases, most of them having very little in common as far as immune complexes are concerned. This drug also has no blanket effect on all immune complex mediated diseases. That it is effective within 24 hours of administration, indicates that its effect is not due to the solubilization or immobilization of immune complexes. It may, on the other hand, be acting on activated T cells reducing their actions and thus its beneficial effect in ENL. It would therefore be interesting to study the effect of this drug in multiple sclerosis, primary biliary cirrhosis and acute graft versus host (GVH) reaction.

In summary, it is proposed that there are 2 phases on ENL, the initiation and perpetuation phases. The initiation phase is proposed to be due to an imbalance of T-cell subsets with a decrease of suppressor T cells. Experiments designed to test this hypothesis by following T-cell imbalances in lepromatous leprosy patients are underway.

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Editorial Note

Dr H W Wheate, OBE joins the Editorial Board of Leprosy Review.

It is with the greatest of pleasure that we welcome Dr H W Wheate as a new member of the Editorial Board of *Leprosy Review*. Following many years as leprosy specialist in Tanzania and later in the All-Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa, Dr Wheate needs no introduction in the world of leprosy. At a stage in the history of this subject when teaching, training and the methodology of leprosy control are becoming of increasing importance, we look forward to the opportunity of benefitting from his wide experience and wisdom.

EDITOR

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Follow-up on short-course 2 months' rifampicin treatment of paucibacillary leprosy

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Summary The results of follow-up for between 1 and 3.5 years of paucibacillary leprosy patients treated with 8 weekly doses of 900 mg rifampicin are presented.

In the pilot trial in Burundi, 8 patients were followed for 3 years and more. All did well, including 1 patient who developed a reversal reaction.

In Addis Ababa, 3 patients on rifampicin developed neuritis at 9–18 weeks after the start of therapy and were excluded from the trial. Three patients treated with rifampicin were followed for 3 years and 5 for at least 2 years. All patients had their lesions healed or considerably improved, there were no relapses and no adverse effects due to the intermittent administration of the drug. Three patients in the dapsone-treated group were followed for 3 years and 2 for at least 2 years. In this group 1 patient developed severe neuritis and 2 others, who absconded for about 2 years, did not improve clinically, or worsen.

Compared with standard dapsone therapy, rifampicin treatment did not accelerate healing; neuritis was not more frequent but it occurred much earlier. Some points to be taken care of in similar future trials are discussed.

Introduction

Dapsone treatment of paucibacillary leprosy is still of very long duration: 2–5 years.

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Lowe[1] recommended 24 months' treatment, Wheate and Pearson[2] 2–5 years, and the third and fourth WHO Expert Committees[3,4] recommend that tuberculoid patients should continue treatment for 18 months after all activity has ceased and the lesions have become quiescent, which could mean a total of 24–36 months.

It is impossible to organize supervised treatment for such long periods, and it is well known that many patients who regularly show up to collect their dapsone tablets, do not necessarily take them.[5]

Rifampicin (RMP) is highly bactericidal for *Mycobacterium leprae.*[6–9] In a previous study on multibacillary leprosy it was found that the morphologic index reached zero values after 2 months of weekly administration of 900 mg rifampicin.[10]

Since it is estimated that paucibacillary (TT–BT) leprosy patients harbour less than 10^6 bacilli,[11] it was thought that it should be possible to cure such patients with a short-term treatment regimen of 8 weekly doses of 900 mg rifampicin.

A pilot trial with this regimen was therefore undertaken in Burundi (JB) and a controlled clinical trial comparing this regimen with dapsone treatment was conducted in Addis Ababa (JW).

Preliminary results with follow-up periods of 39–64 weeks were published previously.[12] We now present the results of follow-up on some of these patients for periods up to 36 months.

Patients and conduct of the trials

Patient selection and pretreatment investigations were described previously.[12] Patients had to have clinical and histologically confirmed TT, BT or indeterminate leprosy, with an IB of 0 or 1 on an earlobe. In Burundi, only patients with minimal or no nerve involvement and living within acceptable walking distance of the clinics were included in the trial.

They were given 900 mg RMP once a week under supervision during 8 weeks and thereafter dapsone placebo tablets. In Addis Ababa all patients presenting with paucibacillary leprosy were included and randomly allocated to one of the following drug regimens:

(1) Dapsone 25 mg/day for 4 weeks, followed by 50 mg/day for 3 years. The patients came to the treatment centre to collect their tablets. RMP placebo capsules were also given. Treatment was not supervised.
(2) RMP, 900 mg once a week during 8 weeks under supervision, followed by dapsone placebo tablets.

In both localities, clinical and neurological examination of patients was carried out at 2 months and thereafter every 3 months for at least 3 years.

Localization of skin lesions and results of neurological examinations were noted in especially devised files. The diameter of one or two patches were measured. Biopsy specimens were taken at the start, after 2 months and thereafter every 6 months. Patients developing reactions, severe enough to require corticosteroid therapy, were withdrawn from the trial and put on standard dapsone therapy.

Results

BURUNDI

In Burundi, 9 patients (1 indeterminate, 1 TT and 7 BT) were taken in. Four patients were observed for 33–36 months, and 4 during 44 months after the start of therapy. One patient (with BT leprosy) died from an intercurrent disease unrelated to leprosy (Table 1). He had been observed for 37 weeks.

Table 1. Results of clinical observations on follow-up in patients in Burundi*

Duration of follow-up	Number	Clinical status	
33–36 months	5	healed	4
		lesions diminished in number, remaining ones wrinkled	1
44 months	3	lesions diminished in number, remaining ones wrinkled	3†

*One patient died of intercurrent disease.
†One patient developed a reversal reaction during the first year of observation.

All patients did well: 4 were considered healed, 4 had their lesions considerably diminished in number and size. The skin lesions of patient 8 (Table 2) were erythematous and active for about 2 years. By the 36th week, she developed mild neuritis of the right radial nerve, which required no corticosteroid therapy. She had no complaints and did her hard work as a rural African woman, normally. At the follow-up examination at 44 months, the lesions had regressed and the skin at the sites of the lesions was wrinkling. One patient of this series, by the end of the rifampicin treatment developed satellite lesions around some macules, but these regressed rapidly.

During the observation period 2 patients went through 1 pregnancy and a third patient through 2 without any harm.

Biopsies of one skin lesion (Table 3) were taken approximately every 6 months. Those taken 2 years after the start of treatment still showed granulomas in all but one patient. Unfortunately, 1 set of 4 late biopsies was lost in transit. As a result, only 4 biopsies taken at 34–35 months after the start of therapy are available. Three of these were from healed or almost

Table 2. Results of histological examination of patients in Burundi

Pt No.	Number of lesions	Start treatment	After ± 1 year		Last examination	
3	4	15.02.77	23.03.78 H +		15.01.80 H ±	h
5	31	15.02.77 (x)	23.03.78 H +	18.07.79 H +	15.01.80 H ±	h
6	34	20.01.77	05.04.78 H ±	07.02.79 H 0	07.11.79 H 0	not h
7	14	24.03.77	25.03.78 H +	11.08.78 H +	14.12.79 H ?	almost h
8		25.03.77	13.05.78 active	16.02.79 active	08.01.80 H +	regression wrinkled
9	23	25.03.77	25.03.78 H +	16.02.79 H +	14.12.79 H ?	lesions diminished
10	29	16.10.77	06.07.78 H 0	06.03.79 H ±	08.01.80 H ?	h
11	2	16.10.77	10.07.78 H ±	06.03.79 H ± 1 lesion	08.01.80 H ?	1 lesion wrinkled
12	24	16.10.77	10.07.78 ± h			died

(x), development of satellite lesions by the end of treatment; H ±, discrete perivascular infiltration; H +, histological lesions present; H 0, histological lesions absent; H ?, biopsies lost; h, clinically healed.

Table 3. Results of clinical observations on follow-up in patients in Addis Ababa

	Dapsone treatment			Rifampicin treatment		
Duration of follow-up (months)	No.	Clinical status		No.	Clinical status	
14–17	1	1 new skin lesion, loss of ulnar nerve function	:1	1	lesions very vague	:1
20–25	1	lesion unchanged (a)	:1	2	lesions vague to very vague	:2
26–29	2	lesion increased in size (b)	:1	1	wrinkled skin (c)	:1
		healed	:1			
30–36	3	healed	:2	7	healed	:2
		fading lesions	:1		very vague (d)	:3
					size unchanged, repigmentation	:2
Total	7			11		

(a) Patient absent for evaluation and drug or placebo collection during previous 22 months.

(b) Patient absent for evaluation and drug or placebo collection during previous 17 months.

(c) Patient absent for evaluation and drug or placebo collection during previous 20 months.

(d) Claimed increase of lesions at 15 months, but this was not confirmed.

healed patients, the fourth from patient 8 who developed the protracted reversal reaction. One biopsy showed no lesion, in two there was still some discrete perivascular round cell infiltrate or some very discrete patches of epithelioid cells. The biopsy of the fourth patient (patient 8, Table 2) showed tuberculoid lesions but of a smaller size as compared with previous biopsies.

ADDIS ABABA

Of the 9 previously studied patients in the DDS group,[12] 7 could be followed for periods between 14 and 36 months (2 indeterminate, 1 TT and 5 BT) (Table 3). Three of these patients did not do well: 1 patient seen at 14 months after the start of treatment had developed new lesions and lost his ulnar nerve function. This might be the result of the patient not taking his dapsone or being infected with dapsone resistent *M. leprae*. Two other patients seen after 24 and 28 months respectively ((a) and (b) in Table 3) had unchanged, active-looking lesions or lesions increased in size. It is remarkable that these 2 patients were the only absconders in this group: 1 patient absconded after 2 months and was not seen again until 24 months, the other absconded at 11 months and was not seen again until 28 months.

Of the 13 previously studied patients in the rifampicin group, 11 could be followed for periods of between 15 and 36 months (Table 3). All patients

did well. Patient (c) in Table 3 when seen 15 months after the start of treatment claimed his lesions had increased in size, but later examinations showed the contrary and by 27 months local wrinkling of the lesions was all that was left. Patient (d) had been absent after his RMP treatment for 20 months without any harm, he has since been followed for 35 months.

Due to circumstances and frequent opposition from the patients to have biopsies these could not be taken as regularly as originally planned.

Discussion

Even long-term dapsone treatment of paucibacillary leprosy can be followed by considerable relapse rates. These depend largely on the duration of therapy, and will in the future, also on the prevalence of primary dapsone resistance.

Lowe[1] during a follow-up study of 6 months–4 years on 69 patients, observed 11.6% relapses. Seven of the 8 relapses occurred within 3–12 months after treatment ceased and 1 at 28 months. Three of the 7 patients had been treated for less than 1 year, 3 for between 1 and 2 years and 2 for 2–2.5 years. Davey[13] reported a relapse rate of 6%, Kandaswamy[14] 4%. Ekambaram[15] found a relapse rate of 1.8% in patients treated for 6 years, with 56% occurring within the first 2 years and 73.5% within the first 3 years. Later on there was a steady decline in the number of relapses. Vellut *et al.*[16] also found that the longer the maintenance therapy was given after the lesions had become quiescent, the lower was the risk of relapses and this risk decreased steadily with time. Touw-Langendijk and Naafs[17] observed 14% relapses among TT patients treated for 1.5 years, among BT patients treated for at least 5 years the relapse rate was 15% against 28% among those treated for less than 5 years. The majority of relapses occurred within 2 years.

It thus seems that follow-up periods of 2–3 years, in trials on paucibacillary leprosy, provide most useful information.

Our original fear that it would not be possible to continue the observation of the patients participating in the present trials for a sufficiently long period of time was not realized.[12] Fourteen patients out of 29 previously analysed[12] have now been followed for 3 years (8 from Burundi and 3 from Addis Ababa on RMP, 3 from Addis Ababa on dapsone therapy), and another 7 for at least 2 years (5 on RMP and 2 on DDS therapy).

It is known that some patients in Addis Ababa died accidentally or moved to other parts of the country. Some patients in the rifampicin group there and in Burundi now show up very irregularly because they consider themselves healed.

Relapses in the rifampicin-treated patients were not observed. However, the confidence limits on such a small number of patients are high.

As far as could be ascertained the rifampicin-treated patients, receiving

placebo dapsone tablets, did not take any specific drug after their 8 doses of 900 mg rifampicin. But clearly, in future trials, provisions should be made to perform dapsone tests on urine samples.

Our previous impression at 1 year,[12] that the improvement after the short-course 8-week intermittent rifampicin treatment of paucibacillary leprosy is not as good as after standard dapsone treatment, has not been confirmed. Patient 8 in Burundi had outspoken erythematous-active-looking lesions at the start of treatment and this situation remained unchanged for about 2 years. This could be interpreted as a reversal reaction although reversal reactions very rarely have such a long duration. One patient in Addis Ababa thought his lesions increased in size but this was not confirmed by further examinations and eventually his lesions healed. Furthermore, in Addis Ababa, 2 patients on dapsone therapy had abandoned therapy for considerable periods of time: 2 years, and showed either no improvement or worsening. In the rifampicin group, 1 patient after the end of treatment, absconded for a comparable period of time, but this did not influence his clinical evolution. These observations illustrate at their best the advantage of short-course chemotherapy, since patients could be released from control at an early stage.

Three patients in the rifampicin group in Addis Ababa developed neuritis, respectively after 9, 12 and 18 weeks,[12] requiring corticosteroid therapy. Later, no further cases of neuritis appeared in this group, but 1 patient in the dapsone group developed neuritis between the eighth and fourteenth month after the start of therapy. The differences in incidence of neuritis between the 2 treatment groups evidently remains statistically insignificant. But it could be that if neuritis develops in rifampicin-treated patients, it occurs at a much earlier stage. Since the development of neuritis during treatment of paucibacillary patients is in a certain way and at present unavoidable, the earlier occurrence of it under rifampicin treatment may again constitute an advantage, as patients could be instructed more precisely what to do when this complication arises.

As was expected at the start of the trial, clinical improvement in the rifampicin-treated patients continued after the administration of the drug was stopped. However, this improvement was as slow as under dapsone therapy. Particularly the skin biopsies, taken most regularly in Burundi, revealed that histological lesions remained visible for 2 years.

This is not at all surprising since symptoms in paucibacillary leprosy are mainly the result of an allergic reaction of the host to the presence of antigens of leprosy bacilli.[18, 19] This means that the treatment of paucibacillary leprosy should be aimed at 2 objectives: killing of the bacilli and stopping the allergic reaction. Evidently, RMP can realize the first objective rapidly and efficiently, since in the present series, no relapses were observed after 8 weekly doses of 900 mg RMP (the second objective will have to be taken care of by other drugs). Whether this regimen can still be shortened or more intensive treatment

regimens (daily or twice weekly or a somewhat longer treatment) would also shorten the healing phase, should be studied in future trials.

The advantages of short-course chemotherapy have been discussed elsewhere[20] and are well known from the chemotherapy of tuberculosis[21]: improved patient compliance, real possibility for supervision of treatment, lower amounts of drugs administered, lower toxicity and cost.

In this study, as in a previous one,[10] when 900 mg rifampicin once a week was administered during 3 months, no adverse effects due to intermittent rifampicin administration have been observed.

The present study has also shown that biopsies should be taken less frequently because, particularly during the early stages, they give no information and patients dislike them for they give rise to a considerable number of keloids, to which the melano-africans are so prone.

Urine samples should be tested for detection of possible additional unprescribed dapsone intake. Finally, patients developing neuritis should not be shifted to dapsone therapy but treated appropriately with corticosteroids. Whether, when this complication arises after rifampicin treatment has ended, this should be done under the cover of a bacteriostatic antileprosy drug, such as dapsone, in order to prevent relapses, remains to be studied.

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The pathogenesis and classification of polar tuberculoid leprosy

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Summary Skin biopsies received from about 1,500 patients of varied ethnic and geographical origins produced 26 cases that fell within the polar tuberculoid (TT) group on the strictest definition, and a further 18 cases that might be considered as TT on histological and immunological grounds.

The 44 cases were of 2 broad types. Nearly half were characterized by many lymphocytes but few other histological features, with no severe nerve involvement, no signs of reaction and good clinical-histological correlation. The remainder were characterized by severe nerve involvement or erosion of the epidermis and often by signs of reaction, all of which are associated with high lymphocyte transformation values; many of these cases were clinically BT.

There was a fairly sharp distinction between these 2 types, with an intermixing of features only in cases that were not truly polar. There was also a partial geographical separation of the 2 types. The first appeared to represent primary lesions with high cell-mediated immunity; the second to have evolved through reactions associated with delaye

Introduction

The history of the classification of tuberculoid leprosy is reviewed by Noussitou,[1] who points to the need for a system which is prognostically reliable enough to serve as the basis for chemotherapeutic trials. It could only be evaluated by reference to the natural outcome of the infection, but whereas Souza Lima and Souza Campos,[2] prior to the introduction of sulphones, were able to follow the natural course of 979 infections for periods up to 5 years, nothing like this is possible today. In the present paper we return to the subject of histology in relation to immunological function, partly for the sake of such value as it may have for classification, but mainly because polar tuberculoid

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leprosy is a subject of immunological interest in its own right, and the only form of established leprosy in which the natural healing processes may be observed.

The only convenient immunological parameter for general use is the lepromin reaction. The original description of the TT group was based partly on it,[3] and it was hoped that it might be sufficiently reliable for use in individual patients.[4] However, these results were based on a small number of TT patients. The lepromin reaction is still possibly the best of the immunological tests,[5] but there is appreciable variation[2] and overlap between TT and BT patients.[6] The introduction of the lymphocyte transformation test (LTT) provided a new parameter which confirmed the spectrum,[7,8] but there were serious individual discrepancies partly due to the preponderant influence of reactions[9] and partly due to serum factors.[10]

The original histological definition of the TT group was based only on lymphocytes and epithelioid cells,[3] and in the light of the LTT results it was broadened to take account of the LTT-associated histological features[4] which probably represent delayed hypersensitivity.[11] The subsequent demonstration that lymphocytes in lesions were not closely correlated with LTT values[9] raised the possibility that there were in fact 2 sorts of TT patient, one in whom cell-mediated immunity predominated, the other delayed hypersensitivity.[11] The subject is explored further in the present histopathological analysis of a larger series of patients. It is regrettable that the need to include cases from as many sources as possible precluded the possibility of full clinical or immunological correlation, but all available data is included.

Material and methods

All biopsies of near-polar tuberculoid leprosy were reviewed from a number of sources as follows; the figures refer to the total intake of leprosy biopsies. (1) Biopsies received from the Medical Research Council Unit at Sungei Buloh, Malaysia, during the 2-year period 1979–80; about 400 biopsies. (2) Biopsies received from the Medical Research Council Unit at Addis Ababa during a 2-year period, which covered a prospective trial of borderline reversal reactions during which lymphocyte transformation tests were carried out;[9] about 400 biopsies. (3) Biopsies received from the Karimui, Papua New Guinea, which were the subject of a study of early lesions in 1973,[12] together with a few more recent cases; about 100 biopsies. (4) Biopsies from a collection at the Hospital for Tropical Diseases, which included amongst others a number of cases of European or Eurasian origin; about 100 selected cases. (5) Biopsies received and reported on by Dr D J Harman at the Leprosy Study Centre during a 2-year period before the material was transferred to this Hospital in 1980; this material was of world-wide distribution; about 800 biopsies.

From this total of about 1,800 biopsies, representing probably about 1,500 cases, 44 cases were found which could be considered to fall within the TT group as previously defined[4] or to show in high degree some of the features on which that definition was based. With 1 or 2 exceptions they had received no previous treatment, and they were not in overt reaction as all such cases were excluded.

The various histological features were indexed on an arbitrary scale from ± to ++. A search was made of two or three suitably stained sections for acid-fast bacilli, which were either scanty (1 or 2 bacilli) or absent. The histology has already been amply illustrated.[4,13]

Results and discussion

HISTOLOGICAL ANALYSIS

All skin biopsies of tuberculoid leprosy were reviewed and assessed in respect of the following features: the presence of an epithelioid cell granuloma; the full development or maturation of epithelioid cells (indicated by large cell size, large nucleus with prominent eosinophilic nucleolus), in at least some areas; the presence of giant cells; the number of large Langhans'-type giant cells; the number of lymphocytes in relation to the size of the granuloma, especially in the deep zone of the dermis, and their relationship to the granuloma; the presence or absence of a clear sub-epidermal zone; the erosion of the basal and squamous layers of the epidermis by granuloma (not just lymphocytic infiltration); the degree of nerve involvement (lymphocytic infiltration, Schwann cell proliferation, nerve enlargement, disorganization of structure, central caseation); the destruction of sweat glands.

The destruction of sweat glands appeared to be related to the envelopment of glands by a large tuberculoid granuloma, and was therefore dependent mainly on the size of the granuloma. There was usually no involvement of glands situated in normal dermis (unlike nerve involvement). It was at least as likely to be seen in BT as in TT lesions, and was not a very useful criterion for classification. The results of the analysis of the other histological features are summarized in Table 1.

It will be seen that there were 19 cases with 2+ lymphocytes together with epithelioid cell granuloma, the lymphocytes encompassing the granuloma and not being situated within it. In 9 of these biopsies there were in some though not all areas compact nests of fully developed epithelioid cells which, since they are known to interdigitate, did not allow the infiltration of many lymphocytes between them (group 1). In the other 10 cases the epithelioid cells were few, immature and often dispersed with lymphocytes in between them. The histology was that of an indeterminate lesion with early tuberculoid

features (group 2). None of the cases in groups 1 or 2 scored 2+ for the LTT-associated features: involvement of nerve or epidermis, or numerous Langhans' giant cells. These features in fact were conspicuous by their near absence in most cases. The slight involvement of the nerves was surprising and could have presented a diagnostic hazard. In line with the lack of epidermal involvement there was often a narrow sub-epidermal clear zone, and in other cases the whole lesion, if small, was confined to the middle and deep zones of the dermis.

There were another 17 cases which scored 2 + for one or other of the LTT-associated features, though never for all of them. None of these cases scored 2 + for lymphocytes; some scored +, others only ± (group 3). However, there were also 8 cases with mixed features, a score of 1 + for lymphocytes and 1 + for the features associated with a high LTT, but not 2 + for either. These also are included in Table 1 (group 4).

EVALUATION OF THE SUBGROUPS

Data for a proper evaluation of the 4 subgroups was lacking, but there was evidence of a maximal immune response in groups 1 and 3. The lepromin reaction was graded as 3 + in 4 out of 4 cases in these 2 groups. AFB were found in 0/9 and 1/17 cases in groups 1 and 3 respectively. Only 1 patient (group 3) was followed up without treatment, and he healed spontaneously. Group-1 patients all had solitary lesions. In group 3 the LTT is known to be maximal.[9]

The results for groups 2 and 4 were not quite so good. There were no lepromin results for group 2; 1 case was recorded in group 4 as 3 +, another only 1 +. AFB were found in 3/10 and in 3/8 cases, respectively. One group-2 patient was proved to be self-healing, but in another the infection was evidently spreading before he was put on treatment.

Small numbers of cases from all 4 of these subgroups were included in an immunoperoxidase study of skin lesions. Immunoglobulins, complement components, lysozyme and plasminogen were all present in greater amounts in all TT subgroups than in BT or BB lesions, despite the low bacterial load in TT and a general correlation of the inflammatory mediators with bacterial load from BT to LL.[14]

DERIVATION OF THE SUBGROUPS

The best clinical correlation obtained in group 1, the original TT group of the Ridley-Jopling classification, but the number of cases found was too small to constitute a useful group for classification purposes. They were all solitary lesions, and the patients were all free of reactional features, clinically and histologically. Destructive nerve lesions were not seen. This appeared, therefore, to be a primary group though it is not known whether or not the patients had passed through an indeterminate phase.

Table 1

Sub-group	Probable derivation	No. of cases	Clinical classification	Lympho-cytes	Develop-ment epitheloid cells	Langhans' giant cells	Nerve involve-ment	Erosion epidermis	Clear SEZ	Reactional oedema	AFB ± No. cases
1.	Primary	9	8 TT 1 mac-ans	++	++	−/±	±	−	−/+	−	0
2.	Post-indeterminate	10	5 TT 4 TT−BT 1 idt	++	±/+	−	±/+	−	−/+	−	3
3.	Secondary	17	3 TT 7 TT−BT 7 BT	+/±	+/++	−/++	±/++	−/++	−/±	−/+	1
4.	Mixed	8	2 TT 4 TT−BT 2 BT	+	+/++	−/+	±/+	−/+	−/±	−/±	3

Group-2 patients presented a slightly less homogeneous clinical picture though it was still strongly tuberculoid, predominantly TT though there was more than 1 lesion in some cases. Only 1 case was clinically indeterminate but the histology in all of them strongly suggested that they were post-indeterminate. The absence of reactional features was consistent with this. It could be that some of these cases would have evolved into group 1, but their evaluation (above) suggested that, as in indeterminate cases, the outcome was not completely predictable.

The majority of group 3 patients were clincally either TT–BT or BT, though a few were TT and solitary skin lesions were not unknown. The presence of reactional oedema in at least half the cases and a clinical history of reaction in some was in keeping with the strong LTT-associated histological features which are known to be associated with reactional or post-reactional states.[9, 15] It is likely, therefore, that in this group of patients the disease evolved through a delayed recognition of bacterial antigen, which in many cases had already become disseminated. On recognition there was some degree of reaction or an overt explosion. In 1 patient with a quiescent solitary skin lesion and in another with several lesions there were associated nerve abscesses; and caseation in nerve bundles of the skin was not uncommon. Even though not all the patients had passed through a borderline phase, it was in some respects an evolved or secondary group comparable to LLs at the other end of the spectrum.

The derivation of the group 4 patients with mixed features is uncertain. Although there were only 8 of them the existence of this group, like that of the much larger BT group, seems to indicate that the distinction between the two forms of response only applies at the extreme immunological level.

INCIDENCE OF THE SUBGROUPS

There were marked geographical variations in the incidence of the two main forms of lesion. The lymphocytic type lesions (groups 1 and 2) were found in equatorial Africa, Papua New Guinea, Fiji, in some Eurasian patients, and there was one Indian maculo-anaesthetic (though some other patients of this type are probably low resistant[16] or BT, or histologically indeterminate.[17]) None were found among the large number of cases from Malaysia and Ethiopia.

The high LTT-associated lesions of group 3 and the mixed group 4 were found in patients from Malaysia, Ethiopia and Papua New Guinea, but not from equatorial Africa.

The incidence of the strong groups 1 and 3 together amounted to 26 cases out of about 1,500. When the slightly suspect groups 2 and 4 are included the total increased to 44, giving an overall incidence of about 3%. Although the types of case varied geographically there were no obvious differences in total incidence in the areas from which material was received. There are, how-

ever, reasons for thinking that the figure of 3% may be too low. It is lower than the 5.7% previously quoted,[12] though the latter may be partly attributed to a higher incidence in early lesions some of which are no doubt self-healing. It may also be that lesions which are thought to be self-healing are less likely to be biopsied. Reacting cases which might have evolved as TT (type 3) were excluded. The main purpose of the more recent material received from Malaysia and Ethiopia was the study of drug resistance in mainly lepromatous patients, which would bias the results against tuberculoid. It seems reasonable, therefore, to accept the incidence in these areas as being between 3 and 6%.

In addition to the TT cases there were about 15 cases that were intermediate between TT and BT, and perhaps more could have been found by careful searching, but the number was appreciably smaller than those included in the TT group.

Conclusion

Although the BT group is so much larger than the TT group it did not appear that there was a large gap in the spectrum between them. Even with a 5-group spectrum there are bound to be intermediate points between adjacent groups, but there does not appear to be any justification for making a special TT–BT group. TT, as it is considered here, seems to include most cases that can usefully be separated from BT, which is the 'normal' tuberculoid group.

The TT group is a small though not insignificant group of much theoretical interest. It is the infection on a knife edge, poised between progression and spontaneous healing, which is why it is uncommon.

At the highest level in the tuberculoid scale there were two fairly distinct forms. Although the immunological relationship between cell-mediated immunity and delayed hypersensitivity has not been clearly elucidated, it is difficult not to think that a measure of separation between these two lymphocyte-mediated functions is not the root cause of the different histological, clinical and geographical patterns observed here. It could be postulated that the distinction determines to some extent the manner of evolution of the infection, or perhaps it is determined partly by it. Truly immune people, who never develop lesions beyond the indeterminate stage, display only the lymphocytic immune process. Those who develop lesions lower down the spectrum display a less-well-developed mixed type of response, which may subsequently become enhanced if (and only if) there is an increase of delayed hypersensitivity.

The lymphocyte-mediated groups 1 and 2 are primary TT in the sense that the patients have never passed through a borderline phase, and to this extent they are the counterpart of the LLp group at the other end of the spectrum. The delayed hypersensitivity group 3 is secondary TT, in the sense that it has evolved as a result of reactional processes, which often commence in the borderline part of the spectrum; it is therefore the counterpart of LLs. Thus

the TT groups could be designated TTp and TTs but, unlike the LL subgroups, there is no evidence that primary and secondary TT equates with polar and subpolar performance. And the group 4 cases which are clearly sub-polar could be either primary or secondary.

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Lepr Rev (1982) **53**, 27–34

A prevalence survey on leprosy and the possible role of village 10-cell leaders in control in Muheza District, Tanzania

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Summary. A total population of 15,029 people in 12 villages was screened for leprosy. The selection of the villages in 2 divisions of Muheza District was based on a proportional cluster sampling method. An overall prevalence estimate of 7.9 per 1,000 was found. Prevalence per village ranged from 0 to 25.8 per 1,000. The prevalence was related to age and sex and a strong male preponderance was found. The type of leprosy was determined and 55% were new cases.

Community participation via village 10-cell leaders is assessed with regard to their ability to assist in leprosy control. Their use in promoting drug compliance of the patients and in diminishing the pool of as-yet-unidentified and possibly infectious cases is discussed.

Introduction

Detection of leprosy patients by means of screening surveys in communities has always been laborious and difficult. Several factors contribute to this. Low prevalence together with a dispersed rural population in a not-always-easily-accessible terrain make such an exercise time consuming and laborious.

This is aggravated by the fact that people affected by leprosy still tend to hide their disease and/or are stigmatized by their surrounding community and condemned to live at the outskirts of their village or completely outside their village.[1]

Besides this, it has often been observed that in many areas leprosy patients are unevenly distributed between villages. This makes sampling of people in such areas for the determination of leprosy prevalence more difficult as the convenient approach of sample taking with a few large clusters will not give a

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reliable prevalence estimate. The above constraints have led many investigators to limit their studies to certain easily accessible groups. Often, school surveys for age-specific prevalence determination and case finding or household-contact surveys for active case finding are done.

A recent review[2] of all surveys and available registration of leprosy patients in Tanzania sums up the situation, but due to limited reliability one has to be careful in drawing conclusions. It seems that the distribution of prevalence is patchy over the regions and even between districts in 1 region. Hyperendemic regions (according to WHO standards more than 10 cases per 1,000 population) appear to be Kigoma, Mwanza, Tabora, Mtwara and Lindi. A major leprosy public health problem (defined by WHO as a prevalence between 1 and 10 per 1,000) appears to exist in Mara, Tanga, Singida, Dodoma, Kagera, Ruvuma and Shinyanga. Other regions either lack data or the prevalence is very low, as in Arusha and Kilimanjaro (Fig. 1).

The National Programme on tuberculosis and Leprosy Control in Tanzania (NTLP) was launched in 1977, being the result of the awareness of leprosy as a public health problem. The control measures put emphasis on an integrated approach at primary health-care level throughout the country. Training of available health cadres and concentrating on case detection, case holding for the treatment period and proper treatment are key aspects of the programme.

To enable proper evaluation of the programmes baseline data on the scope of the problem are of utmost importance. Only in this way can the impact of the programme after a certain time period be measured. It was in this view that an extensive village screening survey on leprosy in Muheza District will be a contribution towards the National Tuberculosis and Leprosy Programme.

In addition we wanted to see whether 10-cell leaders* would be able to detect the leprosy patients in their 10 households, and if they could, we could use them to refer the patients in time to the existing health facilities such as village health posts, dispensaries or health centres.

Methodology

The present study was a part of the training programme in community medicine at the Medical School, Dar es Salaam. A field project on infectious diseases is one of the practical field training programmes which is carried out by medical students in their third year. The aim of the project is to gain practical experience in applied epidemiology. Being in the field, collecting data, analysing it, treating patients at the same time and referring them where necessary adds still more to a community-oriented approach to medical practice.

*Ten-cell leader or balozi: a representative of *c.* 10 households, selected by the villages themselves and spokesman for them within the administrative party structure at village level.

In 1979 leprosy in Muheza District was chosen (Fig. 1). The area is hilly and fertile and agriculture is the main means of subsistence. A few scattered estates also give some regular employment like sisal, red palms or cardamon. The houses are mainly of mud bricks and thatched roofs. The population density is high for Tanzania.

The population of Bwembera and Muheza Division (excluding Muheza township) was our target population. For sampling purposes the population was divided into 60 units or clusters, that is, villages and special category settlements like sisal or cardamon estates.

The average population per sampling unit was 1,523 people. Twelve units covering approximately 20% of the population of Bwembera and Muheza

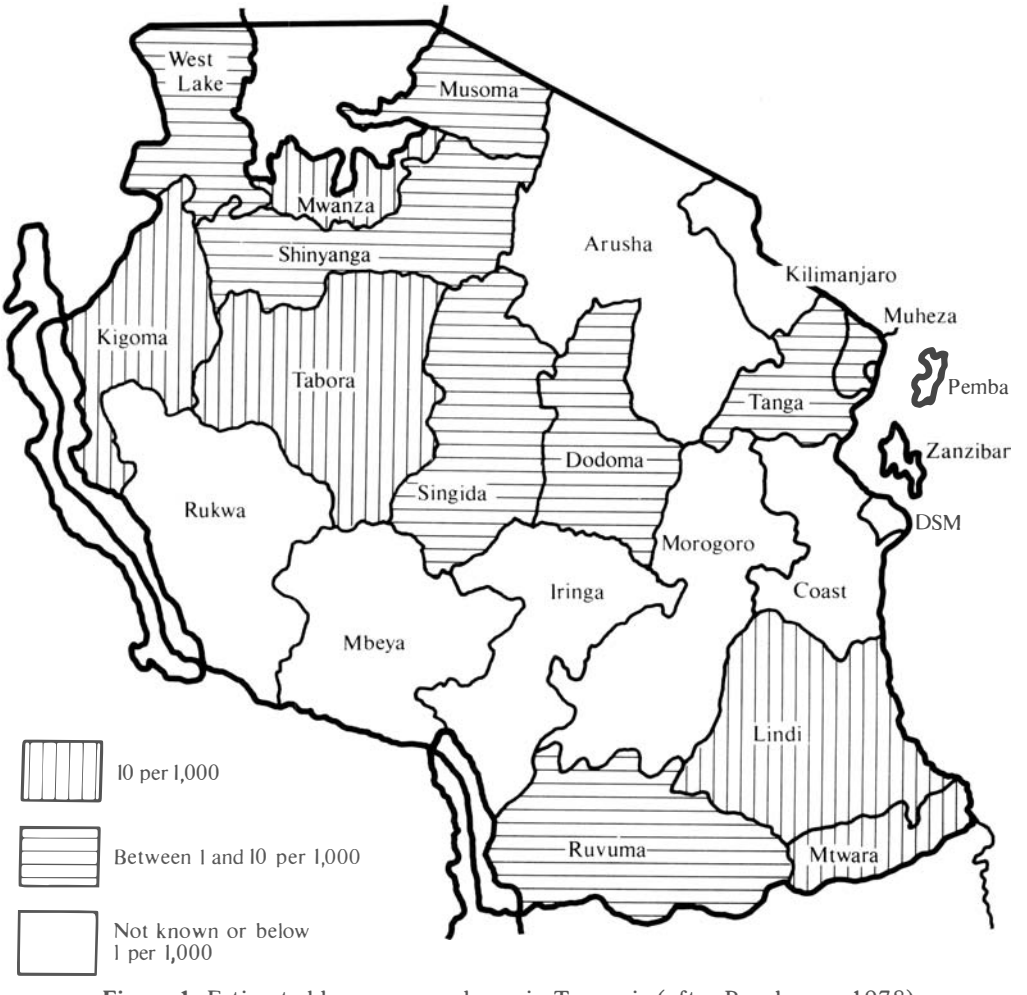

Figure 1. Estimated leprosy prevalence in Tanzania (after Broekmans 1978).

division were selected in such a way that each unit had a chance proportional to its population size to be in the sample. (A technique called proportional cluster sampling.)

A group of 8–10 students with one leprosy expert covered two villages. There were 6 groups altogether covering 12 villages. Each group screened the whole population in that village systematically with the help of the 10-cell leaders.

Diagnosis of leprosy was tentatively made by the students on clinical grounds and confirmed by the expert either clinically and/or by Ziehl–Neelsen staining of skin smears. Before the field stay students underwent a practical session in the leprosy ward of the Muhimbili Medical Centre diagnosing leprosy, based on examination of skin and nerves.

The activities by the 48 students in the 12 villages took a week. Data analyses were done with the students at the Division of Community Medicine, Muhimbili Medical Centre in Dar es Salaam.

Results

A total number of 15,029 people were screened during the 1-week survey, being 93% of the total registered population according to the 1978 census in the villages (Table 1). This shows that considerable coverage can be achieved in such a survey. One cannot expect all people to be at home even if they are informed beforehand.

The under-15 age group in the registered population and in the screened population was 47 and 48% respectively. Which corresponds with the Tanzanian average of the under-15 population being around 45%.

The villages show quite a focal distribution of leprosy prevalence (Table 2). A wide range of 0–25.8% was found. These prevalences differ significantly (Poison dispersion test: $P < 0.001$) with 2 outstanding prevalence rates in Kimbo and Ndondondo villages. Omitting these 2 prevalences the differences between the other villages is not significant ($0.25 < P < 0.5$) and just reflects random variation. The total number of cases found was 116. This number excludes those who do not need treatment any more (10 burnt-out cases). These 116 cases represent a proportion of 7.7 cases per 1,000 of the study population. Taking into account the proportional cluster sampling technique the correct prevalence estimate is 7.9 per 1,000. This makes leprosy a major public health problem in the 2 divisions in Muheza District. It is of interest to note that the 2 villages with the highest prevalences are geographically close to each other.

For determining the different clinical types of leprosy we used the Madrid field classification with 1 modification that indeterminate cases were grouped as tuberculoid. The majority of cases were tuberculoid (Table 3). This compares with findings from other areas in East Africa where a ratio T:B:L of 80:10:10 is generally found.

Table 1. Coverage of screened population by age

Age (years)	Total population in the villages* No.	Total screened population No.	Coverage %
0–1	3,098	3,040	98
6–15	4,435	4,204	95
16–25	2,333	2,139	92
26–35	1,931	1,767	92
36–45	1,513	1,407	93
46–55	1,068	995	93
Above 55	1,435	1,362	95
Unknown	295	115	39
Total	16,111	15,029	93

*According to 1978 census.

Table 2. Distribution of screened population and leprosy cases by villages

Village	Screened population	Leprosy cases	Prevalence per 1,000
Ndondondo	1,738	45	25.9
Kimbo	545	14	25.7
Maduma	908	10	11.0
Mkinga Mpakani	1,341	10	7.4
Bombani	1,217	7	5.7
Magila	1,695	9	5.3
Kicheba	2,477	12	4.8
Kilongo	550	2	3.6
Mwembeni	957	3	3.1
Kibaranga	1,935	3	1.5
Mgambo	671	1	1.5
Makole	995	0	0
Total:	15,029	116	7.7

The sex ratio male:female among the leprosy patients is 1:10, showing a strong male preponderance which is even more explicit from 25 years onwards (Fig. 2).

Many of the patients (55%) had not been diagnosed earlier as having leprosy, and so had never received any kind of medical treatment (new cases – Table 4). Proportionally more males than females were new cases, showing more than twice higher prevalence. This contrasts clearly with the on-treatment

Table 3. Distribution of screened population by type of leprosy (Madrid classification)

Type of leprosy	No	%	Prevalence per 1,000
Tuberculoid	83	71.6	5.5
Borderline	24	20.7	1.6
Lepromatous	9	7.8	0.6
Total	116	100.1	7.7

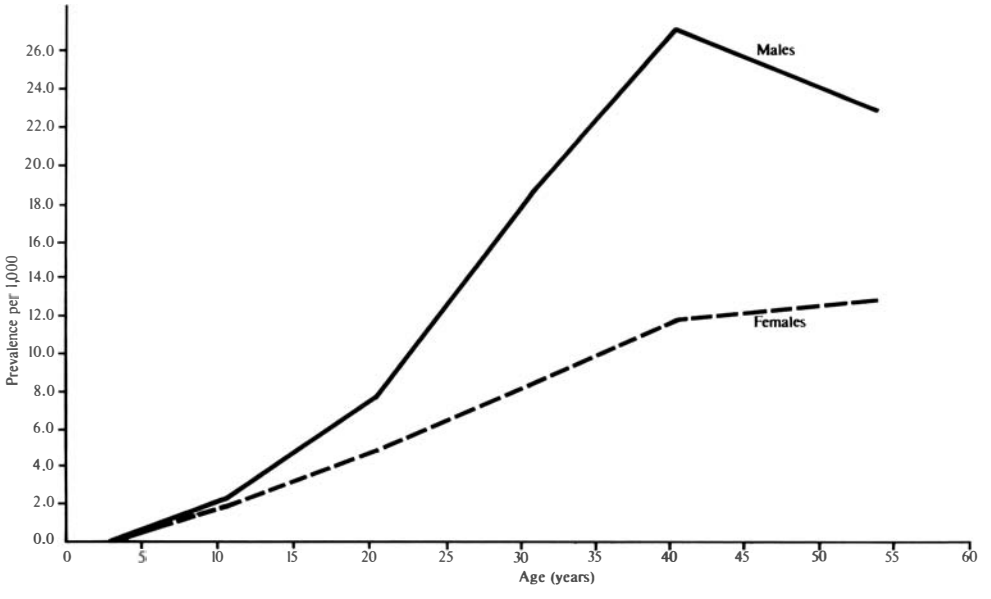

Figure 2. Prevalence of leprosy by age and sex.

cases where the prevalence for males was 4% and for females 2.7%. Clearly, female patients come much more easily to the clinics compared to males.

The age distribution in the newly diagnosed and the old cases differs considerably (Table 5). Most of the new cases are found in the younger age groups, reflecting under-reporting at health facilities, hence a greater risk of developing disability leading to loss of productivity and esteem in this young age group.

Altogether this 55%, the newly diagnosed cases found by the students and confirmed by the leprosy experts, contribute to the continuous infection risk

Table 4. Distribution of new and old cases by sex

	Male		Female		
Leprosy status	No.	Prev. %	No.	Prev. %	T. test on difference
Old cases	30	4.0	20	2.7	$P = 0.16$
New cases	47	6.3	19	2.5	$P < 0.001$
Total	77	10.3	39	5.2	$P < 0.001$

Table 5. Distribution of new and old cases by age

	New cases		Old cases		Total	
Age (years)	No.	%	No.	%	No.	%
25–	16	73	6	27	22	100
26–55	41	61	26	39	67	100
56 +	7	20	27	80	34	100
Total	64	55	52	45	116	

Table 6. The ability of 10-cell leaders to screen for leprosy

		Diagnosis		
		Leprosy	No leprosy	Total
Opinion Balozi	Leprosy	40	6	46
	No leprosy	86	14,897	14,983
	Total	126	14,903	15,029

Sensitivity, 31.7%. Specificity, 99.9%. Positive predictability, 87%.

of the community as, specifically, the multibacillary non-treated cases are the main source of infection.

Tazania has a very organized rural administrative structure, where since 1974 all rural people are living and registered in villages. Approximately for every 10 households an administrative representative, the 10-cell leader or balozi, is chosen. We could use this advantage in seeking the assistance of the balozi to identify leprosy patients. For this reason their screening ability without prior education on leprosy was tested (Table 6). Burnt-out cases, the ones who do not need treatment any more, were also considered as leprosy cases here, as the balozi cannot differentiate between those burnt out or still in need of treatment so has to refer to the health unit for final judgement.

Out of the 40 confirmed leprosy cases identified by the 10-cell leaders 29 were disabled and the others had lesions on the various parts of the body. Five of the 40 were new cases, of which 4 were not disabled and 1 was a burnt-out case.

Discussion

The wide range of prevalences in the different villages confirms the observation of many leprosy workers in Tanzania that the distribution is clustered, even between villages. We looked into reasons for this clustering but could not find any obvious ones as health services, the old/new case distribution, sex and age ratios were roughly similar in all villages studied.

Strong clustering, however, is important for the sampling method as reliability of the prevalence will depend on the increasing number of small clusters one is able to survey.

Sex distribution is another interesting finding for comparison with what is already known in the country.

From the registered cases of most of the regions in Tanzania we find an almost equal sex distribution, but in the community as shown here there is a clear male preponderance. This might indicate that stigma is stronger in males, preventing them from seeking early treatment.

The data are not yet conclusive as to the desirability of using balozis in the national programme.

Obviously, with a high predictability and specificity they will bring true leprosy patients to the clinics. Many of them are already on treatment (87%). The importance of this finding lies in the possibility of further strengthening of the compliance to drug therapy of leprosy patients. The balozi hereby acts as a reminder to leprosy patients to adhere to dapsone taking until otherwise advised by the dispensary. However, balozis miss quite a considerable amount of leprosy patients (low sensitivity) and of the ones they send many are already known or disabled.

Maybe, with a particular emphasis on instructing them on leprosy disease, one can expect that they may be able to refer 'suspected leprosy patient' to the clinic, as the balozi is the man of confidence within his community. The rural medical aid at the nearby dispensary could be asked to hold repeated sessions with all village representatives to explain the problem, request their cooperation and teach them accordingly. In this way we have an important tool by which to diminish the 55% unregistered, which must surely include many infectious cases.

A pilot study to this effect will be needed to test the feasibility of this line of thought.

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Decreased cellular and humoral anti-infective factors in the breast secretions of lactating mothers with lepromatous leprosy

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Summary. Breast secretions from 28 healthy lactating women and 12 lepromatous mothers feeding their children for a varying period (2 days–2½ years) were studied for the total and differential cell counts and immunoglobulin concentrations. It was observed that the total leucocyte count in the milk of the lepromatous mothers was low and also the macrophage count was significantly decreased. The mean secretory immunoglobulin-A level was significantly decreased in the colostrum as well as in the mature milk of the lepromatous mothers as compared to those from the healthy mothers. Acid-fast bacilli could be detected in 9 of the 12 leprosy patients' breast secretions by employing a new technique of coprecipitation of bacteria by 4% polyethylene glycol. The immunologic implications of these findings have been discussed.

Introduction

There is considerable evidence to show that the breast feeding is biologically superior to any other form of infant feeding.[1, 2] Lactating lepromatous leprosy patients are economically underprivileged and are known to excrete leprosy bacilli in their breast secretions.[3] Since breast milk is very often the major source of nutrition to the babies born to these patients, they tend to prolong breast feeding until the child is 2 to 3 years of age. The immunologic profile of the colostrum and mature milk from the lepromatous mothers has not been adequately known. In the present study the cellular composition and immunoglobulin levels in the breast secretions collected from the lactating leprosy

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patients were estimated and compared with those in the healthy mothers from a similar socio-economic background.

Materials and methods

Samples of breast secretions were collected from 12 lepromatous leprosy patients (aged from 19 to 30 years) breast feeding their children for a varying period (2 days–2½ years). The diagnosis of leprosy was established in these patients by clinical features, histopathology, bacilloscopy and lepromin test (armadillo-derived lepromin, WHO Geneva).[4] The disease spectrum varied from borderline lepromatous (BL) in 7 cases to polar lepromatous in 5 cases. One patient in each group developed erythema nodosum leprosum (ENL) during early postpartum period. Twenty-eight healthy mothers (aged from 21 to 30 years) nursing their infants for a varying time (2 days–1½ years) were studied as age- and parity-matched controls.

Five ml of colostrum (breast secretion during first 72 h) or milk was collected by manual expression. A known volume of each sample was centrifuged in a siliconized glassware within ½ hour of its collection at 500 g for 15 min. The fat collected on the top was removed. The supernatant was separated and kept frozen at −20°C and used for the quantitation of immunoglobulins. The volume of the centrifuged deposit consisting of the cells was noted and resuspended in standard white-blood-cell diluting fluid and the total number of cells were counted in an improved Neubar's chamber. The differential cell count was performed after staining the cell deposits by Giemsa technique and various cells were expressed as a percentage after counting all the cells in the smear.

Smears made from the centrifuged cellular deposit were also stained for acid-fast bacilli.[3] In addition, equal volumes of milk and 8% polyethylene glycol in distilled water (PEG, molecular weight 6000, BDH, England) were mixed and allowed to stand for an hour at room temperature. The mixture was then centrifuged at 500 g for 15 min and the precipitate obtained was washed thrice with 4% PEG. A smear made from this washed precipitate was stained as above for acid-fast bacilli. This method of smear preparation has been found by this laboratory to be better in the detection of bacillaemia in the patients with lepromatous leprosy.[5] This PEG-precipitated material was then cultured on Lowenstein Jensen medium for growth of mycobacteria.

Levels of immunoglobulin A, G and M were measured by single radial immunodiffusion technique[6] using commercially obtained monospecific antisera against secretory IgA, IgM and IgG and their reference standards (Meloy Laboratories, USA). Colostral IgA standard, kindly donated by Dr R A Thompson (Birmingham, England), was used for estimation of immunoglobulin A.

The observations were statistically evaluated and paired *t*-tests of significance were done to compare the data.

Results

Colostral secretions were collected from 2 lepromatous mothers, one having ENL reaction and 14 matched controls while mature milk was obtained from 10 patients (one having ENL) and another 14 healthy nursing mothers. Figure 1 shows the scatter of the total cell counts and immunoglobulin levels in the colostrum samples obtained from normal and lepromatous mothers. No overlap was seen between the total cell counts and IgA levels of the 2 groups.

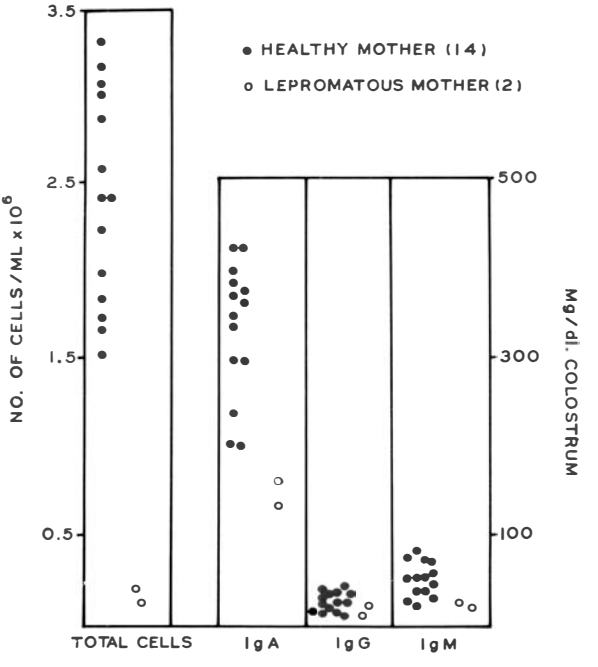

Figure 1. Scattergram showing total cell counts and immunoglobulin levels in the colostrum samples obtained from 14 healthy and 2 lepromatous mothers. No overlap was seen between the cell counts and IgA levels of the 2 groups.

Tables 1 and 2 describe the total and differential cell counts and immunoglobulins A, G and M concentrations in colostrum and milk respectively. It was observed that while the cells seen in the breast secretions of healthy mothers and lepromatous patients were morphologically similar (Fig. 2), the mean total cell count and percentage of macrophages were remarkably lower in the latter than in the former group. The average cell counts were higher in colostrum

Table 1. Total and differential cell counts and immunoglobulin concentrations in the colostrum of the lactating lepromatous patients and healthy controls

Lactating mothers		Total cell count per ml mean ± SD (range)	Mean differential count (%) (range)			Immunoglobulin level (mg/dl) mean ± SD (range)		
Group	Number		Polymorph	Lymphocyte	Macrophage	A	G	M
Healthy	14	24,50,000 ± 4,85,667 (15,50,000–32,00,000)	28 (10–42)	22 (2–42)	50 (25–75)	340 ± 92 (205–425)	25 ± 12 (10–40)	50 ± 29 (20–85)
Lepromatous leprosy	2*	1,55,000 (1,50,000 & 1,60,000)	20 (16 & 24)	68 (64 & 72)	12 (12 & 12)	150 (157.5 & 162.5)	15.5 (10 & 21)	22.5 (20 & 25)

*Both patients showed acid-fast bacilli in milk both by direct as well as PEG-precipitated smear examination.

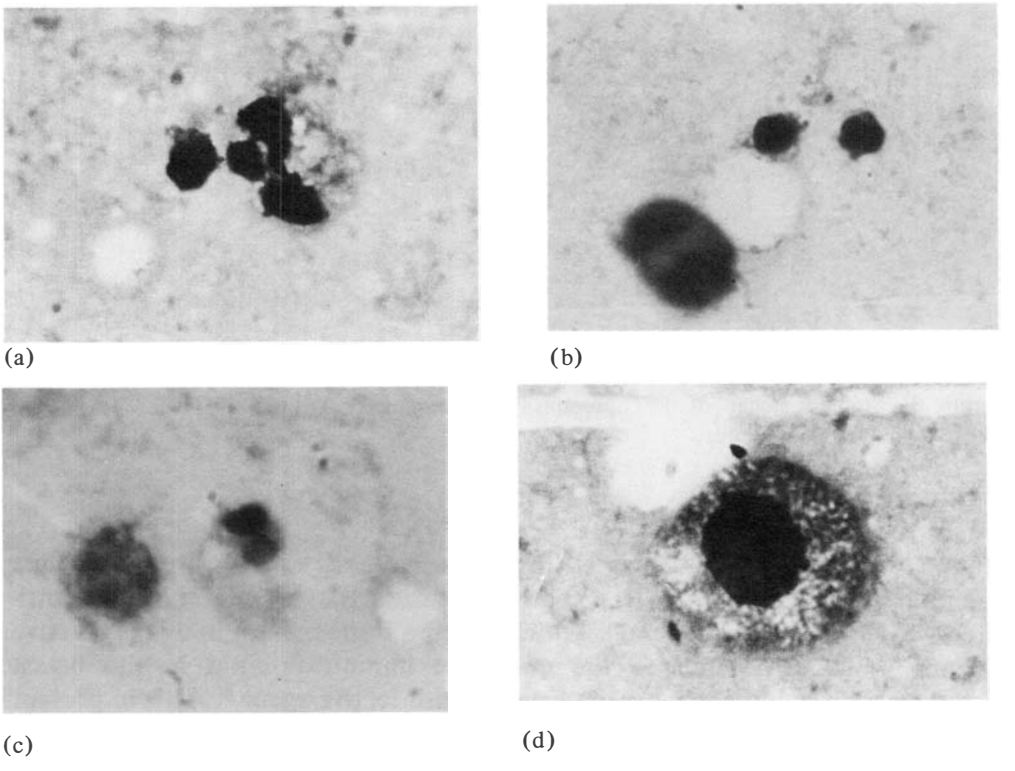

Figure 2. Various types of cells in colostrum of a normal healthy mother. (a) – Four-lobed polymorphonuclear leucocyte; (b) – Two lymphocytes; (c) – Macrophage (left) and a bilobed polymorphonuclear leucocyte (right); (d) – Colostral corpuscle (macrophage).

than that in mature milk. Also, in all cases, there was a higher concentration of immunoglobulin A in colostrum than in milk samples. There was a significant difference in the mean level of IgA in the colostrum and milk of the leprotic mothers as compared to the healthy mothers (Tables 1 & 2). On the other hand, no significant changes were observed in the levels of immunoglobulins G and M in breast secretions of the leprosy patients and age-parity-matched controls. Table 3 analysed the effect of the duration of lactation on the total cell counts and the profile of immunoglobulin levels in the mature milk samples obtained from both healthy as well as lepromatous mothers. As the period of breast feeding increased there was a concomitant fall of the cellular content as well as the immunoglobulin concentrations of the milk samples from both the groups. Interestingly, there was a steeper fall of the cellular contents in the milk of the healthy mothers than in the lepromatous mothers.

Excretion of *Mycobacterium leprae* was demonstrated in the milk of 9 out of 12 leprosy patients. Figure 3 depicts a stained smear of PEG-precipitated breast-milk sample showing an acid-fast bacillus. No acid-fast bacilli could be grown on culture in Lowenstein Jensen medium.

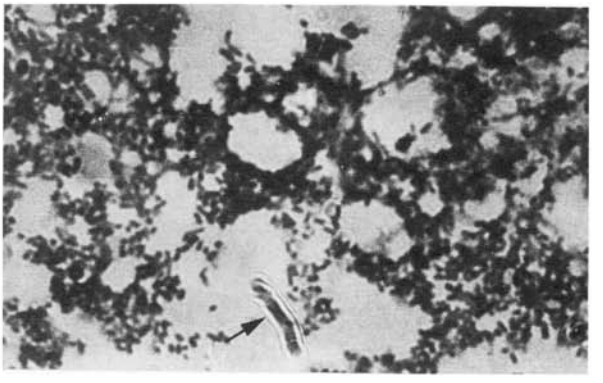

Figure 3. Stained smear of PEG-precipitated breast milk from a lepromatous mother showing an acid-fast bacillus.

Discussion

There are several studies to suggest that breast milk has a protective role against infection in the infant.[7–9] It is now recognized that there are several anti-infective factors in human milk which may be responsible for this protective action.[1] Milk IgA which is the dominant immunoglobulin in the breast secretion is probably the most important anti-infective agent.[10] This important immunoglobulin was found to be significantly low in the colostrum as well as in the mature milk of the lepromatous mothers compared with the healthy mothers. It is now known that the gut shares with other mucosal surfaces, an extensive immunological system that operates somewhat differently and separately from the systemic immune mechanisms.[10] The profile of the immunoglobulin classes in the latter system shows increased levels in the lepromatous patients,[11] while in the former system, as seen in the tears,[12] salivary secretions[13] and intestinal aspirates,[14] there appears a decrease in the IgA concentration. This observed dissociation between these 2 immunological compartments is remarkable. The IgA immunocytes are said to arise from the gut-associated lymphoid tissue and home-in on the mammary glands and also to other mucosal surfaces.[10, 15] This homing is influenced by the presence of antigen[10] and hormones.[16] Acid-fast bacilli have been seen in breast secretions[3] and also in the intestinal aspirates.[14] It is, therefore possible that these specialized committed IgA immunocytes may home 'to see' the similar antigen (*M. leprae* or its products) in the breast. Cooper *et al.*[17] have shown that IgA-antibody production is T-cell dependent. These thymus-derived lymphocytes are functionally impaired in lepromatous patients.[11] For the induction of immunoglobulin-A synthesis by the antigen-sensitive B cell, it is believed[18] that 2 signals are required. Signal 1 is generated after binding the antigen to the B-cell antigen receptor site. This is followed by signal 2 generated as a result of T-cell recognition of foreign determinants on the B-cell membrane. This

Table 2. Total and differential cell counts and immunoglobulin concentrations in the mature milk of the lactating lepromatous patients and healthy controls

Lactating mothers		Total cell count per ml mean ± SD (range)	Mean differential count (%) (range)			Immunoglobulin level (mg/dl) mean ± SD (range)		
Group	No.		Polymorph	Lymphocyte	Macrophage	A	G	M
Healthy	14	16,25,000 ± 2,54,667 (7,25,000–25,25,000)	25 (10–40)	35 (20–25)	40 (20–60)	102 ± 25 (50–150)	14 ± 5 (5–22.5)	6 ± 2.4 (2.5–10)
Lepromatous leprosy	10*	1,27,334 ± 37,794 (74,700–2,00,000)	15 (0–30)	80 (70–90)	5 (0–10)	40 ± 7.5 (25–50)	10 ± 4.5 (5–15)	8.5 ± 2 (5.5–12)
Statistical evaluation								
t value		22.43				8.7		
p value		< 0.001				< 0.001		

*Seven of the 10 patients showed acid-fast bacilli in PEG precipitate only and not by direct smear examination.

Table 3. Effect of duration of lactation on the cell count and profile of immunoglobulins in milk of healthy and lepromatous mothers

Lactating mothers			Total cell count per ml mean ± S.D. (range)	Immunoglobulin level mg/dl mean ± S.D. (range)		
Group	Duration of lactation	No.		A	G	M
(A) *Healthy mothers*						
	up to 6 months	7	21,50,000 ± 4,94,252 (10,00,000–25,25,000)	112 ± 30 (50–150)	18.6 ± 3 (14–22)	7.7 ± 1.6 (5.5–10)
	up to 12 months	5	12,10,000 ± 3,80,000 (7,25,000–17,50,000)	93.5 ± 13 (70–107)	10.7 ± 3.4 (5–14.5)	5.2 ± 1.6 (2.5–7.5)
	up to 18 months	2	8,12,500 (7,25,000 & 9,00,000)	60 (55 & 65)	6.2 (5 & 7.5)	2.5 (2.5 & 2.5)
(B) *Lepromatous mothers*						
	up to 6 months	5	1,60,400 ± 31,752 (1,20,000–2,00,000)	47 ± 4 (40–50)	10 ± 3.5 (5–15)	10 ± 2.5 (5.5–12)
	up to 12 months	2	1,24,666 (1,22,000 & 1,27,334)	42.5 (40 & 45)	15 (15 & 15)	8 (7.5 & 8.5)
	up to 18 months	2	90,000 (80,000 & 1,00,000)	27.5 (25 & 30)	7.5 (5 & 10)	5 (5 & 5)
	up to 18 months	1	60,000	25	5	8.5

sophisticated 2-signal model thus proposes that a single antigenic signal would induce a state of tolerance unless the cell concomitantly received a second (mitogenic) signal from the T cell.[19] The significant decrease in levels of the IgA in lepromatous mothers as reported in the present study supports this notion. The specificity of IgA antibody in the lepromatous leprosy mothers, however, remains to be demonstrated.

The anti-microbial activity of the breast milk is not only mediated by antibodies but also by the lymphoid cells including neutrophils and macrophages.[10] As many as 10^6 cells/ml of colostrum are regularly found during the first 2 weeks after delivery while up to 10^5 cells/ml may still be detected 6 months after.[20] The leucocytes and macrophages play an important part in preventing infection in the mammary gland as well as in the infant's gastrointestinal tract.[21] The observation of a definite drop in the total leucocytic cells and macrophage counts in the breast secretions of the leprosy patients assumes significance in this context. Breast milk is not sterile and may contain bacteria which are mainly harmless.[22] These bacteria are known to promote a normal bacterial colonization of the gastrointestinal tract and also suppress the invasiveness of certain pathogenic micro-organisms.[1]

However, the effect of the ingestion of lepra bacilli by the infant of the lepromatous mothers for a prolonged period is not yet shown. A serious consequence of taking *M. leprae* or its products by these unfortunate babies may be an induction of specific immunologic tolerance[23, 24] by the generation of suppressor T cells.[25]

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Immunological implications of necrotic, cellular and vascular changes in leprous neuritis: light and electron microscopy

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Summary. The fine structural changes and, to a lesser extent, histochemical and histopathological features of biopsy specimens of nerves from patients with non-lepromatous leprosy (mainly very early cases), or lepromatous leprosy (mostly treated cases), have been studied from the point of view of possible immunological response of the host tissues. Using electronmicroscopy and acid phosphatase or B-glucuronidase as markers of lysosomal enzymes, the survival and degradation of *Mycobacterium leprae* by Schwann cells and macrophages in the nerves is compared, both these cells utilizing the lysosomal machinery for such degradation, the macrophages more strongly, once the individual bacillary space has been breached. Long-treated lepromatous patients show relatively fewer intact and more degenerating bacilli. Both live and killed *M. leprae* appear to provide antigenic material, and plasma cells as well as activated macrophages harbouring considerable rough ER, probably produce antibodies and lysosomal enzymes.

Impressive, fine structural changes in clinically well-preserved nerves from patients with very early non-lepromatous leprosy, as well as those with overtly tuberculoid and untreated or treated lepromatous leprosy, included the appearance of products of breakdown of nerve fibres, particularly of myelin, vacuolated macrophages among the fibres; and changes in the intraneural blood vessels such as loosening of the endothelial-tight junctions, proliferation of basement membrane and exudation of plasmatous material perivascularly. On the basis of these findings 3 possible non-bacterial antigens producing damage to nerve parenchyma are considered: (i) myelinogenous proteins which are known to evoke allergic neuritis and further

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myelin destruction, as an autoimmune mechanism; (ii) the vascular basement membrane material which is mainly a protein, like reticulin; and (iii) plasma proteins, especially when containing high, circulating levels of antibodies.

Introduction

This paper represents a revised and updated version of a talk given at the International Symposium on Immunological Aspects of Leprosy and other Mycobacterial Diseases, held at Delhi in November 1977 (unpublished). A considerable amount of nerve biopsy material collected since then is now included. The emphasis in this paper will not be on a mere detailing of the changes in nerves in one or another type of leprosy, which have been reviewed elsewhere,[1, 2] but rather an evaluation of the functional significance of the changes, mainly at the fine structural level, in various constituents of the peripheral nerve. Stress will be particularly laid on the necrotic changes in and around the blood vessels and in the Schwann cells, the nature of the inflammatory reaction evoked, particularly in very early non-lepromatous leprosy and in treated lepromatous patients, and the fine structure of macrophages.

Material and methods

The material is drawn mainly from 4 groups of cases, briefly outlined below, examined clinically and bacteriologically, surgically explored and biopsied at Acworth Leprosy Hospital, during 1976–9.

CLINICAL MATERIAL

Group I included 8 patients with very early non-lepromatous leprosy, mostly of the early macular tuberculoid variety. All patients were bacteriologically negative and had only 1 or 2 small, flat, hypopigmented and hypaesthetic to anaesthetic skin lesions of recent origin. A biopsy of these lesions showed only small inflammatory exudates in the dermis, made up of lymphocytes with stray large mononuclear cells, but no giant cells, lepra cells or bacilli. None of the patients had any signs or symptoms referable to the peripheral nerves, except in 1 patient where there was slight tenderness of the ulnar nerve.

Group II was made up of 3 patients with established untreated tuberculoid leprosy of long duration. They were all bacteriologically negative on examination of smears of full thickness skin biopsies. Two of them had small or large pale anaesthetic skin lesions, occasionally with raised edges. Paraffin sections of

these showed more florid mononuclear cell reaction than in patients of Group I. While 2 patients had moderate thickening and enlargement of nerves in the arms, the third patient had no detectable skin lesions but marked thickening and tenderness of both ulnar and lateral popliteal nerves. The expected sensory impairment and muscular weakness were found in these patients.

Group III consisted of 3 patients with untreated lepromatous leprosy. They were bacteriologically postive with a large number of acid-fast bacilli (AFB) in both skin clip and nasal smears. There were no discrete lesions but there was generalized infiltration of the skin, and therefore biopsy specimen of the skin from the edge of the incision on the hand was examined. This showed a few exudates with lepra cells and small mononuclear cells. The ulnar, greater auricular, sural or other nerves were found to be thickened and tender.

Group IV included only 8 treated lepromatous patients, who had taken the prescribed course of treatment with dapsone (DDS), usually 600 mg a week continuously for $1\frac{1}{2}$–6years. The dapsone/creatinine ratio in urine was found to be within normal limits (35 and over) in 7 patients, and markedly low in only 1 patient, indicating that he was not taking the prescribed dose. All patients showed many AFB, mainly granular forms, in skin clips and nasal smears. The ulnar nerve was found thickened in all patients and in 1 case the radial and lateral popliteal nerves also.

The muscular weakness or wasting encountered in patients of Groups II, III and IV, has been outlined elsewhere.[3]

SURGICAL PROCEDURE

In 5 of the patients of Group I, a medial funiculus of the ulnar nerve just above the elbow was biopsied, since this is the most frequently involved nerve in leprosy. In the remaining patients the index branch of the radial cutaneous nerve on the dorsum of the hand was biopsied, keeping in mind the fact that this nerve is also frequently involved in all types of leprosy. The plastic surgeon (J S S) was assisted at surgery by the Professor of Neuropathology (D K D) and the leprologist (C R R). Care was taken not to stretch the nerve or to lift it completely off its bed just prior to excision of a 5–6 cm length of the funiculus (of the ulnar nerve) or the entire nerve. It was immediately divided and transferred to different vials with appropriate fixatives for histochemical, electron-microscopic and histological examination.

LABORATORY METHODS

Histochemical demonstration of acid phosphatase was carried out by Gomori's method[4] and of B-glucuronidase by the method of Hayashi *et al.*[5] on frozen sections.

Paraffin sections were stained routinely with haematoxylin and eosin,

Gomori's reticulin stain, the picro-Mallory procedure for connective tissue and myelin, Holmes's silver method for axons, and Fite-Faraco's method for acid-fast bacilli.

The essential steps for ultramicrotomy and electronmicroscopy (EM) were the collection and immediate fixation of small longitudinally oriented pieces in cold 4% glutaraldehyde in Millonig's phosphate buffer, post-fixation in OsO_4, dehydration in a graded series of ethyl alcohol and blocking in araldite. The blocks were sectioned by glass knives; semithin (1 μm) sections were obtained and stained with toluidine blue for survey. Silver-grey thin sections were collected on copper grids for examination on a Phillips EM200 Electromicroscope.

Observations

Findings in patients with very early non-lepromatous leprosy (Group I) and in cases with treated lepromatous leprosy (Group IV) will be stressed. A couple of relevant illustrations will also be given from cases not included in the above material.

The material is best considered under 2 headings: reactive and degenerative changes in and around the Schwann cells; and the vascular and inflammatory cell changes, mainly of macrophages.

REACTIVE AND DEGENERATIVE CHANGES RELATED TO SCHWANN CELLS

The metabolic activity which still-viable Schwann cells put forth non-specifically in any peripheral nerve disorder, infectious, allergic, toxic or traumatic, can be readily detected by a delineation of the activity of a lysosomal enzyme such as acid phosphatase. This has been discussed earlier elsewhere.[1, 6, 7] The activity is spotty and mainly paranodal along the Schwann cell pathways, as illustrated in the above references. When inflammatory cells infiltrate the nerves, in both tuberculoid and lepromatous leprosy, the macrophages also manifest lysosomal activity, as seen with B-glucuronidase in Figure 1 (a). If the Schwann cells have degenerated and been replaced by collagenous tissue, as in this specimen, then no activity is seen in the Schwann tubes. The disorganization of the endoneurium and Schwann cell pathways and the proliferation of blood vessels which takes place in such nerves, is well revealed by Gomori's reticulin stain (Fig. 1 (b)).

Even in clinically unaffected nerves, as from very early cases of Group I, where only 1 or 2 skin lesions gave evidence of non-lepromatous leprosy, some reaction and degeneration of the Schwann cells was encountered in nerve biopsy specimens (Fig. 2 (a)). In Figure 2 (b), of a nerve from such a patient,

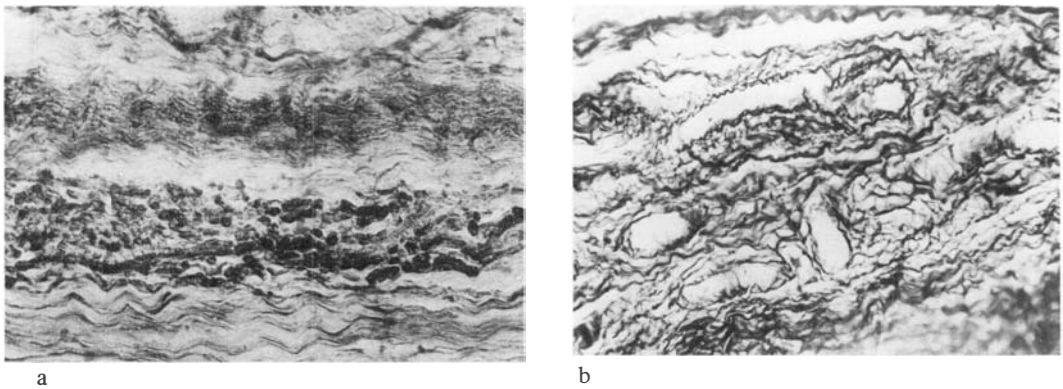

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Figure 1. (K/63) (a) Group II: B-glucuronidase activity in inflammatory cells, mainly large mononuclears, in the centre of a destroyed funiculus from the radial cutaneous nerve, the periphery of which is also fibrosed. (b) Same nerve as above, showing irregular reticulum framework in a nerve bundle which showed both inflammation and fibrosis. Note the vascular and perivascular reticulin of proliferated blood vessels. ((a) Longitudinal section stained by Hayashi's method for B-glucuronidase, on frozen sections, × 158; (b) Gomori's reticulin, × 158).

the greater part of the nerve parenchyma has degenerated and is being replaced by myelinic figures. The change was found to involve Schwann cells of unmyelinated fibres essentially, and some of these fibres also manifested regeneration in the form of dense groups of very small but intact unmyelinated axons (RS, in Fig. 2 (c)). Here a vacuolated macrophage (M) is also seen. Figure 3 shows degeneration of the nerve fibres, particularly of the myelinated fibres, with an accumulation of reticulated osmiophilic material (left border of Fig. 3) within the Schwann cell around a still-existing myelin sheath. With more advanced degeneration the endoneurium becomes studded with tissue breakdown products.

In overtly TT- or BT-type cases of some duration (Group II), small mononuclear cells, representing one or another kind of lymphocyte, and large mononuclear cells with abundant cytoplasm with vacuoles containing finely granular material and a pale indented nucleus, representing epithelioid cells, can be seen within and between nerve funiculi. Such 'tuberculoid' exudate was generally encountered in the midst of necrotic tissue, as in Figure 4 (a). While muscle histology, histochemistry, and fine structure in these 4 groups of cases have been reported earlier,[3, 8] it is relevant to record the frequent neurogenic atrophy and the less frequent inflammatory infiltration between the muscle fibres that occurs in such cases (Fig. 4 (b)). Such infiltrates in the muscle are usually perineural and perivascular (arrows, Fig. 4 (b)), and arise from the main intra-neural inflammation.[2, 3, 9, 10] The ulnar nerve in this case was thickened throughout its extent.

In untreated lepromatous leprosy the heavily bacillated Schwann cells and infiltrating macrophages show a lot of lysosomal enzyme activity (as seen in

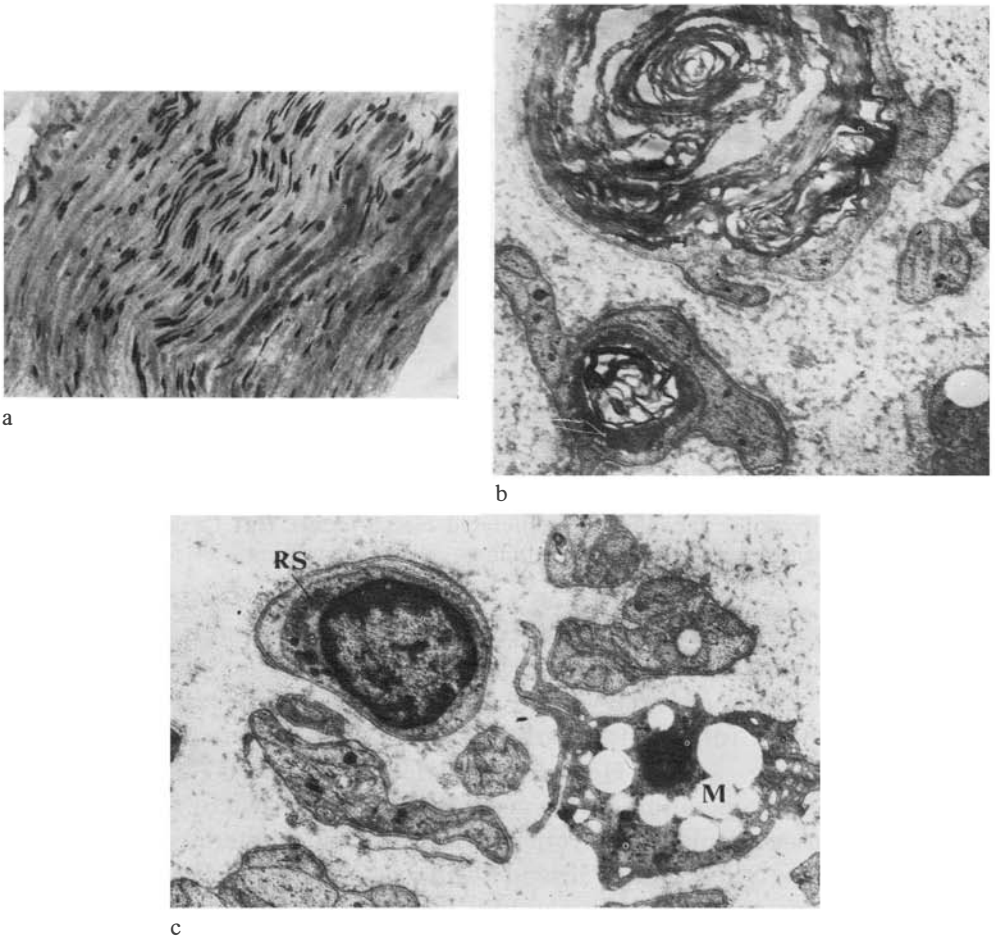

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Figure 2. (a) (I/674) Group I: Medial funiculus of unaffected ulnar nerve at the elbow, showing central stream of increased nerve sheath (mainly Schwann cell) nuclei. (Frozen section, Hematoxylin and Eosin (H&E), × 150.) (b) (J/557) Group I: Radial cutaneous nerve. Large and small myelinic figures occupying the greater part of 2 Schwann cells with only a few small unmyelinated axons remaining along the periphery. (c) Another part of same nerve showing possibly regenerating groups of very small unmyelinated axons. Note the possibly regenerating nucleated Schwann cell (RS) inside the older paler Schwann cell; and the vacuolated macrophage (M) with filipodia. (Osmicated araldite section stained with uranyl acetate and lead citrate; (b) × 9610; (c) × 11,676.)

Fig. 5 in Ref. 7). In treated lepromatous leprosy, where the bacilli tend to be fewer, the enzyme product is often seen along the periphery of distended, at times vacuolated, Schwann cells. This is seen with B-glucuronidase in Figure 5. When a concurrent bacillary stain is carried out on an enzyme preparation the bacilli are seen to remain intact and the Schwann cell alone shows the enzyme product (Fig. 5). There was no evidence of either of these 2 lysosomal enzymes being *on* the bacilli.

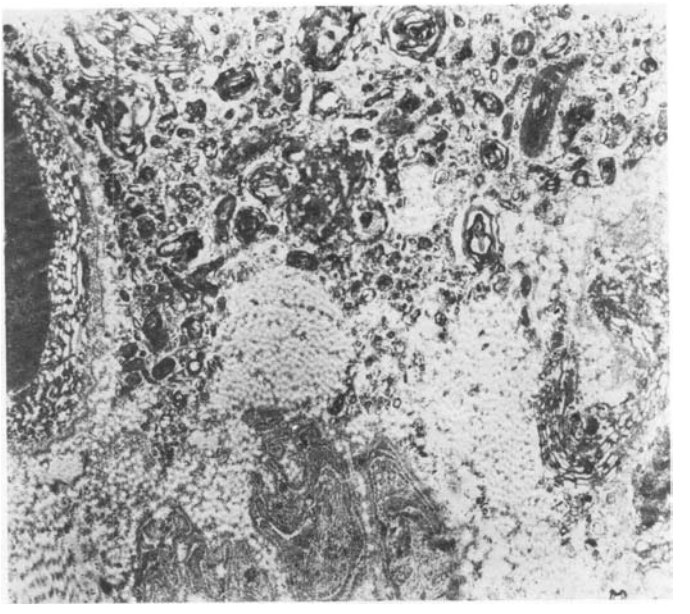
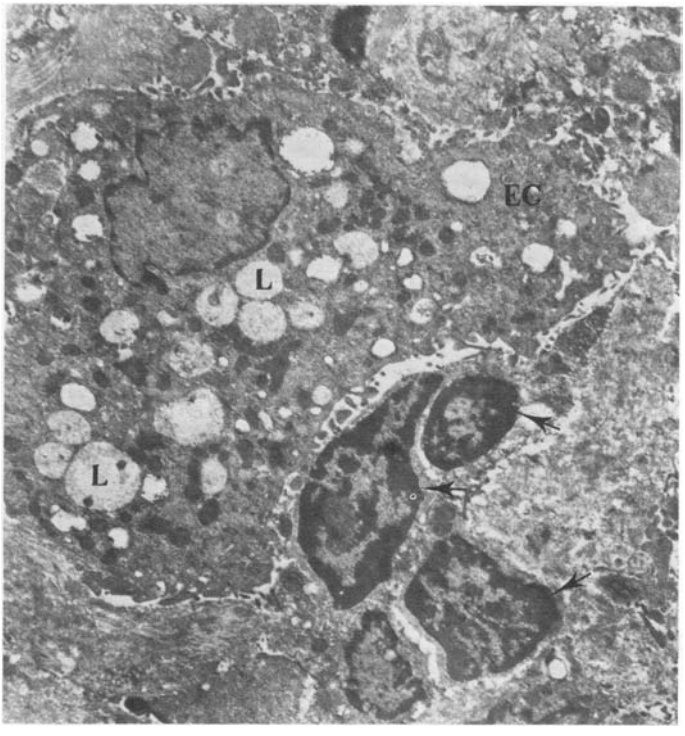


Figure 3. (I/976) Group I: Affected part of the nerve showing more fragmented osmiophilic debris, fewer fibres and more collagen. (Same as in Fig. 2, × 8,820.)

In both treated and untreated lepromatous leprosy, macrophages are sometimes prominent. In Figure 6 there is a macrophage lying free in the endoneurial collagen and containing lysosomal bodies (L). There is also a small, possibly regenerating, Schwann cell (RS) within the larger Schwann cell (arrows) bearing a large axon (A) and a prominent lysosomal profile (L). Bacilli (B) are seen in the peripheral cytoplasm of the old Schwann cell as well as of the new. Such bacilli are still intact in their respective spaces or halos. In some parts of nerves from patients with treated lepromatous leprosy the Schwann cells showed many crumpled degenerate forms of bacilli (arrows, Fig. 7) and a number of empty lysosomal profiles (L, Fig. 7), with relatively few intact bacilli. Moreover, the entire Schwann cell of unmyelinated fibres in such nerves was found distended (SC, Fig. 7) and the clear spaces around individual bacilli had virtually disappeared. At times granular material representing primary lysosomes was also encountered in such distended Schwann cytoplasm of treated cases (Fig. 15 (b) in Ref. 7).

INTRA-NEURAL VASCULAR AND INFLAMMATORY REACTION

Even in early tuberculoid cases, without appreciable perivascular inflammatory cell reaction, the blood vessel showed evidence of increased transport of fluids across the endothelial cells and pericytes in the form of increased pinocytic vesicles in rows and clusters. The endothelial tight junctions tend to remain



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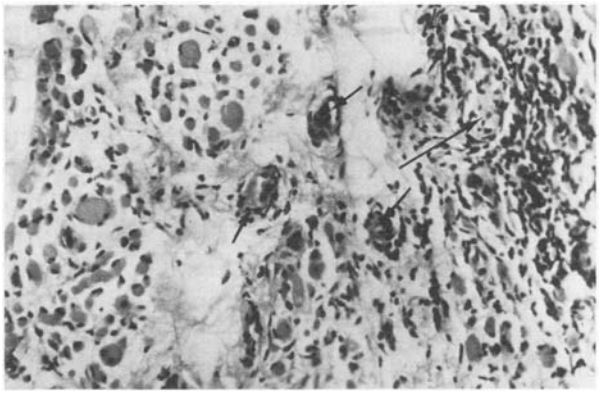

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Figure 4. (a) (J/763) Group II: The large mononuclear cell at the top is probably an epithelioid cell (EC), with many pale bodies containing finely granular material, probably lysosomes (L). The 4 small nuclei grouped together possibly belong to lymphocytes (arrows) and the large indented nucleolated nucleus at lower right to another macrophage. The matrix has necrotic tissue and collagen. (b) (K/63) Group II: First dorsal interosseous muscle. Cross-section showing severe group atrophy of the 2 fascicles on the left. Note disorganization of fascicles on the right by inflammatory mononuclear cell infiltrates, which are predominantly around a small nerve twig (long arrow) or small blood vessels (short arrows). ((a) As in Fig. 2, × 4,830; (b) Haematoxylin and Eosin, × 180.)

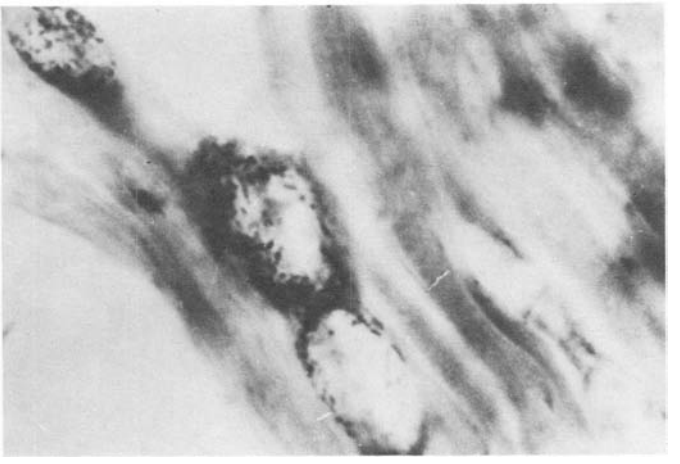


Figure 5. (J/299) Group IV: B-glucuronidase reaction product along the periphery of swollen Schwann cells containing intact acidfast bacilli. (As in Figure 1 (a) with superadded Fite-Faraco stain, × 1,120.)

intact with the zonula occludens keeping shut along most of its course. A more advanced vascular change was the opening of gaps in the tight junctions, i.e. the formation of 'gap-junctions' (Fig. 17 (b) in Ref. 7). The small blood vessel outside the perineurium (P) in Figure 8 shows irregular and loosened contacts between endothelial cells and formation of oedema spaces between endothelial cells and pericytes, in addition to concentric proliferation of basement membranes (arrows).

In lepromatous leprosy, more frequently than in tuberculoid leprosy, mast cells were encountered both within and between the nerve funiculi. At times these cells were really large with abundant fuchsinophilic granulation, and were found in the vicinity of *M. leprae*, as in Figure 9 (a). Plasma cells were also found in lepromatous leprosy, though not as frequently as in the tuberculoid varieties. The perivascular location and pleomorphic appearance of macrophages can be noticed in Figure 9 (b), where the binucleate cell at the top with distended cisterns of endoplasmic reticulin (ER) filling its cytoplasm is quite unusual. Alternatively, it may be a very active plasma cell. In the centre of Figure 9 (b) the macrophage shows the more usual appearance with a number of lysosomes and phago-lysosomes (L), and one small group of probable degenerating bacilli (split arrows).

Dense perivascular inflammatory reaction by large and small mononuclear cells is seen in nerves from both established tuberculoid and established lepromatous cases. Actual infiltration of the vessel wall or vasculitis also occurs as in Figure 10 (a). In this nerve and in all such similar situations, copious perivascular reticulin fibres are laid down, and the endoneurial collagen also tends to be disorganized. A rather dramatic expression of perivascular macrophage reaction (arrows) and heavy bacillation in an area of loss of myelinated fibres

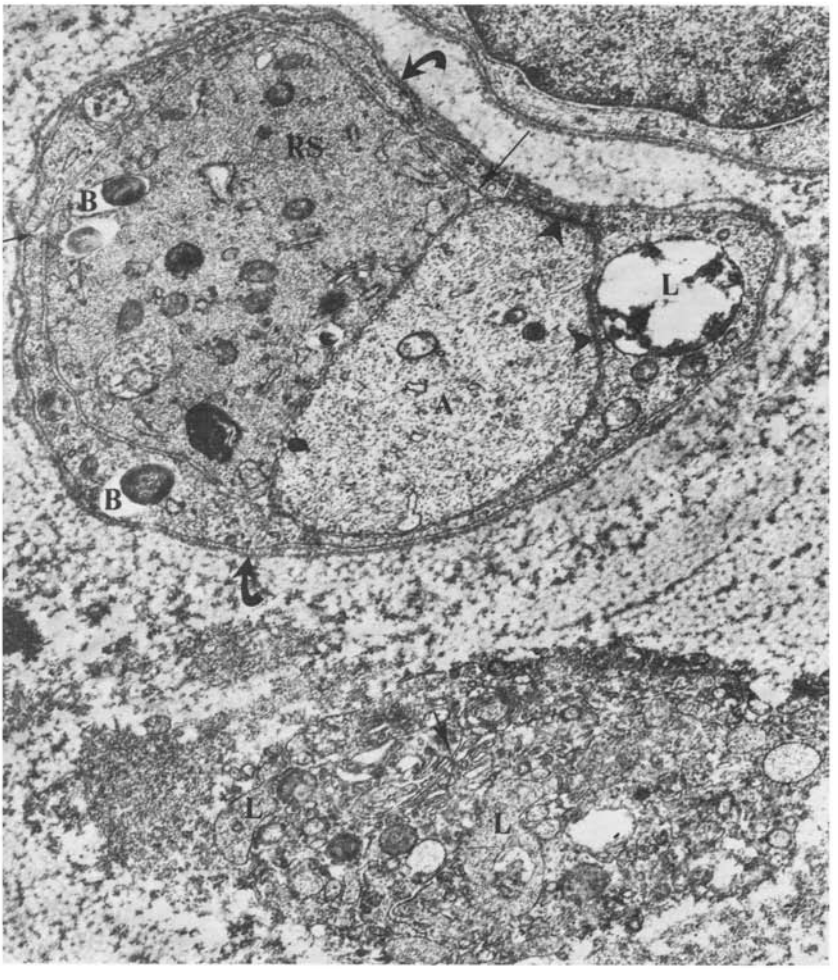


Figure 6. (I/746) Group III: Along the lower part of the picture is a macrophage showing Golgi tubules (arrow), lysosomal profiles (L) and vacuoles. The greater part of the picture is occupied by a large Schwann cell (curved arrows), with a break in its plasma membrane (small arrow), the greater part of it is occupied by another possibly regenerating Schwann cell (RS) with more organelles in its cytoplasm, containing a large axon (A) with possible myelination starting on one side (arrow heads), and the continuous membrane (long arrow) enclosing the cytoplasm and the axon of the inner Schwann cell. Note intact bacilli (B) in their respective spaces in the cytoplasm of both the old and the new Schwann cell and the large lysosome (L) containing osmiophilic debris in the old Schwann cell. (As in Fig. 2, × 14,490.)

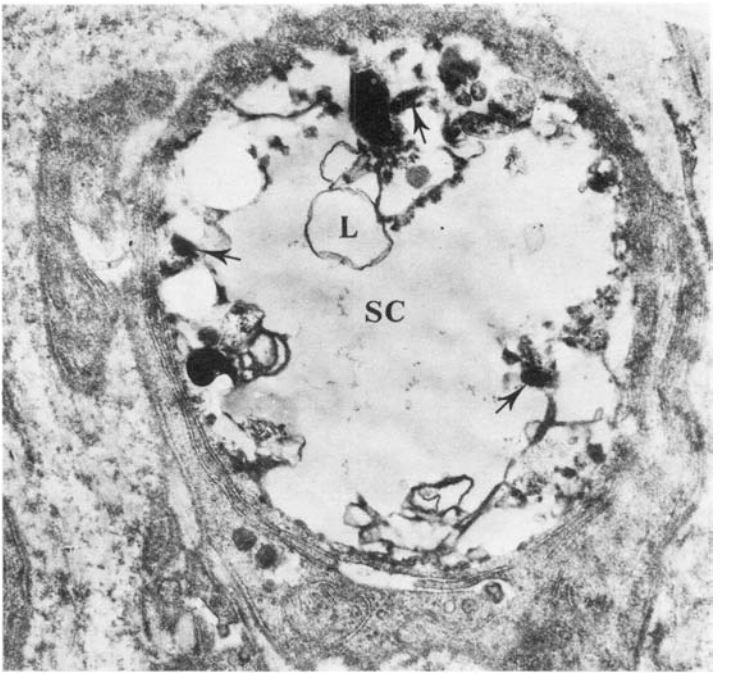


Figure 7. (J/107) Group IV: Nerve from treated lepromatous patient showing entire Schwann cell (SC) converted to a large space containing single membrane profiles of possible lysosomes (L), 2 intact osmiophilic bacilli and several degenerating bacilli (arrows). As in Fig. 2, × 15,498.)

in a nerve bundle is seen in Figure 10 (b). The centre of this lesion is formed of a small, thick-walled blood vessel and the periphery of fibroblast processes. It is of further interest that the large number of *M. leprae* encountered in this specimen was despite its being from a treated patient. At times large empty vacuoles (Fig. 11 (a)) in distended macrophages suggest large phagosomes earlier harbouring either bacilli or products of cellular degradation. The cytoplasm of such vacuolated macrophages (arrows, Fig. 11 (b)) manifests strong lysosomal enzyme activity.

Vascular and cellular reactions in and around the perineurium are a replica of those in the endoneurium, the perineurial cells being structurally comparable to the Schwann cells. An early reaction is a proliferation of perineurial cells which still retain their parallel alignment (P, Figs. 8 & 12 (a)), when the interfunicular mononuclear cell exudate has not yet invaded the perineurium. With advancing disease the perineurial cells and their basement membrane thicken. They may show bacilli or empty vacuoles in lepromatous cases, and tend to disappear with replacement by macrophages, fibroblasts and collagen, as seen in Figure 12 (b), from a treated lepromatous patient (same nerve as in Figs. 5 & 11 (a)).

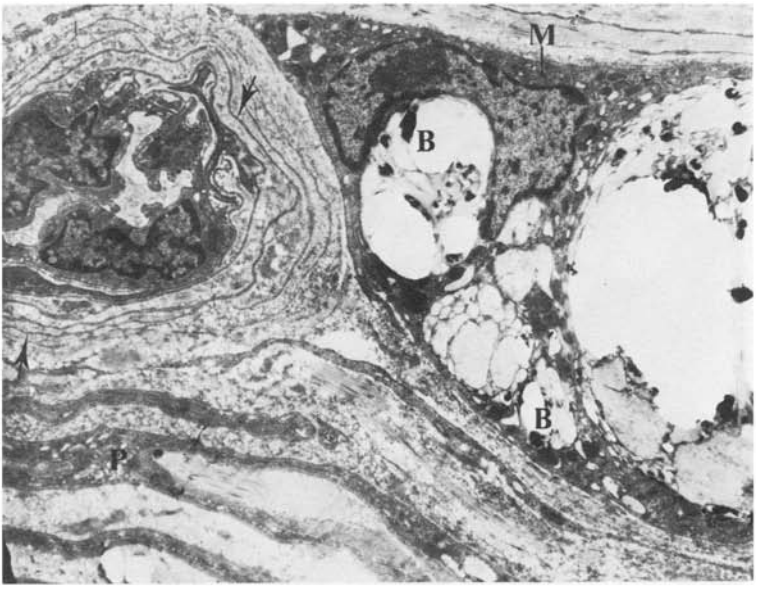


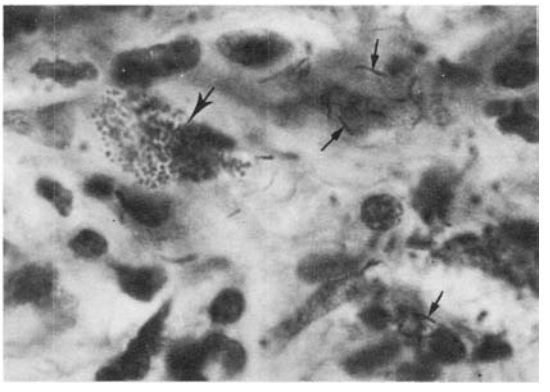
Figure 8. (J/107) Group IV: A blood vessel and a macrophage (M) just outside the thickened perineurium (P) of a nerve bundle. Note the intact bacilli (B) in the large and small vacuoles in the macrophage cytoplasm; and the oedema spaces between the endothelial cells and pericytes of the blood vessel, which shows proliferated BM laminae (arrows). (As in Fig. 2, × 3,535.)

Discussion

HISTOCHEMISTRY AND FINE STRUCTURAL COMPARISON OF MACROPHAGES AND SCHWANN CELLS, WITH SPECIAL REFERENCE TO *MYCOBACTERIUM LEPRAE*

One histochemical finding of the present study which merits a comment is the detection of the lysosomal enzyme B-glucuronidase only in the cytoplasm of the Schwann cells harbouring *M. leprae*, and not in the organisms. This fact was clearly brought out by the chemical analysis of lepromatous mouse tissue by

Figure 9. (a) (C/691): Ulnar nerve of untreated lepromatous patient (autopsy specimen) showing a large mast cell (arrow) with many granules and a number of intact rod forms of bacilli (small arrows). (b) (K/39) Group III: In the lower part of the picture is a small blood vessel with tight junctions (small arrows) between endothelial cells, and narrowed lumen (curved arrows). In the centre is a macrophage with 1 nucleus and cytoplasm showing many lysosomes (L) and a space containing 2 degraded bacilli (split arrows). Along the top of the picture is a large cell with 2 nucleolated nuclei and cytoplasm full of distended rough endoplasmic reticulum (ER): either an active macrophage or an active plasma cell. ((a) Paraffin section, Fite-Faraco's method, × 945; (b) As in Fig. 2, × 5,670.)



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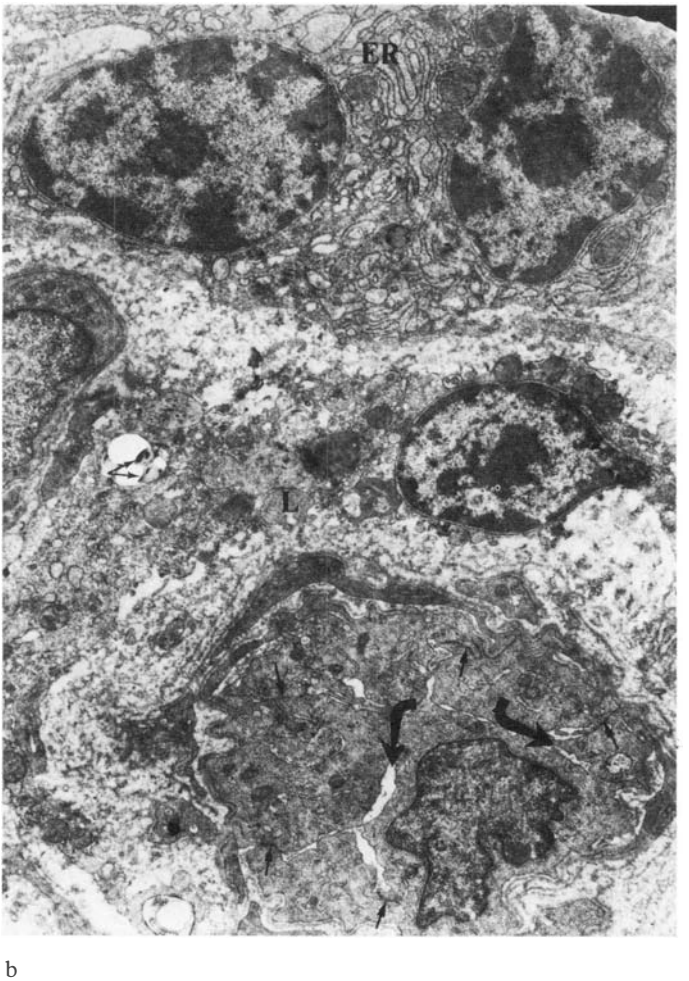

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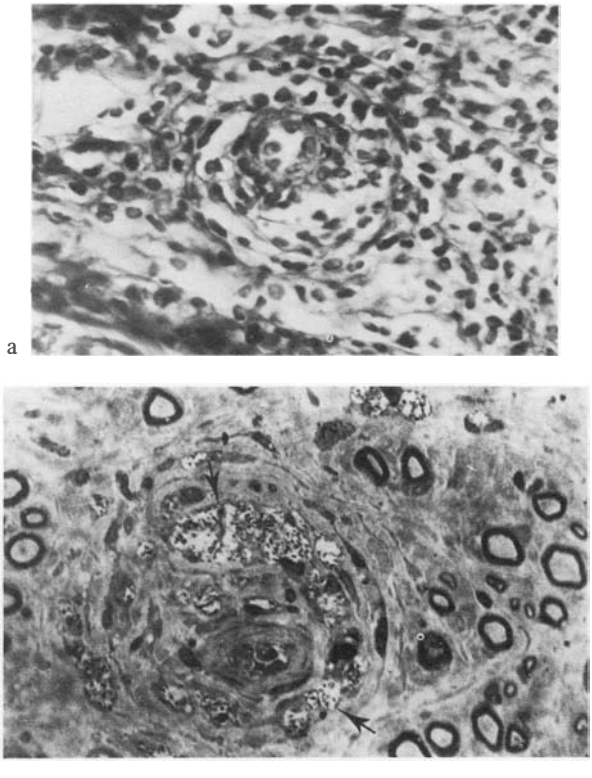

Figure 10. (J/201) Group IV: (a) Dense mononuclear cell infiltration filling a nerve bundle with clear vasculitis of the small central blood vessel. (b) Ring of distended macrophages full of bacilli, around a small central endoneurial blood vessel, in an area where the nerve fibres are destroyed. ((a) Picro-Mallory stain, × 403; (b) 1-μm-thick osmicated araldite section stained with toluidine blue, × 532.)

Prabhakaran *et al*[11] and recently confirmed,[12] when this enzyme was detected exclusively in the host cell cytoplasm and not in the leprosy bacilli as suggested originally, by Skinsnes & Matsuo.[13] Another point of interest is that B-glucuronidase was also detected histochemically in Schwann cells and macrophages from our patients with non-lepromatous leprosy, just as acid phosphatase was found at these sites in our earlier study.[14] Thus these lysosomal enzymes are not related to the presence of bacilli, but rather to the increased metabolic activity of Schwann cells and macrophages in all pathological states.

The fine structure of intraneural phagocytes other than Schwann cells, i.e. the macrophages, has been described here, because most of the earlier reports have confined themselves to macrophages in leprous skin lesions.[15–18] They are described briefly in tuberculoid nerves,[19] lepromatous nerves[20] and in leprous nerves and skin lesions.[2, 14] Very recently, Ridley *et al*[21] have given a comprehensive electromicroscopic account of these mononuclear phagocytes across the immuno-histologic spectrum of leprosy, with emphasis on macro-

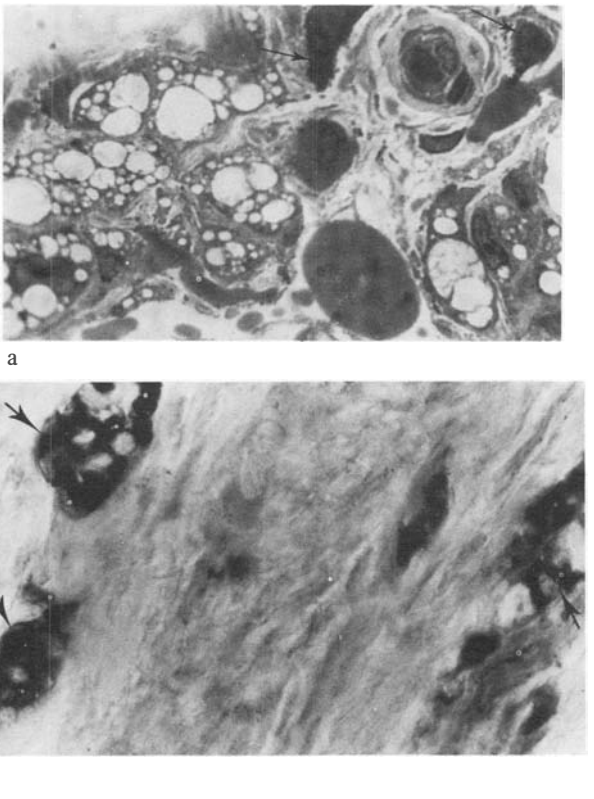

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Figure 11. (J/299) Group IV: (a) Clustering of highly vacuolated interfunicular macrophages. Note the small blood vessel at the top right, ringed by darker non-vacuolated mononuclear cells (arrows); note similarity of vacuoles to those in macrophage in Figure 8. (b) (J/107) Group IV: Acid phosphatase preparation on frozen section showing enzyme product in cytoplasm between vacuoles in the large macrophages (arrows), along the perineurium of the nerve bundle. ((a) As in Fig. 10 (b), × 945; (b) Gomori's acid phosphatase method, × 532.)

phages in borderline tuberculoid (BT) skin lesions. We have now stressed their appearance in the endoneurium and perineurium of nerves from very early non-lepromatous and treated or untreated lepromatous cases.

Irrespective of the type of leprosy, macrophages were usually found perivascularly, suggesting their origin from the blood monocyte; and were in larger numbers in the perineurium or just outside it, rather than in the endoneurium. In the latter situation the phagocytic propensity of Schwann cells for all particulate matter seemed to suffice. They seem capable of picking up virtually anything of a small enough size from their immediate environment. Thus in one study[22] carbon particles were phagocytosed by Schwann cells when injected within the perineurium, the cells of which too can ingest material like ferritin.[23] The Schwann cells from human acoustic schwannomas avidly engulf mycobacteria such as *M. leprae* and the I.C.R.C. bacillus, even in short-term tissue cultures on standard media, and also show a positive reaction to lysosomal

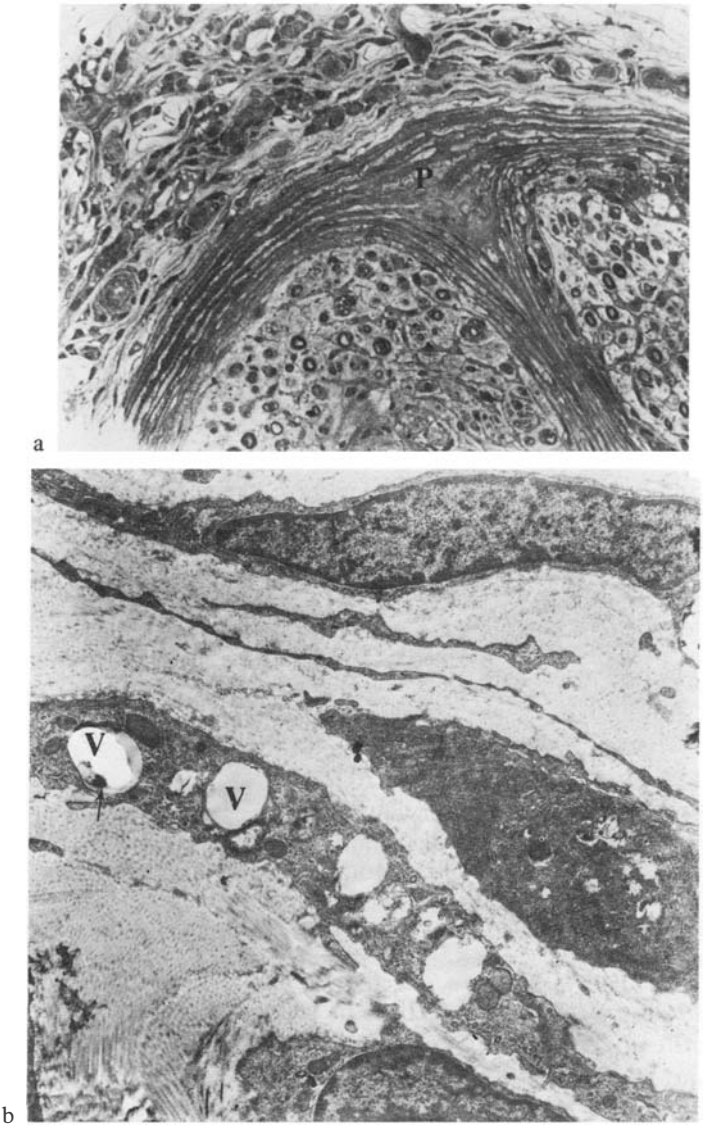

Figure 12. (K/39) Group III: (a) Many distinct layers of perineurial cells (P) enclosing and splitting a nerve bundle with depleted myelinated fibres. Note the dense vascular and mononuclear reaction outside the funiculus. (b) (J/299) Group IV: Perineural cells, increased fibroblasts and macrophages in dense collagenous matrix. Note degenerating bacillus (arrow) in one of the vacuoles (V) in a macrophage. ((a) As in Fig. 10 (b), × 200; (b) As in Fig. 2, × 7,440.)

enzymes.[24] Human acoustic schwannomas rarely become melanotic when the tumour cells take up endogenously-produced melanin pigment.[25]

The cytoplasm of the macrophage was often highly vacuolated, especially in the treated lepromatous cases, where shrunken, possibly degenerating, and

intact forms of bacilli were seen in these vacuoles or spaces. The appearances were comparable to those observed by us in macrophages of borderline lepromatous (BL) type of skin lesions (Figs. 11 (a), (b) in Ref. 14) and to the recent description of BL lesions by Ridley *et al.*[21] However, many of the vacuoles in our treated lepromatous nerves or borderline skin lesions were empty or contained finely granular material and, to some extent, these macrophages came to resemble epithelioid cells in BT-type nerves,[1, 19] in BT-type skin lesions[21] and in the very early non-lepromatous or established tuberculoid nerves (present study). Considered together with our histochemical findings, these vacuolar changes appear, at least in part, to be an expression of lysosomal enzyme activity. The fine structure and behaviour of lysosomes in disorders of infection and tissue breakdown, such as leprous neuritis and cerebral tuberculous lesions, has been discussed elsewhere.[26]

Both live and killed *M. leprae* are probably immunogenic. In fact, Rook[27] has adduced evidence that the killing of leprosy bacilli might enhance their immunogenicity, because killed organisms may not be able to get into 'hiding places' such as the Schwann cells of peripheral nerves, but are taken up into the phagosomes of macrophages. Ever since histopathologists have seen bacilli along nerve fibre pathways, i.e. Schwann cells, by light microscopy[9, 10, 28, 29] they have been impressed by their large number and good preservation. They were believed to be sheltered from drugs and antibodies circulating in the blood. Electromicroscopy with modern methods has revealed, however, both intact and degenerating forms of bacilli within Schwann cells of lepromatous nerves[20, 30] and we have presented evidence of bacterial degradation in and around lysosomes in Schwann cells, perineurial cells and intraneural endothelial cells,[1, 20] to the extent that in both treated and untreated cases the entire Schwann cell may look like a large phago-lysosome[7, 20] (and this paper). Moreover, on histochemical and fine structural evidence the behaviour of Schwann cells towards microbes or tissue breakdown products comes to resemble that of macrophages in nerves or skin lesions.[2, 6, 7, 14] Thus the Schwann cells of lepromatous nerves, especially in treated patients, may be capable at least to some degree, of 'processing' bacillary antigen, especially from killed *M. leprae* which are not insulated in their own little spaces, and presenting them to the antigen-recognizing system of the host, as the macrophages do. Hence the Schwann cell cytoplasm may not be a site as 'immunologically privileged' as believed hitherto.[27]

POSSIBLE ROLE OF NON-BACTERIAL ANTIGENS IN NERVE DAMAGE

One of the features revealed by the foregoing brief illustrated review of fine structure of the nerves in very early non-lepromatous leprosy is that an appreciable breakdown of nerve parenchyma, including axis cylinders, myelin sheaths and Schwann cytoplasm, can take place in the absence of bacilli and

virtually of inflammatory changes or any corresponding sensory or motor deficit. It has also been seen that changes in the vessel wall, including the endothelial cells, tight junctions, pericytes and basement membrane can occur. An attempt has to be made to explain the pathogenesis of these changes. The histopathology of the skin lesions in these cases indicated a dermatitis with chronic inflammatory and occasionally granulomatous reaction, consistent with non-lepromatous leprosy. Despite the absence of acid-fast bacilli in all nerves and skin lesions examined from this group of patients, it has to be conceded that the *initiation* of the pathologic process must be through the host's own cell-mediated immune reactions to *M. leprae*. However, one has to look out for potentially immunogenic non-bacterial tissue components which may *perpetuate* nerve tissue destruction. Two such components which one has encountered in the present material are (i) the myelin sheath, and (ii) the vascular basement membrane.

The myelin sheath's basic protein has been recognized as a potent immunogenic substance since its demonstration as an essential component (along with Freund's adjuvant) in the production of experimental allergic encephalomyelitis (EAE) when CNS white matter is used,[31] and of experimental allergic neuritis (EAN) when peripheral nerve myelin is used.[32] We have found a similar mechanism working in tuberculous encephalopathy in some children with tuberculous meningitis where the brain damage is greater, the meningeal reaction not very severe and tubercle bacilli rarely demonstrated.[33, 34] Wisniewsky and Bloom[35] demonstrated, through an elegant series of experiments using sensitized animals with various forms of tuberculo-protein, that demyelination occurred at sites of lymphocyte and macrophage infiltration in the brain, spinal cord and peripheral nerves. They discussed the implications of the findings to both tuberculous meningitis and leprous neuritis. On the basis of the electronmicroscopic observations on non-lepromatous nerves reported here and published earlier,[1, 19] and supported by our earlier histological and histochemical findings,[6, 10] we have indirect evidence to suggest that products of nerve tissue breakdown, probably proteinous, can evoke a further hypersensitivity reaction and thereby perpetuate the nerve-damaging process initiated by the bacilli. Lumsden[36] had advanced a similar view on theoretical considerations. Thus further nerve damage could result as an auto-immune disturbance without the mediation of bacilli.[1]

Passing reference must be made to the production of granulomatous lesions in rabbits by the use of human sensory nerve extract with Freund's adjuvant.[37] Hardwicke and Crawford[38] have shown recently that the antigenic fraction is located in the 'nuclear' fraction of the non-myelin component of sensory nerve and that it is not a lipid. While it is difficult to appreciate the specificity of nuclear fractions of sensory nerves as against motor nerves or mixed nerves, the fact remains that the earliest change in nerve fibres in any form of neuropathy, including leprous, even when there is no clinical deficit and no inflammatory reaction, is an increase in nerve sheath nuclei (as in Fig. 2 (a)).

In both lepromatous neuritis (Groups III & IV) and in the clinically silent neuropathy of very early cases of leprosy (Group I), another likely non-bacterial antigen that suggested itself is the perivascular basement membrane material that had frequently proliferated in several layers. Vascular and perivascular reticulin is probably the structural substrate of the basement membranes seen on the electronmicroscope.[39] We, too, have evidence from examination of the reactive zone of brain tuberculomas that the copious basement membrane material around blood vessels is mainly reticulin, rather than periodic acid schiff (PAS) positive or alcian-blue positive substance which give fainter reactions.[40]

Among the vascular changes a feature of pathogenetic significance is the loosening of endothelial tight junctions, and at times the formation of gap junctions with the presence of plasmatous material outside such blood vessels. This obvious breaching of the blood–nerve barrier in respect of the endoneurial blood vessels finds a reflection in the experimental demonstration by Boddingius *et al*[41] of the seepage outwards of proteins and dyes injected intravenously in mice heavily infected with *M. leprae*. Moreover, both Boddingius[42] and we[2, 19] have observed the proliferation of basement membranes of intraneural blood vessels in the tuberculoid varieties of leprosy also and, in the present study, even in nerves from cases with very early non-lepromatous leprosy. Such proliferated basal laminae may represent attempts at repair of a 'damaged' vessel in any condition, and one source of their origin may be the blood itself, as discussed elsewhere.[40] Exuded plasma proteins naturally produce intra-neural oedema, which could constitute another mechanism of nerve-fibre damage. This could assume a more ominous significance in lepromatous nerves where the level of circulating antibodies is elevated. We have invoked the possibility of this mechanism as one explanation for the considerable muscle-fibre degeneration we encountered (in addition to atrophy), in the virtual absence of bacilli within muscle fibres, in the lepromatous patients of Groups III and IV, who actually showed higher levels of serum IgG.[8]

Acknowledgements

This work was supported by successive Research Grants from the American Leprosy Missions, Inc., New York; Sir Hormusji and Lady Manekbai Mody Trust, Bombay; and the Bombay Hospital Trust of the Medical Research Centre, Bombay.

Thanks are due to the Tata Institute of Fundamental Research for permitting generous use of their electronmicroscope; to Mr N Solanki for the printing of photomicrographs and electronmicrographs and to Mr A D'Souza for the typing. Special acknowledgement is due to my colleague Dr Daya Manghani for help in organizing the material.

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Lepr Rev (1982) **53**, 67–68

Domiciliary and Field Work

THE TRANSPORT OF HISTOPATHOLOGICAL SPECIMENS BY AIR MAIL

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In a previous publication in this journal, Harman[1] drew attention to the various stages involved in the selection, excision, fixation, despatch and processing of biopsies in leprosy. During the past 20 years, first in the Department of Human Anatomy in Oxford, and later in the Department of Dermatology, a very considerable number of biopsies from human and animal tissues have been handled and it is a remarkable fact – and a tribute to the postal authorities concerned – that there have been virtually no postal losses. There have, however, been a number of breakages of glass and other containers together with many instances of leakage. Although not bacteriologically hazardous, these have resulted in the loss of important clinical and research material. The use of glass, particularly when small bottles were packed together without adequate packing, often accounted for breakage and leakage, and it was also heavy and thus expensive for airmail posting. Plastic containers of high quality have recently become more generally available and we have found them satisfactory for the preservation and despatch of material from Malawi to Oxford in a series which started in 1979 and has now reached a total of 175 biopsies. Originally these were posted with minimal packing and, although successful, we soon realized that the method did not conform to International or UK Post Office Regulations on the transport of 'perishable biological substances in the Overseas Postal Service'.[2] These regulations require that non-living pathological material 'must be packed in an inner impermeable container with an outer protective container and with absorbent material placed either in the inner container, or between the outer and inner containers. This material must be of sufficient quantity to absorb, in case of breakage, all the liquid contained in the inner container, and the contents of both inner and outer containers must be packed in such a way as to prevent any movement.'

More recently, a re-usable, high impact plastic postal box has come on to the market which has been designed specifically for the transport of pathological specimens.[3] This box measures 107 mm × 65 mm × 34 mm deep and will comfortably hold two plastic 'universal bottles'[4] together with sufficient material to absorb all the fixative in the event of breakage. It has a sliding lid which can be opened easily for official inspection and is secured at one end by a plastic clip.

In the above series from Malawi, biopsies have usually been taken with 4-mm punch and then placed in 1.5-ml polypropylene tubes with push-on lids,[5] having a diameter of 9.5 mm and a depth of 39 mm. Each tube need contain only 1 ml of fixative, as this is more than sufficient to keep the biopsy moist. Larger, elliptical biopsies can also be accommodated in these tubes satisfactorily. Three tubes are then placed inside a plastic 'universal' bottle giving a total of 6 biopsies per box (Fig. 1). This added precaution, we believe, may minimize the chances of changing temperature, atmospheric pressure or vibration from loosening the lids of the plastic tubes. We have found this method of transport entirely satisfactory for biopsies, and the box may also be used, with appropriate packing, for the despatch of glass slides carrying cut sections.

0305–7518/82/053067 + 02 $01.00

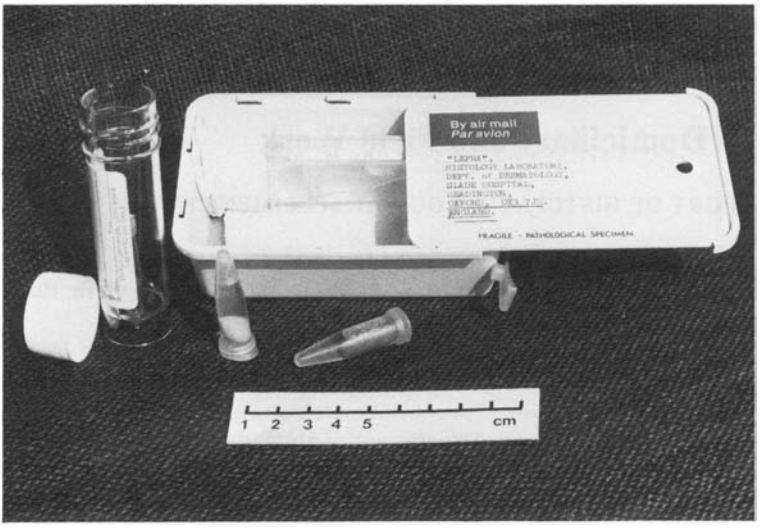

Figure 1. Re-usable plastic postal box, 'universal' containers and polypropylene stoppered tubes. Parcel weight (including 6 biopsies) = 110 g (maximum).

In the UK, the Post Office requires users to submit a sample pack for inspection in order to ensure that it complies with the regulations; unless this is done, packs which do not comply may be intercepted and destroyed. It is advisable for workers in other countries to follow a similar procedure with their own postal service. The costs of the materials involved are as follows:

Re-usable postal box (each)	£1.05
Plastic 'universal' bottle (2)	£0.13
Polypropylene stoppered tube (6)	£0.08

(The latter two items are usually supplied by the manufacturers in bulk only, but they should be obtainable from hospitals and laboratories in small quantities.)

We have found this arrangement to be simple and practical to use and relatively cheap for the despatch of biopsies from Africa to the UK, and of stained slides in the other direction. Needless to say, the plastic boxes and 'universal' containers can be used over and over again, but we advise discarding the stoppered tubes when leprosy is suspected in view of the possibility of contamination of further specimens by acid-fast bacilli.

Acknowledgement

Jones RL and Ponnighaus JM are both supported by grants from LEPRA.

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2 Post Office Regulation PHQ 3845M, 200.11.75. LE (The United Kingdom).
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Lepr Rev (1982) **53**, 69–73

News and Notes

INTERNATIONAL LEPROSY CONGRESS, NEW DELHI, INDIA, NOVEMBER 1983

Dr R H Thanaraj, the Organizing Secretary of the XIIth International Leprosy Congress, 1 Red Cross Road, New Delhi 110 OOL India, has issued details of 'Preliminary Congress and Social Programmes' as follow:

Sunday 20 November
Registration

Monday 21 November
A.M.: Inauguration followed by Scientific Sessions in the morning and afternoon.
7.30 p.m.: Dinner hosted by the Chairman of the Local Organizing Committee and President of the ILA.

Tuesday 22 November
A.M. & P.M.: Scientific Programme.
7.00 P.M.: Cultural Show.

Wednesday 23 November
A.M.: Scientific Programme.
P.M.: City Tour.

Thursday 24 November
A.M. & P.M.: Scientific Programme.
7.00 P.M.: Banquet (Contributory).

Friday 25 November
A.M. & P.M.: Scientific Programme.

Saturday 26 November
A.M.: Closing Session.

The accompanying form also gives an opportunity to submit an intention to register and there is advance information on airline travel and hotel accommodation. The scientific programme includes free papers, poster displays, film presentation and exhibits. Further information from the above address.

CHINESE MEDICAL JOURNAL: ACHIEVEMENTS IN LEPROSY TREATMENT

The following account is taken from Vol. 94, No. 7, page 440 of the *Journal of the Chinese Medical Association,* 42 Dongst Xidajie, Beijing, People's Republic of China:

Efforts are being made in China to eradicate leprosy throughout the country by the year 2000, a spokesman for the Ministry of Health said.

0305–7518/82/053069 + 05 $01.00

More than half of the 500 000 to 600 000 cases, a number estimated in 1949, have fully recovered thanks to positive medical measures and efficient, regular treatment.

China now has a nationwide leprosy control network made up of about 2000 specialized hospitals and institutes. More than 10 000 special medical workers have been trained in diagnosis and treatment of the disease. This number does not include barefoot doctors and primary medical workers who received short periods of training. A special research institute under the Chinese Academy of Medical Sciences is responsible for leading the national research effort.

A publicity campaign on leprosy, its control and treatment, together with repeated surveys among people in endemic areas, have been carried out since 1949. New drugs such as diaminodiphenylsulfone, rifampicin, clofazimine and acedapsone are used in treating the disease and dispensed free. Non-infectious cases are generally treated at home and cared for by special medical workers. Infectious cases are isolated in special leprosy hospitals or villages where they received regular treatment. Patients who live in these special villages participate in productive labor and cultural activities and lead a normal life. They are visited by their families regularly. The patients return to their homes and working posts after they are completely cured.

Ambulatory treatment of infectious leprosy cases is being tried out in some areas.

PAHO: LEPROSY AMONG CHILDREN IN TRINIDAD AND TOBAGO

The Bulletin of the Pan American Health Organisation, Vol. 14, No. 3, 1980 has a short but useful account of leprosy in children in these countries, which includes the following interesting observations: (1) of the total of 848 patients in both areas, only 10% were in the 0–14 age group, whereas the vast majority (87%) were in the 10–14 year age group, with none in the 0–4 year age range: (2) 88% of affected children had either TT or BT leprosy – below the age of 10 years, all affected children had only these milder forms: (3) the sex difference in children was not significant: (4) since 1973 there has been a steady decline in the number and proportion of children diagnosed as having leprosy. The report, which comes from the *Caribbean Epidemiology Center (CAREC),* concludes with the expressed belief that the decline in the number of new cases among children has occurred 'primarily through reduction of the reservoir of *Mycobacterium leprae* – a reduction fostered by providing infectious adults with effective chemotherapy and by securing earlier diagnosis and treatment of new cases'.

[It would, however, be of interest to know if such a decline has also been influenced by (1) segregation and/or (2) a general improvement in environmental conditions, and we would welcome further comment from those working in these areas. *Editor.*]

MOLOKAI; *NATIONAL GEOGRAPHIC MAGAZINE,* VOL. 160, NO. 2, AUGUST 1981

Pages 196 onwards of this well-known magazine contain an account of the origin and development of the leprosy colony first established in 1866 at Kalawao, together with photographs of Sila Siloama Church (1871) on the site of the original colony and of Father Damien from the Damien Museum and Archives. The text records that the 118 remaining patients live in a cottage-community in Lalaupapa. Admissions stopped in 1969 and new patients are now treated as outpatients in clinics in Honolulu.

SYMPOSIUM ON LEPROSY; MADRAS CITY, JUNE 1981

In collaboration with the sub-faculty of the IMA College of General Practitioners, the

Madras City Branch of the Indian Medical Association and the Hind Kusht Nivaran Sangh-Tamil Nadu Branch, a Symposium on leprosy was organized on 26–27 June 1981.

The objective of the Symposium was to expose the general practitioners of Madras (who form the bulk of the doctors in the city) to the widening horizons of leprosy, so that they could detect cases of leprosy at the earliest stage and advise suitable treatment: also to create in the minds of the general practitioners an awareness of the early signs and symptoms of neuritis, reactions, etc. so that they could take advance action to forestall such sequelae. Further, it was also intended to give them an idea of the control measures through which the Government and Voluntary Organizations in the State are trying to control and ultimately eradicate this disease.

Various distinguished contributors from India gave papers on – clinical aspects and diagnosis; differential diagnosis; epidemiology and control; the prevention and treatment of disabilities.

INTERNATIONAL SYMPOSIUM ON *MYCOBACTERIUM LEPRAE* AND OTHER ATYPICAL MYCOBACTERIA

This symposium was organized by the Association of Microbiologists of India and the Foundation for Medical Research, Bombay, February 1981. We acknowledge with thanks receipt of their abstracts, summarizing 25 papers from eminent workers in India and elsewhere on the above subjects. Copies may be obtained from Dr P R Mahadevan, General Secretary, AMI, The Foundation for Medical Research, 84-A R G Thadani Marg, Sea Face Corner, Worli, Bombay 400 018.

JAPANESE NIHON KENSHO-KAI FOUNDATION AWARD, 1981 TO DR W F KIRCHHEIMER

While in Asuncion, Paraguay as a consultant on Hansen's Disease Research to the Health Ministry of Paraguay from 1 September 1981 to 2 October 1981 Dr Waldemar F. Kirchheimer, Chief, Laboratory Research Branch, National Hansen's Disease Center Carville, LA, was honoured for his research accomplishments by the Japanese Nihon Kensho-Kai Foundation. This Foundation awards individuals for outstanding contributions of significance to human progress.

A plaque of appreciation and a scroll in Japanese signed by Mr Ryoichi Sasakawa, President, Nihon Kensho-Kai Foundation and President of the Sasakawa Memorial Health Foundation in Tokyo was handed to Dr Kirchheimer by Professor Dr Nishiura from Kyota University, Japan, at a ceremony attended by personnel of the Health Ministry of Paraguay and representatives of the WHO.

ERRATA

Please note the following corrections to the paper by M ELIZABETH DUNCAN, R MELSOM, J M H PEARSON & D S RIDLEY. *The association of pregnancy and leprosy, Papers I and II, Lepr Rev* (1981) **52**, 245–270 for which we apologize.

page 250, two lines up, for *of margins or* read *of margins of.*
page 258, line 10, for *significant* read *insignificant.*
page 260, ref 15, line 2, for 173–80 read 263–70.
page 267, Table 2, heading BL, for *34* read *36.*
page 270, ref 4, line 2, for *155–72* read *245–62.*

SCHIEFFELIN LEPROSY RESEARCH & TRAINING CENTRE, KARIGIRI, SOUTH INDIA. SCHEDULE OF TRAINING COURSES FOR 1982

Course	Qualification	Duration	Commencing Date	No. of seats	Fees in Rs
For Doctors					
(a) Condensed course in leprosy	Doctors & senior medical personnel	1 week	18–23 January 5–10 April 6–11 September	20	25
(b) Medical students course	Undergraduates	1 week	Pooja Holidays	20	--
(c) Medical officers course	Medical personnel engaged in leprosy work	6 weeks	1 February to 13 March 5 July to 14 August	16	75
(d) Ophthalmic aspects in leprosy	Qualified medical personnel (included in 6 weeks' course)	3 days	15–17 March 16–18 August	4	10
For non-medical personnel					
(a) Non-medical supervisors course	Fully qualified para-medical workers with a minimum of 3 years' experience	4 months	7 June	12	200
(b) Orientation course in leprosy	For paramedical personnel (nurses, physios, OT and administrators) 1 week condensed course + 3 weeks' in-service training	1 month	18–23 January 5–10 April 6–11 September	6	--
(c) Paramedical workers course	SSLC passed, graduates preferred	6 months	6 September	20	200
(d) PMW refresher course	Qualified PMWs	1 month	7 June to 3 July	20	50
(e) Leprosy for general health workers	Persons now working or trained as general health workers	1 week	22–27 November	20	30
(f) Physiotherapy technicians course	SSLC passed. graduates preferred	9 months	14 June	8	250
(g) Laboratory technicians course	SSLC passed P.U.C preferred	12 months	5 July	4	250

SCHIEFFELIN LEPROSY RESEARCH & TRAINING CENTRE, KARIGIRI, SOUTH INDIA. SCHEDULE OF TRAINING COURSES FOR 1982 (*Continued*)

Course	Qualification	Duration	Commencing Date	No. of	Fees in Rs
Inservice training					
(a) Prosthetic Technicians	SSLC passed, P.U.C. preferred	18 months	4 January 5 July	3	--
(b) Shoemakers course	V standard with knowledge of English preferred	6 months	4 January 5 July		
(c) Smear technicians	SSLC passed (regular qualified laboratory technicians	3 months	4 January 7 June 6 September		
	(refresher)	1 month	April, May, December		75
(d) Medical record-keepers	SSLC with proficiency in typing and good English	2 months	by arrangement		50
(e) In-service training in medicine surgery pathology, control & laboratory technology	For qualified medical personnel	9 months	by arrangement		

All correspondence to the Training Officer, SLR and T Centre, SLRS PO via KATPADI 632 106, North Arcot District, South India

Lepr Rev (1982) **53**, 74–76

Letters to the Editor

INTRACEREBRAL *MYCOBACTERIUM LEPRAE* DO NOT ENHANCE SUBSEQUENT FOOTPAD INFECTIONS IN MICE

Sir,

Dr Stoner has elegantly proposed[1] an hypothesis to explain the selective cell mediated immune (CMI) anergy of lepromatous leprosy patients. In this hypothesis, Dr Stoner proposes that lepromatous leprosy may result, not from a genetic defect, but from active suppression of a genetically normal response capability. Effective CMI to *Mycobacterium leprae* follows the presentation of bacillary antigens by the intradermal route to regional lymph nodes, i.e. the peripheral lymphon compartment. If bacillary antigens are first presented intravenously to the spleen, thymus and bone marrow, i.e. the central lymphon compartment, then a humoral immune response occurs accompanied by a suppressed CMI response. A primary infection of peripheral nerves with *M. leprae* results in the bacilli being isolated from peripheral lymphatic channels but having direct access to the central lymphon compartment. Thus, it is reasoned, continuous leakage of bacilli directly into the circulation, with their concomitant exclusion from draining lymph nodes, preferentially stimulates the central compartment producing a humoral immune response and suppression of CMI.

We reasoned that an experimental approach to testing this hypothesis might be the intracerebral inoculation of viable *M. leprae* into mice followed by standard footpad challenge[2] with viable bacilli. To the extent that the central nervous system is isolated from the peripheral lymphon compartment, to the extent that *M. leprae* antigens might leak from the central nervous system into the central lymphon compartment and to the extent that the mouse footpad infection is analogous to the human infection, this experimental approach seems appropriate.

BALB/c mice, 2–3 weeks of age, of either sex were divided into 3 groups. One group

Table. The effect of intracerebral *M. leprae* on subsequent mouse footpad infection with *M. leprae*

Inoculation		Footpad harvests of *M. leprae* [Mean ± S.D. (N) × 10^6 AFB/Footpad] at days after footpad inoculation:		
Intracerebral	Footpad*	237	270	365
1.0×10^6 *M. leprae*	5.0×10^3 *M. leprae*	0.30 ± 0.31(6)	1.17 ± 0.22(3)	0.16 ± 0.18(3)
Normal saline	5.0×10^3 *M. leprae*	0.93 ± 0.11(6)	3.26 ± 0.33(3)	0.93 ± 0.68(3)
1.0×10^6 *M. leprae*	Normal saline	Not done	Not done	$< 0.016^{\dagger}$(3)

*Footpad inoculation 43 days after intracerebral inoculation.

†Less than 1.6×10^4 AFB/footpad, the lower limit of detectability in this system.

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was injected with 1.0×10^6 *M. leprae* in a volume of 5.0 µl suspended in Hanks's Balanced Salt Solution with 1% bovine serum intracerebrally using a 27 gauge needle, 2.5 mm in length. Forty-three days later the animals were challenged with 5.0×10^5 viable *M. leprae* of human origin in a volume of 30 µl, using standard methodology.[2,3] A second group received sterile normal saline intracerebrally and 5.0×10^3 *M. leprae* of human origin in the footpad 43 days later. A third group received the identical intracerebral inoculation as the first group but received normal saline in the footpad 43 days later (to control for dissemination from the intracerebral site).

Results are given in the Table. At all 3 time-intervals mice inoculated intracerebrally with *M. leprae* had fewer bacilli in their footpads than the control animals. As with any negative experiment, these findings by no means invalidate the hypothesis being tested. On the other hand, the data do not support the hypothesis that intracerebral inoculation of viable *M. leprae* enhances subsequent footpad infections in mice and, thus, the data do not support Dr Stoner's hypothesis regarding the pathogenesis of human leprosy.

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REPLY TO 'INTRACEREBRAL *MYCOBACTERIUM LEPRAE* DO NOT ENHANCE SUBSEQUENT FOOTPAD INFECTIONS IN MICE'

Sir,

The hypothesis[1] states that suppression develops as a result of continuous and steadily increasing leakage of antigen into the circulation from foci in peripheral nerves. It seems unlikely that a single intracerebral injection of *Mycobacterium leprae* would meet this requirement unless the bacilli were to multiply unchecked in the central nervous system of the mouse. To my knowledge this has not been previously demonstrated, nor has the Carville group reported the counts of bacilli in the brain at the conclusion of the present experiments.

It is of interest that intracerebral inoculations of allogeneic cells into mice[2] and rabbits[3] are capable of sensitizing the host. Therefore, the apparent immunizing, rather than tolerizing, effect of intracerebral *M. leprae* is not surprising.

It might be possible to duplicate the hypothesized venous drainage of increasing amounts of antigen in a developing leprosy infection by direct intravenous injection of *M. leprae.* Mackaness and Lagrange[4] attempted to simulate the immunological effect of a leprosy infection by injecting sheep red blood cells (SRBC) into CD-1 mice in daily 2-fold increments starting with 50 and reaching a cumulative dose of 10^9 25 days later. The first 10 injections were intravenous and the remainder were intraperitoneal. They found that a weak delayed-type hypersensitivity to SRBC appeared by day 10, but was soon replaced by a

persistent unresponsiveness. They concluded that 'the cell-mediated attack on a source of antigen, whether it be a tumour or a slowly enlarging population of microorganisms (as in leprosy), may be interrupted prematurely if the antigenic stimulus develops too slowly'. Similar repetitive injections of increasing numbers of viable *M. leprae* might produce a suppressed CMI leading to an enhanced footpad infection, if the injections are properly times in relation to the footpad inoculation.

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Lepr Rev (1982) **53**, 77–78

Book Reviews

Medical Laboratory Manual for Tropical Countries, by Monica Cheesbrough. Vol. 1. Introduction to the Laboratory; Anatomy and Physiology; Clinical Chemistry; Parasitology; Wall Charts; SI Unit Tables. Cambridge, U.K., 1981. 496pp. Cloth, 18 × 26 cm, 2.5 cm thick. Extensive index.

In the Foreword, Dr Waldemar Ferreira, formerly Chief Medical Officer of the Health Laboratory Unit of WHO, refers to the WHO publication 'Manual of Basic Techniques for a Health Laboratory' and indicates that the present manual by Monica Cheesbrough has 'a more ambitious purpose; to fill the gaps existing today in manuals for laboratory technicians in intermediate and referral hospitals in tropical countries, where the need for laboratory support is greater than in the peripheral areas. Such a manual could be used both for training and for reference, as many of the technicians work in isolated conditions.'

The main sections are as listed above. There are also 3 appendices entitled 'Preparation of Reagents, Addresses of Manufacturs and Useful Tables'. In addition, there is a 'pocket' with colour charts for wall display, SI unit conversion tables, labels to mark dangerous chemicals and colour charts for use with urine reagent strips. Obtainable on direct application of Miss Monica Cheesbrough, FIMLS, Tech RMS, 14 Bevills Close, Doddington, Cambridgeshire, England PE15 OTT. Cost: £4.70 in developing countries, plus £1.25 for postage and packaging; £7.60 in other countries, plus £1.75 for postage and packaging.

This remarkable manual, at an almost unbelievably low price, should be in the possession of all laboratory workers and teachers in tropical laboratories, at any rate in English-speaking areas. This statement is made on the understanding that such workers, even at beginner level will have achieved a reasonable level of literacy. In fact, the book contains a wealth of detailed information, which should be of great value at all levels, from junior technician to qualified pathologist.

AC MCDOUGALL

Leprosy—Tuberculosis Eradication. Principles, Practical Implementation, by Enno Freerksen and Magdalena Rosenfeld. Excerpta Medica. Amsterdam, Oxford, Princeton, 1980.

Despite its title, this book is largely concerned with leprosy. It includes 31 pages on the scientific principles of leprosy and tuberculosis eradication, but only 8 pages on the practical implementation of such programmes. It represents the accumulated experience of the Borstel Institute; of the 68 references quoted, 38 refer to work undertaken by, or in association with that Institute.

The main emphasis of the book is in the area of leprosy; the standpoint may be summarized as follows:

1. Leprosy can only be eradicated by chemotherapy; hopes for an effective vaccine are misplaced.
2. All (or almost all) cases of leprosy can be cured by treatment for 150 days with a course of combined therapy, including rifampicin, isoniazid, prothionamide and dapsone.

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3. This short-course treatment offers for the first time a realistic possibility of leprosy eradication in defined areas.

The book ranges widely, and one can agree with much of what is written. The emphasis on the need for multiple drug therapy, and on the possibility that leprosy can be cured by periods of treatment shorter than those advocated for dapsone monotherapy, is most welcome, as is the insistence that the only sound assessment of the value of a drug or drug combination is to discontinue treatment and, by careful follow-up, measure the relapse rate. Similarly, the Borstel Institute pioneered study of the use of surrogate mycobacteria to study the chemotherapy of leprosy, and this approach seems likely to become fruitful.

The reports of treatment of leprosy patients with the combined regimen are optimistic; however, only 1 such trial (the Malta project) has undergone detailed independent follow-up. The patients in that programme have developed remissions of disease for encouragingly long periods, but one can take serious issue with the statement 'relapses in leprosy are usually seen within the first 12 months after discontinuation of therapy' (p. 48). It would be most unwise to consider a patient with lepromatous leprosy as proved to be cured merely because there was no sign of relapse 5 years after stopping treatment.

Some statements on clinical matters must cause concern. They include: 'immunosuppressants have proved ineffective in leprosy reactions' (p. 17); 'dapsone initially has some effect in halting the spread of the disease, and can delay its course a little' (p. 26); 'punch biopsies are not feasible because of the cosmetic consequences' (p. 36); 'the occurrence of leprosy reactions requiring additional treatment. Thalidomide is added in such cases' (p. 44). Statements such as these must cast doubt on the reliability of the clinical findings reported by these workers.

Similarly, many workers will take issue with the statement 'the widespread view that only "solid forms" are viable is unfounded; some other forms can be viable' (p. 15). The view, though ultimately probably impossible to prove, is well founded on quantitative experimental findings; and its general validity is implicitly accepted by the authors when they state 'if only killed, acid-fast material is present or demonstrable, there is no point in continuing chemotherapy' (p. 46).

The authors make many general pronouncements that are hard to justify. They include: 'the aspirations [of a vaccine] based on work done in armadillos are utopian' (p. 16); 'controlled trials [are] in principle, unsuitable in the field of chemotherapy research, and [have] not led to any unequivocal findings either in tuberculosis or leprosy' (p. 21); 'clofazimine . . . was considered to be unsuitable in tuberculosis as early as 1960. Obviously, the arguments against its use in tuberculosis also apply to leprosy (p. 25).

Finally, one must comment on the authors' presentation of the development of their combined therapy regimen. This was selected on the basis of its efficacy against *Mycobacterium marinum*. But, consisting as it does of the 3 most active anti-leprosy drugs in full dosage, it would probably have been selected empirically as the most active drug combination. The addition of isoniazid is still of unproven benefit in terms of the chemotherapy of *M. leprae*. and may well increase the toxicity of a potentially toxic drug combination. The success of this combination, though gratifying, would not be unexpected; but much longer follow-up will required to substantiate the claim that it can, in 5 months, usually cure lepromatous leprosy. One must hope the claim proves to be true, for this would indeed be a major breakthrough in the control and possible eradication of leprosy.

JMH PEARSON

Lepr Rev (1982) **53**, 79

Abstracts

The following are reproduced with our grateful acknowledgement to the Bureau of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

5. Epidemiology

PROST A, NEBOUT M, ROUGEMONT A (1979) **Lepromatous leprosy and onchocerciasis.** *British Medical Journal* **1** (Mar. 3), 589–590

The authors begin by quoting previous publications on the striking impairment of cell-mediated immunity in patients heavily infected with onchocerciasis, in areas where onchocerciasis is hyperendemic. Since cell-mediated immunity is known to be low or absent in lepromatous leprosy, they decided to compare the prevalence of leprosy, in districts with and without a high prevalence of severe onchocerciasis, in the Republic of Upper Volta.

They conclude that their results indicate that the prevalence of lepromatous leprosy is about twice as high in the areas where onchocerciasis is hyperendemic, and that this agrees with the hypothesis that '. . . a highly infected patient with onchocerciasis is more likely to develop the lepromatous form of leprosy and any other infection.' [This very short communication does not do justice to the potentially great importance of such an assertion; one could wish for much more data on the immunological state of the patients studied, including a comparison between those with severe onchocerciasis only, and those with severe onchocerciasis plus fully developed lepromatous leprosy. If this important work is to be reported at greater length elsewhere, some information on the incidence of tuberculosis of various types in patients heavily affected by onchocerciasis would also be of interest.

This paper is concerned with clinical findings and immunology, but it would be valuable if further studies in this and similar areas, endemic for onchocerciasis and leprosy, could pay some attention to the ingestion and fate of bacilli by Simuliidae from patients with untreated lepromatous leprosy.]

A C McDougall

FINE PEM *et al.* (1979) **HLA-linked genes and leprosy: a family study in Karigiri, South India.** *Journal of Infectious Diseases* **140** (2), 152–161

'The evidence for a genetic determination of susceptibility to leprosy is reviewed. To test the hypothesis that an HLA (histocompatibility leukocyte antigen)-linked gene is associated with such susceptibility, the association between the distribution of leprosy within a family and the segregation of HLA haplotypes was investigated among 72 families who lived in Karigiri, Tamil Nadu State, South India. A statistically significant association was found for families in which siblings had tuberculoid leprosy and in which neither parent had leprosy. The findings from the data of this study agree with those of two previous studies carried out among smaller populations in Surinam and Wardha, Maharashtra State, India. Such an agreement suggests that a genetic determinant which is linked to the major HLA locus on chromosome 6 and which is probably recessive affects susceptibility to tuberculoid leprosy in humans.'
[See also *Trop. Dis. Bull.*, 1977, **74**, abstr. 585.]

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CONTENTS

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