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SPECIAL ARTICLE

The cellular exudate–*Mycobacterium leprae* relationship and the critical reading of skin smears

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Summary A careful reading of conventionally stained Ziehl–Neelsen skin smear preparations in leprosy provides a number of insights into the patient's situation, including his approximate position in the spectrum. This data serves as a cross-check on the primary results of the smear examination, and aids their interpretation for the purposes of diagnosis, assessment of the response to chemotherapy and the possible onset of relapse.

The use of the paucibacillary–multibacillary (PB–MB) classification of leprosy for the management of patients on multidrug therapy (MDT) relies heavily on the correct processing and interpretation of slit-skin smears. This, on the view of a recent WHO report,[1] is the weakest link in the whole control programme. Attention to technique will minimize, although it cannot eliminate, the problems inherent in this classification. For this reason the report recommends that all bacteriologically positive patients should be treated as if they were MB. But the determination of smear-positivity or smear-negativity still depends on technique. Error may arise at any stage of the taking, preparation, staining, counting and interpretation of smears, and some technical guidance has been well laid out by Leiker & McDougall[2] and Kim.[3]

In this paper we consider in detail some hitherto unreported data that can be gleaned from a thorough examination of a skin smear made under controlled laboratory conditions, taking account of cellular exudate as well as bacilli. Slit-skin smears are normally undertaken for the purpose of diagnosis, and to monitor the patient's response to treatment and the possible onset of relapse. The results also provide the definitive PB–MB classification (using the terms in their customary sense of scanty or many bacilli), and although they are not used directly in the Ridley–Jopling classification, the bacterial–cellular relationship as seen in smears, and the morphology and distribution of the bacilli, all provide some clues to the position in the spectrum. A proper understanding of the full data serves to cross-check the main results and gives a better insight into the patient's situation. It is necessary first to review the various modifications of acid-fast stain technique in relation to their effects on bacterial counts and morphology, and their appropriateness to skin smears as opposed to bacterial suspensions. We comment also on the measurement of leprosy bacilli.

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Methods

ACID-FAST STAINING

The smear is assumed to be well prepared, and composed of host tissue cells and bacilli spread evenly over an area about 5 mm in diameter on a clean glass slide. The modifications of technique that have been recommended have been numerous: fixation by mild heat or formalin vapour, the use of hot or cold staining solutions, the length of staining time, and differentiation by 1% acid–alcohol or 1–5% sulphuric acid, which may or may not be followed by a rinse in ethanol. The choice is determined partly by the composition of the smear: ideally, homogenates composed of a clean suspension of bacilli would not be treated in exactly the same way as an exudate containing proteinaceous, lipoidal or cellular elements that may interfere with the precise staining of bacilli.

Oxidation in 10% w/v periodic acid prior to staining in carbol-fuchsin[4] is not recommended for smears because of detachment of cells and bacilli. It has some advantages in histology but bacterial morphology is distorted.

Fixation by gentle heat on the under surface of the slide is the most effective, simple and quick to perform. Even under field conditions smears should be allowed to dry for a few seconds before fixation. Heat eliminates the precautions necessary in dealing with noxious formalin vapours, which may in any case cause detachment of bacilli from the slide or render staining more difficult. Phenol-gel protection before formalin fixation has some advantages,[5] but it is messy.

Stain temperature. The choice of hot or cold staining solutions usually depends on the facilities available, and on the relative importance attached to the numbers as opposed to the morphology of the bacilli. To ensure 100% detection of bacilli, and when diagnosis is the primary criterion, there is no better alternative to the use of hot solutions. Because the estimation of stain temperature is crude, it is better to err on the high side and aim for 60°C, the point at which steam begins to rise, but at this temperature, as well as during fixation by heat, there may well occur artifacts in bacterial morphology. Redistribution of cytoplasm may produce false solid staining, or globular condensation of cell wall and cytoplasm may produce a false granularity. In the latter situation bacilli may be lost through over-decolourization. At all stages excessive heat is to be avoided. The ideal temperature, satisfactory for both total count and bacterial morphology, is 45°C.[6] This is the point at which a change in surface tension leads to the formation of a metallic sheen on the surface of the stain.

Staining time is necessarily influenced by the method of fixation, the choice of hot or cold solutions, and the differentiating agent. But a standard time of 15 minutes is recommended, which is long enough to allow penetration of the dye when exudates are unavoidably thick or contaminated with protein or fat, and not so long that the stain precipitates.

Differentiation. Since *Mycobacterium leprae* is only weakly resistant to acid and very poorly resistant to alcohol, the use of either sulphuric acid or absolute ethanol is not to be recommended. Sulphuric acid has other disadvantages. It reacts with the peroxidase of macrophages and with haemoglobin to produce a brown or a prussian blue discolouration which impairs the recognition of bacilli, and attempts to reverse this reaction by washing are liable to cause detachment of some organisms. The differentiating agent of choice is 1% hydrochloric acid in 70% alcohol, the action of which is relatively mild. It is erroneous to think that prolonged staining will minimize the risk of losing the bacilli by overdifferentiation, rather the reverse. Overstaining of the cellular exudate impairs fine control of the differentiating process.

In general, in paucibacillary smears it is vital to stain every bacillus lest they fail to be detected. In multibacillary smears the loss of a few bacilli is less important, but in regressing patients, difficulties may be caused by the release of lipids from disrupted foam cells. The use of hot staining solutions overcomes this problem and aids penetration of the dye.

The following method is recommended as a routine procedure.

STAINING METHOD

1 Fix by gentle heat, using the flame of a spirit lamp or the pilot light of a bunsen burner on the under side of the slide until a ring of condensation forms around the flamed area (up to 6 sec).
2 Flood the slide with carbol-fuchsin to cover it completely, to allow for loss by evaporation. Heat gently until a metallic sheen appears on the surface of the stain, and stop just before steaming commences (45°C). Leave to stain for 15 min without further heat.
3 Wash the slide, directing the flow of water to one end and draining off at the other end. Tip off the water.
4 Differentiate in 1% acid–alcohol allowing the reagent to fall directly on the smear. When the excess dye flows from the smear, rinse in water. Repeat the differentiation and rinsing until a pink colouration in the thin parts of the smear, the end point, is obtained. A red colouration of any areas of thick exudate may be ignored. The time is variable.
5 Counterstain in 0·5% methylene blue in water.
6 Rinse in water.
7 Dry in air.
8 Examine under oil immersion.
The application of oil or mountant is irreversible, for attempts to remove it may cause the loss of large numbers of bacilli. Restaining entails exposure to oil and turpentine mixtures or prolonged immersion in xylene to detect all bacilli, but this distorts the cytology of the exudate. It is essential to stain correctly in the first instance.

MEASUREMENT OF BACILLI

Leprosy bacilli in fixed-stained smears were measured using a standard binocular microscope with × 100 oil immersion objective, and a × 10 eyepiece with graticule calibrated against a stage micrometer. The measurements obtained were further checked in a few instances by means of an image analyser (Mr C Souter, Histopathology Department, St Bartholomew's Hospital, London). The measurements obtained by the two methods were in close agreement with one another. It is of interest that the measurements of length are appreciably lower than those commonly quoted for *M. leprae*: in the slit-skin smears the length did not exceed 5 μm, although in the nude mouse longer forms of up to 7 μm might be seen. Very long forms may be the result not only of rapid unchecked growth but of an abnormality of internal filaments. The width of bacilli was too small for accurate measurement, but in LL it was of the order of 0·5 μm, in BT and BB about 0·2 μm.

In practice, the exact measurement of leprosy bacilli is not important for the examination of skin smears, and the measurements given are intended mainly as a guide to indicate the relative lengths of organisms observed in different types of case. The differences in length are easily perceptible without measurement. The figures are at a magnification that is close to that of the visual image under the microscope.

Cellular exudate

The cellular exudate in a skin smear usually includes keratinocytes and epithelial cells derived from the epidermis, in addition to the inflammatory cells, if there is a lesion, comprised of macrophages, monocytes, lymphocytes and perhaps polymorphonuclear neutrophils. When cells are disrupted in preparing the smear only the nuclei may be visible, but on searching around whole cells can be seen.

Epidermal cells. Keratinocytes and epithelial cells are recognized by their polygonal shape, rounded nuclei and copious pale cytoplasm (Figure 1). These cells are often clumped together and resemble endothelial cells, though the latter are much smaller. Keratin and eleidin granules are acid-fast. Rarely melanocytes with brown melanin pigment can be seen. These are not to be confused with granular bacilli in decrepit macrophages.

Table 1. To show a summary of the reading of smears

Classification	Acid-fast bacilli			Cellular exudate			Expected outcome after effective therapy
	Numbers	Morphology	Distribution	Macrophages	Lymphs	Pmns	
Untreated TT–BT	0 to 3+	Slender, solid 2 μm × <0·5 μm	Separate, ingested AFB are granular	Large, elongated nucleus, intact uniformly dense	Many or moderate	None	1 No AFB 2 Persisting cellular exudate
BB	0 to 3+ varies at each site	Slender, solid or fragmented. 2 μm × <0·5 μm	Separate, close proximity, often intracellular	Medium size, dark oval nucleus, intact	Moderate	None	1 No AFB, but increased lymphs, may suggest reversal reaction 2 Granular AFB with few lymphs may stabilize at BB 3 Previously negative sites may be positive
BT–BB–BL (downgrading)	3+ at every site	Majority slender few long forms, solid or fragmented 2 μm × <0·5 μm 3·5 μm × 0·5 μm	Single or small clumps, AFB arranged in parallel	As for BB, also some rounded nuclei of disrupted cells, intracellular solid AFB	Few	None	1 Granular AFB 2 Fall in BI As for BB
Smear negative Paucibacillary (PB, WHO) Idt, TT, BT	0			Large, intact, few monocytes	Few, none, many or moderate	None	1 Temporary rise in lymphs
BL	3+ to 4+	Slender, solid fragmented and granular, most are 2 μm long	Small clumps, AFB arranged in parallel or free, no globi	Medium size, round nucleus, dense, many intact, no foam cells	Numerous	None	1 Granular AFB spread over slide. No globi 2 Rapid fall in BI, may suggest reversal reaction 3 No foam cells
BL (continued)	4+ to 5+	Mixed slender and long thick AFB, solid fragmented, granular 1–2 μm or 3 μm rarely 4 μm long	No globi, small clumps of AFB in parallel, many free over slide	Medium size, round nucleus, pale nucleus and cytoplasm, often intracellular AFB, disrupted	Few	None	1 Fall in BI, granular AFB in small clumps, but more free, spread over slide indicates stability in BL and slow clearance 2 As above for BL

Table 1. (*continued*)

Classification	Acid-fast bacilli			Cellular exudate			Expected outcome after effective therapy
	Numbers	Morphology	Distribution	Macrophages	Lymphs	Pmns	
BL–LL (downgrading)	4+ to 5+ wide variation between sites	Mixed slender and long thick AFB, most solid 1–4 μm	Small or medium clumps, no globi	As for BL, also pale poorly formed foam cells, disrupted cells, round nuclei, monocytes	Few	None	1 Granular AFB with rise in lymphs may suggest reversal reaction. As for BL. 2 Poorly formed foam cells
LL	4+ to 6+	Solid or fragmented, majority 3 μm × 0·5 μm. But fragmented and granular AFB may predominate most being 3 μm. Or, mixed short and long solids 1–5 μm × 0·5 μm	Globi, round clumps and free AFB spread over slide	Very large pale vacuolated or foam cells, pale round nucleus, disrupted cells, free nuclei, fatty exudate, monocytes	None	None	1 Granular AFB 2 Slow fall in BI 3 Globi conspicuous 4 Intact foam cells at late end stage 5 Usually no lymphs except before reversal reaction and rapid fall in BI
ENL	2+ to 5+	Granular or debris	Globi with degraded AFB	Large foam cells often empty with shrivelled dark nuclei, fatty	None	Many	1 After subsidence of ENL there may be lymphs though no Pmns
LL (exacerbation and reaction	6+	Solid 3–5 μm × 0·5 μm	Globi, and clumps of AFB in parallel	As for LL, also large intact cells with solid AFB	None	Many	As for LL
LL (relapse in early stage)	0 to 6+ variable between sites	Few sites affected with solid AFB, most are 2 μm long, range = 1–5 μm × 0·5 μm other AFB may be granular	Globi and clumps of solid AFB in parallel at affected sites	Large pale, often intact with solid AFB, round nucleus, foam cells, and monocytes	None	None	1 Granular AFB As for LL 2 Not usually followed by reversal reaction 3 No lymphs
LL (resistance)	3+ to 6+ all sites similar	Solid at most sites, little variation in length, 3·5 μm–4–5 μm × 0·5 μm	Globi and clumps of solid AFB in parallel at most sites	Often intact, with pale round nucleus, ingested AFB, foam cells, monocytes	None	None	As for LL 1 No lymphs

AFB numbers, bacterial index;
lymphs, lymphocytes;
Pmns, polymorphonuclear cells;
LL relapse refers to relapse from causes other than primary drug resistance;
Morphology measurement is that of the majority of AFB.

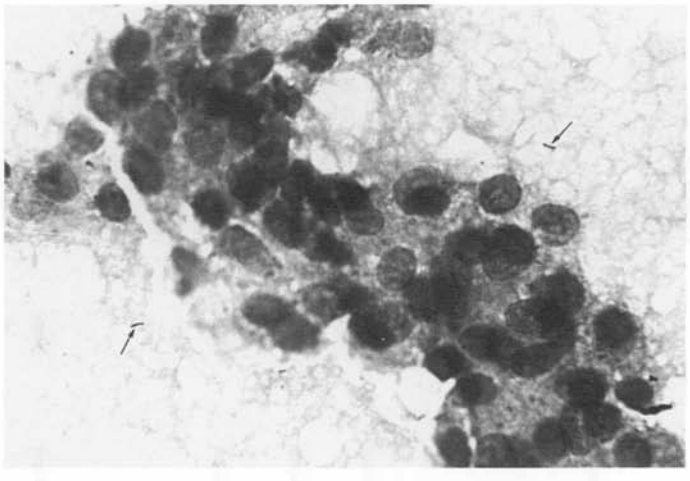

Figure 1. BB. Epidermal cells, and two solid-staining AFB 2 μm long (↑). Acid-fast stain × 1200.

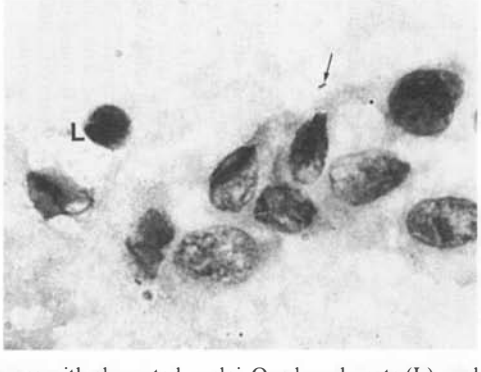

Figure 2. BT. Intact macrophages with elongated nuclei. One lymphocyte (L), and one ingested granular AFB 2 μm long (↑). Acid-fast stain × 1200.

Inflammatory cells. More important are the inflammatory cells in their various forms. Cellular identification is based on visual experience as in a blood film. Experience can be gained by studying the appearances of macrophages with ingested bacilli and similar cells without bacilli taken from patients with different forms of leprosy. Initially special stains like Giemsa, or better still immunohistological methods, help in identifying the inflammatory cells whose nature provides a useful check on diagnosis and the position in the spectrum (summarized in Table 1), which is of clinical and prognostic value.

Macrophages. In TT, BT and BB, the macrophages are large, fleshy, uniformly blue-stained cells with prominent elongated nuclei (Figure 2). Treatment may diminish their numbers but, especially when bacilli are present in the untreated smear, macrophages may persist after the bacilli have disappeared. In BL the macrophages have a dark rounded nucleus and dense blue-stained cytoplasm (Figure 3), and these are the cells that are associated with clumps of bacilli. In other cells poorly distinguishable phagosomes may be seen. After treatment only poorly delineated foam cells

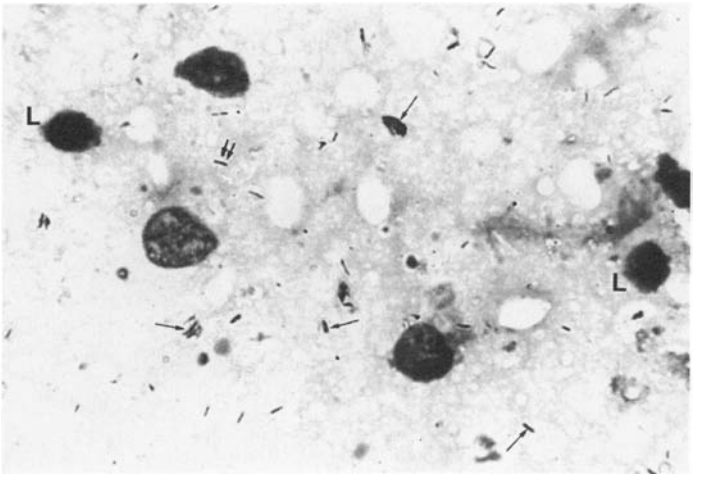

Figure 3. BL. Dark rounded macrophage nuclei and two lymphocytes (L). There are many solid-staining AFB 2–3 μm long (↑↑), and some in clumps arranged in parallel (↑). Note also one fragmented bacillus (top left), but no granular organisms. Acid-fast stain × 1200.

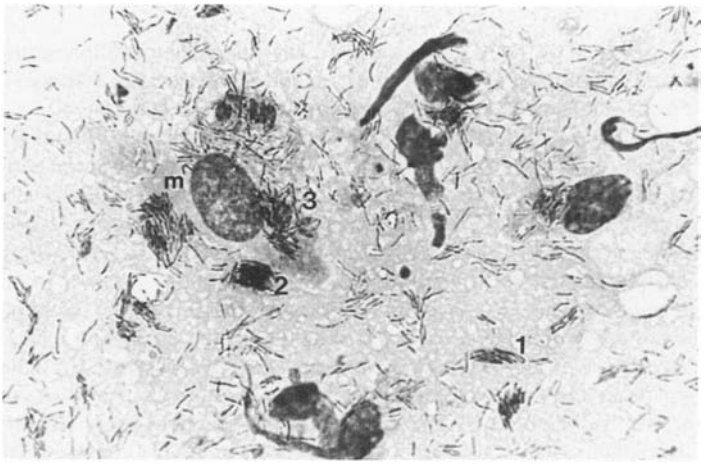

Figure 4. LL. Large vacuolated macrophages with pale round nuclei and ingested bacilli (M). There are several free nuclei. AFB are mostly 3·5–4 μm long with a range of 2–5 μm. Globi contain about 25 (1), 50 (2) or 100 organisms (3). Acid-fast stain × 1200.

may be present among larger numbers of empty, darkly stained macrophages. In LL the macrophages are pale, vacuolated, with characteristically large rounded nuclei, and sometimes contain ingested bacilli (Figure 4); often these cells are disrupted in the preparation of the smear. Bacilli, globi and nuclei are then dispersed over the slide. Foam cells are not inconsistent with active bacterial multiplication, but they are more prominent in the presence of non-solid organisms. Following treatment foam cells, sometimes with dark shrivelled nuclei, are the commonest cell type found in the smears of LL patients (Figure 5). In long treated LL patients, foam cells are not so fragile and remain intact in the smear.

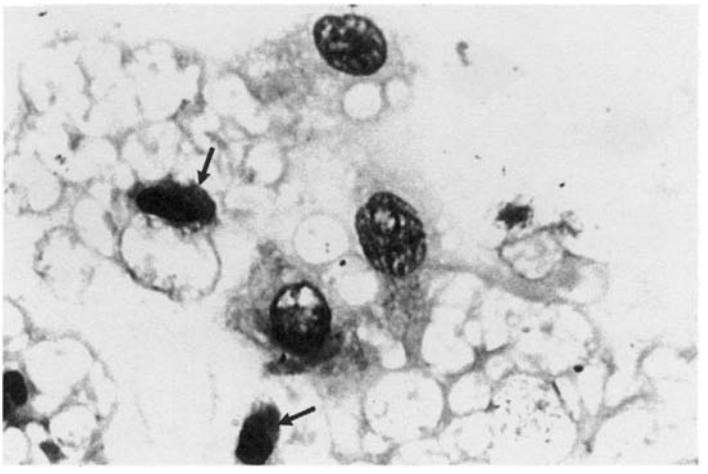

Figure 5. LL after 10 years treatment. Intact foam cells, two with dark shrivelled nuclei (↑), not to be confused with lymphocytes. Acid-fast stain × 1200.

Monocytes are identified by their kidney-shaped nuclei which almost fill the entire cell. They are plentiful in active untreated leprosy especially in LL and in drug resistance. Monocytes are also seen in relapsing lesions, together with a pleomorphic bacterial morphology.

Lymphocytes are moderately numerous in smears from tuberculoid TT and BT patients, and they may be very plentiful in BL (Figures 2 and 3), but they are not a feature of active or quiescent LL. Reacting LL patients who upgrade towards BT after treatment may show few lymphocytes, as do patients recovering from ENL after the disappearance of polymorphs. Lymphocytes never outnumber macrophages in any of the leprosy groups. When blood is present lymphocytes may be more conspicuous, but they are to be ignored.

Neutrophil polymorphs, seen in association with signs of highly active bacterial proliferation, with many solid-staining bacilli spread over the slide due to the disruption of macrophages, are usually a sign of an exacerbation reaction (or a reactional area in a histoid lesion); they are seldom if ever present in hyperactivity except as a result of such a reaction. In less active and more borderline patients, polymorphs may be present in the smears in type I reactions especially during upgrading from BL to BT. In many patients this occurs after commencing MDT. In regressing lepromatous patients with granular bacilli, many polymorphs signify ENL. Polymorphs present in smears from the ear lobes but not in those from other sites are of no significance.

Bacterial morphology

In acid-fast stained preparations *M. leprae* is a rod-shaped bacillus with parallel sides and rounded ends, and of variable size and characteristic mode of degeneration.[2] The disintegration of most bacilli, at least in LL, first affects the cytoplasm of the organism, and the fragmented and granular appearances are due to the irregular staining of cytoplasm within mainly intact cell walls.[7] This degenerative process is often related in part to the effects of chemotherapy, but it is also related to some extent to the patient's position in the spectrum. Bacterial morphology in the reading of smears is summarized in Table 1.

BEFORE TREATMENT

Bacilli, when detected in untreated patients in the TT to BB region of the spectrum, are typically solid-staining, and situated either singly or in a group of 2 or 3 in close proximity but separate. They are notably more slender, about 0·2 μm, than the organisms in LL and somewhat shorter, about 2 μm (Figure 1). Only rarely are granular bacilli detected, usually in macrophages and in patients who are immunologically unstable and liable to react, and especially in BB patients with a low BI.

In smears of BL and LL patients, solid-staining rods are usually conspicuous among a great variety of forms, although it is not uncommon to find a preponderance of fragmented and granular forms even before treatment.[8] In the latter case, smears from the ear lobes will almost certainly show solid bacilli, and numbers may be lower than at other sites.

BL and LL. The solid-staining bacilli of BL patients with a low BI are about 2 μm long, and characteristically slender like those of BB rather than LL patients (Figure 3). When large numbers are present some bacilli may be longer (3–4 μm) and thicker, like those in LL patients. In LL the bacilli are normally about 3 μm long and 0·5 μm wide, but the length may range from 1 to 5 μm (Figure 4). The longest bacilli are seen under conditions of unchecked growth. In BL the bacilli are often arranged in parallel in clumps, but the clumps are never large (Figure 3). Strictly speaking these clumps are not globi, which are more conspicuous, large and rounded in smears from LL patients, in which they tend to be spread over the slide. Bacterial degeneration is the same in BL as in LL. The distinction between these two groups is further aided by the nature of the cellular exudate (Table 1).

AFTER TREATMENT

In tuberculoid and borderline leprosy (TT to BB) bacilli should disappear relatively quickly, due to simultaneous degradation of cell wall and cytoplasm. Degenerate bacilli are fragmented rather than granular. Residual acid-fast debris is quickly cleared by immunocompetent cells.

In BL and LL fragmented and granular forms are predominant. In BL, if the initial BI was low it may quickly fall with treatment but if it was initially high the fall will often be slower. Globi are never found in BL, and the separate bacilli are spread over the slide. This contrasts with LL, in which most bacilli are found in globi. The elimination of organisms here proceeds very slowly, and even after years of monotherapy on DDS it is possible to find a few granular bacilli that still attain the full length of 4–5 μm. Under these conditions the finding of even a single such bacillus among highly granular organisms is a firm indication of the LL group. After MDT bacilli become more shrunken and darkly stained. MDT may be more damaging to the cell wall than DDS monotherapy.

In LL, bacilli with the same morphological characteristics (e.g. long solid forms, or fragmented and granular) tend to be concentrated around the disrupted macrophage from which they were released, so that the distribution of bacilli is not entirely uniform over the smear, at least as far as the morphological characteristics are concerned.

The number of lymphocytes present in smears with granular bacilli complement the fall in the BI as a monitor of the patient's response to treatment. Few lymphocytes, granular bacilli and a slow fall in the BI are the common findings in immunologically stable BL patients. Large numbers of lymphocytes and a rapidly falling BI are suggestive of upgrading.

SIGNIFICANCE OF BACTERIAL FORMS

Solid-stained bacilli are equated with viability. Nevertheless, recently dead bacilli which have not yet undergone morphological change will also appear solid, as will dormant but moribund bacilli. The circumstances under which such organisms might regain activity are unknown. In activity associated with drug resistance solid-staining bacilli are likely to be found at multiple sites, whereas

in relapse due to the recrudescence of 'persister' or dormant organisms fewer sites are likely to be involved.

Short solid bacilli, 1 μm $\times$ 0·5 μm, are common in new or relapsing LL lesions when bacterial proliferation is marked. Some of them are too short to qualify as solid under the definition of the solid ratio,[9,10] which is more strictly defined than the morphological index, yet they are not to be confused with non-solid forms, from which they are morphologically distinct. If all the bacilli present are short solids they are likely to be viable, and the same is true of course of short solids amongst long solid bacilli. Short solids amongst fragmented and granular organisms usually show, with careful scrutiny, some irregularity of staining, especially at one pole, so that they are most probably fragmented and non-viable. Short solid forms are frequently found in smears from the fingers, where they often appear to be viable but dormant.[11] They may be seen in the early stage of relapse.

Club-forms are bacilli characterized by metachromatic swellings that may be situated at any position within the rod. The bacillus is usually solid-staining, or the club may be isolated or in possession of a short acid-fast tail. The exact significance of club-forms is not clear. Our recent experience shows that in some patients on sulphone monotherapy they may be associated with bacterial proliferation; in other such patients, and those on MDT, the association is with past activity, recognizable bacilli being no longer present. This might suggest that club-forms are slow to decay, but that their future evolution is impossible to determine. It is suggested that until more evidence is available these organisms should be discounted from a morphological assessment.

The indices

BACTERIAL INDEX

The logarithmic index of Ridley is widely used for the enumeration of bacilli,[2,12] and because of the 10-fold differential between the steps of the index, agreement between observers should be good, given standardization of technique for the staining and preparation of the smear. Large discrepancies between observers, such as BIs of 1 and 5,[13] are due to serious technical shortcomings. This should not happen if due attention is paid to the cellular exudate as outlined above and in Table 1. An acceptable error would be one division on the log scale. It is possible for bacilli to be selectively concentrated at the periphery of smears of homogenates although there is no evidence that this occurs in skin smears.

The mean BI of all sites is the guide for monitoring the response to treatment, but in view of the variation between sites the individual indices must also be reported. The reason for differences in the rate of clearance of bacilli between different patients within the LL group is not known.

It is usual for patients in the TT–BT region of the spectrum to show no bacilli, or an index of up to 3+ at a few sites. A finding of 3+ at all sites including the ear lobes would suggest downgrading, perhaps associated with a larger bacterial deposit in peripheral nerve. In downgrading from BB to leproma the index is usually higher than 3+ at some sites.

On occasion one might find, say, 50 bacilli in a clump when there were no other organisms in 100 fields. The BI would be 2+, but the unusual distribution of the bacilli should be reported. If the organisms included solid-staining forms, more bacilli might be found in subsequent smears at the same site; and even in the first month of treatment the index might rise to, say, 3+ before falling to zero. This situation may be seen in relapse. If the bacilli were granular subsequent smears would probably be negative. The estimation of numbers of bacilli present in a clump or globus is somewhat arbitrary, being based on the size of the clump. A small clump is estimated to contain about 25 bacilli, a medium sized clump about 50 and a large one at least 100 (Figure 4).

MORPHOLOGICAL INDEX

The MI is the index of morphology in most general use,[2,12] its object being to indicate the percentage of viable forms of bacilli. However, it produces problems of standardization and reproducibility that are difficult to eliminate,[14] the chief difficulty being the determination of the bacilli to be considered as solid-staining.[2] When the total number of bacilli in the smear is less than 100 it is desirable to record the actual number of solid-staining or other organisms present.

SOLID-FRAGMENTED-GRANULAR (SFG) INDEX

The approximate ratio of each class of bacillus, solid, fragmented and granular, is estimated by assigning a value of 2 to the predominant class, 1 to any other class or classes present in significant numbers, and 0 to any class represented by very few or no bacilli. Permutations ranging from 2–0–0 (all solid) to 0–0–2 (all granular) correspond to SFG indices of 10 to 0. The SFG for each field of view is determined, and the mean score for several fields is taken.[2] The SFG ratio takes account of all bacilli (except those in globi which cannot be seen). It does not attempt to indicate the actual percentage of solid-staining organisms, but the results are usually more reproducible than those of the MI. An SFG of 3 (reading 1–1–2) is the lowest score to signify the presence of solid forms. LL patients on effective therapy would show readings of 0–1–2 (SFG = 1) or 0–2–2 (SFG = 2). An SFG of 3 after several years treatment could indicate failure of control or relapse, but not necessarily. The solid-staining organisms whose presence was indicated might be dormant or moribund and only potentially capable of regeneration.

MULTIPLE SITES

We support the recommendations of Leiker and McDougall[2] for the selection of sites for smears, which should number at least 4 and preferably 6.

Variation in the BI from multiple sites may be considerable in some untreated BL patients, but not usually in LL even after effective treatment. Large discrepancies in the BI usually arise as a result of relapse and enhanced activity at one or more sites, though not at others, and there are corresponding differences in bacterial morphology. Smaller variations in the indices can possibly be due to the position from which the smear was taken within the lesion. Smears from the active edge of the lesion are most likely to show solid-staining bacilli in LL patients; in TT, BT and BB they are the most likely to yield bacilli and large numbers of macrophages and lymphocytes. These cells are visible also in smear negative cases.

The ear lobes in tuberculoid patients may be negative, but they are positive in some BB cases in which bacilli are numerous, especially in those downgrading to BL. The ear is the first site to show large numbers of bacilli, and the first to be cleared of them in BL. The ear lobes remain positive for many years after effective therapy in LL.[15,16] In drug resistance solid bacilli quickly reappear at this site, more so than in relapse from other causes. Good vasculature and circulation no doubt explain the early appearance of bacilli in MB patients, and their clearance due to the circulation of drugs and immunocompetent cells in BL. Good circulation could also explain the lack of ear lobe involvement in tuberculoid patients, bearing in mind the high susceptibility of *M. leprae* to serous and other immunoreactive substances.

Nasal smears (or nose blows) are usually positive in LL patients, and to a less extent in BL or BB downgrading to BL, but not in BB or BT. The nasal site is quickly cleared following treatment. The morphology of the nasal bacilli is not a reliable guide to the general bacteriological status of the patient.

The fingers are to some extent a preferential site for bacilli on account of their low temperature and the superficial situation of the nerve supply.[11]

Acknowledgments

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