Chapter 3
Microbiological Investigations and Histopathology

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Abstract The diagnosis of leprosy requires demonstration of lepra bacilli. Slit skin smear and stain are helpful in diagnosing multibacillary cases. In paucibacillary cases, where the bacterial load is low and demonstrating lepra bacilli is very difficult, biopsy may be helpful in establishing the diagnosis. Tuberculoid leprosy shows epithelioid granuloma, usually centered on the nerves and the appendages. Lepromatous leprosy is characterized by diffuse infiltration of the dermis with foamy histiocytes laden with plenty of bacilli. The histopathology of histoid leprosy is distinctive, and spindle-shaped histiocytes arranged in bands or whorls are seen.

Keywords Leprosy · Histopathology · Granuloma · Epithelioid granuloma · Langhans giant cell · Foamy histiocytes

Introduction

The "gold standard" for the diagnosis of an infectious condition is demonstration and culture of the microorganism. *M. leprae* is usually not identified in paucibacillary cases and is not cultivable. The search for another diagnostic tool is fulfilled by histopathology which not only provides enough diagnostic clues but also helps in classifying the disease as per five-part Ridley-Jopling classification. Slit skin smear is a fact technique to demonstrate acid-fast bacilli (AFB), but its usefulness is limited in clinical practice.

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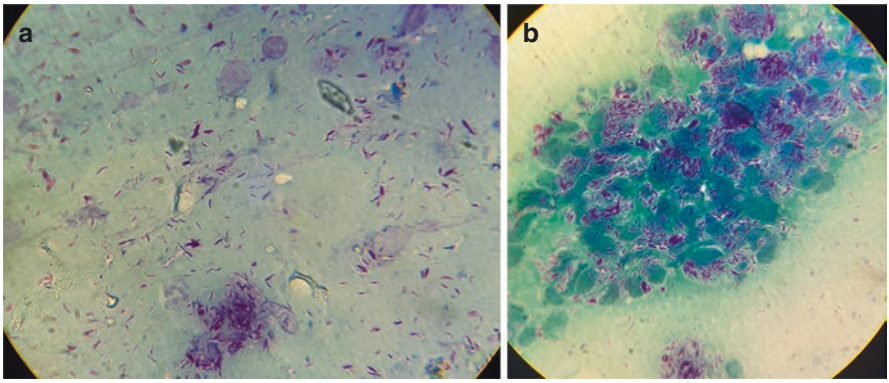

Fig. 3.1 (**a**) *M. leprae* is seen as rod-shaped, bright red colored bacilli (Ziehl-Neelsen, ×1000). (**b**) Clumps of *M. leprae* (globi) (Ziehl-Neelsen, ×1000)

Slit Skin Smear (SSS) [1, 2]

- Tissue fluid from the skin lesion and sites with expected high bacterial load (e.g., earlobe) is taken on a glass slide and is dried and stained with Ziehl-Neelsen stain or Fite stain (Fig. 3.1a, b).
- The number of bacteria is counted under light microscopy at high magnification with oil immersion, and the bacterial index is calculated as per Ridley's logarithmic scale.
- The sensitivity and, hence, diagnostic usefulness depend on the bacterial load and are low in paucibacillary cases.
- Hence, the clinical utility of SSS is limited, as SSS will be positive in multibacillary cases where clinical diagnosis itself is obvious, but it would not help in paucibacillary cases where clinical diagnosis is doubtful and requires diagnostic support.

Histopathology

For diagnostic purposes, deep biopsy including the complete dermis from the most active part of lesion should be obtained. Tuberculoid leprosy is characterized by well-formed granulomas, often perineural in location and assuming a serpentine shape. On the other hand, in the lepromatous spectrum, there is a diffuse infiltration of histiocytes laden with bacilli throughout the dermis, sparing the upper papillary dermis (grenz zone). However, in several cases, the clinical features do not correlate with the histological findings. Clinically, tuberculoid lesions may show lepromatous features histologically, and vice versa. Further the histological features and

Table 3.1 Histopathological classification of leprosy

	TT	BT	BB	BL	LL
Grenz zone	–	+	+	+	+
Epithelioid granuloma	+++ (well formed)	++ (less well developed)	± (variable)	–	–
Foamy macrophages	–	–	–	++ (focal/ nodular)	+++ (diffuse)
Location of granuloma	Perineural, perivascular	Perineural, perivascular, periappendageal	Perivascular	Perivascular	Diffuse
Langhans giant cells	+++ (large well developed)	++ (smaller)	±	Rare	–
Lymphocytes	+++ (periphery of granuloma)	+ (within granuloma, when present)	+	++ (seen throughout the macrophage granuloma)	+ (focal aggregates)
Acid-fast bacilli (AFB)	±	±	+	++	+++ (Globi)

bacteriological index may vary from lesion to lesion, even within the same patient. Clinicopathological correlation is essential. The salient features of different poles of leprosy are summarized in Table 3.1 and are discussed below. While H&E stain provides details of infiltration and architectural changes, bacilli are visualized on Wade-Fite stain or Fite-Faraco stain [3, 4].

The salient histopathological features of leprosy are discussed below [5, 6, 7].

Tuberculoid Leprosy (TT)

- Multiple, well-formed, noncaseating granulomas in the dermis rimmed by lymphocytes.
- Granulomas are composed of epithelioid histiocytes and some multinucleate Langhans giant cells (Fig. 3.2a–c).
- Caseous necrosis is rare but may be seen in granulomas involving the nerve.
- Granulomas originate in perineural locations and expand to involve other areas.
- No grenz zone. Granulomas may erode the undersurface of the epidermis.
- AFB not seen.
- When visualization of nerves on H&E staining is difficult, S-100 immunostaining would help.
- Granulomas may extend to and damage arrector pili muscles, hair follicles, and sweat glands.

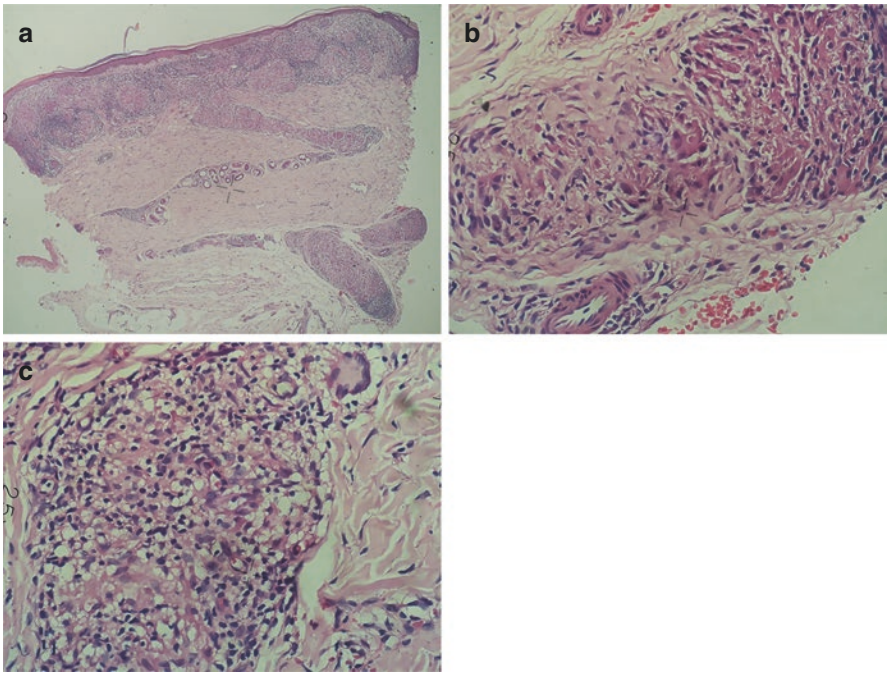

Fig. 3.2 (**a**) Epidermal thinning with well-formed epithelioid granuloma (H&E ×100). (**b**) Well-formed, elongated epithelioid granuloma (H&E ×400). (**c**) Langhans giant cell, histiocytes, and epithelioid cells in granuloma (H&E ×400)

Borderline Tuberculoid Leprosy

- Grenz zone seen (Fig. 3.3a–c).
- Well-formed granulomas.
- Lymphocytes are less than those in TT.
- Swollen nerve bundle may be identified within the granuloma.
- BI is 0–2+.

Borderline Leprosy

- Epithelioid cells present in a diffuse manner.
- Lymphocytes are present diffusely.
- Langhans giant cells usually not present.
- Nerve bundles identified—Schwann cell proliferation. Perineural fibrosis may be noted.
- BI is 2–3+.

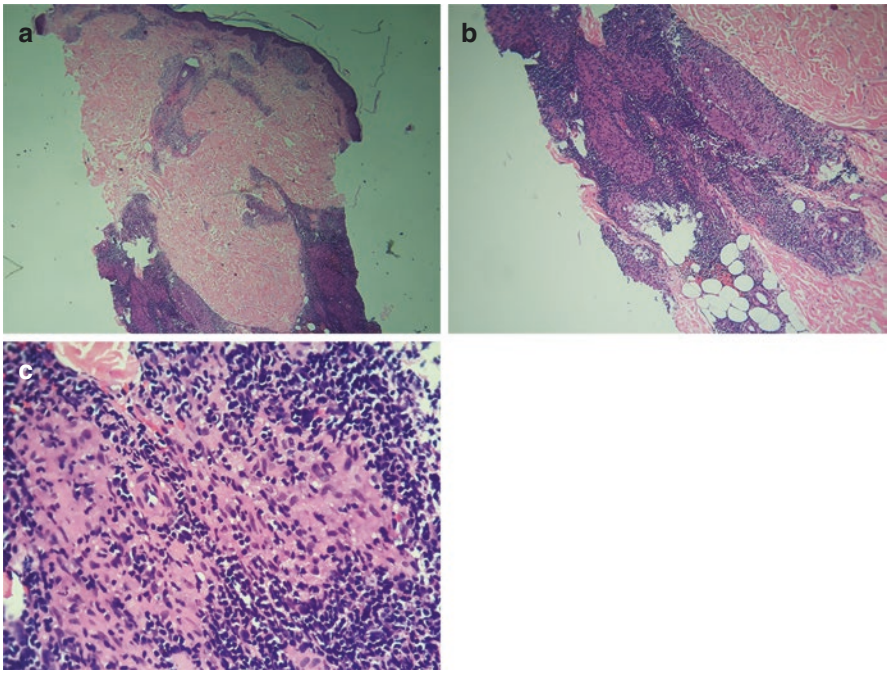

Fig. 3.3 (**a**) Epidermal thinning, grenz zone, and well-formed granuloma reaching up to deeper dermis (H&E ×100). (**b**) Oblong-shaped well-formed granuloma with lymphocytes at the margin (H&E ×400). (**c**) Well-formed granuloma with histiocytes and epithelioid cells in the center and lymphocytes at the margin (H&E ×400)

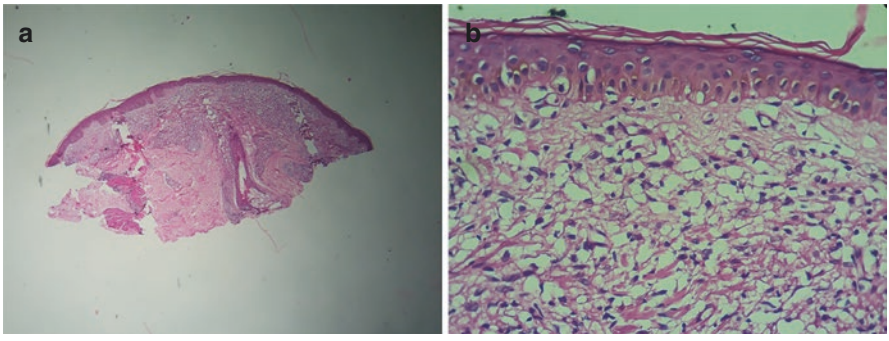

Fig. 3.4 (**a**) Foamy macrophages infiltrating the dermis (H&E ×100). (**b**) Sheets of foamy macrophages (H&E ×400)

Borderline Lepromatous Leprosy

- Foamy macrophages with granular cytoplasm diffusely present (Fig. 3.4a, b).
- Lymphocytes variable—present around the nerves.
- Grenz zone.
- BI is 2–4+.

Lepromatous Leprosy

- Grenz zone.
- Sheets of foamy histiocytes containing numerous AFB. Globi seen. Presence of numerous bacilli gives a grayish tinge to the cytoplasm on H&E stain (Fig. 3.5a–c).
- Nerve bundles lack cellular infiltrate but may show damage.
- BI 5+.

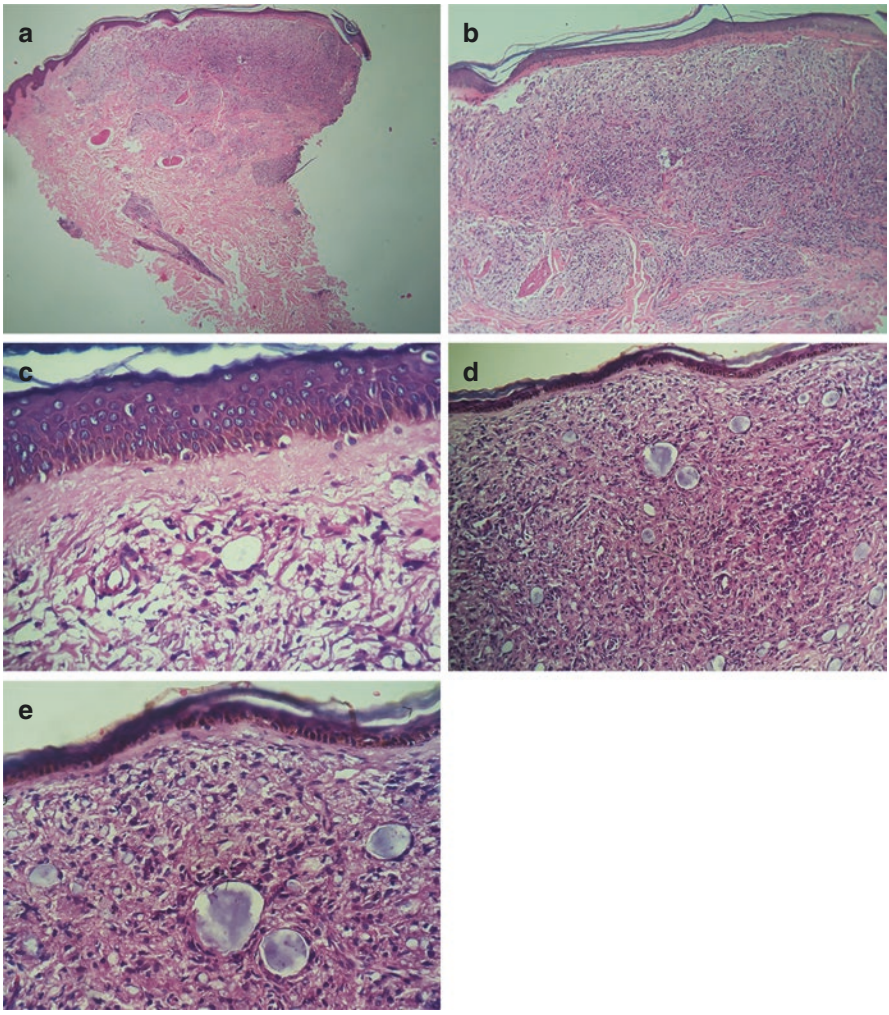

Fig. 3.5 (**a**) Grenz zone and foamy macrophages in the upper and mid dermis (H&E ×40). (**b**) Foamy macrophages with interspersed lymphocytes (H&E ×100). (**c**) Higher magnification (H&E ×400). (**d**) Rarely, acid-fast lepra bacilli seen as blue-gray intracytoplasmic mass (H&E ×400). (**e**) Higher magnification (H&E ×400)

Indeterminate Leprosy

- Sparsely perivascular, perineural, and periadnexal infiltrate of lymphocytes and a few histiocytes.
- Bacilli are usually absent. AFB may be found when a dermal nerve is followed on serial sections.

Histoid Leprosy

- Atrophic epidermis.
- Grenz zone may be seen.
- Circumscribed nodular mass in the dermis consisting of spindle cells filled with bacilli. Cells are arranged in a storiform pattern. Central necrosis may be seen.
- Pseudocapsule may be noted.

Pure Neural Leprosy

- Epithelioid granulomas, mononuclear cell infiltrate, perineural/subperineural edema, fibrosis, and a decrease in myelinated fibers.
- Immunohistochemistry using antibodies against lipoarabinomannan and phenolic glycolipid 1 (PGL-1).

Type 1 Reaction

Upgrading Reaction

- Granulomatous destruction of the nerves and dermal edema.
- Increased granulomatous organization, increased lymphocytes, and an increased number of multinucleated giant cells.

Downgrading Reaction

- Macrophages replace lymphocytes and epithelioid cells.

Type 2 Reaction (Erythema Nodosum Leprosum)

- Superimposed upon the chronic inflammation of lepromatous leprosy.
- Dermal neutrophils with vasculitis.
- In older lesions, neutrophils may not be found.
- Predominantly lobular panniculitis may be noted.

Resolution [8]

Resolution of histopathological changes in leprosy is seen after treatment or, sometimes, even without treatment. The fate however differs in tuberculoid and lepromatous poles.

- In tuberculoid poles, the granuloma usually disappears within a year. Nonspecific lymphocytic infiltration may be the only residual change.
- In lepromatous lesions, degenerative changes cause lipid accumulation, reflecting as foamy changes. These foamy residues resolve very slowly and may persist for years.

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