HANSEN'S DISEASE AS A RESEARCH MODEL*

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Mycobacterium leprae (M. leprae), the etiologic agent of leprosy,[1] produces a chronic infectious disease with diverse clinical appearances. The variety of clinical forms is determined by the host-parasite relationship, which depends on the patient's cell mediated immune response to *M. leprae*. Clinical manifestations range from a single, often self-healing lesion as seen in polar tuberculoid leprosy, to a disseminated and progressive disease involving almost the entire skin and many peripheral nerves, manifested in lepromatous leprosy. Epidemiologic studies suggest that only a minority of those exposed to the infectious agent get the disease. Most probably have an effective immune response which arrests the multiplication of *M. leprae* at a subclinical level.

M. leprae is an obligate intracellular parasite which multiplies within the phagocytic vacuoles of the monocyte/macrophage series and in Schwann cells of peripheral nerves. They have an apparent generation time of 16 to 20 days[2] and cannot yet be cultivated *in vitro.* Man appears to be the major reservoir of leprosy and the mode of transmission, although unknown, is most likely respiratory[3] and/or through the broken skin.[4] Both the mouse footpad[5] and the nine banded armadillo[6] are used as animal models for experimental work.

CLASSIFICATION

Attempts to produce a systematic classification of the disease have generated three current systems. These are: the International classification,[7] the Indian classification,[8] and the Ridley-Jopling classification.[9]

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The most commonly used is the Ridley-Jopling classification, based on the patient's immunity and heavily dependent on the histopathological evaluation of the lesions. According to this classification system, six main categories of Hansen's disease are recognized: indeterminate, tuberculoid, borderline tuberculoid, midborderline, borderline lepromatous, and lepromatous. A modified Ridley-Jopling classification has been suggested by Job and Chacko,[10] which is widely used in the United States. This includes midborderline patients with the borderline lepromatous patient group, and a sixth purely neural group is added.

Indeterminate disease is considered the earliest clinical manifestation of the disease. Lesions are asymptomatic hypopigmented skin macules. The histological appearance is that of a nonspecific chronic dermatitis. Lesions can resolve spontaneously or progress to one of the other forms of the disease if not treated.

At the tuberculoid end of the spectrum are individuals with strong cellular immunity against *M. leprae* antigens. Highly ordered granulomas contain epithelioid cells, multinucleated giant cells, and large numbers of lymphocytes. Few bacteria are present. At the other end of the spectrum are individuals with the lepromatous type of leprosy. These patients show no or minimal cellular immunity against the bacteria. The lepromatous lesions have infiltrates with poorly organized, foamy macrophages and few lymphocytes. Large numbers of bacteria are found in the phagocytes. Many patients do not satisfy all criteria of polar tuberculoid or polar lepromatous disease and fall into the borderline group. The group includes a wide variety of clinical disease which is subdivided according to the histological similarities to the polar groups and the degree of immune responsiveness and/or control of bacterial growth and bacterial load.

Immunological Responses

The different clinical forms of leprosy are clearly associated with varying host defense and cellular immunity to *M. leprae*. As mentioned earlier, the bacterial load of leprosy patients is highest in lepromatous patients and reduced as one approaches the tuberculoid pole (Table I). The large number of bacteria found in the phagocytes of lepromatous lesions suggest that the macrophages are not activated and cannot effectively kill the intracellular bacteria. The lack of intracellular bacteria in the tuberculoid state suggests that mononuclear phagocytes are activated and can kill the organisms.

TABLE I. IMMUNOLOGICAL FEATURES OF LEPROSY PATIENTS

	Tuberculoid	*Borderline tuberculoid*	*Mid borderline*	*Borderline lepro-matous*	*Lepro-matous*
Acid-fast bacilli in skin lesion	−	−/+	+	++	+++
Lepromin (Mitsuda) reaction	+++	++	−	−	−
Lepromin (Fernandez) reaction	+++	++/−	+−	−	−
Lymphocyte transformation test[11]	15%	5.7%	2%	0.4%	0.3%
Leukocyte migration index[11]	0.76	0.84	0.89	0.92	0.96
Anti-*M. leprae* antibodies[12-14]	−/+	−/++	++	+++	+++

Monitoring of cell mediated immune responses is often done by evaluating delayed type hypersensitivity through skin testing. The lepromin reaction, the local delayed granulomatous response to intradermal inoculation of autoclaved suspensions of human lepromatous tissue, varies from strongly reactive in patients with paucibacillary infections (borderline tuberculoid and tuberculoid) to defective or absent in multibacillary infections (borderline lepromatous and lepromatous). This is the case whether the test is read after 48–72 hours (the Fernandez reaction) or after three to four weeks (Mitsuda reaction) (Table I).

In addition, a number of tests for T lymphocyte function *in vitro* have been established with *M. leprae* antigens of armadillo origin. They include the lymphocyte transformation test and macrophage and leukocyte migration test.[11] Both tests have been found to correlate with delayed type hypersensitivity reactions in leprosy patients. Antibody responses to intact *M. leprae* have been tested throughout the entire range of leprosy[12-14] and correlated with the bacterial load, and appear to be non-protective. Antibodies directed specifically against a phenolic glycolipid from *M. leprae* have also been identified in patients' serum,[15] and may be useful as a specific diagnostic test for leprosy infection.[16]

While lepromatous patients are regularly found to be delayed type hypersensitivity negative to *M. leprae* antigens, their responses to other antigens may or may not be depressed,[17] suggesting a specific rather than a general immune nonresponsiveness or immune suppression. Mehra and coworkers[18] have suggested that suppressor T lymphocytes mediate a specific nonresponsiveness in patients with lepromatous leprosy.

The Nature of the Infiltrating Cells in the Cutaneous Lesions of Leprosy

While most studies on the cellular function in leprosy have been carried out with peripheral blood mononuclear cells, little immunological information on the cells of the skin lesions is available. With the availability of highly specific reagents, we decided to study the cells found in the biopsies obtained from patients representing the full spectrum of leprosy. The percentage of macrophages, T lymphocytes, and their subsets were identified and enumerated by specific monoclonal antibodies.[19] A summary of some of these results is presented in Table II.

As one progresses from the lepromatous pole through intermediate to tuberculoid forms of the disease, the percent of T lymphocytes in the lesions increases and the relative numbers of the T lymphocytes subsets change. In patients with lepromatous leprosy only about 10 to 30% of the cells stained with anti T cell antibodies. Of these, about 90% or more were identified as Leu 2a/OKT8 (suppressor/cytotoxic) positive T lymphocytes. These cells were found dispersed among the phagocytes. No obvious T lymphocyte clusters or mantles were observed. In intermediate patients, as one approached the tuberculoid pole a rise in the percent of Leu 3a/OKT4 (helper) T lymphocytes was observed. In patients with tuberculoid disease, 25 to 55% of the inflammatory cells were T lymphocytes. Here the T lymphocytes were predominantly Leu 3a/OKT4 positive. The T lymphocytes were found in clumps and in mantles around the mononuclear phagocytes of the granulomas.[19] No such changes in total or relative numbers of T lymphocytes and their subsets were found in the peripheral blood of leprosy patients. We suggest that the evaluation of the OKT4/OKT8 ratio of T lymphocytes in skin lesions may be a useful diagnostic tool to determine the immune status of patients.

After 18 months of treatment the patients had a second biopsy and the inflammatory cells in their lesions were identified and counted. Preliminary results indicate that although the bacterial load is reduced drastically in lepromatous patients, significant changes in the T cell numbers and/or the OKT4/OKT8 ratios do not follow treatment (Sarno et al., *Int. J. Lepr.*, ms. submitted for publication). This suggests that treatment arrests the clinical development of the disease but does not change the cellular immunological status of the patients.

The same population of patients provided material for transmission electron microscopic studies of the skin lesions.[20] At the tuberculoid end

TABLE II. IMMUNOFLUORESCENT STUDIES: PERCENTAGE OF CELLS REACTIVE WITH MONOCLONAL ANTIBODIES AGAINST T CELLS AND THEIR SUBSETS IN CUTANEOUS INFILTRATES OF 25 PATIENTS WITH LEPROSY.

	Diagnosis		*Percent of total cells*	*Percent of total T-cells*	
Patient No.	*Clinical**	*Histology†*	*Leu 1‡*	*Leu 3a/OKT4‡*	*Leu 2a/OKT8‡*
1	L	LL	20	5	>90
2	L	LL	15	5	>90
3	L	LL	20	5	>90
4	L	LL	30	5	>90
5	L	LL	15	5	>90
6	L	LL	15	15	85
7	L	LL	30	5	>90
8	L	BL	30	40	60
9	L	BL	15	5	>90
10	B	BL	10	10	90
11	B	BL	20	25	75
12	B	BL	20	35	65
13	B	BB	50	50	50
14	B	BB	30	50	50
15	T	BB	50	80	20
16	T	BT	25	85	15
17	T	BT	55	85	15
18	T	BT	40	40	60
19	T	BT	30	35	65
20	T	TT	30	60	40
21	T	TT	25	60	40
22	T	TT	45	55	45
23	T	TT	25	65	35
24	T	TT	35	75	25
25	T	TT	55	90	10

*Clinical diagnosis: L=lepromatous leprosy, B=borderline, T=tuberculoid.

†Histologic diagnosis: Ridley-Jopling scale; LL=lepromatous, BL=borderline lepromatous, BB=borderline, BT=borderline tuberculoid, TT=tuberculoid.

‡Leu 1—recognizes all T lymphocytes. Leu 2a/OKT8—recognize T lymphocytes of the suppressor/cytotoxic subset. Leu 3a/OKT4—recognizes T lymphocytes of the helper subset.

of the spectrum, well developed granulomas with clear evidence of an exuberant lymphocyte response was evident. Large numbers of unique T lymphocytes with extremely long and complex filipodia were closely associated with epithelioid and multinucleated giant cells (Figure 1). Bacillary remnants were scarce and the cytoplasm of the epithelioid cells contained many stacks of endoplasmic reticulum and mitochondria. Many mononuclear phagocytes appeared nonviable, and areas of necrosis were evident.

Lepromatous leprosy was characterized by cutaneous infiltrates containing predominantly parasitized foam cells with large, multibacillary vacuoles. These cells had the appearance of a stable, quiescent population.

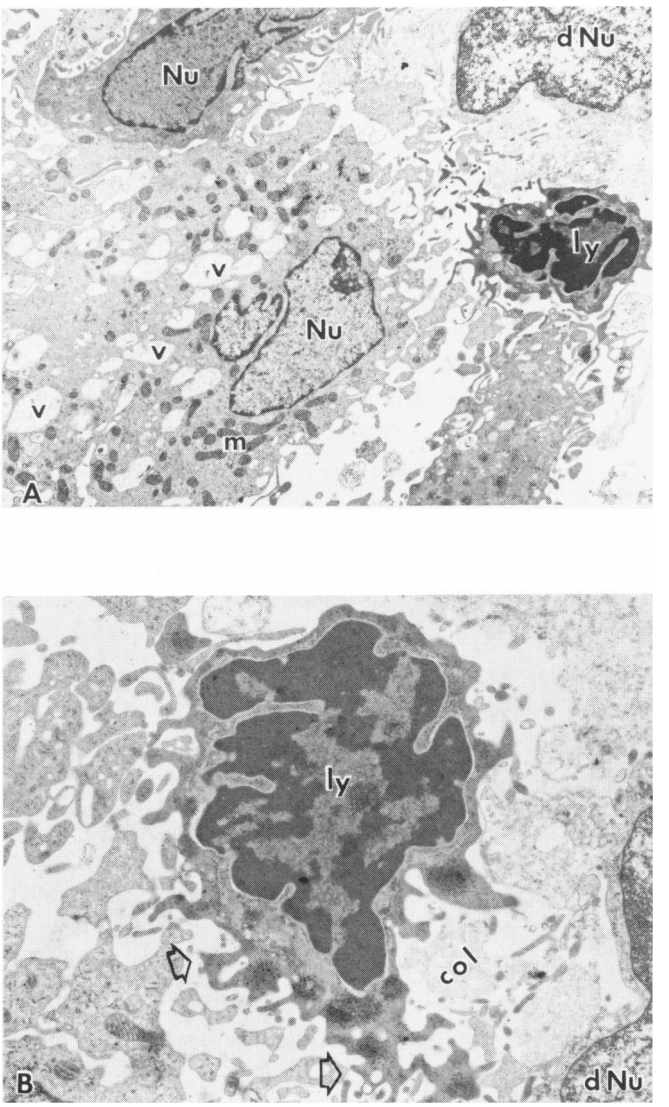

Fig. 1. Transmission electron micrograph of cutaneous infiltrate from patient with tuberculoid leprosy. A. The granuloma cells consist mostly of large epithelioid cells and lymphocytes (ly). The epithelioid cells contain a large irregular nucleus (Nu) and many mitochondria (m) and vacuoles of various sizes (v). Many damaged or dead epithelioid cells were found (dNu) × 4,800. B. The lymphocytes of the granulomas (ly) are very irregular in shape with lobed nuclei and dense chromatin. The cytoplasm is electron dense and the plasma membrane is extruded into villous processes (arrowheads). The cells are surrounded by a collagenous matrix (col). Damaged or dead epithelioid cells and nuclei (dNu) are found in parts of the lesions. × 10,000

The intact and partially degraded osmiophilic *M. leprae* were embedded in an electron lucent matrix. No extracellular bacteria were evident. The Schwann cells of peripheral nerves were also parasitized and demyelination was prominent (Figure 2). Only small numbers of scattered lymphocytes were found. As one progresses from one pole to the other, the intermediate borderline forms of the disease show characteristics between the polar forms.

These observations are consistent with the idea that both macrophages and T cells are activated in tuberculoid lesions. We suggest that Leu 3a/OKT4 helper T lymphocytes may be capable of driving the effector function of mononuclear phagocytes. This would lead to macrophage activation and a significant microbicidal effect on *M. leprae*.

T-LYMPHOCYTE MEDIATORS

Supernatants from antigen- or mitogen-stimulated lymphocytes have been shown to augment macrophage antimicrobial activity against such intracellular pathogens as *Trypanosoma cruzi, Toxoplasma gondii, Leishmania, Candida* sp., and mycobacteria.[21] Recent reports have demonstrated that IFN-γ is probably the macrophage activating factor which is secreted into the medium. When in pure form, IFN-γ has been shown to render macrophages cytotoxic to tumor cells.[22] The addition of IFN-γ to macrophages infected with *Toxoplasma gondii* induced intracellular killing by the macrophages.[23] The failure of lepromatous patients' macrophages to restrict the multiplication of *M. leprae* suggests that the immune defect of these patients may be associated with the activation of macrophages to the bactericidal state.

In a recent study carried out in our laboratory, Nogueira et al.[24] showed that *M. leprae* stimulation of the PBMC's from patients with borderline tuberculoid and tuberculoid leprosy induced release of IFN-γ whereas borderline lepromatous and lepromatous patients failed to release IFN-γ. Release could be partially restored by a combination of purified IL-2 and *M. leprae*, but not by IL-2 alone.

The above observation, together with data reporting that peripheral blood mononuclear cells of lepromatous leprosy patients fail to produce IL-2 in response to *M. leprae*,[25] suggest that the immune defect could be at the level of the IL-2 producing T lymphocytes. Such patients would therefore not produce IFN-γ in response to *M. leprae*. This might result in a lack of macrophage activation with uncontrolled intracellular bacterial growth.

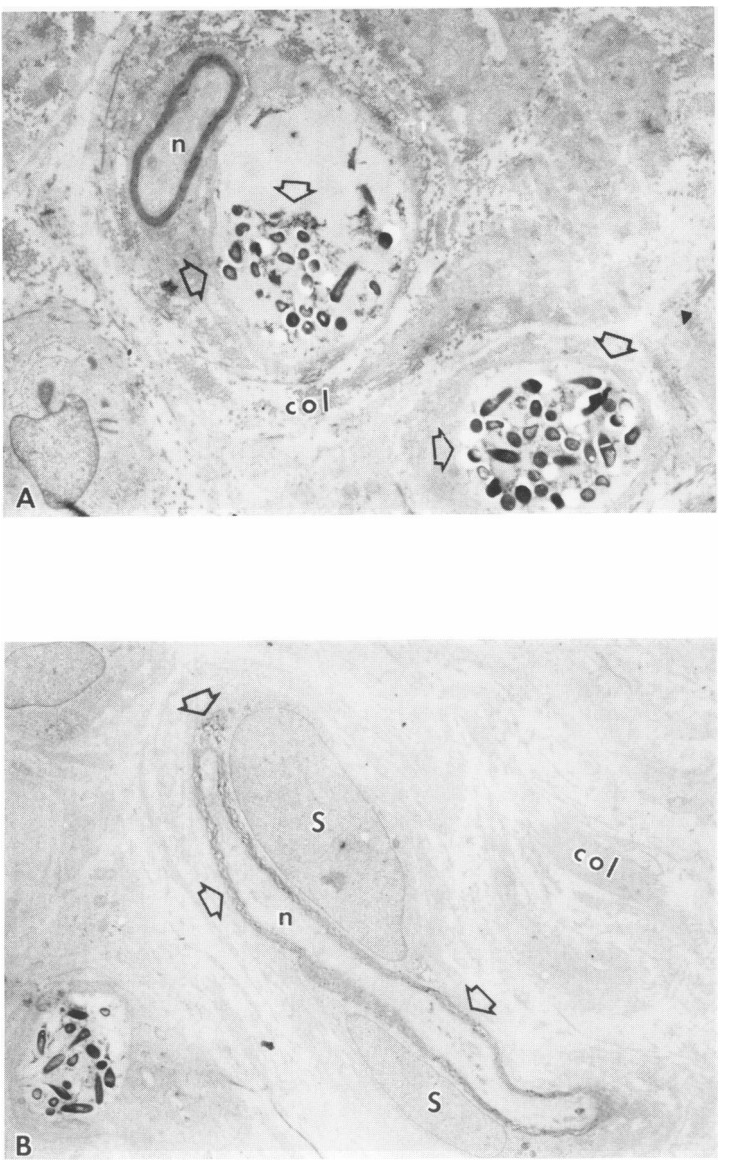

Fig. 2. Transmission electron micrograph of a cross section through a peripheral nerve from a patient with lepromatous leprosy. A. The nerve is infiltrated with inflammatory cells. *M. leprae* (arrowheads) are found within vacuoles of perineural Schwann cells and macrophages. A myelinated neuron (n) surrounded by an infected Schwann cell is found embedded in a collagenous (col) matrix. × 6,000. B. A longitudinal section through a neuron (n) with two Schwann cell nuclei (s). Demyelination of the nerve is prominent (arrowheads). × 4,500

Conclusions

These observations suggest that the severity of the disease is related directly to the extent of macrophage activation. An important factor which complicates the disease is the chemical complexity of the *M. leprae* bacteria. Even after they are killed, the bacteria are digested only very slowly and a storage-like disease ensues. It is important to establish the nature of the vacuolar content in the bacteria laden phagosomes of lepromatous infiltrate cells. That treatment does not cure the disease, and a viable bacterial reservoir remains adds to the complexity of leprosy. Determining where and how the viable bacteria survive awaits the discovery of a rapid method to identify viable as compared to dead bacteria.

It would be of interest to establish the exact phenotype of the unusual lymphocytes found in the tuberculoid lesions and identify the cause of the micronecrosis in these lesions.

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