Revista Latinoamericana de Patología Clínica
MEDICINA DE LABORATORIO

New observations on the polar spectrum of human leprosy

1. Clinical types, histopathology, immunology

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Key words: Leprosy, *Mycobacterium leprae*, clinical types, histopathology, immunology.

Palabras clave: Lepra, *Mycobacterium leprae*, tipos clínicos, histopatología, inmunología.

ABSTRACT

Leprosy is a chronic granulomatous infection of the skin and peripherial nerves, caused by Mycobacterium leprae. Leprosy clinical diagnosis should be considered whenever skin lesions and sensory loss occur. Recent immunologic research, classic bacteriological methods and histopathology, molecular bacteriology and the Ridley-Jopling (RJ) classification which recognized clinical-types not as separate entities, but merely segments of a wide spectrum ranging from purely localized, hyperergic, tuberculoid (TT) pole, trough the borderline (B) types, to the generalized, anergic lepromatous (LL) pole. Reactive episodes continue to be a serious complication, but the availability of thalidomide and clofazimine to control erythema nodosum leprosum, has much improved the prognosis. This review summarises recent advances in understanding the clinical features and pathogenesis of the disease.

RESUMEN

La lepra es una infección granulomatosa crónica de la piel y los nervios periféricos, causada por Mycobacterium leprae. El diagnóstico clínico de la lepra debería ser considerado cuando se observe la presencia de lesiones cutáneas con trastornos de la sensibilidad. Con la investigación imunológica, los métodos clásicos de la bacteriología e histopatología, la micobacteriología molecular y la clasificación propuesta por Ridley-Jopling (RJ), se reconoció que formas clínicas diversas no son entidades separadas, sino partes de un espectro vasto que recorre desde el polo tuberculoide (TT) más localizado e hiperérgico a través de los tipos intermedios-dimorfos (BB), hasta el polo opuesto lepromatoide (LL) generalizado y enérgico. Los episodios "reaccionales" son todavía una complicación grave, pero con la disponibilidad terapéutica de la talidomida y la clofazimina para apagar el eritema nodoso leproso, se ha logrado mejorar el pronóstico. Esta revisión presenta los avances recientes de la clínica y la patogenia de la enfermedad.

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Recibido: 02/12/2012.
Aceptado: 07/02/2013.

DEFINITION

Leprosy (Hansen´s disease) is a chronic infectious disease caused by *Mycobacterium leprae*.[1,2] It principally affects the peripheral nerves and the skin namely the cooler parts of the body: nose, ears, and cheeks. Less commonly the eyes, bones, lymph nodes, nasal-oral structures, testis, liver and spleen may also be involved.[3] Hanseniasis is most prevalent in developing tropical countries; however, this is not due to climate, because disease was formerly very prevalent in the North of Europe cold countries.[2] In the psycho-social aspects, it has been associated with stigma, fear and the shame, *figure 1* hence, it is underreported and the exact number of patients is unknown.[4-6] Certainly, it is an ancient affliction of human kind and has persisted into contemporary times, despite the fact it is not highly transmissible and effective chemotherapy has been available for 60 years.[7] Research of human genetics and immunology over the past 40 years, strongly suggest both factors (genes *versus* social economic environment) may influence susceptibility and development of diverse clinical forms, and histo pathological polar responses (Th-1 *versus* Th-2), to the same intracellular pathogen, Hanseniasis research offers a unique opportunity to link innate-adaptive pathogenesis versus specific host-genes functions.[8,9]

BIOLOGY OF *MYCOBACTERIUM LEPRAE*

The causative organism is an acid-fast (AFB), Gram positive-bacillus, an obligate intracellular parasite with tropism for macrophages and Schwann peripheral nerves cells. The mean generation time of 12.5 day, therefore, it multiplies very slowly.[2] It grows best at 27-30 ºC, hence its predilection for cooler areas of the human body.[10,11] The bacteria can replicate in the mouse foot-pad, athymic nude-mouse and the body of nice-banded armadillo,[12-17] which have provided bacterial-mass for research studies. It has never been cultured in artificial media, but it was found in wild armadillos in the south-central United States and, occasionally in a chimpanzee, sooty Mangabey monkeys, and the African green monkeys.[18]

Through not proven, it most probably spread by the nasal mucus discharges *(figure 2)*, from untreated lepromatous patients, often containing large numbers of infectious bacilli, it may occasionally enter through broken skin, full of isolate infectious AFB and globi *(figure 3)*. Contact with armadillos and soil may result in new cases, but this has not been proved. *Mycobacterium* DNA can also be identified from osseous-nasal lesions, in the archeo pathological analysis of human remains, recorded in ancient India, and the Northern-Central Medieval Europe.

The *M. leprae* genome includes 1,605 genes encoding proteins and 50 genes for RNA-molecules.[19] More than half functional genes of the *M. tuberculosis* are absent and have been replaced by pseudo genes (inactivated genes).[20] *M. leprae* have jettisoned several genes required for fast replication *ex vivo*, and it has a unique ecological niche, with limited host-range and the need for growth within infected cells, such as macrophages.

This "genetic decay" has removed several catabolic pathways and regulatory genes, however, the genes essential for the structural formation of *Mycobacterium* cell wall have been retained.[21] The leprosy bacillus is highly dependent on several host´s metabolic products, which could explain the long generation-time and inability to grow in culture.[19-21]

There appears to be some genetic diversity within the specie *M. leprae*,[22,23] and there is some good evidence that the observed genetic differences may also influence the virulence of *M. leprae*, as compared to *M. lepromatis* apparently causing diffuse lepromatous leprosy and Lucio´s leuko cytoclastic vascular-hemorrhagic, systemic reaction.[24]

Mycobacterium cell-wall contains some selected antigens *(figure 4)*, involved in the host immune responses: the plenolic glycolipid 1 (PGL-1)[25] which stimulates a potent Ig-M response,[25] in proportion to bacterial burden and tends to fall after chemotherapy; the lipo arabino mannan which can inhibit gamma interferon activation of macrophages,[26] and several proteins involved in cell wall synthesis, which may function to stimulate cellular-immunity, because they are potent Th-1 cells antigens.[27,28] Specific genes have been identified for various proteins

Figure 1. Leprosy a devastating, chronic disease can inflict extreme physical deformity and discomfort on its victims. The Canon of the Lateran Council 1179, stated: "Lepers" should not dwell among the healthy. Victorian scholars moulded the spiritual stigma linked to sin, and forged the image of the unclean outcast. (Courtesy of Prof. Virender N. Sehgal, New Delhi, India).

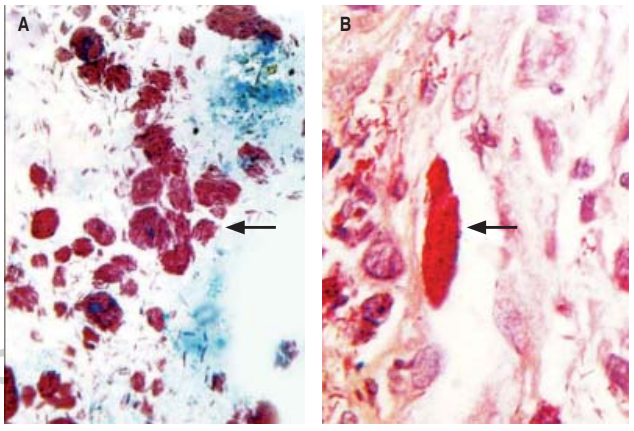

Figure 2. *M. leprae* has been found **(A)** in the nasal mucus secretion (arrow). **B:** The nasal mucosa (arrow). The infective capacity of multi bacillary lepromatous patient is 4-11 times than pauci bacillary, tuberculoid cases. After contact susceptible persons may be infected, probably by a respiratory route.

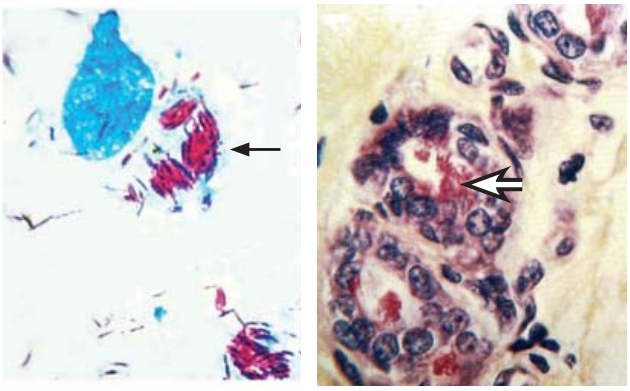

Figure 3. *M. leprae*. **A:** Living leprosy bacilli appears as bright pink rods with rounded ends, length 3-8 microns. ZN stain 1000x. **B:** Bacilli has been also found in sweat glands (arrow) and ducts, arrector pili muscle, hair follicles, muscular media of arterioles and endothelial cells. HE and Fite-Faraco stains 800x.

shared with the environmental *Mycobacterium* and *M. tuberculosis*, however, the specific and restricted leprosy genome includes several open reading frames not present in *M. tuberculosis*.[29-31] Future comparative genetic analysis of the *Mycobacterium* group,[32] may provide the much needed information to generate highly specific skin test reagents for epidemiologic research, and better diagnostic assays to detected subclinical, indeterminate or tuberculoid (TT), leprosy early, pauci bacillary infections.[33,34]

The *Mycobacterium* mass can also be purified from heavily infected fresh-frozen autopsy tissue, and the paraffin-embedded tissue can also be used as source for DNA-extraction. To identify the molecular structure of acid-fast bacilli (AFB), the 16 S ribosomal RNA (rRNA) can be amplified by polymerase chain reaction (PCR), cloned and then sequenced. Other selected genes can also be sequenced such as the housekeeping hsp65 (65-kDa heat shock protein), and rpoβ (for RNA polymerase β subunit) and rpoT gene (for RNA polymerase σ factor), for the analysis of tandem repeats. The genome of *M. leprae* from Tamil Nadu (TN) Southern India, contained 3 repeats of GACATC in rpoT,[19] and a recent study of 600 northern Indian strains also reported 89% having three repeats, and this molecular data correlate well with the predominance (90%) of tuberculoid (TT) localized leprosy in India.[35] In contrast, an analysis of 27 strains from Mexico, showed 25 (93%) with four repeats, paralleling the vast majority (90%) of lepromatous anergic, disseminated leprosy (LL) recorded in Mexico.[36,37]

The 3.3 mega-bp of *M. leprae* genome has been exceptionally stable over time, and the genetic-reductive evolution must have antedated the global spread of *M. leprae* from Africa and North India, to Europe and the Americas.[38,39] Future research may address its relationships with human archeo pathology, history and spread of infectious agents, and the migrations.[40-43]

M. LEPRAE HAS A PREDILECTION FOR PERIPHERAL NERVES

M. leprae has a unique predilection for the glial (Schwann cells) of the peripheral nervous system, and this leads to the neurological damage that underlines the sensory motor loss and subsequent deformity and associated disability. Most affected is the posterior tibial nerve (medial maleolus), followed by, ulnar (elbow), median (wrist), lateral popitleal (neck of fibula), and facial nerves (*figure 5)*. A small sliver biopsy of the thickened radial nerve just above wrist, dorsum of hand or lateral border of foot is suitable for histopathology *(figure 6)*. Early nerve involvement can be detected by electro-microscopy or the use of specific monoclonal antibodies.

Initially, the Mycobacterium PGL-1 of the cell wall has a selected binding to the α2LG module of the G-domain of α2 chain of laminin 2, which is a component of the basal lamina in the Schwann cells *(figure 7)*. This laminin is restricted to the peripheral nerves, which explains specific neuro tropism of *M. leprae*. Subsequent uptake of *M. leprae* by Schwann cell depends on α-dystroglycan, receptor for laminin over the cell membrane, and other intracellular components *(figure 8)*. Once inside the Schwann cell, the leprosy bacilli replicate slowly and generate a chronic mononuclear inflammation produced by T cells, which can recognize the bacterial-superficial antigens. The Schwann cell can also express HLA-class-2 molecules which play an active role in the reaction by presenting *Mycobacterium* peptides to CD4 positive T cells. Swelling within the perineurium leads to increased intra nerve pressure, edema, ischemia, hypersensitivity granuloma and vascular changes, with damage, fibrosis and axonal death *(figure 9)*, and reverse reaction is regarded as the main cause. However, nerves are often functionally impaired without obvious symptoms such as skin lesions or nerve pain, this condition is called "silent neuritis".

THE SPECTRUM OF CLINICAL LEPROSY

The fascinating and unique clinical-immune-histo pathological spectrum of hanseniasis,[51] reflects the advances

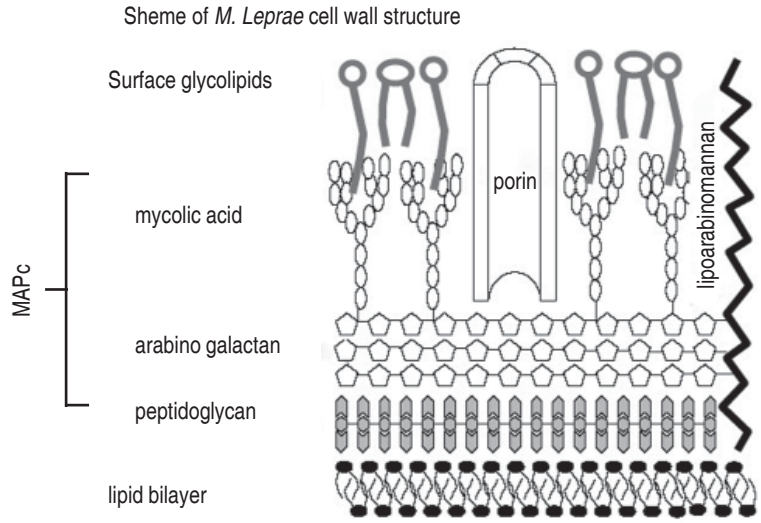

Figure 4.

Structure of M. leprae cell wall. The peptidoglycan layer is linked to the robust core of mycolyl-arabinogalactan complex (MAPc), overlaid by polypeptides and glycolipids. Other components: lipoarabinomannan and phosphatidyinositol mannosides (PIM). When stained by the acid fast procedure, it will resist decolorization and stain red with carbol fuchsin.

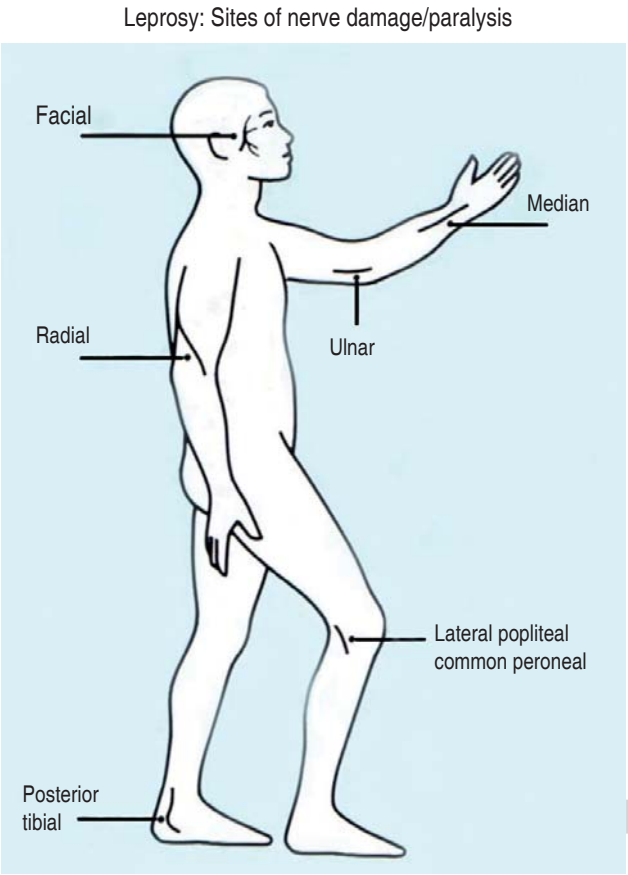

Figure 5. Aproximately 25% of untreated patients, develop muscular weakness or deformities of the hands and feet, plantar ulcer or foot-drop, produced by inflammation and destruction of nerve trunks and fibers, which lead to motor paralysis, hipo aesthesia or anaesthesia, or lack of sweat (anhidrosis).

of medical science,[51] regarding the pathogenesis and natural history of intracellular bacterial parasites *(figure 10)*. Including the Ridley-Jopling and the World Health Organization cardinal signs: a) Skin lesion (*sl*) single or multiple, usually less pigmented than surrounding skin some time reddish or copper colored. A wide variety of *sl* may be seen as flat macules, raised papules or nodules. Sensory loss to pin prick and/or light touch is a typical feature of leprosy. In absence of these signs, nerve thickening by itself without sensory loss or muscle paresia is often not a reliable sign of Hanseniasis.[52] Positive skin and/or nasal smear with rod-shaped, red stained AFB of *M. leprae* are diagnostic of the disease, when examined under a microscope by an experienced clinical pathologist. Disease is likely a rare result of infection by *M. leprae*, conditioned by genetic susceptibility and social economic strata: leprosy is a disease of poverty, overcrowding, slums and lack of good medical services. (Lockwood DNJ. Leprosy and poverty. Inter J Epidemiol 2004; 33: 269-270).

PAUCI BACILLARY, HYPERERGIC DISEASE

The mildest from of leprosy is classified as indeterminate (*ID*)[53-55] and may require a dermatologist to even make the diagnosis, as bacilli are rarely seen.[56] It usually presents as single or multiple, asymmetrical, slightly hypo pigmented (pale) of faintly erythematous and ill-defined, hazy skin macules[57] *(figure 11)*. Sensation in affected area is normal or slightly impaired, but sweating and hair growth is unaffected. Skin biopsy shows dermis with minimal accumulation of lymphocytes around nerves[58] *(figure 12)*. *ID* is

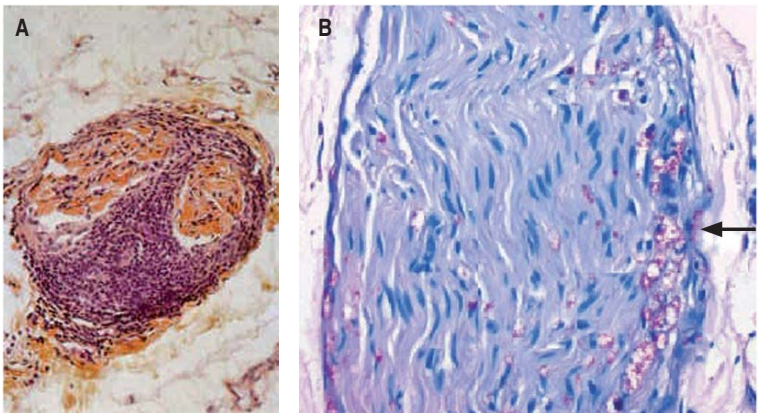

Figure 6.

A: Dermal nerve showing cellular infiltration by CD4+ activated lymphocytes and epitheliod cells, in tuberculoid leprosy "reverse reaction". **B:** Nerve fiber with a high burden of acid-fast bacilli, identified as *M. leprae* with the use of monoclonal specific antibodies, in diffuse lepromatous leprosy of Lucio-Latapí. Courtesy of Prof. Dr. Fernando Latapí, Centro Dermatológico Pascua, México DF.

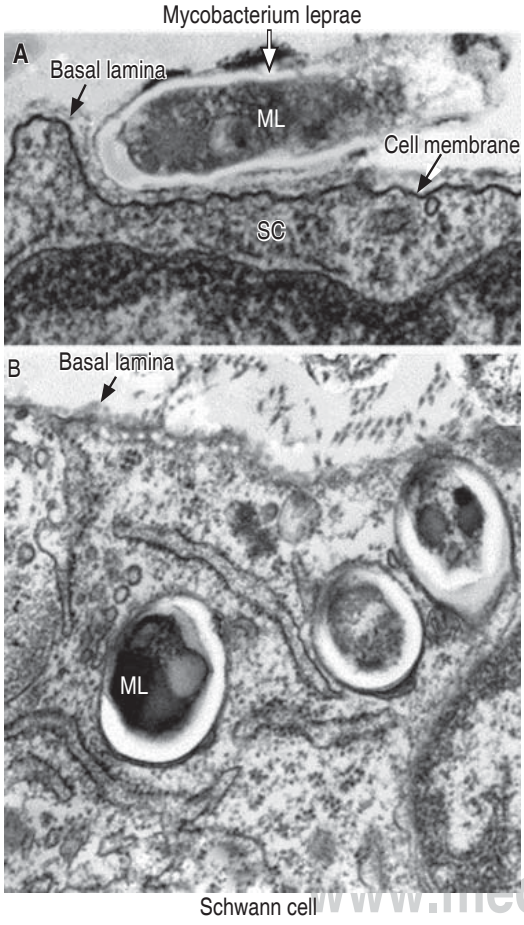

Figure 7. Infection of Schwann cell (Sce) by *M. leprae* (ML). **A:** Electron micro graph of time-course analysis, shows the specific attachment of *M. leprae* to basal lamina of the Sce axon unit, it is the first step of the selective bacterial neuro predilection. **B:** After binding of *M. leprae* leads to internalization and multiplication of the parasite, causing neuritis and neurolysis of peripheral nerves.

often self-curing, but may also progress to other forms of disease, where the presence or absence of T-lymphocytes cell-mediated-immunity (CMI), is critical to pathogenesis.

Tuberculoid (TT) polar case is usually a single *sl*, but there may be two or three asymmetrical lesions, seldom over 10 cm in diameter. *sl* may be reddish, brownish or hypo pigmented, but never completely de pigmented as in vitiligo.[59] TT-leprosy is usually oval or rounded well demarcated lesions from surrounding skin, by a distinct edge[60] *(figure 13)*. ***Sensory loss i.e. feeling for pain and/or touch and temperature and well defined, raised edges, are characteristic features of TT.***[61] The presence of serrated, amoeboid margin or scaling indicates a high level of activity. Some patients may have enlarged cutaneous nerve; the examiner should run his finger lightly around the patch to detect inflamed nerve cord. Biopsy shows a low burden of AFB in skin and striated muscle. Main immunological features are: strong cytokine production or gamma interferon (IFN-γ), interleukin 2 (IL-2), IL-6, IL-12 and tumor necrosis factor (TNF).[62] Active CMI is reflected in the positive Mitsuda-lepromin skin test. Histopathology shows well organized granuloma formation, consisting of epitheliod cells (epc), of with indistinct nuclear chromatin and faintly stained cytoplasm, and Langhans giant cells surrounded by a large accumulation of lymphocytes *(figure 14)*. CMI is "high" while the B-lymphocyte antibody response to *M. leprae* is virtually nonexistent. TT in not always a benign disease, but rather a double-edged sword, it apparently affords "protection" against uncontrolled growth of *M. leprae*, but it is also the likely mechanism of peripheral nerve damage[63] *(figure 15)*.

MULTIBACILLARY (MB) ANERGIC DISEASE

Lepromatous leprosy (LL) is at the opposite end of the spectrum.[64] The first evidence of disease is often ignored

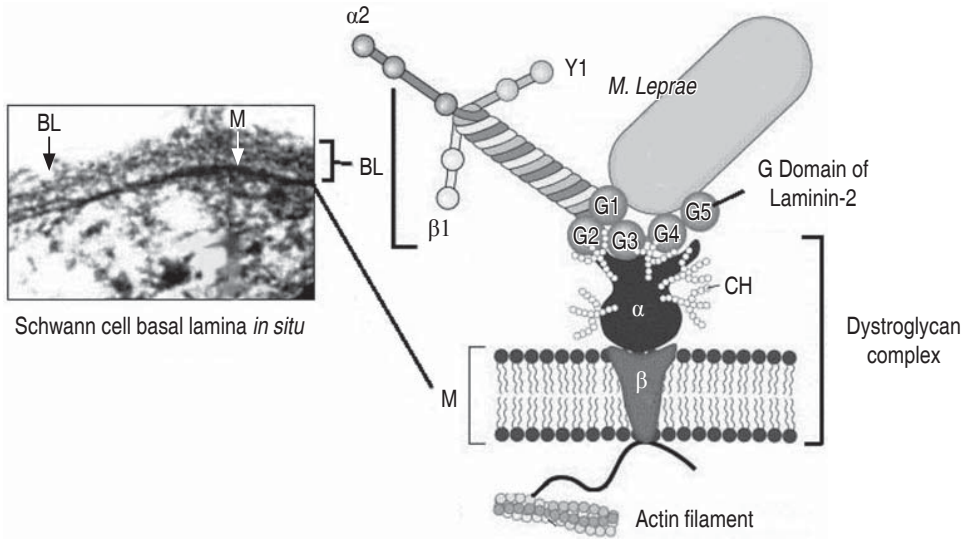

Figure 8.

Left: Electro-micrograph of Schwann cell (Sce) in a sciatic nerve showing the basal lamina (BL) just outside the Sce cell-membrane (M) *in situ*. Right: A molecular model representing the *M. leprae*-Sce interaction, of the G-domain of laminin-2 and the complex structure, of α-distroglycan, with a high content of carbohydrates (CH).

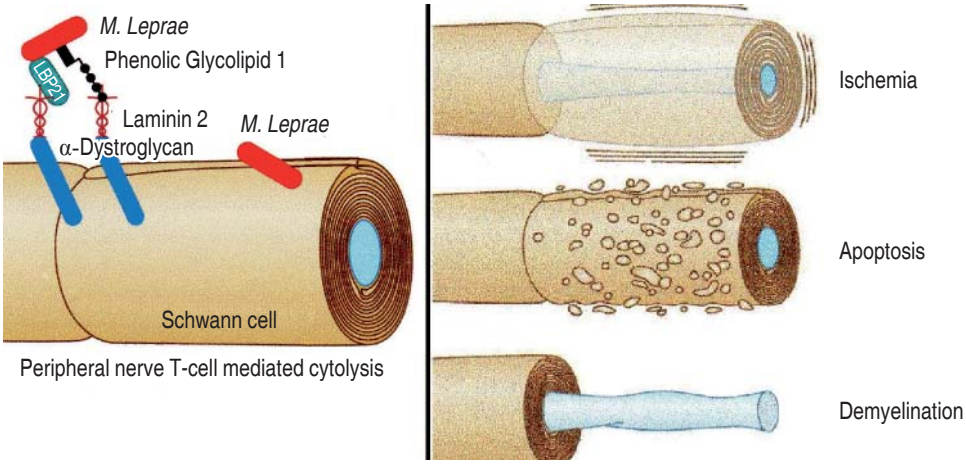

Figure 9.

A: Laminin binding protein 21 and phenolic glycolipid-1 in the *M. leprae* cell wall bind to the α2 chain of laminin 2 and α-distroglycan on the Schwann cell membrane, with entry and subsequent damage to peripheral nerve. **B:** Nerve damage is T-cell mediated inflammation and cytolysis, due to release of cytokines TNF, ischemia result after edema within neural sheath, and apoptosis or demyelization with motor paralysis and loss of sensation.

or barely noticed by the patient *(figure 16)*. In Hawaii, Wayson followed carefully for three years 108 children born of parents with leprosy and observed evidences of leprosy in 10 (9.26%). Neurological disturbances generally preceded cutaneous lesions, described as slight thickening of one or more nerve trunks/branches, and small areas of hipoaesthesia or anesthesia. Atony or slight atrophy of inter osseous muscles of hand and the thenar-hypothenar eminences, weakness of facial muscles, localized anhydrosis, skin glossines-hypopigmentation and sensory loss.[65]

An analysis of clinical histories from the US. Public Health Service Hospital at Carville from the States of Florida and Louisiana, for the period 1921-1950, showed 80% came from families in which no other cases of leprosy have been diagnosis. Louisiana's patients 65% denied knowing of any relative or acquaintances with the disease.[66]

Porrit and Olsen reported the curious cases of two Marine veterans, inhabitants of Michigan, who developed skin lesions at the site of tattooed emblems. The tattooing of each had been done by the same "artist" in Melbourne, Australia, 2.5 years before the lesions appeared. Diagnosis of leprosy in these two patients was well documented. Prolonged contact evidently is not needed for infection to take place, if all factors needed for inoculation of a well person by bacilli from an active-case are present.[67]

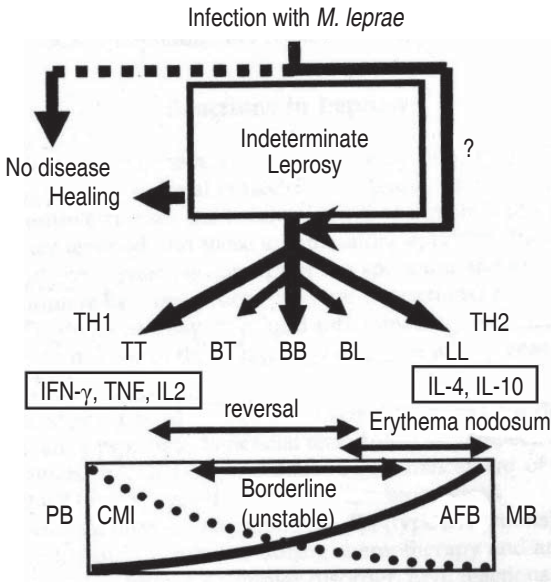

Figure 10. The spectrum of leprosy. After exposure to *M. leprae*, most persons will not develop disease. Susceptible individuals have indeterminate leprosy, which may heal spontaneously or progress. The RJ classification combines clinical-immunological-histopathological evidence with two polar forms: tuberculoid (TT) and lepromatous (LL), and three borderlines BT, BB, BL. WHO-classification has two therapy categories: pauci bacillary (PB) and multi bacilliary (MB). Towards TT pole lesions display cell mediated immunity (CMI) and have few AFB bacilli as Th-1 interpheron gamma mediated response. Towards LL, response is mediated by IL-10, IL-4 and Th-2 profile, poorly developed CMI, and abundant AFB. Borderline are unstable, BT and BB are prone to disfiguring reversal reactions, while BL and LL are subject to painful erythema nodosum (ENL) reactions. RJ=Ridley-Jopling; AFB=acid fast bacilli *Mycobacterium.*

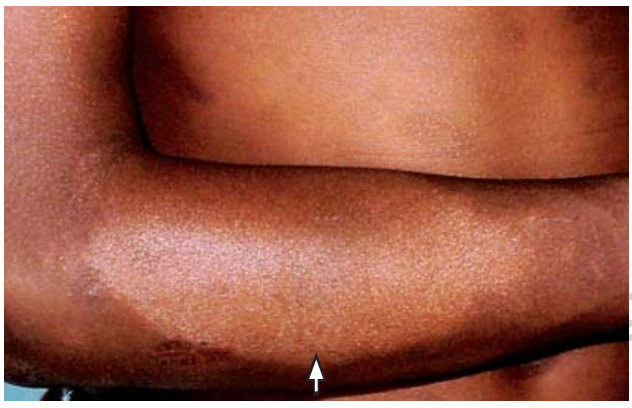

Figure 11. Indeterminate leprosy (ID) a single, asymmetrical, ill-defined and hip pigmented macula, with faintly erythematous areas. Slight sensory impairment (Courtesy of Prof Dr. Fernando Lataí, CDP México DF).

In the laboratory, Rees et al have reported success in obtaining disseminated lesions in mice inoculated with *M. leprae* after thymectomy and total irradiation,[68] Kirchheimer and Storrs have reported severe disseminated lepromatoid leprosy, developed in nine banded armadillo *Dasypus novemcinctus*, 15 months after inoculation of human leprosy bacilli, and showed histological evidence of systemic infection.[69]

Early skin changes in LL are widely and asymmetrically distributed as macules, poorly defined with hypo pigmentation and erythema, papules and nodules, peripheral edema of the legs and ankles due to stasis when left untreated, skin may thickens due to dermal infiltration, giving the leonine facies *(figure 17)*. Hair is lost from eyelashes and eyebrows (madarosis). Involvement of nasal mucosa gives rise to sensation of stuffiness and epistaxis, the massive infiltration of nasal structures may lead to a saddle nose deformity *(figure 18)*, due to septal perforation and destruction of the anterior nasal spine. Enormous numbers of leprosy bacilli are found in skin, up to 10^{10} *M. leprae* per g of tissue.[1]

Histopathology shows thinning and flattening of rete ridges. A granuloma free, clear sub epidermal zone of Unna is seen *(figure 19)*. Dermal lesion is comprised almost enterily of foamy and vacuolates macrophages, collected around nerves.[58] The AFB often in clumps may be found into macrophages, testis and perineural cells. Peripheral nerves are affected asymmetrically, in advanced cases nerves become thin and hard, due to fibrosis and result in extensive anesthesia.

The lepromin Mitsuda test is always negative, LL is characterized by low cell-mediated immunity with a humoral Th-2 response, poor granuloma formation; mRNA production is predominantly for cytokines IL-4, IL-5 and IL-10. IL-4 has been show to downregulate Toll-like receptors (TLRs) on the surface of monocytes and macrophages (*Mac*) which recognize mycobacterial lipoproteins, this leads to low monocyte differentiation into *Mac* and dendritic antigen presenting cells. IL-10 will suppress production of IL-12 this is associated with a preponderance of $CD8^+$ lymphocytes in LL, and the anergic response, absence of organized granulomas and failure to restrain *M. leprae* growth. Spontaneous regression of disease does not occur in LL cases, prone to erythema nodosum (type 2) reactions in as much as 50% of individuals.[70]

The eyes involvement is cause of blindness in 3.2% of those affected with lagophthalmos (inability to close the eyes), corneal ulceration, irido cyclitis and secondary cataract, with frequent damage to zygomatic-temporal branches of the facial (7th) nerve and exposure keratopathy. Reduced corneal conjunctiva sensation is due to

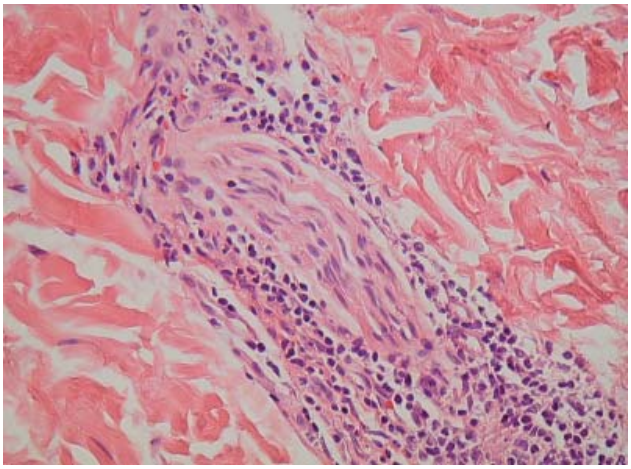

Figure 12. Indeterminate leprosy punches skin biopsy. Center shows a dermal nerve fibril, infiltrated by peripheral lymphocytes. No granuloma formation and rare AFB. HE stains 100 x. (Courtesy of Prof. Dr. Fernando Latapí, CDP, México DF).

Figure 13. Tuberculoid (TT) leprosy, clinical case. **A)** This young man, had a single, large, facial and oval skin lesion, with raised and sharp, erythematous margins sloping toward the center, a scaly surface, and anesthesia confined to just the area occupied by the lesion. **B)** After chemotherapy the skin lesion disappeared. Courtesy Centro Dermatológico Pascua, México DF.

involvement of the ophthalmic branch of the trigeminal (5th) nerve, predisposes to corneal ulcers. Blindness has devastating consequences for those who have sensory loss of hands and feet.[1,2]

Testicular atrophy results from bacillary infiltration of structures and the repetitive orchitis of type-2 (ENL) reactions, with hypo gonadism and secondary osteoporosis.

The borderline (B) part of the spectrum is dynamic between two polar types. These reactive changes are conditioned by the interactions of T and B lymphocytes and *Mac*, mediated by cytokines, chemokines, adhesion molecules and their receptors. All together play a role in ultimately determining immune response of the infected person.[70]

DIFFUSE AND SUCCULENT (PRETTY) LEPROSY

A diffuse form of LL (DLL) known as leprosy of Lucio and Latapí, is characterized by diffuse-systemic skin infiltration without formation of nodules, total loss of eyebrows and eyelashes, highly bacilli ferous dermal infiltration which may smooth out the facial wrinkles, resulting in appearance of "pretty leprosy", but the hand´s dorsum may be atrophic and wrinkled[71] *(figure 20)*. Histologically is characterized by massive mycobacterial invasion into endothelium along andotherial proliferation *(figure 21)*, vasculitis in the dermis and sub cutis and vascular occlusion.[72] The recurrent crops of sharply demarcated purpuric lesion, particularly on the lower extremities *(figure 22)* and even generalized, with development of anemia and high levels of creatinine-kinase. Postmortem, a massive burden of leprosy bacilli has been recorded.[73]

IMMUNE RESPONSE TO *M. LEPRAE*

The immune system is divided in two categories:

a) Innate (In) refers to nonspecific mechanisms occurring immediately or within hours of a new antigen appearance into the body, including physical barriers such as epidermis, effector molecules in body surface as nasal mucus secretion and blood, and participation of myeloid cells linnage, the response is activated by chemical properties of the bacterial antigens.[74-78]
b) Adaptative (Ad) immuty is more complex, refers to antigen-specific (Ag) responses. The Ag first must be processed and presented; a residual "memory" renders more efficient. In an Ad responses are much interconnected. Macrophages (Mac) and dendritic cells are recognized by T-lymphocytes through their major histocompatibility complex (MHC) presentation of Ags.[79,80] Initially bacteria are phagocyted in a process after contact with the macrophage mannose receptor

(MRC-1) and C3-complement receptor (CR1)[81] *(figure 23)*. The human toll-like receptors (TLR 1, TLR 2 and TLR-4) also play a rol in *M. leprosy* uptake.[82,83] After entry into host Mac, the bacteria reside in endocytic vacuole called phagosome-lysosome, where it can encounter a hostile environment including acid pH, reactive oxygen intermediates (ROI), other, bactericidial mechanisms are dependent on the arginine production of NO, plus lysozomal enzymes, and toxic peptides. ROI, produced by activated Mac are major weapons in antibacterial activity.[84] Mice with mutations in the gene encoding the macrophage-localized-cytokine-inducible nitric acid synthase gene are more susceptible to *Leishmania major*, *Listeria monocyto genes* and *M. leprae*, and the, RNI resistance demonstrated among various strains of pathogenic *Mycobacterium* correlates well with virulence.[85,86]

M. leprae probably enters the body via the nose (airborne), and then spreads to the skin and nerves via the circulation. Spontaneous healing cannot be measured not excluded, but certainly exists. The bacterial residues in early phagosomes, blocks the maturation to phago lysosomes formation. However, the arrest is incomplete and some bacteria are killed, fragmented or impaired in replication through antimicrobial effectors. Iron restriction is another mechanism to control the infection.[87]

The highly conserve LTRs recognize mycobacterial lipoproteins, mainly through TLR2/1 heterodimer, and lead to monocyte differentiation into active macrophage and dendritic cells causing secondary activation of naïve T cells by IL-12 secretion, because the specific membrane receptor IL-12β R2 is expressed more on Th-1 lymphocytes, shifting the immune response to the TT-hyperergic-granuloma formation pole. TLR stimulation can also activate the nuclear

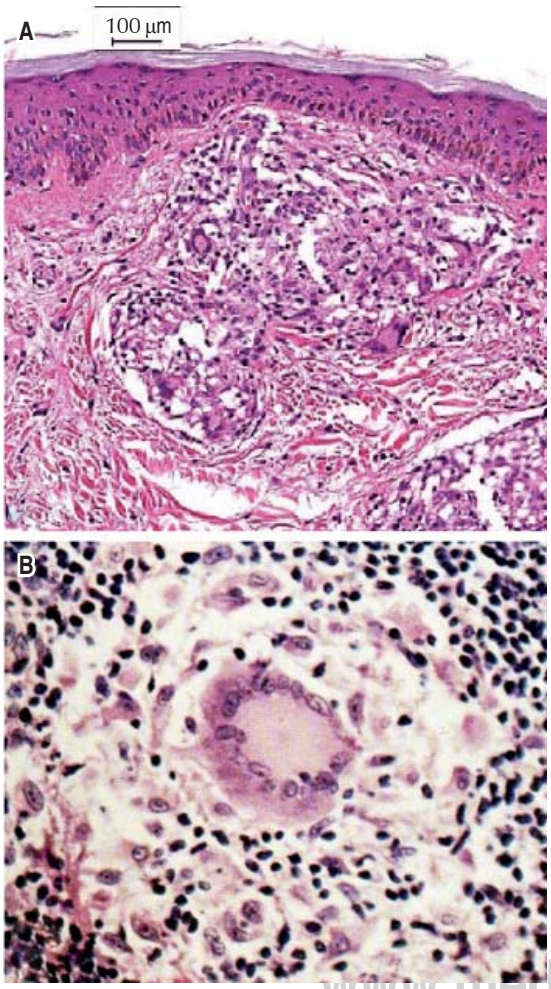

Figure 14. Tuberculoid leprosy histopathology. **A)** Dermis occupied by organized granulomas, erosion of the basal layer of epidermis, and infiltration of cutaneous nerves. **B)** A Langhans giant cell (center), surrounded by pale epitheliod cells and a peripheral zone of mononuclear activated lymphocytes. Interpheron-γ is always present in this type of leprosy, with few AFB. Courtesy Prof. Dr. Fernando Latapí, Centro Dermatológico Pascua, México DF.

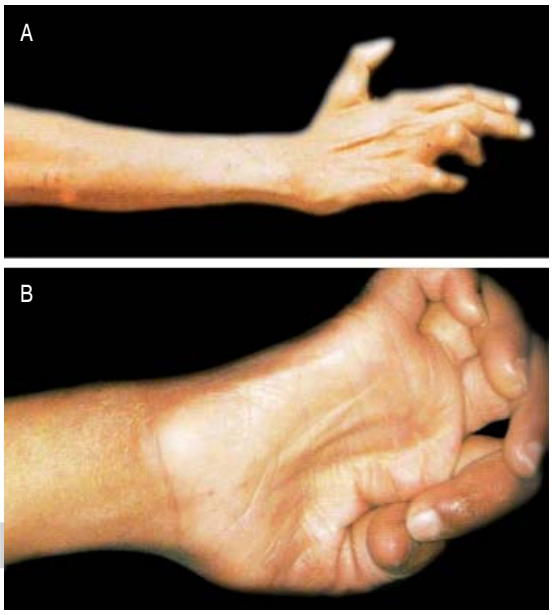

Figure 15. A: Tuberculoid leprosy, clawing of little and ring fingers, due to paralysis of the ulnar nerve. **B:** Claw-hand due to paralysis of both ulnar and median nerves. It is feasible to save these hands with early diagnosis, physiotherapy and tendon surgery. Courtesy of Prof. Dr. R. H. Thangaray, Bombay, India.

Figure 16.

Lepromatous leprosy evolution in the pre sulfone period. In Aug 1931 a 19 year old boy was described with infiltrated macules, elevated above skin and few facial lesions. On April 1933, lesions become larger and confluent, covering much of the skin surface diffusely infiltrated, and obstruction-destruction of nasal structures. (Courtesy of Dr. Chapman H. Binford, Armed Forces Institute of Pathology, Washington DC).

Figure 17. Lepromatous leprosy. Infiltrated skin is thickened, erythematous and shiny, with glossy plaques, soft and the slope toward the periphery. Eyebrows are lost. Multiple nodules on face are due to marked aggregation of the infiltrate, after starting on the ears, they appeared on the face, extremities, trunk and genitalia, leading to the "facies leonina" a lion-like appearance. Courtesy of Sasakawa Memorial Health Foundation Tokyo, Japan.

transcription factor NF-kB, to modulate a more extensive inflammatory response.[88]

TT-pole is mediated by INFγ and lymphotoxin-α, resulting in an intensive Mac phagocytic activity, plus TNF which promotes the production of $CD4^+$ in the well organized granuloma, and small amount of $CD8^+$ cells in the surrounding mantle.[89-91]

Bacterial containments and destruction is focused namely on granuloma formation, where Mac and different T-cells populations participate. These include a) $CD4^+$ cells recognizing antigenic peptides in the context of gene products encoded by MHC class II b) $CD8^+$ cells recognizing peptides in the context of MHC class I c) γ8 cells recognizing Ag-ligands independent of specialized presentation molecules-namely phospholigands and d) CD1 restricted T-cells recognizing glycolipids abundant in the mycobacterial cell wall presented by CDI molecules.[92]

LL is characterized by Th-2 response (IL-4 and IL-10) robust antibody complex formation occur but is not protective, cell mediated immunity is conspicuously absent. Lack of $CD4^+$ T cells, numerous $CD8^+$ cells and foamy *Mac* absence of granulomas and failure to restrain *M. leprae* growth.[93,94]

At least some of the CD8 T cells, γδ, and CD1 restricted cells, secrete perforin and granulysin, thereby directly killing mycobacteria within Mac. Most of this basic knowledge was obtained from laboratory experiments with mouse; several murine mutants with well defined immune deficiencies (knockout strains) are available. Furthermore, the

Figure 18.

Leprosy nasal deformities. These are due to invasion and destruction of the nasal septum, and septal perforation destroying the cartilage and the anterior nasal spine. Both lepromatous patients had a collapsed nose structure (arrows). **A:** Lepromatous multi nodular. **B:** Diffusely infiltrated leprosy of Lucio-Latapí. Courtesy of Prof. Dr. Fernando Latapí, Centro Dermatológico Pascua, México DF.

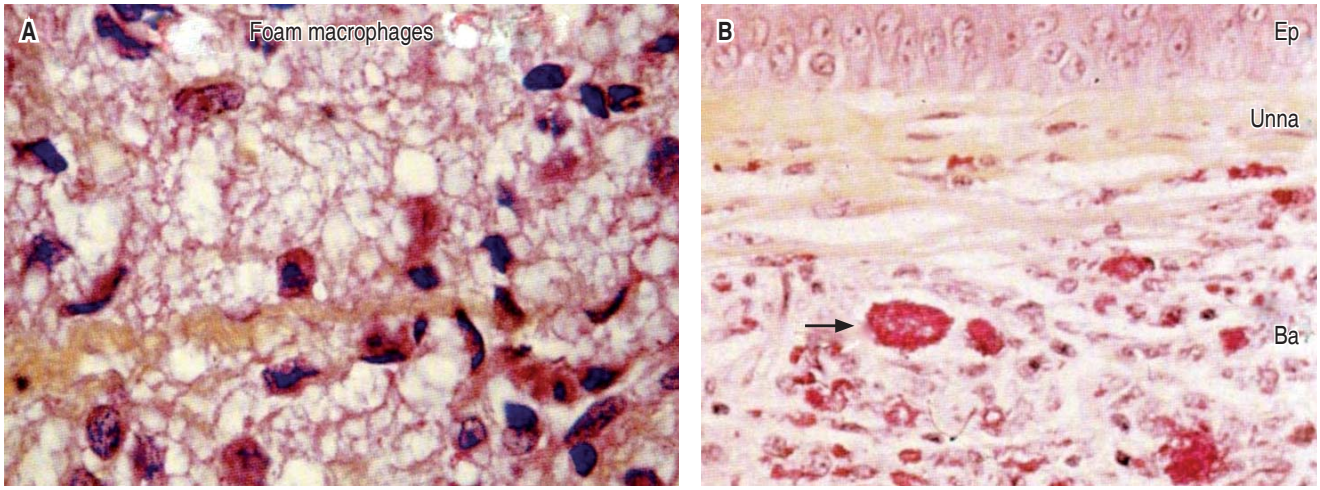

Figure 19. Lepromatous leprosy histopathology. The epidermis shows thinning and flattening of rete ridges, a granuloma free clear sub epidermal zone first described by P. G. Unna, a German dermatologist. Dermis has vacuolated foamy cells, containing multiple globi of leprosy bacilli, lymphocytes are scanty, no granuloma formation. Histological evaluation is essential for accurate classification of leprosy skin lesions, including the full depth dermis. Biopsy is usually taken from most active edge of lesion.

specific functions of IFNγ, IL-12, TNFα or $CD4^+$ cells is similar in mouse and humans, therefore, laboratory animals are critical to gain a clear insight of natural resistance and immunologic functions. For example, preference for cooler temperatures is clearly seen in the mouse model of infection, where growth occurs only in the cooler footpad.

LEPRA REACTIONS

Leprosy is by no means a static disease. Type-1 reversal reaction, occurs in 30% of borderline cases, characterized by acute inflammation and oedema in skin lesions *(figure 24)*, nerves of both, frequently recurrent and when untreated may lead to further nerve damage; It has been commonly recorded after starting chemotherapy or during puerperium.[95,96]

M. leprae Ags were localized to Schwann cells and *Mac,* with selective expression of major-histocompatibility complex (MHC) II on the surface of these cells and better Ag-presentation, which triggers $CD4^+$ lymphocyte-killing of infected cells[97] Immune-histochemical studies showed greater TNF-staining in the skin samples,

as compared with non-reactional controls.[98] There is a marked shift towards increased Th-1 response, with expression of pro-inflammatory cytokines IFN-γ, IL-12 and nitric acid synthasa, as well as some chemokines such as IL-8, a monocyte chemoattractant protein-1 and secreted (RANTES), however, the measured levels of circulating cytokines do not reflect the magnitude of skin observed lesions.[99] Medical treatment should be aimed at controlling acute inflammation, easing neuritic pain and reversing nerves damage.[100]

Type II (erythema nodosum = ENL) reaction is a systemic disorder, occurs in half the lepromatous cases and 10% of borderline lepromatous *(figure 25)*. The risk is greater with higher bacterial burden and massive skin infiltration, his onset is acute, but it may pass into a chronic, recurrent problem.[101] ENL generates fever, painful skin nerves, tender red papules or nodules in crops, distributed on the face and extensor surface of the limbs. It may also curse with panniculitis, bullous lesions and ulcerations, uveitis, arthritis and orchitis, finally leading to blindness and sterility.

In vitro, the peripheral mononuclear cells may secrete increased amounts of circulating plasma levels of TNF; this serious reaction has been treated with thalidomide, a drug inhibiting macrophage-TNF-production.[102] Treatment of leprosy reactions should be managed by an experienced specialist.

Figure 20. Diffuse leprosy of Lucio-Latapí. First described in 1852 by Lucio in Alvarado in Mexico. It is characterized by diffuse widespread infiltration of skin, without formation of nodules, loss of eyebrows and eyelashes and, widespread sensory loss. The skin contains numerous leprosy bacilli. The smooth skin infiltration may result in a youthful appearance called "pretty leprosy" (lepra bonita). Courtesy of Prof. Dr. Fernando Latapí, Centro Dermatológico Pascua, México DF.

DISCUSSION

Everything we have to know about *M. leprae*, a close relative of *M tuberculosis*, is encrypted in the genome. The complete genetic-DNA sequence of the TN=laboratory-strain pass aged in the armadillo was used to obtain the cosmid-basic-library,[103] then a comparative genomic-analysis with *M tuberculosis* H37Rv[104] was a powerful approach to uncover the biochemistry-physiology of *M. leprae*.[105]

M. leprae has a circular chromosome and no plasmids, containing 3, 268 203 base-pairs (bp) with an average content of 6+C 57.8%. comparative bio-informatic-genomic-sequences predicted the existence of 1, 605 genes, 49.5% of the *M. leprae* genome was occupied by protein-coding genes, while 27% of the sequence were inactive-pseudo genes reading frames with functional counterparts in the tubercle bacillus, the remaining 23.5% did not appear to be coding at all. The process by which a large-scale loss of gene-function arises has been termed reductive evolution (re), which results in decreased fitness and little genetic variability, and as a consequence of its highly specialized macrophage niche, the only organism with which *M. leprae* can exchange DNA is the human host. Leprosy bacilli has the lowest G+C content of all mycobacteria, and is noteworthy that the genomes of organisms which have undergone re are generally richer in A+T.[106,107] If one makes the assumption that genomes of *M. leprae* and *M tuberculosis* were once topologically equivalent and roughly 4.4 Mb in size, then extensive downsizing must have occurred during millenary evolution of leprosy infection, since genome is < 75% the size of *M. tuberculosis*. On proteomics analysis: When pair wise comparison of the gene and protein sts of the leprosy and tubercle cacilli were performed, 1,433 proteins were found to be common to both pathogens. After removal of proteins shared with all other prokaryotes (except Actinomycetes) and eukaryotes the sample contains only 333 proteins. Since these pathogenic mycobacteria occupy similar niches in the human body, where they encounter the same physiological stresses and immune responses, it is conceivable that the producs of some of these genes may affect highly specialized functions that could be essential for intracellular growth of mycobacteria. If this was the case, corresponding proteins or enzymes might represent novel drug targets. The 333 candidates identified by comparative genomics can be subdivided into those proteins that are confined to the genus *Mycobacterium* (there are 219 of these), and a second group of 114 polypeptides that also occur in *Streptomyces* or *Corynebacteria* spp, related members of

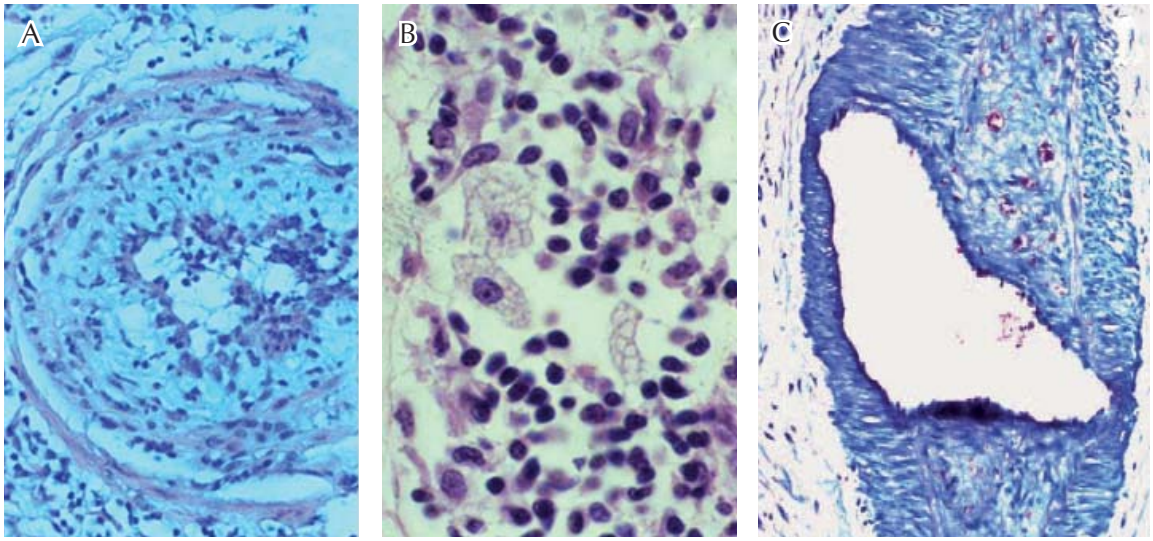

Figure 21. Skin biopsy of Lucio´s phenomenon. **A:** Endothelial proliferation and vasculitis. **B:** Panniculitis with presence of vacuolated macrophages and lymphocytes. **C:** Vascular invasion with a high burden of red stained Mycobacterium Fite-Faraco stain 100 x. The molecular study of the 16 S rRNA genes showed a 19-bp sequence TAATACTTAAACCTATTAA known previously for *M. leprae*.

Figure 22. A 50 year old-homeless man from Sinaloa, Mexico. He came to the Hospital with multiple, tender, well demarcated purpuric lesions with scabs on all four extremities. There were loss of eyebrow hairs and hypoesthesia. No pulse from dorsalis pedis was felt, and hepato megaly. Hemoglobin 10.7 g/dL; creatinine-kinase 4334 U/L. High fever of 40 ^{o}C, skin biopsy showed vasculitis and necrosis. Diagnosis was: Lucio´s phenomenon, vasculitis-panniculitis, and massive burden of AFB.

the Actinomycetales kingdom. It is reasonable to assume that those restricted to mycobacteria may play an even more specialized role.

The systematic study of the complete set of genetic material in the mycobacterial cell, through deoxyribonucleic acid (DNA) sequencing and bio informatic analysis, offering vast future potential in terms of drug target and antigen discovery to enhance development of new antibacterial agents, diagnostic tools and vaccines. For a relative modest, single investment the entire complement of genes present in *M. leprae* and *M tuberculosis* was defined and their sequences compared versus other microbes, mice and men.[108]

Leprosy is an ancient and widely misunderstood disease.[109] Early diagnosis is often missed, though it need not be if physicians will remember that skin lesions plus sensory loss represent leprosy- case, until proven otherwise.[110] Management is not difficult. All skin lesions are noted and described in detail, and usually photographed. A complete sensory examination is done, emphasizing evaluation of light, touch and pain perceptions, and temperature discrimination. Motor strength should be evaluated in hands and feet, and nerves palpated: the great auricular, ulnar, median, radial, and common peroneal.[111] It should be followed by skin scraping, by pinching skin to diminish blood flow, wiping area with an alcohol sponge, and making a small, slit with sterile razor blade or scalpel; the slit edges are pressed gently, and the drop material is smeared on a microscopic slide to be stained by the Fite-method for acid-fast bacilli.[112] Good medical education, an open mind and research-interest from physicians and health-team-worker will help in the best interest of the leprosy patient and the affected family, to be treated near his home on a long-term basis, although an initial evaluation by an experienced dermatologist is advisable for lepromatous and borderline cases.

Host-genetic factors have a partial effect on both development of leprosy and pattern (type) of disease. Whole human genome screening has identified susceptibility loci in chromosome 10 p 13 close to gene for mannose-receptor C-1 a phagocytic receptor on macrophages, and on chromosome-6 within MHC. Linkage has been showing with HLA class II genes in India and for TNF in Brazil. Polymorphism in promoters for genes both TNF and IL-10 are associated with development of LL-leprosy. The HLA locus also affects the disease-pattern: HLA DR2 and DR3 are associated to TT-disease; and HLA DQ1 is linked to the LL-pattern. A mutation in the toll-like-receptor TLR-2 is more common in LL-patients, which indicates the TLR-2 signal contributes to susceptibility. Polymorphism in the NRAMPI gene has been associated with LL-linked in practice to cell-mediated-immunity to *M. leprae*.[38] (See also: Misch EA, Berrington WR, Vary Jr JC, Hawn TR. Leprosy and the Human Genome. Microbiol Mol Biol Rev 2010; 74: 589-620).

If you work-hard to understand leprosy pathogenesis, you will learn bacteriology, immunology, molecular biology, human genetics, histopathology an good management of chronic patients then, you will be in a better position to advise medical studies, other physicians

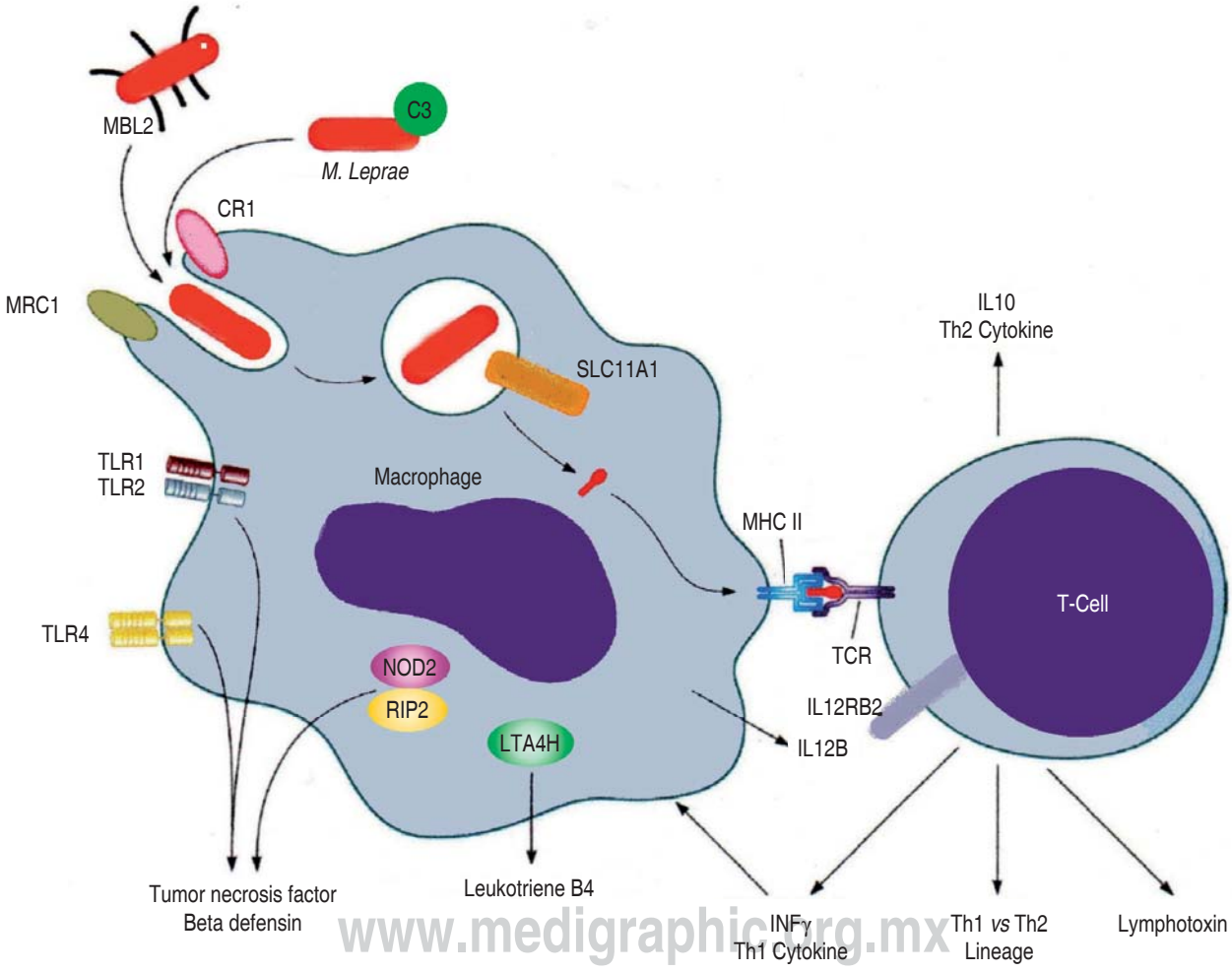

Figure 23. Cellular immunology of leprosy. *M. leprae* is initially recognized by Toll-like innate receptors (TLR-2), a family of highly conserved trans membrane proteins that orchestrate recognition of 19-kDa protein and the lipo peptides of the cell wall. On monocytes PGL-1 mediates phagocytosis via the complement receptors CR1 and CR3, and the mannose receptor (MRC1) which binds mannose and other carbohydrates moieties present on mycobacteria. The Mac principal function is phagocytosis of antigens (Ag) and presentation of processed Ag to the T-cell receptor (TCR), with production of several cytokines and pro inflammatory mediators such as tumor necrosis factor (TNF-α). MHC II = major histo compatibility complex class II; IL-12 RB2 = receptor of interleukin-12.

and public health workers, regarding the importance of epidemiologic-research, and the solution of clinical and therapeutic complicated problems, the long terms results surely will be rewarding. *M. leprae* is a unique intracellular parasite, and leprosy is a fascinating disease. To expend your time in the study of leprosy in a good-way to learn Medicine, and much of the social-economic problems. Link to extreme poverty.[114]

The overwhelming majority of people are apparently able to resist this infection. However, there are no test to detect early exposure or to diagnose-infection in advance of clinical symptoms. Even the route of transmission is uncler.[36] Untreated lepromatous patients expel large numbers of bacilli from their nasal discharges, which conceivable invade new susceptible host via the respiratory route[114] or through cuts and skin abrasion.[1,3] In permissive laboratory animals (mice, armadillo) *M. leprae* has been show to multiply very slowly, with an estimated generation time of ~13 days. In humans, the incubation period ranges from less than a year to decades, with an average from 3 to 5 years,[115] so linking a new case with a contact are impossible.

It is no wonder that leprosy epidemiology is so poorly understood.[116,117] However, population-studies have established some risk factors, the strongest of which is genetic relatedness and close contact with lepromatous patients, low educational level,-and poverty, lack of BCG-vaccination, and in most publicized-studies there was a 2 to 1 ratio of affected males to females.

Another small percentage, however, go on to develop indeterminate leprosy as hypo pigmented macula with or without sensory loss. In as much as this lesion is inconspicuous and asymptomatic this stage probably is missed in most patients.[59] It may, heal spontaneously, but it diagnosed it should always be treated. Those with the greatest relative degree of resistance produce IFN-γ and develop tuberculoid (TT) while those with the least resistant (anergic) produce IL-4 and IL-10 and develop lepromatous, disseminated disease. The amazing complement, composition, and configuration of the tissue granulomas, where immunity response to the pathogen results from intricate interaction of various T and B lymphocytes subsets, macrophages and other cell types, and their release of modulating cytokines in response to *M. leprae*

Figure 24. Tuberculoid-borderline (BT) leprosy with facial lesion. Six months after chemotherapy, the patient showed signs of acute inflammation: pain, tenderness, erythema and oedema, with sharp pain of facial nerve and partial palsy. Type I reverse reaction was diagnosed, hypersensitivity type IV and increase in cell mediated immunity and a shift toward TT-pole.

Figure 25. Borderline lepromatous (BL). One year after treatment, a sudden appearance of pink colored crops, tender nodules and plaques, evanescent, some became vesicular, with fever and malaise, signs of iridicyclitis and bone pain. The trigeminal right nerve branches showed tenderness without loss of function. Diagnosis erythema nodosum leprosum (ENL) type 2 reaction, a good example of humoral hypersensitivity due to precipitation of antigen-antibody complex in skin and vascular tissues. The patient was successfully treated with thalidomide.

antigens.[118-121] The polar forms as presented in this paper, appears to be classical manifestation of TH1 and TH2 cell mediated immunity, with a poorly understood unstable "borderline" response in between.[44,75]

The opportunity remains for young researches to investigate in deep immune regulation and other susceptibility factors of the human host in a unique and fascinating, nonfatal human infectious disease.[87] At a minimum, to eradicate leprosy will be: The maintenance of teaching and expertise in leprosy in all countries, political and financial support to promote training of primary-health staff in leprosy diagnosis and treatment, and early referral of patients with reactive-leprosy or complications. One positive outcome of multidrug therapy has been the wide recognition that Hanseniasis is **curable**, and may help to reduce impairment of nerves function and disability.[3]

The Kerr-Pontes study in Brazil, showed the very-close link between the high level of inequality in endemic populations, with overcrowded households, and excessive population grow so as to facilitate aerosol-transmission of leprosy, hence extreme-poverty and social inequality produce unmet social need, poor education and unemployment, and leprosy should perhaps be seen as an affliction of an **unhealthy society.**[122,123] Hanseniasis never will be eradicated unles an effective poverty-reduction programmed is operating in the entire marginalized slum. The aim should be to improve the standard of living-education and the income of the families, as well as the index of human development.[124] Leprosy should now be included in the big portfolio of poverty-associated diseases.[125,126]

In a trial in Malawi, BCG-vaccination induced 50% protective efficacy against tuberculoid and lepromatous forms, are immunization with BCG increased the protective effect by a further 50% against tuberculosis and leprosy. Therefore, widespread immunization of children is certainly a cheap and practical way to leprosy-control.[127] The future development of tools to recognize early infection with *M. leprae* before disease manifestation surely will help to target the vaccination and chemoprophylaxis of the household contacts, with full involvement of the educated patient, the family and the community, in the regeneration of the poverty-broken social networks.[54]

ACKNOWLEDGMENTS

This paper is dedicated in memoriam to my mentor in Dermatology Professor Dr. Fernando Latapí, he was an outstanding Teacher, hard work Leprologist and exemplary investigator. I also want to express my gratitude to Professor Amado Saul and the staff of the Centro Dermatológico Pascua, México DF, and to Professor D. A. Mitchinson, Director of the Mycobacteria Research Unit, the Royal Postgraduate Medical School, University of London, under his wis direction, I started my early work of experimental mycobacteriology, supported by the British-Medical Council-Scholarship which provided a generous three year postgraduate training in Medical Bacteriology at London Cardiff and Glasgow. Mr. Miguel Ivan Olvera Macias helped elaboration of texts and figures with much dedication and outstanding enthusiasm.

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