MINI REVIEW

Leprosy pathogenetic background: a review and lessons from other mycobacterial diseases

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Abstract Leprosy is a disease caused by Mycobacterium leprae that initially affects the peripheral nervous system with patients exhibiting contrasting clinical, immunological, and pathological manifestations despite minimal genetic variation among bacilli isolates. Its clinical manifestations are related to M. leprae survival, innate and acquired immune responses, and interactions between host and bacterial proteins, preventing their invasion and infection, or promoting their development and pathogenesis. The complex molecular interactions in affected individuals influenced by the pathogenetic background will be explored in this review. However, the great genetic diversity imposes difficulty for understanding disease development, and it is likely that many factors and metabolic pathways regulating the immense and contrasting symptomatology will yet be revealed. Four pathways may play a central role in leprosy, including the TLR/LIR-7, VDR, TNF-α, and TGF-β1 for which a large amount of gene polymorphisms have been described that could potentially affect the clinical outcome. Cross-talk pathways may significantly change the course of the disease, depending on the specific disequilibrium of genic homeostasis, which is highly dependent on the environment, antigens that are presented to the host cell, and specific polymorphisms that interact with other genes, external factors, and pathogen survival, culminating in leprosy occurrence. Currently, the microarray-based genomic survey of gene polymorphisms, multiple gene expression analyses, and proteomic technologies, such as mass spectrometry and phage display applied in the discovery of antigens, represent a great potential for evaluating individual responses of leprosy patients and contacts to predict the outcome and progression of the disease. At present, none of the genes is good prognostic marker; however, in the near future we may use multiple targets to predict infection and leprosy development.

Keywords Leprosy · Pathogenetic background · Molecular interactions · TLRs · VDR · TGF · TNF · Gene polymorphisms

Introduction

Microbes and host defense systems have developed intricate genetic pathways and the complex interactions between them are termed pathogenesis. The immune system has evolved primarily to combat infection, but in leprosy, the immune response is responsible for the broad clinical spectrum of the disease [83] and, similar to an autoimmune disease, seems to trigger further complications such as nerve damage [71]. Therefore, the search for a better understanding of the molecular mechanisms involved in leprosy onset and progress is the most important challenge to be met to control the disease and reduce the rate and severity of disabilities.

The disease displays a clinical spectrum that correlates with the level of the immune response to the pathogen [83].
Patients with the tuberculoid form are relatively resistant to the pathogen; the infection is localized and lesions are characterized by the expression of type 1 cytokines characteristic of cell-mediated immunity [121]. In contrast, in the most severe, or lepromatous form, the specific cell immune response against *Mycobacterium leprae* is absent, with diffuse dermal lesions characterized by poorly differentiated young macrophages with a heavy load of bacilli and a small number of T-cells predominantly of the Th2 type [121]. In the spectrum of borderline leprosy, there are varying degrees of cell-mediated immune response characterized by patients with a low response to the bacillus [82].

Various genes and genomic regions of the human genome have been linked to or associated with susceptibility to leprosy or a particular clinical form of leprosy [1, 3, 25, 38, 58, 59, 77, 113]. Many of these associations, however, may not be reproducible in different populations, probably due to the many confounding factors such as lack of population stratification, small sample size, mixed ethnic factors, and many others [25]. These results suggest that susceptibility to leprosy is multigenic, with a high degree of heterogeneity among different populations studied. In this sense, a complex genetic model of susceptibility to leprosy could make linkage detection difficult [1].

Survival of the *M. leprae*, innate and acquired immune responses, and interactions among host proteins and bacterial antigens preventing invasion and infection by the bacilli have been associated with many genetic factors, and the high complexity of all these molecular events may explain the wide spectrum of clinical forms of leprosy. We will explore some of the molecular and immunological mechanisms that may be of great importance during the development of infection and pathogenesis.

### Genetic relationship of *M. leprae* and its human host

An extensive review has been presented elsewhere [24] reporting specific genes and their polymorphisms that may be involved in genetic susceptibility to mycobacterial diseases in humans.

Elucidation of the genetic control of susceptibility to leprosy is expected to provide new and more effective tools for prevention and control of the disease [96]. Both the development of the disease upon exposure to *M. leprae* and the pattern of clinical manifestations displayed by leprosy patients are highly dependent on human genes that may be linked to several chromosome regions, such as chromosome 6q25 [59], 10p13 [102], 17q11-q21 [38], 20p12 [113]. A genome-wide scan for leprosy susceptibility has provided data with evidence of linkage to nine regions on chromosomes 6, 9, 11, 12, 13, 15, 16, 17, and 20 [58], corroborating previous reports and providing novel chromosome regions, demonstrating the high complexity of this disease.

Another review has been presented elsewhere [98] reporting specific genes that may be involved in the innate and acquired immune responses in leprosy. We have chosen some of those genes to explore their potential interactions and their involvement in the development of the disease. The genes studied were: Parkin and the Parkin co-regulated gene (PARK2/PACRG), toll-like receptors 1 and 2 (TLR2/1), solute carrier protein 11A1, iron transporter (SLC11A1), tumor necrosis factor alpha (TNFA), interferon gamma (INFG), interferon-gamma receptor (INFGR), interleukin-10 (IL-10), human leukocyte antigens (HLA class I and II), transporter associated with antigen processing (TAP), interleukin 12 (IL12) and its receptor (IL12RB1), heat shock protein 70 kDa protein 1A (HSPA1A), and vitamin D receptor (VDR).

### Genetics of host defense: resistance versus susceptibility

One of the most recent contributions to the genetics of susceptibility to leprosy has been reported elsewhere [59, 61, 96], in which variants of the shared promoter region of the PARK2 and PCARG genes have been identified as major risk factors for leprosy susceptibility.

PARK2 codes for Parkin and E3 ubiquitin–protein ligase that is involved in the delivery of polyubiquitinated proteins to the proteasome complex [101] and may be involved in other neurodegenerative diseases characterized by involvement of abnormal protein ubiquitination. The function of PACRG is unknown, but it is linked to the ubiquitin–proteasome system [117]. Both genes are expressed in different tissues, including Schwann cells and monocyte-derived macrophages. Skin macrophages and Schwann cells are the primary host cells for *M. leprae*, which suggests that ubiquitin-mediated proteolysis, a biochemical pathway that has so far received little attention in the study of leprosy pathogenesis, plays an important role in the control of *M. leprae* infection [2, 96, 97]. Ubiquitination is a general regulatory process for proteins, and several E3 ubiquitin ligases have been recently shown to interact with proteins of the immune response, including those involved in the TLR signaling pathway [18] and T-cell anergy induction [39].

One possible link between *M. leprae*-triggered host responses and Parkin activity driven by promoter alleles is the cellular oxidative stress pathway. Reactive oxygen intermediates (ROI) and NO (nitric oxide) can be produced by both Schwann cells and inflammatory macrophages following neural damage or exposure to inflammatory cytokines. Production of ROI or NO induces apoptosis of Schwann cells, which is detrimental to *M. leprae* survival,
although involved in the pathogenesis of nerve damage [96]. It has also been hypothesized that Parkin down-regulation may interfere with intracellular phagosome trafficking or in the regulation of protein degradation involved in the immune response to *M. leprae*.

Single nucleotide polymorphisms (SNPs) located in the shared promoter region of PARK2 and PACRG have been identified as major risk factors for leprosy susceptibility in two ethnically distinct populations (Vietnamese and Brazilian). A multivariate analysis has detected two SNPs (PARK_e01, position –697; PARK2_e01, position –2599, rs1333955 and rs1040079, respectively) that could explain the entire association of all tested SNPs with leprosy [61]. Nevertheless, it is not known to what extent the linkage disequilibrium pattern and the SNPs are maintained across different ethnicities. Even so, negative findings with SNPs of both PARK2 and PACRG genes in an Indian population were not able to include the variants as universal risk factors for leprosy [54], possibly because of the smaller number of SNPs used [97]. Therefore, replication studies of different ethnic groups of leprosy patients are an efficient way to narrow down, by genetic means, the location of the true functional variants [97]. However, noninvolvement of the major risk SNPs in the regulatory region of PARK2 and PACRG loci with leprosy susceptibility in the Indian population may also indicate a differential effect of these SNPs in regulating genetic susceptibility to leprosy in different populations [54].

Single nucleotide polymorphisms within genes coding for TNFA, lymphotoxin-α, and IL-10 have been associated with several infectious diseases. The minor haplotype –3575A/–2849G/–2763C in IL-10 promoter has been defined as a marker of resistance to leprosy and its severity in the Brazilian population [93]. Conversely, the IL-10 haplotype –3575T/–2849G/–2763C/–1082A/–819T/–592A was associated with the risk of development of a severe form of leprosy in contrast to the minor risk in the Brazilian population [53]. This suggests that there is an important involvement of the IL-10 locus in the outcome of leprosy, but apparently, there may be other genes interacting with this gene, as evidenced by the differential ethnic background of the two populations investigated.

Linkage disequilibrium mapping has also identified the low-producing lymphotoxin-α (LTA)+80 A allele associated with leprosy risk and possibly with early onset of the disease [3]. However, this risk factor has not shown a significant role in leprosy susceptibility in individuals over the age of 25 years. It is important to emphasize that this polymorphism was not significantly associated with leprosy in the Brazilian population [3], reinforcing the ethnic problems when polymorphisms are considered.

Another important gene that has been directly associated with infectious disease susceptibility is the iron transporter natural resistance-associated macrophage protein 1 (Nramp1), coded by the SLC11A1 gene, which confers resistance to the growth of a variety of intracellular pathogens, including mycobacteria. Iron is an important element for the growth of microorganisms as well as for the defense of the host by serving as a catalyst for the generation of free radicals via the Fenton/Haber–Weiss reactions [124].

It has been reported that susceptibility may be influenced by polymorphism in the promoter region of the SLC11A1 [12]. Segregation analysis through the LOD score has shown that the SLC11A1 gene haplotypes were associated with susceptibility to tuberculosis [31] and with positive Mitsuda test results [4]. However, other investigations have found no correlation between SLC11A1 genotypes and the Mitsuda response [25, 91].

The lack of association between the SLC11A1 genotypes and Mitsuda tests have lead investigators [25] to consider them as two independent events that may interact with each other. The SCL11A1 promoter genotypes 22 and 23 were found to be unfavorable genotypes when in combination with the negative Mitsuda response, showing an approximately 7-fold higher chance of developing leprosy. The hypothesis that the differential SLC11A1 gene expression is related to *M. leprae* survival [12] instead of microbial proliferation [9] is supported by a study [25] that demonstrated no association between SLC11A1 promoter genotypes and the bacilloscopic index. Hence, it has been proposed that the low expression of the solute carrier protein, conditioned by allele 2, may function in microbial persistence and survival [25], probably by inefficiently transporting iron ions, as was also shown for the transferrin receptor [124]. However, it will only affect the outbreak of clinical symptoms if in association with other immunological and genetic factors, as demonstrated by the interaction with a negative Mitsuda test [25]. This hypothesis is corroborated by two recent investigations that have provided evidence that the Mitsuda reaction is controlled by a recessive major gene [76]. Additionally, linkage was found at chromosomal regions 2q35 (at the SLC11A1 locus) and 17q21–25, which have been previously linked to mycobacterial infection and other granulomatous diseases [77], confirming that the SLC11A1 gene is not associated with the positive Mitsuda test.

Because iron is not freely available in the host, pathogens must actively compete for this metal to establish an infection, but they must also carefully control iron acquisition as excess free iron can be extremely toxic. Recent studies have demonstrated that failure to assemble the iron acquisition machinery or to repress iron uptake has deleterious effects for *Mycobacterium tuberculosis* [86]. Therefore, there is compelling evidence that iron acquisition is critical.
for *Mycobacterium* survival and replication in the infected host. However, given the potential danger involved in bearing excess iron, each aspect of the metabolism of this metal is tightly regulated [87]. This is a very important mechanism considering that a high iron concentration in the host, found in some SLC11A1 genotypes, may be linked to the oxidative killing mechanisms encountered during macrophage infection.

This hypothesis is also supported by experiments with *Salmonella typhimurium*, in which, following bacterial invasion, macrophages exhibited a significantly increased iron export mediated by the induction of Fpn1 expression (ferroportin 1, the only mechanism for cellular iron export identified so far, is carried out by the transmembrane protein). This resulted in a depletion of cytosolic and ferritin-associated iron pools, and thus, in a reduced availability of this essential nutrient for invading microbes [66]. These results complement a recent report demonstrating that overexpression of wild type, but not of mutant Fpn1, reduces the intracellular growth of *S. typhimurium* in J774 macrophages [17].

Further evidence has also been noted in hereditary hemochromatosis, an inherited disease in which most cells become overloaded with Fe. However, hereditary hemochromatosis macrophages have lower than normal levels of intracellular Fe. This is associated with reduced growth of *M. tuberculosis* since its growth within macrophages from subjects with hereditary hemochromatosis acquired less Fe compared to healthy controls [69].

Slc11a1 in iron turnover has been examined in macrophages transfected with SLC11A1 (Gly169) (wild type) or SLC11A1 (Asp169) (mutant = functional null) alleles following direct acquisition of transferrin (Tf)-bound iron via the Tf receptor (TfR). In wild type macrophages, most iron was in the soluble (60%) rather than insoluble (12%) fraction, with 28% ferritin (Ft)-bound. With activation, the soluble component increased to 82% at the expense of Ft-bound iron (<5%). In mutant macrophages, 40–50% of iron was in insoluble form, 50–60% was soluble, and <5% was Ft-bound. These data suggest that iron acquired by phagocytosis and degradation is retained within the phagosomal compartment in wild type macrophages, and that NO triggers iron release by direct secretion of phagosomal contents rather than via the cytoplasm [65]. This evidence explains the possible link between SLC11A1 gene polymorphism and mycobacteria survival, which is favored by the soluble iron in phagosomes.

In the host–microbe interaction, little transferrin-associated iron is available within the cell to stimulate the growth of the surviving microorganisms. At the same time, however, the host cell finds itself starved of an essential supply of biologically active iron to mediate the generation of free radicals necessary to eliminate the intracellular pathogens. It has been shown that infection with *M. avium* differentially regulates mRNA expression of the proteins associated with iron transport in murine peritoneal macrophages. Both Nramp1 and Nramp2 mRNA levels increase following infection, while the expression of transferrin receptor mRNA decreases [124].

Interestingly, both *M. avium* infection and treatment of cells with IFN-γ, independently and in combination, resulted in a decrease in TIR mRNA expression and stability in macrophages [37, 68, 114, 124].

Therefore, the proposed hypothesis is that the low expression of Slc11a1, conditioned by SLC11A1 allele 2, may function in microbial persistence and survival [25], probably by inefficiently transporting iron ions, as also shown for the TIR [124], and that it will only affect the outbreak of clinical symptoms if in association with other immunological and genetic factors, as demonstrated by the interaction with a negative Mitsuda test [25], and possibly with genes involved in the immune response, such as the INFG and its receptor, culminating with pathogen entrance into the body.

Interferon gamma (IFN-γ) is believed to be crucial for host defense against many infections. In leprosy, the IFN-γ is present in the granuloma lesions of the tuberculoid pole [118] and is the main cytokine of the Th1 response, because it is responsible for inducing the production of free radicals in the macrophage [22]. Gene polymorphisms in the interferon-gamma (IFNG) and the interferon-gamma receptor (IFNGR) are good candidates for susceptibility genes because they display dominant and recessive mutations, both of which confer the genetic vulnerability underlying most mycobacterial diseases [40].

To test the hypothesis that a polymorphism in IFNG was also associated with susceptibility to tuberculosis, two independent studies were conducted [90]. In a case–control study of 313 tuberculosis cases, a significant association was noted between a polymorphism (+874A-T) in IFNG and tuberculosis in a South African population. This finding was replicated in a family-based study in which the transmission disequilibrium test was used in 131 families. The transcription factor NF-kappa-B (NFKB1) bound preferentially to the +874T allele, which is overrepresented in controls, suggesting that genetically determined variability in IFNG and expression might be important for the development of tuberculosis, and may also be associated with leprosy, a hypothesis that should be investigated [90].

The genetic vulnerability to *Mycobacteria* sp may be further complicated by mutant alleles that encode cell surface IFN-γ receptors that lack the intra-cytoplasmatic domain, which through a combination of impaired recycling, abrogated signaling, and normal binding to IFN-γ exert a dominant negative effect [40].
Since the mutant chains are unable to transduce any IFN-γ-induced signaling following interaction with its ligand, and this deficiency is able to form paucibacillary and mature granulomas in response to BCG [40], it is possible that the Mitsuda response may also be partially regulated by IFNGR polymorphisms; this hypothesis, however, has not yet been investigated.

It is clear that mutations in the IFNG and IFNGR genes may predispose to leprosy, but they cannot account for most of the cases in the population. Other genes may also regulate the IFN-γ pathway, and may be involved in susceptibility to the disease as well. In fact, the complexity of this disease requires two or more molecular mechanisms, including genomic alterations, acting in a cascade of events that could lead to the final clinical outcome.

Thus, another important molecule associated with IFNG regulation is the VDR, traditionally described as responsible for calcium regulation and bone metabolism mediated by 1,25-dihydroxyvitamin D3 (VD). Recently, it was reported that TLR2 activation leads to the use of vitamin D3 as a mechanism to combat M. tuberculosis [50, 51]. TLR activation of human macrophages up-regulated the expression of the vitamin D receptor and the vitamin D-1 hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular M. tuberculosis [50].

It has also been demonstrated elsewhere that blocking the vitamin D receptor, inhibiting CYP27B1, or limiting 25D3 availability prevented TGF-beta 1 from inducing cathelicidin, CD14, or TLR2 in human keratinocytes, while CYP27B1-deficient mice failed to increase CD14 expression following wounding. The functional consequence of these observations was confirmed by demonstrating that 1,25D3 enabled keratinocytes to recognize microbial components through TLR2 and respond by cathelicidin production. Therefore, the role of vitamin D3 in innate immunity has been demonstrated, enabling keratinocytes to recognize and respond to microbes and to protect wounds against infection [95].

Toll-like receptor down-regulation and CD14 up-regulation were substantially inhibited by the VDR antagonist ZK 159222, indicating that the immunomodulatory effect of 1,25(OH)2D3 on innate immunity receptors requires VDR transcription factor activation. These data provide strong evidence that 1,25(OH)2D3 primes monocytes to respond less effectively to bacterial cell wall components in a VDR-dependent mechanism, most likely due to decreased levels of TLR2 and TLR4 [92].

Intracellular pathogens are recognized by the innate immune system. TLRs represent one mechanism by which the innate immune system recognizes biochemical patterns displayed by infectious agents [45]. There are two different mechanisms by which TLR activation can contribute to host defense. First, activation of TLRs can directly mediate responses by regulating phagocytosis and triggering antimicrobial activity [46, 49]. Second, activation of TLRs can trigger the release of cytokines and the differentiation of immature dendritic cells, enabling the innate immune system to instruct the adaptive response [46].

The intricate aspects among VD, TLR, and other signaling pathways, including polymorphisms of the intermediate genes, as demonstrated in the convergence of pathways shown in Fig. 1, seem to work in synergism/antagonism or in redundancy to maintain cellular homeostasis or may be due to genetic disequilibrium in one or more of these pathways. This explains the lack of agreement among several reports associating gene polymorphisms with leprosy susceptibility or resistance.

Ethnic background may also play an important role in frequency of different gene polymorphisms, and the balance among these pathways may lead to infection, disease development, progression, or immunity.

**Infection and disease progression: interactions of genetic and immunological aspects**

*Mycobacterium leprae* probably enters the body by way of the nose and then spreads to the skin and nerves via the circulation [98]. The host’s immunological response determines the clinical forms that develop. Leprosy patients show a spectrum of five clinical forms with two poles, tuberculoid and lepromatous. Tuberculoid disease is the result of high cell-mediated immunity with a largely Th1 type immune response, and lepromatous leprosy is characterized by low cell-mediated immunity with a humoral Th2 response [62].

In tuberculoid leprosy, interferon IFN-γ, IL-2, and lymphotoxin-α are secreted in lesions and these result in intense phagocytic activity [104]. Macrophages under the influence of cytokines, particularly TNF-α together with lymphocytes, form granulomas [6]. CD4+ cells are found mainly within the granuloma and CD8+ cells in the mantle area surrounding it [63]. T-cells in tuberculoid granulomas produce the antimicrobial protein granulysin [67]. Lepromatous disease is characterized by poor granuloma formation. MessengerRNA production is predominantly for cytokines IL-4, IL-5, and IL-10 [118]. IL-4 has been shown to down-regulate TLR2 on monocytes [15], and IL-10 will suppress production of IL-12 [48]. This is associated with a preponderance of CD8+ lymphocytes in lepromatous lesions.

There is strong genetic epidemiological evidence that leprosy subtypes have been linked to the HLA region in several populations, as reviewed elsewhere [30, 60]. A segregation study has demonstrated that the HLA/TNF region is linked to the clinical spectrum of leprosy, but none of the
HLA/TNF haplotypes of the candidate region showed significant evidence for linkage with immune responsiveness as measured by the lepromin skin test. The non-significant linkage results with leprosy subtypes obtained for the TNF-308 (G/A) promoter and the TNFR polymorphisms argue against a major role of the TNF alleles in the pathogenetic process. Therefore, the bias segregation of this region into offspring with different clinical forms of leprosy within the same family is mainly caused by genes in the HLA region, most likely HLA class II genes [60].

The latter results are corroborated by the evidence that HLA-DRB1*10 is associated with susceptibility to leprosy, and HLA-DRB1*04 is associated with resistance to leprosy, both in Brazilian and Vietnamese populations [114], suggesting that these alleles may play an important role in the activation of cellular immune response against *M. leprae*. This is further evidenced by the fact that specific peptide/HLA class II complexes can also determine the quality of the immune response by selectively activating the regulatory (suppressor) T-cells [30].

However, the HLA class II complex effects seem insufficient to explain the differential immune response of the disease’s clinical forms, and other genes such as TLRs, VDR, TGFB, and many others, may also affect both innate and adaptive immune responses.

Toll-like receptors activation triggers the release of cytokines and the induction of co-stimulatory molecules that can influence the nature of adaptive T- or B-cell response. A variety of microbial pathogen-associated molecular patterns (PAMPs) is able to induce cytokine release and dendritic cell maturation: LPS through TLR4, CpG through TLR9, and bacterial lipopeptides through TLR2 [41, 110]. The stimulation of specific TLRs results in the release of IL-10 or IL-12, leading to skewing of the T-cell response toward Th1 or Th2 cytokines [73]. Thus, TLR2-mediated signals seem to induce preferentially a Th2 profile, whereas TLR4 activation mainly leads to a Th1 response [78].

The highly conserved TLRs on the surface of monocytes and macrophages recognize mycobacterial lipoproteins [15], in particular, TLR2 homodimers [7, 15] and TLR2-TLR1 heterodimers [5, 108]. TLR2 and TLR1 were more

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strongly expressed in lesions from the localized tuberculoid form as compared to the disseminated lepromatous form of the disease [45]. The TLR2/1 heterodimer leads to monocyte differentiation into macrophages and dendritic cells in M. leprae [45, 46]. The latter presents antigens and causes the activation of naive T-cells by IL-12 secretion [21]. The IL-12βR2 portion of the IL-12 receptor is expressed more on Th1 lymphocytes, preferentially shifting the immune response further towards a Th1 response. In a Moroccan study [80], two promoter polymorphisms in IL-12RB1 have been found to be in strong linkage disequilibrium with pulmonary tuberculosis, indicating that there is a gradation of genetic defects predisposing to severe infections with low virulence mycobacteria [24].

Toll-like receptor stimulation also activates the nuclear transcription factor NF-κB, which modulates the transcription of many immune response genes [109]. A mutation substituting arginine with tryptophan at residue 677 in one of the conserved regions of TLR-2 was shown to have a role in susceptibility to lepromatous, but not tuberculous leprosy [42]. Later, this mutation proved to abolish intracellular signaling and activation of NF-κB after exposure of different cell types to M. leprae and M. tuberculosis [14].

Toll-like receptor activation of human macrophages also up-regulated expression of the vitamin D receptor and the vitamin D-1-hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular M. tuberculosis [49, 50].

Vitamin D receptors were identified on activated T and B lymphocytes and monocytes and this sparked an interest in vitamin D as an immune system regulator. Regulation is mediated via the active form of vitamin D, 1,25-(OH)2D3, which carries out its immunoregulatory role by binding to the VDR [84]. Vitamin D maintains the balance between Th1 and Th2 cells by negatively regulating Th1 cells or by positively regulating Th2 cells [16]. It has been suggested that since VDRs are found on Th cells, vitamin D may play a role in the function or development of Th cells.

Vitamin D may be a physiological regulator of T-cell development. In vitro, 1,25-(OH)2D3 has been shown to be a differentiation factor for monocytes and other cell types. In T-cells, 1,25-(OH)2D3 seems to preferentially down-regulate Th1 cells both by increasing proliferation and by decreasing cytokine production, such as IL-2 and IL-12, important T-cell differentiation factors. Vitamin D also acts as a regulator of other transcription factors, such as the Stat1, Stat4, Stat6, and the GATA-3in Th1 and Th2 cells. In vivo, 1,25-(OH)2D3 treatment increased the proportion of Th2 cytokines IL-4 and TGF-β1 [16].

Vitamin D receptor knockout mice experiments [56] and the inhibition of M. tuberculosis growth in human macrophages treated with VD have demonstrated its essential role in the maturation of host cells [19]. Furthermore, VD was able to inhibit the expression of IFN-γ [79], IL-12 [20], and HLA-DR [112], and the Th1 response [72], while activating the CD8 and Th2 cells [23], and seems to stimulate the expression of the NRAMP1 gene in maturing phagocytes [88], which is an another important non-MHC gene related to leprosy resistance [1].

Therefore, the activation of the VDR pathway by TLRs [50, 51], the strong expression of TLR2/1 in the tuberculoid pole [45], and the interaction between VDR polymorphisms and the negative Mitsuda response favoring leprosy occurrence [35] are consistent findings that demonstrate vitamin D synthesis as a key factor in increased mycobacteria susceptibility and its clinical manifestations.

The link between altered vitamin D metabolism as an intermediary for an important pathway of innate immunity (induction of antimicrobial peptides by TLR activation) opens exciting new horizons for vitamin D research in general and the epidermal photoendocrine vitamin D system in particular. This is true not only for the study of anti-infectious immune defense mechanisms, but also for a better understanding and treatment of inflammatory skin diseases or compromised wound healing [99].

The effects of the lipophilic hormone vitamin D are directly controlled by VDR polymorphisms, which regulate cell proliferation and differentiation and may cross-talk with other growth regulatory factors, such as the transforming growth factor-β (TGF-β).

The transforming growth factor-β is a potent regulatory cytokine with diverse effects on hemopoietic cells. The pivotal function of TGF-β in the immune system is to maintain tolerance via the regulation of lymphocyte proliferation, differentiation, and survival [47]. In addition, TGF-β controls the initiation and resolution of inflammatory responses through the regulation of chemotaxis, activation, and survival of lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes [34]. The regulatory activity of TGF-β is modulated by the state of cell differentiation and by the presence of inflammatory cytokines and co-stimulatory molecules. Collectively, TGF-β inhibits the development of immunopathology to self or non-harmful antigens without compromising immune responses to pathogens [47]. TGF-β causes activation of SMAD proteins acting as co-activators or transcription factors in the nucleus [122].

Vitamin D controls the transcription of target genes through the VDR. Smad3, one of the SMAD proteins downstream in the TGF-β signaling pathway, was found in mammalian cells to act as a co-activator specific for ligand-induced transactivation of VDR by forming a complex with a member of the steroid receptor co-activator-1 protein family in the nucleus. Thus, Smad3 may mediate cross-talk between vitamin D and TGF-β signaling pathways [122]. Results elsewhere also indicate that Smad3 co-activates
VDR to enhance further TGF-β signaling and vitamin D3 signaling pathways [123].

TGF-β probably plays different roles in leprosy: (1) mediation of a suppressive action locally, associated with the presence of PGL-1, and (2) induction of proinflammatory effects when secreted systemically by monocytes, thereby acting as a modulatory cytokine in the acute inflammatory reactions of ENL and associated with the Th2 immune response in multibacillary forms of leprosy [32, 33]. Therefore, reactions of the immune system can result in protection against the invading pathogen; however, if this response is excessive or inappropriate, it may harm the host and benefit the pathogen. A balance between the various responses must occur, and it depends on both genetic and environmental factors.

We believe that due to the intense cross-talk among genes and pathways, patients’ molecular and immunological profiles must be determined through analyses of microarrays (proteins and mRNAs) instead of DNA polymorphisms, which may finally indicate specific expression patterns that might explain resistance or susceptibility as well as the development of different clinical forms of leprosy.

An elegant gene expression analysis using cDNA microarrays between tuberculoid and lepromatous leprosy patients has demonstrated a distinct gene expression profile [13], and although relevant gene clusters were demonstrated only for the clinical forms from both poles, the gene expression profile across the five clinical forms of the disease spectrum still remains to be revealed. The gene expression profiles were consistent in showing that type 1 cytokines associated with cell-mediated immunity predominate in tuberculoid lesions, whereas type 2 cytokines predominate in lepromatous lesions, which correlates with immunologic unresponsiveness. However, the high expression of inhibitory receptors in lepromatous leprosy patients, especially the leukocyte immunoglobulin-like receptor 7 (LIR-7), may have also contributed to the unresponsiveness in those patients. LIR-7 was almost 6-fold higher in lepromatous patients in relation to tuberculoid patients. It has also been shown that activation of LIR-7 interferes with TLR-induced antimicrobial activity and modulates the balance of IL-12 and IL-10 [13].

A metabolic schematic map for the immune response against M. leprae, including interactions among different signaling pathways (TLRs, VD, SCL11A1, IFN-γ, and TGF-β and LIR-7) cross-talking through activation or inhibition of important genes is proposed in Fig. 1, based on experimental evidence in literature. Balance among these pathways and the relationship among genes are essential for the development of Th cells and for the switch between Th1 and Th2 responses, as well as in the host resistance process. However, this balance is also dependent on the interaction with virulence factors, which according to the host’s genetic background may also be responsible for the different clinical manifestations.

**Virulence factors**

Some bacteria survive inside phagocytic cells, in either neutrophils or macrophages. Bacteria that can resist killing and survive or multiply inside phagocytes are considered intracellular parasites. The phagocyte environment may be a protective one, shielding the bacteria during the early stages of infection or until they develop a full complement of virulence factors [111], and this seems to be the case in leprosy.

The intracellular environment guards the bacteria against the activities of extracellular bactericides, antibodies, drugs, etc. Intracellular parasites usually survive by virtue of mechanisms that interfere with the bactericidal activities of the host cell. Some of these bacterial mechanisms include inhibition of phagosome–lysosome fusion, survival inside the phagolysosome, and/or escape from the phagosome [111].

Vaccination with Bacillus Calmette-Gérin (BCG) has been used as a tool to study the pathogenesis of tuberculosis [10]. A genomic region, called the RD1 locus, is absent in the attenuated M. bovis BCG vaccine strains. The potential importance of RD1 was reinforced when more comprehensive genomic comparisons failed to find a more compelling candidate for the attenuation of BCG [10, 106]. An important clue was that the genes within RD1, encoding the early secretory antigenic 6 kDa (ESAT-6) and the culture filtrate protein 10 (CFP-10), two small proteins that lack traditional signal sequences, were useful in the immunodiagnosis of latent tuberculosis infection [106]. This virulence region was therefore identified as a new specialized secretion system with an unknown purpose.

This specialized secretion system of pathogenic bacteria commonly transports multiple effectors that act in concert to control and exploit the host cell as a replication-permissive niche. Both the M. marinum and the M. tuberculosis genome contain an extended region of difference 1 (extRD1) locus that encodes one such pathway, the ESAT-6 system 1 (ESX-1) secretion apparatus. ESX-1 is required for virulence and for secretion of proteins ESAT-6, CFP-10, and EspA. The result establishes EspB as a substrate of ESX-1 that is required for virulence and growth in macrophages [57].

ESAT-6, CFP-10, and the MAPKs (mitogen-activated protein kinases) encoded outside the locus are mutually dependent for secretion [28]. Each protein (ESAT-6 and CFP-10) contains two z-helices that interact to form a
heterodimeric four-helix bundle. Despite reports of association with and damage to host cell membranes, the complex presents no hydrophobic faces, suggesting that these two proteins alone are unlikely to form a transmembrane structure [43].

Mutations affecting the synthesis or secretion of ESAT-6 or CFP-10 attenuate the virulence of *M. tuberculosis* in murine models of infection. These results suggest that the ESAT-6/CFP-10 secretion system plays a role in preventing phagolysosomal fusion, a novel function that accounts for the ability of bacteria to survive inside host cells. This finding provides a mechanism by which the ESAT-6/CFP-10 secretion system potentiates the virulence of pathogenic mycobacteria [108].

The immunodominance of the *M. leprae* ESAT-6 ortholog, ML0049, has been confirmed, but it has shown that problems of potential cross-reactivity arise in individuals likely to have been infected by or exposed to *M. tuberculosis* [29]. The sequence of the *M. leprae* ortholog of CFP-10 has 40% identity (60% homology) at the protein level with *M. tuberculosis* CFP-10, and may also be subject to cross-recognition. Nevertheless, the ESAT-6 protein ML1055 (EsxK1), but not ML1056 (EsxL1), appears to cross-react with sera from healthy individuals [8].

Macrophages infected with *M. tuberculosis* secrete less tumor necrosis factor alpha and interleukin-12 than those infected with nonpathogenic mycobacteria, indicating that the RD1 locus provides *M. tuberculosis* with a means to alter host responses actively [106].

Other recent findings have added more complexity to this system: two parallel independent studies have demonstrated that genes Rv3614c–Rv3616c and genes immediately upstream of the RD1 locus shared some similarities [94], and strains lacking Rv3616c were unable to secrete ESAT-6 [27].

The genome project on *M. tuberculosis* H37Rv has also revealed four mammalian cell entry (*MTmce1*-4) operons putatively involved with entry and survival of mycobacteria in host cells. An operon homologous to the *MTmce1* operon was identified in cosmid B983 of *M. leprae*. The corrected *MLmce1* operon sequence seems to be highly homologous to the *MTmce1* operon, and similarly encodes eight potential genes. Thus, both *M. tuberculosis* and *M. leprae mce1* operons may be functional and involved in host cell targeting [118].

Other non-protein and cell wall components have shown important biological activities, acting as virulence factors [85], including:

1. Lipoarabinomannan (a major lipoglycan of the cell wall envelope)—resistance to oxidative metabolites;
2. Mycolic-acid glycolipids, wax D, and trehalose dimycoclate (cord factor)—granuloma formation and adjuvant activity;

The persistence of strong staining for LAM in biopsies of treated cases with leprosy reactions indicates that this cell wall-associated glycolipid is excreted in copious amounts by mycobacteria and is probably essential for the intracellular survival of mycobacteria within macrophages [52]. It also has a wide range of biological effects, such as inhibition of interferon-γ activation of macrophages, induction of TNF-α release, and inhibition of antigen presentation by antigen-presenting cells as reviewed elsewhere [52].

Other important virulence factors are the phenolic glycolipids (PGLs), which are polyketide-derived molecules, produced by *M. tuberculosis*, *M. leprae*, and other mycobacterial pathogens, and in leprosy, the PGL-1 seems to determine the bacterial predilection for the peripheral nerve [67]. A study reported elsewhere [26] has elucidated the mechanism of chain initiation required for the assembly of the phenolthiocerol moiety of PGLs, which has allowed the development of an inhibitor of PGL assembly with potent activity in several mycobacterial pathogens. This result provides important support for the feasibility of targeting PGL biosynthesis to develop new drugs for treating mycobacterial infections.

Therefore, the identification of separate antigens in leprosy lesions is of major interest because one may then postulate a role for individual antigens in the development of leprosy immunopathology [36, 52].

### Autoimmunity

The triggers of autoimmune diseases remain elusive. However, it is suggested that repeated infections with common pathogenic and even nonpathogenic organisms could expand T-cells specific for conserved protein domains that are able to cross-react with tissue-derived and ubiquitous autoantigens, generating an autoantibody (autoAb) response [103]. This could be one of the explanations for the generation of autoantibody response in leprosy, as observed in the erythema nodosum leprosum (ENL) reaction, which is evidenced by the production of humoral autoAb, such as rheumatoid factor, anti-nuclei factor, and increased levels of immunoglobulins G and M, and complements C2 and C3 [64, 89]. Moreover, immune complexes have also been found in ENL lesions associating it with the Arthus phenomenon [116].

Autoantibodies detected in leprosy may be categorized as (1) AutoAb directed towards antigens expressed on the membrane of infected cells (e.g. antiendothelial cell autoAb); (2) AutoAb driven by a molecular homology between infectious agents and host proteins, such as heat shock protein (HSP), DNA and cytoskeleton; (3) AutoAb...
binding to a given epitope present on the microbe and the host (e.g. HSP 65 kDa and lactoferrin); (4) AutoAb neutralizing the pathogen (e.g. anti-phospholipid Ab); and (5) AutoAb contributing to its elimination (e.g., rheumatoid factor). Finally, autoAb production in such chronic infections may reflect a specific activation of autoreactive B-lymphocytes rather than a generalized polyclonal B-cell activation [81].

So far, it is not known which antigens are recognized by lesional T-cells [105]. Mycobacterium leprae-specific T-cells can be isolated from blood, inflamed skin [114], and nerve tissue [105]. It is possible that nerve damage may be caused or enhanced by autoreactive T-cells, in addition to M. leprae-specific T-cells. Such autoreactive T-cells may be primed by cross-reacting mycobacterial antigens (molecular mimicry) [118, 119, 120]. An obvious candidate autoantigen is HSP60. Mycobacterial HSP60-reactive murine T-cells are indeed able to lyse uninfected macrophages [44, 107] and IFN-gamma-stressed target cells [125]. Furthermore, transfer of an HSP60-specific CD8+ T-cell clone into immunocompromised mice leads to severe immunopathology [107]. These data indicate that the presentation of peptides from endogenous proteins can be responsible for cell-mediated autoimmune-like tissue destruction. However, it is also possible that, during inflammation, T-cells are primed against non-cross-reacting Schwann cell or nerve-associated autoantigens, perhaps by cross-priming, and that such autoreactive T-cells can contribute to peripheral neuropathy in leprosy. Analysis of the antigen specificity of lesion-infiltrating T-cells will be needed to address the role of self-antigen versus M. leprae antigen recognition in the immunopathology of leprosy neuritis [105].

Mechanisms of nerve damage by Mycobacterium leprae

The identification of the molecular basis of M. leprae neurotropism has had high priority in recent leprosy research, since the neuropathy in leprosy is caused in part by invasion of peripheral nerves by M. leprae. It has been shown that the G domain of the alpha 2 chain of endoneurial laminin 2 (LAMA2) is crucial in the invasion of peripheral nerves by M. leprae [74]. Furthermore, alpha-dystroglycan has been identified as the laminin alpha2-G receptor on the Schwann cell and a candidate protein receptor on the surface of M. leprae [75]. Therefore, the Schwann cell is an important target for bacterial invasion of nerves. This fact is a great contribution to our understanding of the pathogenesis of leprosy and may have important implications for the design of interventions to control leprosy-induced nerve damage.

The extent of the expression of α2-laminin-binding properties among mycobacteria was investigated [55], and species of Mycobacterium tested (M. tuberculosis, M. chelonae and M. smegmatis) also expressed laminin-binding capacity, suggesting that the ability to bind α2-laminins is conserved within the Mycobacterium genus. This report also demonstrated that not only M. leprae but also all the mycobacterial species tested readily interacted with the ST88-14 cells, a human Schwannoma cell line, and that the addition of soluble α2-laminins significantly increased their adherence to these cells. These results indicate that the ability of M. leprae to adhere to Schwann cells via α2-laminins should not be considered the reason for the uniqueness of M. leprae among pathogenic mycobacteria in its ability to invade Schwann cells inside the host, as had been previously suggested [74]. In fact, the tropism is mediated by a complex of host and microbe-derived factors, most of which are still unknown [55].

Evidence has also been provided for the involvement of the specific trisaccharide unit of the phenolic glycolipid-1 (PGL1) of M. leprae in determining the bacterial predilection for the peripheral nerve. PGL1 binds specifically to the native laminin-2 in the basal lamina of Schwann cell-axon units. This binding is mediated by the LG1, LG4, and LG5 modules present in the naturally cleaved fragments of the peripheral nerve LAMA2 chain, and is inhibited by the synthetic terminal trisaccharide of PGL1. PGL1 is involved in the M. leprae invasion of Schwann cells through the basal lamina in a laminin-2-dependent pathway [67]. The results indicated a novel role of a bacterial glycolipid in determining the nerve predilection of a human pathogen.

Activation of Schwann cells in vitro with a synthetic lipopeptide of the putative M. leprae 19 kDa lipoprotein lqphH, ML1966, triggered nuclear apoptosis, and Schwann cells in leprosy lesions were also demonstrated to have undergone apoptosis [70]. These observations indicate that activation of TLR-2 on Schwann cells contributes to nerve damage in leprosy and is of great interest when comparing the significance of innate and adaptive immunity for development of nerve damage.

Nerve damage, a hallmark of leprosy, has continued to happen even when pathogens are contained and TLR2 activation is strong. It is quite possible that while TLR activation is a quick response to infection, subsequent non-stop inflammatory reactions due to deregulated TLR signaling might cause tissue (nerve) damage in leprosy and deserve further investigation [11].

A single cell wall protein of 21 kDa has also been identified, which seems to be the major adhesin of M. leprae for interaction with peripheral nerves [100]. This study represents a major breakthrough in understanding the molecular mechanisms involved in the interaction between M. leprae and the peripheral nerve. Despite indications that parallel (e.g. non-laminin-mediated) mechanisms may also be at work, these findings represent a crucial step towards
developing strategies to prevent *M. leprae*-induced nerve damage.

Conclusions

Modern genomics can reveal the sets of genes that correlate with protective responses or inappropriate responses leading to disease progression and tolerance, providing unanticipated insights into pathogenesis and targets for therapy [13]. It is quite improbable that a single marker could provide an efficient prognosis, because adaptive immunity is a cascade of molecular events that requires the interaction of many factors. The contribution of sets of genes that lead to a specific clinical form and the primary events that trigger the immune response remain to be demonstrated.

There is no longer any doubt that the TLRs are capable of sensing a wide range of microbes and quickly produce anti-microbial chemokines, cytokines, interferon, and reactive oxygen species to protect the host against invading pathogens. Nevertheless, at the same time, hyper- or hypo-responsiveness of TLRs or uncontrolled and improper signaling from these receptors may occur, which can lead to serious consequences for the host [11]. Four important pathways have been chosen in this review, TLR/LIR-7, VDR, TFG-β1, and TNF-α, which may interact with each other with different expressions, according to the environment and as an immune response to specific antigens that are presented. The balanced expression of the genes involved in those pathways may lead to the development of a specific clinical form.

The selected set of gene polymorphisms that are tightly linked to each other through cross-talk pathways could significantly change the course of the disease, according to a specific disequilibrium of genic homeostasis, which is highly dependent on the environment, antigens that are presented to the host cell, specific polymorphisms that interact with other genes, external factors, and pathogen survival, culminating in leprosy occurrence.

Currently, the microarray-based genomic survey of gene polymorphisms, multiple gene expression analyses, and proteomic approaches, such as protein fingerprinting by mass spectrometry or phage display technology for epitope mapping, may constitute the most adequate procedures for evaluating individual responses of leprosy patients and contacts to predict the outcome and progression of the disease. At present, none of the genes is good prognostic marker; however, it is quite possible that in the near future we will use multiple targets to predict the clinical outcome of leprosy.

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