Immunopathology of leprosy: towards the search for diagnostic and prognostic biomarkers to elucidate pathobiology and their utility in patient care

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Introduction and outline of the thesis
Leprosy is widely regarded as one of the oldest diseases known to man. References to leprosy have been found in the Bible, although Cochrane [1] argued that the biblical usage of the word probably indicated a defiling or cursed disease rather than leprosy per se. Leprosy was prevalent in ancient Egypt, with indisputable evidence for leprosy in a mummy dating from the 2nd century BCE [2; 3]. In ancient India, leprosy was a common disease and was mentioned in the Vedas [4]. The Sushruta Samahita provides a detailed account of the various clinical features and treatment of leprosy [5]. Inscriptions from ancient China dating from around 600 BCE also reveal the prevalence of the disease there [6]. In ancient Greece, clinical descriptions of what was probably the lepromatous form of leprosy was included under the name ‘Elephantiasis graecorum’ [7; 8]. The disease is thought to be introduced to Europe by the soldiers of Alexander the Great returning from the Indian campaign [7; 8] or Pompey’s soldiers returning from Egypt [3]. Leprosy was introduced in the Americas largely as a result of colonization by Europeans and to some extent by the slave trade from West Africa in the 18th century [9].

Much has improved in our understanding of leprosy since the causative bacterium, Mycobacterium leprae, was discovered by Armauer Hansen in 1874 [1]. However, despite this fact, leprosy is one of the few diseases where Koch’s postulates have not yet been fulfilled due to a lack of a suitable in vitro culture medium and the absence of good animal models. Leprosy is a chronic infectious disease primarily affecting the skin and the peripheral nerves. Mycobacterium leprae is primarily an intracellular rod-shaped bacterium, which occurs in groups resembling cigar bundles called globi in highly bacilliferous patients. The presence of high molecular weight mycolic acids in the cell renders the bacillus resistant to decolourization by acid-alcohol using the Ziehl-Neelsen Carbol Fuchsin method and hence its acid-fast nature. It is on basis of this acid-fast nature that the Hansen’s bacillus was included in the genus Mycobacterium, together with other closely related species like Mycobacterium tuberculosis and Mycobacterium kansasii [10]. The 8th edition of the Bergey’s Manual classifies the organism as: Class II – Schizomycetes; Order VI – Actinomycetales; Family II – Mycobacteriaceae; Genus – Mycobacterium; Species – M.leprae.

**Magnitude of the leprosy problem**

Leprosy is still far from being a disease of the past, although recent data indicate stabilization and even a steady declining trend in the yearly new case detection rate (NCDR) [11]. The globally registered prevalence of leprosy at the beginning of 2007 was 224,717 cases, with 259,017 new cases detected in 2006, a decline of about 13.4% compared to 2005 [11]. This is a decline of almost 98%, from roughly 12 million cases in 1985 [12; 13]. With this declining trend, the goal of eliminating leprosy as a public health problem (defined as
having a prevalence rate of < 1 per 10,000 population) has been reportedly achieved in most countries except Brazil, the Democratic Republic of Congo, Mozambique and Nepal [11]. This is largely attributed to the introduction of multi drug therapy (MDT) in 1982 [14; 15] and the WHO-led campaign for elimination of leprosy as a public health problem [16; 17]. On the other hand, the high number of new cases still being detected may indicate the presence of a hidden reservoir involved in the continued transmission of leprosy. What remains alarming is the high number of multibacillary leprosy cases, ranging from 27-91% in different regions, amongst the new cases detected which is a potential source for the continued transmission of the bacterium [11]. The WHO global strategy for leprosy 2006-2010 aims for a further reduction in global leprosy numbers, for improved diagnosis, management and information systems with leprosy services integrated into the general health care system and provision of adequate tools for disability management and rehabilitation [18; 19]. However, despite the undoubted success of the WHO global strategy in the control of leprosy, the progress towards leprosy “elimination” needs to be reviewed cautiously. This is especially important since doubts exist about the reliability of statistics from some of the highly endemic countries such as India and Madagascar which have registered a decline of almost 30% between 2003 and 2006 [11]. This is despite no significant changes in the policies and anti-leprosy activities in these countries compared to previous years and in the face of a much slower decline globally. Hence, in order to consolidate the successes of global strategy, the focus of future efforts needs to be on accurate and reliable assessment of the global prevalence and incidence to pursue the goal of leprosy control further.

Pathogenesis of leprosy

Sources and routes of infection

Although M.leprae-like, non-cultivable, acid-fast organisms have been found in Mangabey monkeys [20], wild armadillos from the southern United States [21] and sphagnum moss from coastal Norway [22; 23; 24], the only known sources of infection are human beings disseminating viable bacilli in nasal secretions [25], sputum [26] or from skin [27]. M.leprae is known to remain viable for several days in droplets from the infected upper respiratory tract, in dried discharges from ulcers or on fomites such as bedding or clothing. Although several routes of infection have been proposed, the skin and respiratory tract appear to be the most likely routes of entry. Studies in mice demonstrating the development of disseminated infection in mice exposed to either aerosols [28] or droplet instillation of M.leprae suspensions [29; 30] along with the high bacillary load in the nasal secretions of lepromatous patients [25] support the role of the nasal tract as a major port of entry and exit of M.leprae. Another plausible route of entry is the skin with epidemiological studies
showing the development of the first leprosy lesions on the extremities where injury to the skin is common [31].

Clinical features of leprosy – the leprosy spectrum and reactions

The cardinal sign of leprosy is a hypopigmented or erythematous skin lesion accompanied by loss of sensation. Patients commonly present with skin lesions, numbness or weakness caused by peripheral nerve involvement, or more rarely, a painless burn or ulcer in an anaesthetic hand or foot [32]. Patients may also present initially with a leprosy reaction [33]. During the chronic course of leprosy, acute increases in disease activity called reactions may occur which may be either type I (reversal reaction, RR) or type II (erythema nodosum leprosum, ENL) as will be described subsequently.

The wide range of clinical manifestations of leprosy forms an unique spectrum which correlate with the magnitude of the host immune response. The pathology of leprosy has been fairly well characterized on basis of clinical, bacteriological and histopathological characteristics and forms the basis of the Ridley-Jopling system of classification of leprosy [34]. The Ridley-Jopling system still remains the basis of a classification of leprosy for scientific purposes. However, the need for histological facilities and skilled, trained personnel meant that this system was complicated for use under field conditions. More recently, a simplified system of classification has been recommended by the WHO for use in the field where slit-skin smears for bacteriology are not available [35].

Figure 1. Leprosy is classified according to the Ridley-Jopling (R-J) 5-group classification (LL – TT) for scientific purposes or according to the simplified WHO 2 group system (multibacillary-paucibacillary) for treatment purposes. Recently, the WHO system has been further modified into a 3 group system based on the number of lesions. The lepromatous pole of the R-J classification is associated with the abundant presence of M.leprae specific antibodies (humoral immunity, HI) whereas cell-mediated immunity (CMI) becomes stronger towards the tuberculoid pole. Chronic leprosy is also associated with acute reactions – erythema nodosum leprosum (ENL) or Type II reaction mainly in LL/BL patients and reversal reaction or Type I reaction associated with the borderline leprosy forms (BL/BB/BT).
Introduction and outline of the thesis

Ridley-Jopling classification, 1966

The Ridley-Jopling classification is based on the spectral manifestation of leprosy and has at its one pole lepromatous leprosy (LL), which shows multiple, symmetrically distributed lesions throughout the body (skin, nerves, eyes and internal organs). Histopathologically, LL lesions show an infiltrate largely composed of macrophages, showing varying degrees of foamy changes, and few, scattered lymphocytes, predominantly of the CD8+ subset [32]. Acid-fast *M. leprae* are numerous within and outside macrophages in LL lesions and may aggregate to form globi. Nerves may show some structural damage and cellular infiltration but not cuffing. In addition, Schwann cells, perineural cells, axons, intraneuronal macrophages of dermal nerves may contain bacilli. Nerve destruction is gradual, slow and frequently unnoticed until late. Immunologically, LL is associated with a complete absence of *M. leprae* specific cell mediated immune responses (CMI) [36; 37]. This is also apparent in the lack of delayed type hypersensitivity (DTH) response to subcutaneous challenge with lepromin, a preparation from highly bacilliferous LL lesions or from *M. leprae* infected armadillo tissues [38]. Interestingly however, patients with LL leprosy are able to mount a normal CMI response against other infectious agents including other mycobacteria [36; 37] suggesting that the immune deficiency is leprosy-specific. Furthermore, antibodies to *M. leprae* are abundant in LL sera but are ineffective in controlling the progress of the disease.

On the other hand, tuberculoid leprosy (TT) shows few lesions with well defined margins, which are markedly anaesthetic and do not show the presence of acid-fast bacteria. Lesional infiltrate primarily consists of foci of well developed epithelioid macrophages, with or without Langhans’ type of multi-nucleated giant cells surrounded by a cuff of lymphocytes. The T-lymphocyte subsets are predominantly of the CD4+ type. Within the granulomas, small nerves may be destroyed beyond recognition and a thickened peripheral nerve is usually palpable in the vicinity of a lesion. Immunologically, TT shows a strong CMI response to *M. leprae* with an intense DTH response to lepromin [39]. This is also reflected clinically and histopathologically in tuberculoid lesions, which are restricted in distribution and may even heal spontaneously [34]. *M. leprae* specific antibodies are usually absent or present at very low levels in these patients.

In-between the two polar leprosy forms are the immunologically unstable borderline forms including borderline lepromatous (BL), mid-borderline (BB) and borderline tuberculoid (BT), showing clinical and histopathological characteristics intermediate to the polar forms. Within these groups, there is a gradual decrease in CMI from BT to BL and this is inversely correlated with the bacillary load within the lesions. About 20-30% of these borderline patients develop acute reactions during or after the course of the disease [40; 41].
Immunopathology of leprosy


Due to the complexity of the Ridley-Jopling classification, the WHO recommended an operational classification to serve as a basis for chemotherapy [42]. Since the transmission of leprosy was thought to occur largely through the highly bacilliferous cases of leprosy the WHO recommended all LL, BL and BB leprosy under the Ridley-Jopling classification with a bacterial index (BI) in slit-skin smears ≥ 2 to be grouped as multibacillary leprosy (MB) [42]. On the other hand, I, BT and TT leprosy with a BI < 2 under the Ridley-Jopling system were thought to pose fewer problems of transmission due to the lower bacterial load and were grouped as paucibacillary (PB). For further convenience, revised recommendations were issued by the WHO in 1988 whereby all smear positive cases were included as MB whereas I, BT and TT cases except those with smear positivity were included as PB [43]. In 1998, based on the fact that services for processing skin smears may not be available or reliable in the field, the 7th WHO expert committee on leprosy recommended a further simplification of classification [35]. This new classification groups leprosy patients into (a) paucibacillary (PB) single lesion leprosy - only one skin lesion, (b) PB leprosy - 2-5 skin lesions; and (c) multibacillary (MB) leprosy - more than 5 skin lesions. A case of leprosy was defined as a person with one or more of the following features – i) hypopigmented or reddish skin lesion(s) with definite loss of sensation, ii) involvement of the peripheral nerves, as demonstrated by definite thickening with loss of sensation, iii) skin smear positive for acid-fast bacilli [35]. This case definition did not include cured persons with late leprosy reactions or with residual disabilities.

In practice, the different diagnostic criteria used complicate the comparison of data among countries and even within a country, and increase the risk of misclassification as has been suggested from various studies [44; 45; 46]. Hence the use of a standard classification such as the clinico-histopathological Ridley-Jopling system should be a pre-requisite to allow comparison of different studies across the globe and even over time [47].

Reactions in leprosy

A significant proportion of the leprosy patients, especially in the borderline region of the spectrum, develop leprosy reactions either sometime during the course of the disease or even after the completion of multi-drug therapy (MDT) [48; 49; 50; 51]. Reactions are thought to be immune exacerbations in response to *M. leprae* and its antigenic components [51; 52] and may be either type I/reversal reaction (RR) – with acute increase in *M. leprae* specific CMI or type II/erythema nodosum leprosum (ENL) – an immune complex mediated condition with some involvement of CMI [51; 52].
**Type I reaction or Reversal reaction**

About 30% of individuals with borderline leprosy are at risk for type 1 reaction which is characterized clinically by acute inflammation of skin and/or nerves leading to nerve function impairment if not treated rapidly [32; 53]. The characteristic signs of RR are an erythematous swelling of existing lesions, appearance of new lesions and the onset or worsening of neuritis [51; 52]. Histopathologically, RR is characterized by a shift of classification towards the tuberculoid end of the spectrum with increased infiltrate of lymphocytes, epitheloid cells, giant cells, oedema and a decrease in bacterial load. A sudden increase in $M.\text{leprae}$-specific CMI accompanied by an influx of CD4+ T cells at the lesional site are some of the immunological characteristics of RR [51; 52]. The immune response is characteristic of a delayed-type hypersensitivity (DTH) reaction with peripheral blood lymphocytes demonstrating an increased reactivity to $M.\text{leprae}$ antigens in a lymphocyte transformation test (LTT) [54]. This may lead to local decrease in bacillary load and augmentation of T cell reactivity leading to nerve damage [51].

Incidence of Type I reactions is significantly higher in BB and BL patients as compared to BT patients [55; 56; 57]. De Rijk et al. [56] noted that 88% of reactional events in MB patients and 52% in PB patients involved neuritis. Moreover, in 40-50% of both groups, neuritis occurred in the absence of skin involvement [56]. Furthermore, in 80% of the cases, RR occurred within the first year of treatment or the first 6 months after completion of MDT [56; 58; 59].

**Type II reaction or Erythema Nodosum Leprosum**

ENL, on the other hand, affects 20% of lepromatous and 10% of borderline lepromatous cases, with a high bacterial load and greater infiltration of lesions as important risk factors [32; 53]. ENL is characterized by painful and tender red papules or nodules of the skin accompanied by fever, joint pain, oedema of the hands, feet, and face, proteinuria and malaise [32; 52; 53]. Other manifestations include iritis, episcleritis, arthritis, dactylitis, lymphadenopathy, organomegaly and orchitis [33]. Neuritis may be part of ENL but is usually milder than in RR [60]. Most patients experience multiple acute episodes or chronic ENL lasting more than 6 months or even years [61; 62]. The inflammatory infiltrate is usually in the deeper layers of the dermis and subcutis [60]. In acute lesions within 72 hours the predominant cell type is the polymorphonuclear leukocyte (PMN) whereas between 72-96 hours equal numbers of neutrophils, lymphocytes and plasma cells are seen along with persistent mast cells [60]. Chronic lesions > 9 days on the other hand have fewer neutrophils and eosinophils but an increased number of lymphocytes, plasma cells and histiocytes [60]. Vasculitis appears to be a major pathological event in ENL along with interstitial oedema and
necrotizing changes [63; 64]. A large proportion of patients with ENL showed deposition of Ig and complement in the skin, with 70% showing *M. leprae* antigens within the complexes, which was absent in patients without ENL [65]. Furthermore, immune complex deposition was significantly higher in skin but similar in serum to patients without ENL [65], suggesting that ENL was caused by local deposition of immune complexes. Laal *et al.* [66] provided evidence for CMI in ENL by demonstrating a strong leukocyte migration inhibition and antigen-induced lymphoproliferation in PBMCs from ENL patients. In addition, several studies have shown an important role for TNF-α in the pathogenesis of ENL [67; 68; 69; 70] suggesting that CMI may be a crucial component of this type of reaction. Later studies have shown that IFN-γ and IL-12 are also important in addition to TNF-α and may contribute to the deleterious effects classically attributed to TNF-α [71].

A wide variation has been reported in the incidence of ENL in leprosy patients globally ranging from 2% to 31% of MB patients [55; 72; 73; 74]. The most important risk factor was found to be a high bacillary load while HIV positivity was found to be another risk factor [74]. A majority of the cases presented 2-3 years after diagnosis of leprosy with some patients developing episodes as late as 7 years after starting treatment [74]. Sixty-three percent of the cases had multiple episodes of ENL, which required management with long courses of prednisolone and additional clofazimine for periods of up to 5 years [74].

**Host cell parasitism and bacterial survival**

Macrophages are known to be important host cells for *M. leprae* and *M. tuberculosis* [75]. On the other hand, *M. leprae* is also known to promote its survival in the mammalian host by entry into immunologically privileged sites such as the peripheral nerve and selectively parasitizing non-professional phagocytic cells such as MHC Class II negative Schwann cells [76; 77]. Rambukkana and co-workers [78; 79; 80] elegantly demonstrated one possible mechanism of *M. leprae* invasion of the Schwann cells of the peripheral nerves using laminin α2 (LN-α2) as a bridge [80]. A 21-kD laminin binding protein on the *M. leprae* cell wall was shown to bind specifically to LN-α2 in the basal lamina [78; 81] which in turn bound to α-dystroglycan on the Schwann cell [82]. In addition, other non-laminin receptors such as the 25-kD glycoprotein from the human peripheral nerve [83] may serve to strengthen this interaction with Schwann cells [78]. Later studies showed a role for the *M. leprae* specific PGL-I antigen in binding to the native laminin-2 in the basal lamina of the Schwann cell-axon units through specific trisaccharide units [84]. PGL-I was also shown to play a role in *M. leprae* induced contact-dependent demyelination of nerve fibres in a time and dose dependent manner, thus contributing to the pathology of leprosy [85].

Within the host the bacterium may adopt different mechanisms to avoid or
circumvent the host immune responses [86; 87; 88]. Phagocytosis thorough receptors which bypass bactericidal activities of macrophages may provide an opportunity for pathogens to manipulate the host environment to their own advantage. Caron and Hall [89] demonstrated the advantage of using complement receptor (CR) 3 as a receptor for bacterial entry which avoided the usual inflammatory response associated with the uptake of IgG-opsonized targets through FcγRII. Phagocytosis using CR1 [90; 91] or the mannose receptor (MR) [92] prevented fusion of lysosomes with phagosomes preventing O₂⁻ radical production thus may allow for survival of mycobacteria. Inhibition of phagosome-lysosome fusion by M.leprae was demonstrated by Sibley et.al. [86] and confirmed by Frehel and Rastogi [93] who also suggested a role for mycobacterial surface components in this event since antiserum coating of M.leprae partly reversed the fusion inhibition event. Furthermore, Sturgill-Koszycki et.al. [94] reported a selective inhibition of fusion of Mycobacterium containing vacuoles with vesicular proton ATPase preventing phagosome acidification and hence mycobacterial killing. Another mechanism of mycobacterial escape suggests a progressive translocation of M.tuberculosis and M.leprae from phago-lysosomes into the cytoplasm of phagocytes dependent on the secretion of the mycobacterial antigens CFP-10 and ESAT-6 [88].

**The host immune response in leprosy**

Intracellular pathogens, which include different species such as *Listeria, Leishmania, Mycobacteria, Salmonella, Chlamydia, Rickettsia* and *Trypanosoma*, live inside the host cells where they are protected from the effector cells of the immune system. The preferred host cells for the pathogens are the mononuclear phagocytes, which are also an important component of the innate immune system. In a constant competition between the pathogen and the host these phagocytes can restrict the intracellular growth of the pathogen by phagosome-lysosome fusion, generation of reactive oxygen and nitrogen intermediates or restricting nutrient availability to the pathogen. At the same time, these cells secrete soluble mediators such as cytokines and chemokines to increase trafficking of T- and B-lymphocytes and concentrate them at the sites of infection. Moreover, the phagocytes along with other specialized antigen-presenting cells like the dendritic cells ensure that protein and lipid antigens from the pathogens are processed and presented leading to activation of the host T-lymphocytes [95]. These T-lymphocytes are thought to be crucial in determining the resistance, susceptibility, and often, the immunopathogenesis of intracellular infections. Although the CD4⁺ lymphocytes are primarily implicated in acquired immunity, increasing evidence points to a role for CD8⁺ T-cells as well as unconventional T-cells such as the CD4⁺CD8⁻TCRαβ⁺ T cells recognizing lipid antigens, TCRγδ⁺ T-cells or natural killer (NK) T-cells [96; 97; 98].
Interestingly, *M. leprae* in itself is a rather innocuous organism as suggested by the fact that about 95% of patients infected with the bacterium do not develop overt disease [99]. This also suggests the importance of the host immune response in controlling disease progression although much of the pathology of leprosy is also thought to be related to an inappropriate immune response.

**Genetics of the host response in leprosy**

The low genetic diversity of *M. leprae* and the fact that only about 0.1 – 1% of the infected population develops overt disease [100], indicate the capacity of the host immunity to control the infection. This suggests differences in host susceptibility to the pathogen which may also explain the clinical spectrum of leprosy. There is increasing evidence for association of the genetic background of the host with development of leprosy or its different spectral forms [100]. A strong argument in favour of the role of host genetic background comes from twin studies where a higher concordance rate for leprosy was found amongst monozygotic twins [60-85%] than dizygotic twins [5-20%] [100; 101]. A previous report suggested that populations with different ethnic backgrounds living in the same endemic areas exhibit distinct prevalence rates for leprosy [100]. Moreover, vaccine studies showed the highly variable protection rates of BCG amongst different populations [102]. Two approaches have been commonly used to test genetic associations in leprosy – the candidate gene approach and, more recently, genome wide scans [100].

**The candidate gene approach**

Since the host immune response plays a crucial role in the development of the disease, the most common candidate genes studied in relation to leprosy are genes related to the immune system, such as the human leukocyte antigen complex (HLA), cytokines and receptors. The HLA loci are traditional candidates for association studies with leprosy with the class I HLA-A*1102-B*4006-Cw*1502 haplotype showing a highly significant association with leprosy susceptibility [103]. Among the class II genes the HLA-DRB1 locus, more specifically DRB1*15 and DRB1*16 are associated with leprosy susceptibility in India [104; 105], Thailand [106] and Brazil [107] while the HLA-DQw1 locus was found to be associated with LL patients [108]. A single nucleotide polymorphism (SNP) resulting in a G-A substitution in the promoter region of TNFA gene encoding for TNF-α showed protection against leprosy [109; 110; 111]. However, contradictory results were shown for this SNP in other studies [112; 113]. Similarly, an SNP at the +252 position (A-G) in the
first intron of the lymphotoxin (LT)-α gene together with the -308A SNP as a haplotype was also associated with protection against leprosy in a Brazilian population [111] possibly by production of higher levels of TNF-α and LTα [100]. Similarly a -819T SNP in the promoter region of the IL-10 gene was found to be associated with susceptibility to leprosy [110; 114]. However, while there is confirmatory epidemiological evidence for the association of this SNP in linkage with other haplotypes in leprosy susceptibility [114], no biological evidence linking these genotypes/haplotypes with IL-10 production in leprosy has been obtained to date [100]. The vitamin D receptor (VDR) was associated with leprosy in an Indian population where a T-C substitution at codon 352 at the 3’ gene region resulted in susceptibility to lepromatous and tuberculoid leprosy in the presence of genotypes TT and CC respectively [115] whereas in a Malawian population the CC genotype was associated with susceptibility to leprosy per se [113]. However, the biological relevance of this SNP on VDR function in leprosy is not known. The NRAMP-1 gene has also been studied as a candidate gene for leprosy resistance/susceptibility based on studies in mice [116]. Within humans however, evidence for association of this gene with resistance to leprosy is controversial [117; 118; 119; 120] although a 4 nucleotide deletion in the 3’ untranslated region (UTR) was more frequently encountered in multibacillary leprosy patients [121]. Other candidate genes tested for leprosy susceptibility include the IL-12Rβ1 and 2 [122; 123; 124], laminin [125] and Toll-like receptor (TLR)-2 [126]. Studies on a small Indonesian population revealed that a polymorphism at the +7809 position in the laminin-α2 gene substituting valine for alanine (V2587A) resulted in a missense mutation which was strongly associated with tuberculoid leprosy [125]. On the other hand, whereas missense mutations of IL-12Rβ1 were not associated with LL [122], IL-12Rβ2 was found to be highly expressed in TT leprosy [124] and missense mutations in the 5’ flanking region of the gene could affect its expression and hence increase susceptibility to the LL form of leprosy [123]. Despite the initial enthusiasm about the association of the Arg677Trp polymorphism in the toll-like receptor (TLR)-2 gene with approximately 25% of LL patients by Kang and co-workers [127], it was subsequently shown to be the result of an exon duplication event rather than a genuine polymorphism [128]. However, more recent studies have shown the association of a TLR2 microsatellite marker with increased risk for development of reversal reaction [129] suggesting that TLR2 may indeed have a role in leprosy.

**Genome wide scan**

In recent years, the use of genome wide scans have enabled genetic linkage studies to be performed in leprosy to uncover regions of the genome which may harbour potential candidate genes. Early studies showed major leprosy susceptibility loci in the 10p13 region,
containing the macrophage mannose receptor gene [101] and the 20p12 region [130], also found to be associated with atopic dermatitis and psoriasis in other studies [131; 132; 133]. Later studies showed linkage of the 10p13 region with TT leprosy rather than leprosy in general [134; 135]. A recent study mapped the leprosy susceptibility locus to the chromosome 6q25 in Vietnamese families affected with leprosy [134]. Further analysis using a systematic association scan showed a significant association between leprosy and 17 markers located in a block of approximately 80 kilobases overlapping the 5' regulatory region shared by the Parkinson’s disease gene PARK2 and the co-regulated gene PACRG [136]. Possession of as few as two of the 17 risk alleles was highly predictive of leprosy which was also confirmed in unrelated leprosy cases and controls from Brazil. The 17q11 locus, which contains the NOS2A and CCL2 (MCP-1) genes among others, was implicated in leprosy susceptibility in other genome wide scans [137]. However there is no independent confirmation of the association of these genes with leprosy.

A major limitation of genetic association studies are the conflicting results, primarily as a result of biases of design including the choice of appropriate statistical tools, selection of controls and low power of the statistics due to insufficient sample size. However, at least part of the discrepancies may also be attributed to the ethnic specificity of the susceptibility/resistance variants being selected by different environmental pressures, the outcome of which is the disease leprosy. Moreover, relatively few studies have explored the physiological relevance of the associated genetic variants to susceptibility/resistance in leprosy.

The immune system and leprosy

Innate immunity
The innate immune response is composed principally of phagocytic cells and is capable of immediately recognizing and responding to microbial invasion. While professional phagocytes such as macrophages and neutrophils act as effector cells to resolve the infection, macrophages and professional antigen-presenting cells like dendritic cells (DC) are also crucial for the initiation of adaptive immunity and the generation of specific immunity.

Macrophages
Macrophages play a crucial role in host defence against intracellular pathogens including M.leprae. LL leprosy is characterized by the accumulation of macrophages which are unable to restrict the multiplication of M.leprae. Attempts to induce activation of LL macrophages by local administration of antigens or IFN-γ have proven to be ineffective [138]. In contrast,
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within TT lesions, hardly any bacteria are seen within macrophages [34]. In recent times, fundamental differences have been reported in the macrophages from LL and TT lesions apart from differences in functional capacities [139; 140]. Expression of toll-like receptor (TLR)-2 and 1 are stronger in macrophages from TT than LL [139]. On the other hand, macrophages from MB lesions reportedly expressed higher levels of DC-SIGN which was suggested to be associated with a Th2 environment in these lesions [140]. Thus, the MB lesions represent a progressive reduction of Th1 activation resulting in a state of antigen-specific tolerance [141; 142]. Macrophages in LL lesions may downregulate CMI by reducing antigen-presenting function and secreting T-helper (Th) 2 cytokines or by secreting suppressive factors [142; 143]. In this regard, adherent cells from MB lesions have been shown to reduce proliferation of lymphocytes from healthy individuals through secreted factors [143]. Similarly, monocytes from LL patients were shown to be involved in the IL-10 and PGE-2 mediated T-cell suppression [142]. Recent advances have suggested a dichotomy of macrophage phenotypes mimicking the Th1-Th2 paradigm of the T-cells with classical activation of the macrophage by microbial products leading to a pro-inflammatory phenotype (MΦ1) while alternative activation may result in an anti-inflammatory phenotype (MΦ2) characterized by IL-10 as the hallmark cytokine [144]. Using in vitro generated MΦ1 and MΦ2 from healthy donors, Verreck and co-workers showed that while mycobacteria can grow in both macrophage subtypes, they induce IL-23 in MΦ1 in contrast to IL-10 in MΦ2 [144]. These results suggest an inherent plasticity of the human macrophage component which along with active interference by pathogens in macrophage signalling pathways may critically affect host defence against mycobacteria. In other studies, M.leprae infected macrophages were refractory to IFN-γ induced activation [145; 146] and manifested aberrant effector functions including impaired microbicidal capacity, decreased oxidative state and lower MHC Class II expression which was related to the length of the infection [146; 147]. A similar phenotype was also associated with M.tuberculosis infection of macrophages where it was shown to be a consequence of disrupted signal transduction, impaired CD64 expression and IL-6 induction [148; 149; 150]. However, although such a mechanism might be plausible in leprosy, evidence for such a hypothesis is yet to be presented.

Neutrophils

Although neutrophils are generally the first cell type to reach the site of inflammation, the chronic course of leprosy is characterized by an absence of neutrophils in situ. ENL lesions, however, show a massive infiltrate of polymorphonuclear (PMN) cells, especially within 72 hours after onset of reaction, suggesting a functional role in this phenomenon [60; 151]. Oliveira et.al.[151] showed that apoptosis of PMNs is significantly increased in
ENL patients. Furthermore, PMNs could be stimulated by *M. leprae* and *M. leprae* derived-lipoarabinomannan (LAM) to secrete TNF-α and IL-8 [151]. This suggested that PMNs could contribute to the recruitment of lymphocytes to the lesional sites and also to the TNF-α induced tissue damage. However, at present, it is unclear if a more significant role is associated with neutrophils in the pathogenesis and the immune response in leprosy.

**NK cells**

Similar to neutrophils, the role of NK-cells *in vivo* in leprosy patients is not well established. One of the earliest reports observed no significant difference in NK cell activity between lepromatous and borderline leprosy and healthy individuals [152]. However, a depression of NK cell activity was reported in LL patients with ENL which appeared to be a result of monocyte activity but not due to NK cell dysfunction [152]. In contrast, a later study reported lower NK activity in untreated and non-reactional lepromatous leprosy patients compared to healthy controls [153]. However patients with RR had normal NK activity whereas no significant difference was found between RR and non-reactional BT patients with respect to NK activity [153]. Similarly, Chipulkar *et al.* [154] also observed that NK cells from untreated and MDT treated LL patients were less effective in mediating antibody-dependant cell cytotoxicity (ADCC) compared to TT patients or healthy controls. Treatment of the NK cells with IL-2 or IFN-α was shown to increase cytotoxicity in these patients [154]. Similarly, intradermal administration of IL-2 in LL patients resulted in an 8-fold increase in the NK-cells in these patients, along with increased T-cell and monocyte infiltration into the dermis, upgradation of the lesional infiltrate to a granulomatous tuberculoid type but no reversal in the anergy to *M. leprae* antigens [155]. Viable *M. leprae*, on the other hand, were found to confer resistance to NK-cell mediated killing on macrophages and Schwann cells probably by a mechanism involving heat shock protein (hsp) induction although this was not further elaborated by the authors [156]. In contrast, heat shock protein 10 could induce cytotoxic activity in normal individuals and PB patients, whereas MB patients showed variable lytic activity depending on bacterial load [157]. This was related to the ability of hsp10 to induce both Class I and Class II restricted effector αβ and γδ T cells and/or CD16^+^CD56^+^ NK cells in PB patients, normal individuals and bacteriologically negative MB patients. In contrast, only Class II restricted CTL activity was observed in bacteriologically positive MB (MB+) patients [157]. Moreover, IL-4 generated in MB+ patients may play a crucial role in downmodulating the activity of the CTLs and NK cells by antagonizing IL-12 induced IFN-γ production [157]. Another important cytokine, which, along with IL-12, plays an important role in induction of IFN-γ production, is IL-18 [158; 159]. IL-18 mRNA expression is elevated in PBMCs of TT but not LL patients stimulated with *M. leprae*, which
also correlated with expression in lesions [158]. Moreover, IL-18 induced IFN-γ production in PBMCs from TT but not LL patients [158] and this could be reduced by 50-60% with anti-IL-12 antibodies suggesting a collaborative role for these cytokines in inducing an IFN-γ response [158; 159]. However, in contrast to the results from Garcia et al. [158], the later study [159] reported that IL-18 was a strong inducer of IFN-γ in response to hsp65 in LL patients which may be related to the different antigens used in the two studies. NK cells were involved early (within 24 hrs) in the IFN-γ response while at later intervals (48 hrs to 5 days) CTLs played a more significant role [158; 159]. De la Barrera et al. [159] elaborated these results further showing that endogenous IL-13 production by NK cells and T cells in MB patients resulted in a decreased CTL activity and IFN-γ production in these patients. They further speculated that the presence of IL-4 in MB patients may lead to accumulation of IL-13+ NK cells due to an impaired maturation of NK cells or a decline in the IFN-γ+ NK cells [159].

**Mast cells**

As with other aspects of immunity in leprosy, various studies have presented contradictory results of the role of mast cells in leprosy [160]. Higher numbers of mast cells were found in lepromatous as compared to tuberculoid leprosy by some researchers [161; 162] while the opposite was reported by Cree et al. [163]. Antunes et al. [160] speculated that an immunohistochemically observed increase in the tryptase-positive subset of mast cells as compared to the tryptase- and chymase-positive subset in reactional lesions may be related to the onset of the reaction. However these observations have not been validated by other reports and the role of specific mast cell subsets in reactions and in chronic leprosy remains enigmatic.

**Complement**

The complement system (CS) represents an important first line of defence in resistance to pathogens and is involved both in recognizing pathogens and in inducing inflammation [164]. Initiation of the CS results in a series of protein-protein interactions, some involving enzymatic activation of key CS proteins, with the release of biologically active peptides and the deposition of fragments on target cells. The activation of complement through either the classical, lectin or alternative pathway converges on the deposition of C3b on the pathogen surface, a key event in complement mediated lysis. The activation of the CS also results in the release of small peptides such as C3a and C5a derived from the cleavage of CS proteins which have important biological properties such as mast cell degranulation and
release of vasoactive amines resulting in vasodilatation and also chemotaxis of immune cells. Deposition of C3b on the pathogen/target cell initiates a cascade resulting in the assembly of the membrane-attack complex (MAC), forming pores on the surface of the target cell and its eventual osmotic death.

A recent study reported on the levels of complement components and the total complement haemolytic activity in the sera of leprosy patients [165]. A reduction in complement haemolytic activity was observed in the sera of LL patients as compared to TT, dimorphous leprosy (DL – including the borderline phenotypes) and healthy controls. Moreover, while a lower level of C4 was observed in LL patients, a lack of significant difference in the different leprosy patient groups in a haemolytic assay using non-sensitised rabbit erythrocytes under conditions favouring activation of the alternate pathway of complement activation suggests a greater role for the antibody-dependant classical or the lectin pathway in leprosy [165]. The lectin pathway of complement activation involves direct interaction of mannan-binding proteins/lectins (MBP/MBL), which have a structural similarity to C1q of the classical pathway, with carbohydrates on the target cell leading to C3 cleavage. Interestingly, the highest median levels of mannan-binding lectin (MBL) were observed in the sera of LL and DL patients which had lower haemolytic activity whereas TT patients with lower MBL levels showed higher haemolytic activity [165]. These results are in concordance with previous reports of increased protection against leprosy [166; 167] and tuberculosis associated with MBL deficiency in the sera [168]. Similarly, a recent genetic analysis showed the association of haplotypes/compound genotypes resulting in low levels of MBL with protection against development of LL and BL [169]. Moreover a strong association was found between the haplotype LYPA, associated with high MBL protein levels, and leprosy per se and its LL and BL forms with a 2-fold increased risk of developing the MB forms of the disease [169]. Functionally, MBL has also been shown to bind *M. leprae* and enhance phagocytosis and uptake of mycobacteria *in vitro* [166; 170] suggesting a role in uptake, pathogen spread and establishment of leprosy.

Opsonization of microbes by complement protein components promote their uptake by complement receptors on phagocytic cells such as monocytes, macrophages, neutrophils and B cells [171; 172]. CR1, CR2 and CR3 show specificity for partially cleaved C3 fragments which are deposited on the microbial cells on activation. With respect to leprosy, whole *M. leprae* bacilli incubated with human serum as well as circulating immune complexes from leprosy patients [173] have been shown to activate the complement *in vitro*. As a consequence, the bacteria are opsonised by C3 fragments and taken up by monocytes expressing CR1 and CR3 [174].
Dendritic cells: the bridge between innate and adaptive immunity

Figure 2 Dendritic cells act as bridges between innate and acquired immunity by recognizing conserved sequences called pathogen associated molecular patterns (PAMPs) on pathogens through pattern recognition receptors (PRRs). This information is then transmitted on to naive T cells together with co-stimulation and a polarizing signal resulting in the development of the different T cell subsets.

To activate or to suppress

Dendritic cells (DCs) are the sentinels of the innate immune system, sensing pathogen and host derived signals through various pattern recognition receptors (PRRs) to influence the subsequent development of innate and adaptive immune responses [175]. DCs are the main antigen presenting cells involved not only in T cell priming but also in establishing tolerance even in mature T cell compartments [175; 176; 177]. How antigen presentation selects between productive immunity and the non-responsiveness associated with tolerance induction is still a matter of considerable debate. Two important schools of thought prevail. Whilst it has been suggested that this choice is determined by the maturation state of the DC [178; 179], other reports have shown evidence to the contrary [176]. DC subsets have been identified which respond to pathogenic antigens by differential production of cytokines [180]. However whether the DC subsets are differentially selected between immunity and tolerance or act at a more subtle level of modulation of type 1 or type 2 helper T cell responses is at present a matter of considerable debate.

Receptors associated with antigen recognition

The most extensively studied PRRs is the TLR family comprising 10 members (in humans) with different ligand specificities [181]. LPS is known to signal through TLR4 whereas
mycobacterial lipopeptides signal through TLR2 [182]. TLRs can recognize antigens like mycobacterial lipoarabinomannan (LAM) including uncapped (AraLAM), mannose-capped or phosphoinositide-capped LAM (PILAM), the 19-kDa lipoprotein, soluble tuberculosis factor (STF) and whole mycobacteria [183;184]. Mycobacterial PILAMs induce production of TNF-α and IL-12 in DCs [185] whereas phosphatidylinositide dimannoside (PIM), abundantly found in M. bovis, BCG and M. tuberculosis, was shown to activate macrophages through TLR2 to secrete TNF-α [186]. Another major immuno-stimulatory component of M. tuberculosis, the 19-kDa lipoprotein was shown to trigger TLR2 to activate NF-κB and secrete IL-12 [187]. Tri-acylated lipopeptides, representing the 19- and 33-kDa lipoproteins of M. leprae, can also activate DCs through TLR2 [139].

Recent data seem to suggest that distinct TLRs may be associated with specific groups of the leprosy spectrum. Expression of TLR2 and TLR1 was found to be much stronger in skin lesions from the resistant tuberculoid form of leprosy as compared to the lepromatous form [139]. On the other hand, an Arg677Trp mutation in the intracellular domain of hTLR2 resulting in an impairment of NF-κB activation was associated with subset (22%) of lepromatous leprosy (LL) patients from Korea [126;188].

Other receptors such as the C-type lectin DC-SIGN are important in the binding to mycobacteria and viruses such as HIV [189]. DC-SIGN may also be involved in suppression of TLR mediated inflammation in response to mycobacterial infections [189]. DC-SIGN has been shown to bind to the mannose-capped cell wall component lipoarabinomannan (ManLAM) of slow growing virulent mycobacteria such as M. tuberculosis and M. leprae [190] triggering production of IL-10 and inhibiting IL-12 and TNF-α production by DCs or monocytic cell lines [189; 191; 192]. In another study, M. tuberculosis ManLAM could also induce production of TGF-β, another immunosuppressive cytokine, by human monocytes [193]. Recently, ManLAM was shown to block BCG mediated maturation of DCs and this effect could be reversed by antibodies specific for DC-SIGN [191]. This suggests that pathogen recognition through TLRs or DC-SIGN could have opposing effects on DC maturation [189].

In contrast, other C-type lectins such as dectin-1 can act together with TLR2 to enhance the production of IL-12 and TNF-α by DCs facilitating a Th1 response [194]. This suggests that the inflammatory responses may be fine-tuned by the balance between TLR and C-type lectin activation. Recent studies have also showed association of DC-SIGN with lepromatous leprosy (LL) involved both in the uptake of M. leprae and the Th2 environment associated with LL [140; 195]. Other receptors on immature DCs such as the mannose receptor (MR) [196], CD11b and CD11c [197] were reported to mediate to mediate binding of mycobacteria to macrophages [198; 199] and probably DCs.

It is estimated that 25% of circulating monocytes differentiate into migrating DCs whereas others give rise to resident macrophages [200; 201]. It was recently reported that in
vitro incubation of inflammatory monocytes with *Salmonella typhimurium* blocked nearly 50% of their differentiation into DC [202]. Another study reported that pre-incubation of monocytes with BCG did not affect their differentiation into DCs, but resulted in IL-1β mediated blocking of their further maturation (CD83 and CD86 expression) and IL-12p70 production by these DC’s [203]. However, pre-incubation of monocytes with *M.leprae* cytosolic protein up to a concentration of 1µg/ml failed to block maturation of DCs and the production of IL-12p70 [203]. In contrast, our preliminary results showed that pre-incubation of monocytes with *M.leprae* sonicate antigen (MLS) blocked differentiation to CD1a+ DCs and further maturation in response to LPS stimulation. The effect was not so pronounced with LPS or BCG. A recent study [195] showed that activation of human monocytes through TLR2/1 heterodimer induced their rapid differentiation into two distinct subsets: DC-SIGN’CD16+ “macrophages” and DC-SIGN’CD1b+ “DCs”. The latter induced stronger T-cell proliferation and IFN-γ production as compared to the DC-SIGN’CD16+ cells. Moreover, the *in vivo* association of DC-SIGN+ cells predominantly with LL lesions and the CD1b+ cells with TT and reversal reactions suggested an association with the spectral pathology of leprosy [195]. Similarly, DC-SIGN was reported to be associated with Th2 environment associated with LL in another study [140]. A subsequent study [204] reported that macrophage derived DCs (MACDC) were more efficient than monocyte derived DCs in stimulating T cell responses at low bacterial multiplicity of infection (MOI) with *M.leprae* [204]. Hence the origin of DCs may also influence the subsequent response to infection.

The presence of CD1+CD83+ mature DCs in tuberculoid leprosy lesions suggests a role for DCs in the immune response against leprosy [97; 195]. In *in vitro* cultures integral *M.leprae* caused down-regulation of MHC class I and II on DCs and induced expression of the maturation marker CD83 only at very high bacterial doses [205]. Furthermore, *M.leprae* was less efficient in DC-mediated induction of T cell responses as compared to *M.bovis* BCG or *M.avium* [205]. In contrast, our preliminary observations showed strong induction of DC maturation by *M.leprae* whole sonicate (MLS). Moreover, these mature DCs were able to induce proliferation of autologous T cells and could produce IL-12 on CD40 ligation. Other groups studying different sub-cellular fractions of *M.leprae* found the cell wall fraction to be toxic to DCs while the cytosol fraction was less efficient in comparison to the membrane fraction in stimulating DCs [206]. The cell membrane fraction, on the other hand, was shown to upregulate MHC Class II and CD86 expression in DC. Furthermore, it could induce strong IFN-γ production in CD4+ and CD8+ T cells and perforin production in *M.leprae* specific CD8+ cytotoxic T lymphocytes [206].
The adaptive immune system

The adaptive immune response requires the specific recognition of foreign antigens which activates the humoral response, resulting in B-cell maturation and antibody production, and the cell-mediated mechanisms, focussing mainly on T-cell activation. These divergent arms of the host response to pathogens are not mutually exclusive as T-cell help is required for antibody maturation and isotype switching while B cells can function as antigen-presenting cells in the induction of specific T cells.

Humoral Immunity (HI)

The study of the humoral immunity in leprosy has largely been restricted to antibodies in immunodiagnosis or monitoring of leprosy and are dealt with in the subsequent sections. Although the in situ presence of plasma cells and B-cells has been reported in leprosy [207; 208; 209], not much is known of the relation to the pathology of leprosy lesions. Recently, the presence of B-cells were reported in lesional skin from both the lepromatous and tuberculoid regions of the leprosy spectrum and this was associated with the detection of *M.leprae*-specific antibodies in supernatants of organotypic cultures of the corresponding lesional biopsies [209]. It was speculated that these lesional B-cells could influence T cell-responses and/or play a role in maintaining the inflammatory reaction in leprosy partly through the local secretion of antibodies [209]. However, functional data supporting such a hypothesis are lacking.

It is generally thought that antibodies against *M.leprae* components do not play a significant role in protection against leprosy. However antibodies may play a role in the uptake of *M.leprae* by mononuclear phagocytes and hence the pathogenesis of the disease [199]. Natural antibodies from non-immune serum were found to be critical for the binding of complement component C1q to PGL-I and the fixation of C3 to *M.leprae* [199]. Previously, C3 was shown to be fixed to PGL-I on *M.leprae* and mediate its uptake through complement receptors on mononuclear phagocytes [174; 210; 211]. These studies together showed that natural antibody promoted complement receptor-mediated uptake of *M.leprae* by host cells. This also suggests that the natural antibody repertoire of an individual may determine the predisposition to leprosy and may even determine the form of leprosy [199]. In this regard, Caron and Hall [89] showed that uptake of IgG-opsonised targets by FcγRII activated the Cdc42, Rac, Rho cascade of Rho GTPases triggering an inflammatory response and respiratory burst within macrophages. In contrast, uptake of complement C3bi-opsonised organisms through CR3 activates only Rho, but not Cdc42 and Rac, with a resultant lack of inflammatory response which might be a plausible mechanism in mycobacterial infections like leprosy.
T-cell subsets

The induction of a specific T-cell response occurs primarily in the peripheral lymphoid organs, the lymph node and the spleen, where circulating naïve T cells from the blood come in contact with antigens presented by APCs such as dendritic cells. CD4+ T cells which are the dominant players in both the induction and effector phases of the adaptive immune response are activated by presentation of peptides derived from exogenous antigens in the context of MHC Class II molecules. These cells differentiate into T-helper (Th) cells which carry out their effector functions by producing distinct patterns of cytokines as is discussed subsequently. CD8+ T cells comprise the other arm of the effector response of the CMI which recognize antigenic peptides in the context of Class I MHC. Effector CD8+ T cells, also called cytotoxic T lymphocytes (CTL), exert antimicrobial effects either by secretion of cytokines such as IFN-γ and TNF activating T cells or by direct mechanisms such as the perforin/granzyme pathway-mediated lysis of pathogen-infected cells. However, at least a subset of the CD8+ T cells was considered to be of the “suppressor” (Ts) phenotype [212; 213; 214; 215], although, in practice, CTLs and Ts were distinguished primarily on the basis of their phenotype since with the exception of, possibly, the CD28 marker, no specific surface markers have been described for these two subtypes [215; 216].

Histopathologically, CD4+ T cells are the predominant subset in TT leprosy whereas the majority of the T lymphocyte population in LL leprosy are of the CD8+ subtype [212; 213; 214]. Moreover, CD4+ T cell clones from TT individuals proliferated in response to M.leprae antigens whereas CD8+ T cell clones from lepromatous individuals did not proliferate but suppressed the proliferation of CD4+ cells [215; 217; 218].

Cell-Mediated Immunity (CMI)

Figure 3. Naïve T cells may develop into various T cell subsets under the influence of polarizing signals (Signal 3, in grey). Furthermore, cytokines secreted by the T cell subsets may act in autocrine or paracrine manner to reinforce the polarization in addition to their effects on other cells.
Effector mechanisms of cell-mediated immunity: The Th1/Th2 paradigm

Since the initial description of T-helper 1 (Th1) and Th2 patterns of cytokine production in mouse CD4+ cell subsets [219] and in humans [220; 221] attempts have been made to delineate all immune responses in disease along these lines. In general, Th1 cells are thought to be involved in CMI-DTH reactions and are important in responses against intracellular pathogens. In contrast, Th2 cytokines encourage antibody production, particularly IgE responses, enhance eosinophil proliferation and are effective against extracellular pathogens, parasites and in development of allergic responses [221]. Although the proper balance of Th1 and Th2 immunity is as important for the success of an immune response as its specificity and overall magnitude, it still remains unclear how the Th1/Th2-response profile is matched to distinct pathogens and to particular affected tissues [177]. Leprosy, which displays a spectrum of immune responses ranging from a strong CMI at the TT pole to an almost exclusively humoral response at the LL pole, has been analysed as a model system to study the Th1/Th2 paradigm. Yamamura et al. [222] demonstrated an increased expression of T-helper 2 (Th2) cytokines interleukin-4 (IL-4), IL-5 and IL-10 in skin lesions from LL patients at the mRNA level. In contrast, “protective” Th1 cytokines IL-2, IFN-γ and TNF-α are associated with TT leprosy [222]. Contemporary studies with M.leprae responsive CD4+ T-cell clones (TCC), generated from peripheral blood or lesional skin of polar TT patients showed a distinct Type 1 cytokine secretion profile, producing IL-2, IFN-γ and GM-CSF, but little or no IL-4 and IL-5 [215; 223]. In contrast, M.leprae non-responsive CD4+ TCC produced little IL-2 and IFN-γ but showed a Type 2 profile secreting IL-4, IL-5 and GM-CSF [215]. Interestingly, polar LL patients showed a subset of IL-4-producing CD8+ clones which suppressed proliferation of M.leprae responsive CD4+ clones in an IL-4-dependant manner [215]. Immunohistochemical studies on cytokines in leprosy lesions showed significantly higher expression of IFN-γ in tuberculoid lesions but not lepromatous lesions [224; 225]. The latter studies, however, need to be interpreted cautiously since the specificity of some of the antibodies used may not be optimal [226]. The seemingly clear cut relationship between Type 1- and Type 2-like T cell phenotypes and the polar forms of leprosy has however been disputed subsequently with studies reporting that almost half of the patients showed co-expression of both IL-4 and IFN-γ irrespective of clinical classification [227; 228; 229]. It must be noted that these latter studies involved bulk peripheral blood mononuclear cells stimulated with various M.leprae antigens and may not necessarily reflect the situation of cells at the clonal level or within lesions. Our own studies on in situ cytokines within borderline leprosy lesions showed the presence of both IFN-γ and IL-4 mRNA and protein [unpublished observations]. RR lesions on the other hand showed increased expression of both cytokines while in ENL, the IL-4 expression was relatively higher than IFN-γ [unpublished observations]. At the clonal level, apart from the polarised Type 1- or Type 2-like phenotypes, a significant
A proportion of the lesional *M. leprae*-responsive TCC showed a Type 0-like cytokine profile, producing both IFN-γ and IL-4 [230]. This shifted to a polarised Type 1-like phenotype during the development of RR, whereas, the *M. leprae* non-responsive T cells from the same lesions were heterogeneous consisting of either Type 1-, Type 0- or Type 2-like phenotype [230; 231]. The above results suggest that although there is some evidence for association of polarized Type 1 or Type 2 responses with the leprosy spectrum especially in isolated clonal T cell populations, such distinctions may be less discernable *in vivo* due to the complexity of the induced immune response.

**Regulatory/suppressor cell responses in leprosy**

In addition to the “classical” functional subsets of CD4+ T cells, two additional populations have attracted growing attention, the T-regulatory (Treg) cells and the IL-17 producing Th cells. Tregs represent the anti-inflammatory or suppressive group of CD4+ T cells which may be divided into two main groups: the adaptive Tregs consisting of the IL-10 producing Treg 1 (Tr1) cells and the TGF-β-producing Th3 cells, and the naturally occurring CD4+CD25+ Tregs [232]. Tr1 and Th3 cells are the primary mediators of anti-inflammatory responses against exogenous antigen such as those associated with mucosal immunity. Tr1 and Th3 cells develop through the differentiation of uncommitted naïve Th precursors whereas exposure of CD4+CD25- naïve T cells to TGF-β can mediate their transition towards a CD4+CD25+ regulatory T cell phenotype with similarly potent immunosuppressive qualities, and are more often associated with tolerance to self antigen [232; 233]. While Tregs require antigen specific activation to carry out their effector function, their suppressive effects are mediated in a non-specific manner inhibiting both innate and adaptive immune responses.

Although studies on the association of the “classical” regulatory T cell phenotypes with leprosy are lacking, a *M. leprae* specific suppression of effector cell responses has been described in LL [234]. One of the early studies to report active immunosuppression in leprosy observed the suppression of proliferative responses to Con A in the presence of lepromin in LL and BL patients which was attributed to adherent monocytes and Tγ cells [235]. A different set-up was used by Nath *et al.* [236] who studied suppression of lymphoproliferative responses to ConA by *M. leprae* specific lymphocytes from leprosy affected HLA-D identical siblings. Interestingly, lymphocytes from TT but not LL patients whereas macrophages from LL but not TT patients suppressed lymphoproliferation in HLA-D identical normal siblings [236]. Stoner *et al.* [234] demonstrated the specific suppression of lymphoproliferative responses to *M. leprae* antigen with some degree of cross reactivity to BCG but not to an unrelated antigen in healthy contacts of leprosy patients. They suggested T-suppressor (Ts) cells as possible candidates associated with this suppression, although they did not directly
demonstrate presence of such cells in their cultures. In a histopathological study, Van Voorhis et al. [212] showed that the T cell infiltrate in LL lesions consisted almost exclusively of OKT8/Leu-2a cells which they considered as “suppressor” T cell populations in contrast to the predominance of OKT4/Leu-3a helper T-cells in TT lesions. This skewed distribution of T cell subsets was also demonstrated in subsequent studies [213; 214]. However no attempt was made in the above mentioned studies to demonstrate the “suppressor” phenotype of the T cell subset in question. Using _M.leprae_ stimulated Th and Ts clones from a BL patient, Ottenhoff et al. [237] showed that Ts clones suppressed responses of Th clones to _M.leprae_ and other mycobacteria but not to unrelated antigens or mitogens demonstrating the specific nature of the suppression. Subsequent studies however demonstrated that the CD8+ population consisted of distinct subsets which may either be cytotoxic or mediate suppression through IL-4 [215]. Apart from the suppressor role of CD8+ subsets, the induction of CD4+ suppressor T cell subsets by a specific hsp65 peptide (between amino acids 439-448) – HLA-DRB1*1503 combination was also demonstrated indicating that specific peptide-HLA Class II combinations could exclusively activate Ts cells [238]. Despite these indications, the presence and activity of the conventionally accepted Tr1, Th3 and Treg subsets has not been conclusively demonstrated thus far.

**Th17 cells in leprosy**

Recently, a subset of non-classical IL-17 producing Th cells were shown to contribute to the development of some Type 1 organ specific autoimmune [239] inflammatory or allergic diseases including systemic lupus erythematosus [240]. These cells may represent a separate lineage of Th cells distinct from either of the classical Th1 or Th2 subsets [241; 242]. IL-17 is a member of a family of cytokines with at least 6 members in the human genome, including IL-17, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F [243]. IL-17 is considered a pro-inflammatory cytokine because it increases IL-6, IL-8, nitric oxide, TNF-α and IL-1β production by various cell types [244; 245; 246; 247; 248]. In addition, IL-17 elicits the secretion of granulocyte-colony stimulating factor (G-CSF) and CXC chemokines that stimulate granulopoiesis and recruitment of polymorphonuclear neutrophils into tissues [249; 250]. IL-17 was expressed in psoriatic lesions; a disease associated with increased expression of Type 1 cytokines [251]. Interestingly, with respect to leprosy, preferential expression of IL-17 mRNA was seen in association with LL lesions [251]. This probably suggests that IL-17 is secreted by Type 0, Type 1 and Type 2 T cells [245; 246]. An alternative explanation could be the lack of specific production of Th1 cytokines such as IFN-γ in LL patients which are involved in the reported suppression of IL-17 [252]
Cross-talk between the cells

Cytokines and chemokines as signaling molecules

The generation and maintenance of immune responses is controlled by a network of small, non-structural, intercellular regulatory proteins, the cytokines and chemokines, which mediate multiple immunologic and non-immunologic functions and are involved in the cross-talk between the different cells of the immune system. These molecules play a crucial role in the recruitment of the immune cells, the clonal expansion of lymphocytes as well as in the innate immune response and the effector response of most immune cells. This results in a complex, fine-tuned regulatory network of cytokines which often determines the clinical course of the infection and the outcome. The role of cytokines in immune regulation and inflammation is fairly well studied as is the role of inflammation as a driver of pathology.

Cytokine cascades and their role in leprosy pathology and immunity

With respect to leprosy, research has focussed on the association of differential cytokine profiles with the spectral pathology [215; 222; 253]. However results from the studies have been varied and conflicting and in retrospect it is difficult to associate distinct cytokine patterns with different spectral forms of leprosy or reactions. Initial studies looked at the association of leprosy spectral pathology with the Th1-Th2 cytokine profiles [215; 222; 253], which has been described in detail in the preceding sections. However later studies increasingly demonstrated the lack of a clear cut dichotomy in the cytokine profiles associated with the leprosy spectral forms and reactions [227; 228; 229; 230; 231]

Analyses of leprosy sera showed increased expression of cytokines (except IL-2 in all patients, IFN-γ in LL patients and IL-10 in TT patients) as compared to healthy controls [254; 255] suggesting activation of the immune cells by M.leprae antigens in all leprosy patients. IFN-γ and TNF-α were elevated in TT patients as compared to LL patients which also showed a significant negative correlation with BI in these patients [254]. These results correlated with in vitro studies showing that a vast majority of the T cells in tuberculoid leprosy are of the CD4+ subset and of the Th1 phenotype producing IFN-γ, IL-2 and TNF-α but little or no IL-4, IL-5 and IL-6 on in vitro stimulation with M.leprae or its antigenic fractions [215; 223; 256]. Furthermore, in vivo analyses showed the presence of cytokine mRNA for IFN-γ, IL-2, lymphotoxin (LT), TNF-α and GM-CSF [222; 253; 257] whereas, in mice, mRNA for IFN-γ, IL-2 and TNF-α is detected in the lymph nodes draining the site of vaccination with killed M.leprae [258]. This suggested that mycobacteria preferentially induce Th1 cells for an effective response in both humans and mice. Interestingly, no significant difference was found between levels of IL-2 in TT and LL patients in serum [254], in contrast to the results...
using PCR and in vitro stimulation of PBMCs.

TNF-α is considered to be crucial in host defence against intracellular pathogens [259] and anti-TNF-α antibodies have been shown to suppress CMI in vivo [260]. Moreover, TNF-α was shown to be necessary in granuloma formation and mycobacterial elimination [261]. LL patients showed higher levels of IL-10 and IL-1β as compared to TT patients [254]. In vivo studies have demonstrated the predominance of IL-4, IL-5 and IL-10 in LL lesions previously [222]. IL-10 has been shown to suppress macrophage-mediated destruction of intracellular microorganisms [262]. This was in line with the positive correlation between IL-10 levels and BI in LL patients [254]. Interestingly, IL-4 was reportedly one of the important cytokines in LL patients which induced a suppression of M.leprae induced immune reactivity of CD4+ T cell clones in vitro [215]. However such a suppressive activity of IL-4 has not been confirmed by other studies [256], although IL-4 has been shown to play a role in the Th2 polarization of CD4+ T cells [263; 264].

**Recombinant cytokines as an adjunct to MDT in leprosy**

Since activation of macrophages is important in killing of the intracellular M.leprae, the role of macrophage activating cytokines like IFN-γ have been studied in this respect. Initial studies suggested that the defective CMI in LL was caused by defective IFN-γ activity in these patients [224; 265]. This defective activity was rather a result of the failure of lymphocyte to produce macrophage activation products in response to M.leprae rather than the inability of macrophages to get activated by these products [266; 267; 268]. This was supported by experiments where monocytes from LL patients could inhibit growth of Legionella pneumophila when treated with supernatants of Con A activated lymphocytes suggesting that monocyte-activating molecules like IFN-γ from these cultures could activate the LL monocytes normally [266]. Further direct evidence was provided by a study in which in vitro monocyte-derived macrophage cultures from LL patients responded to treatment with recombinant human IFN-γ (rhIFN-γ) in the presence of M.leprae by producing H₂O₂ to the same extent as normal healthy donors [267]. Intralesimal injection of rhIFN-γ caused an accumulation of T-cells predominately of the CD4+ helper phenotype and monocytes at the site which was increased by a second injection of the cytokine, although this could not conclusively be attributed to a direct effect of the IFN-γ [138]. Interestingly, prolonged treatment with rhIFN-γ induced ENL in 60% of the LL patients as compared to 15% of the patients receiving MDT alone [269]. In vitro analyses showed a 3 - 7.5 fold increase in IFN-γ induced TNF-α production by LL patient monocytes which could be suppressed by thalidomide [269]. Similarly, low dose inoculation of rhIL-2 intradermally had clear systemic effects including an elevated number of circulating mononuclear cells, generalized
infiltration into the skin, and subsequent rapid five-fold increase in clearance of *M. leprae* bacilli as compared to MDT alone, without significant side-effects [270]. Considering the reported beneficial effects of these recombinant cytokine therapies, it is surprising that such a line of therapy has not been pursued further.

**Chemokines in cell migration and tissue immunity in leprosy**

Chemokines are potent chemoattractants of various leukocyte subsets and play an important role in migration of effector cells in inflammatory diseases such as tuberculosis [271], sarcoidosis [272], cutaneous leishmaniasis [273] and psoriasis [274]. However, not much is known about the chemokine profiles in tissues and in circulation in leprosy patients. Some of the early studies looked at the induction of IP-10 (CXCL10) on local administration of recombinant human IFN-γ or PPD in LL lesions [275]. Although LL lesions did not express IP-10 constitutively, administration of PPD or IFN-γ into the lesions resulted in a strong induction of IP-10 expression especially by keratinocytes followed by monocytes, fibroblasts and endothelial cells. In contrast, TT lesions and lesions of cutaneous leishmaniasis showed intense IP-10 expression by keratinocytes and infiltrating cells suggesting a differential expression of IP-10 across the leprosy spectrum associated with IFN-γ expression [275]. A subsequent study showed IP-10 to be important in the migration of monocytes and activated T-lymphocytes and also enhanced T-cell adhesion to endothelial cells [276]. However, in contrast to the chemokine RANTES (CCL5), which was a chemoattractant for unstimulated and stimulated T cells, IP-10 appeared to be specific for the CD4<sup>+</sup>CD29<sup>+</sup> activated T cell subtype [276] suggesting a distinct role in inducing migration of these cells to the site of inflammation. More recently, Kirkaldy *et al.* [277] studied the expression of the chemokines MCP-1 (CCL2), RANTES and IL-8 (CXCL8) within leprosy lesions across the spectrum by *in-situ* hybridization (ISH). Although all chemokines were elevated in leprosy lesions, no differences in the level of expression were noted across the spectrum. However, MCP-1 and RANTES were elevated in reversal reactions as compared to non-reactional leprosy suggesting a role for these chemokines in migration and activation of the monocytes and T-lymphocytes in these lesions [277]. In subsequent studies, MCP-1 and IL-8 were found to be elevated in circulation in LL patients [278; 279]. Hasan *et al.* [280] recently demonstrated elevated levels of MCP-1 in sera of LL patients as compared to endemic healthy controls (EC) or pulmonary tuberculosis patients which was probably related to the extent of dissemination of the disease. In contrast, RANTES levels were significantly lower in the LL patients as compared to EC or TB patients suggestive of a shift away from the Th1 phenotype of these patients [280]. Mendonca *et al.* [281] reported elevated levels of only CCL3 (MIP-1α) and CCL11 (Eotaxin) but not CCL2, CXCL9 or CXCL10 in leprosy patients as compared to non-
infected individuals in a Brazilian population. They suggested the utility of CCL11 monitoring in plasma as an aid to the diagnosis of leprosy patients from non-infected populations.

Treatment of leprosy and reactions

The present treatment of leprosy using multiple drug treatment (MDT) was in response to increasing rates of primary and secondary dapsone resistance and was based on the experience with tuberculosis. Over the years, the duration of intake of the drugs has been shortened from treatment to skin smear negativity [WHO, 1982] to the present fixed-duration treatment (FDT) [32; 53]. The present recommended standard regimen for MB leprosy is: rifampicin 600 mg. once a month, dapsone 100 mg. daily, clofazimine 300 mg. once a month and 50 mg daily for a duration of 12 months. The treatment regimen for PB is: rifampicin 600 mg. once a month and dapsone 100 mg. daily, whereas PB single lesion is treated with a single dose of rifampicin 600 mg., ofloxacin 400 mg. and minocycline 100 mg. (ROM) [35].

Prompt and adequate treatment of leprosy reactions is essential to avoid permanent damage to peripheral nerves and deformities. Prednisolone is widely used in the control of reactions both RR and ENL. RR and neuritis are treated with a standard 12-week course of prednisolone starting at 40-60 mg. daily and then in gradually reduced dosages every week till the end. Mild ENL is treated with non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin while severe ENL is treated with prednisolone as for RR. Alternatively, thalidomide is also used in the treatment of severe ENL although it is contraindicated in women of childbearing age due to its teratogenic effects. Clofazimine, which is a component of MDT is also effective against ENL although it is less potent than corticosteroids. The dose of clofazimine used is 300 mg. daily for a duration not exceeding 12 months [35].

Biomarkers in leprosy

Antibody detection in diagnosis and monitoring

Serology in leprosy has largely focussed on identifying markers that facilitate the diagnosis of cases with few or no clinical symptoms since early detection and treatment can significantly reduce the risk of deformities and disease transmission [282]. Antibody levels have been used as a surrogate marker of bacterial load in numerous studies with widely varying but positive correlation between seropositivity and BI [283]. A significant decrease in misclassification of MB patients was achieved by combining serology with lesion counting [284]. Moreover, since antibody levels decline with treatment at the rate of about 25-50% per
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year, measurement of serum antibodies may have some value in monitoring the treatment in leprosy [285; 286; 287; 288; 289].

Detection of serum antibodies directed against various *M. leprae* components have been evaluated for their utility in the diagnosis and monitoring of treatment in leprosy. The most widely used antigens include the *M. leprae* specific phenolic glycolipid (PGL)-I, the 35kD and the cross reactive antigen 85 complex (Ag 85). Since the demonstration of the species-specific PGL-I in the cell wall of *M. leprae* [290], various studies have shown seropositivity in 90% of untreated MB patients (BL/LL) and upto 50% of PB (BT/TT) patients while the proportion of seropositive healthy endemic controls was around 5-10% [282]. The demonstration by Brett *et al.* [291] and Fujiwara *et al.* [292; 293] that the major epitope of PGL-I was the 3,6-di-o-methyl-glucopyranosyl residue situated at the distal disaccharide region led to the development of test systems using synthetic analogues of PGL-I such as ND-o-BSA and NT-p-BSA. In general, the PGL-I ELISA is highly effective in diagnosis of MB patients (LL/BL) showing 90-100% sensitivity whereas its sensitivity for PB (BT/TT) patients is low [282]. Moreover a high variability in seropositivity rates for PGL-I antibodies in different studies may be a reflection of the different antigen types used (native antigen vs neo-conjugates), the criteria used to define a positive result and the inclusion of treated cases in the patient groups [282]. Modifications of anti-PGL-I antibody assays including the *M. leprae* gelatin particle agglutination test (MLPA) [294; 295] and the latex agglutination test (LAT) [296] were evaluated and found to have a good concordance with the conventional PGL-I ELISA. Recently, a simple and rapid immunochromatographic flow test was developed for detection of IgM anti-PGL-I antibodies in serum and whole blood samples from leprosy patients. The flow test was comparable to the PGL-I ELISA in detection of 97% MB patients and 40% PB patients whereas only 9.8% of endemic controls showed seropositivity [297]. However, apart from the PGL-I ELISA, which is used mainly in research settings, none of the above mentioned tests have been implemented widely.

The monoclonal antibody ML04 reacting against the My2a epitope on the 35kD antigen [298; 299], widely used in an inhibition ELISA [287; 300] or a serum antibody competition test (SACT) could detect almost 100% of active BL/LL patients, 40% tuberculoid patients while showing seropositivity in 10% of healthy contacts, It also correlated better with the BI and clinical score when compared with a PGL-I ELISA. A modified dipstick assay for the detection of anti-35kD IgG showed a higher sensitivity as compared to the anti-PGL-I dipstick assay although the latter demonstrated a higher specificity as compared to the former [301]. However, as with the *M. leprae* flow test, these dipstick assays were not developed further probably on account of the limited commercial value, as leprosy is still largely a disease associated with poverty [302].

With the sequencing of the complete *M. leprae* genome [303] a comparative genomics
approach was used to select *M. leprae* candidate antigens which had no known homologue in other mycobacteria and that contained peptide binding motifs covering more than 75% of the known HLA-DR alleles [304]. Five of the twelve selected candidate genes recognised by patient T cells induced significantly higher IFN-γ production in PB and reactional patients as compared to MB patients and negative controls. The additional value of the five post-genomic antigens lies in the ability to detect 71% of exposed healthy contacts which were not identified by the PGL-I IgM antibody assay. Such tests may facilitate development of new tools for the detection of specific *M. leprae* infection.

**DNA and RNA markers in leprosy**

Standard immunological and histological approaches for assessing leprosy have limited value for diagnosing new cases at early stages and for monitoring treatment whereas the availability of the *M. leprae* genome sequence has led to the development of molecular techniques which can reportedly confirm 40-50% of the cases missed by standard histology [305]. Several polymerase chain reaction (PCR) methods have been developed to amplify genes encoding various *M. leprae* proteins such as the 18kD [306], 36kD [307], 65kD [308], leprosy serum reactive protein (LSR) [309], rRNA [310; 311] and repetitive sequences [312]. These assays have been reported to be sensitive to 1-10 organisms and to be positive in 95-100% of BL/LL and 50-70% of TT, BT and I specimens. Using a combination of multiplex-PCR to amplify the 16S rDNA and reverse cross-blot hybridization technique with mycobacterial and species specific probes, Kox et.al. [313] could rapidly identify mycobacteria as the species level including *M. leprae* with a sensitivity of 97.9% and a specificity of 96.9%. However, comparison of conventional PCR with more recent quantitative real-time PCR techniques did not suggest a significant increase in detection of *M. leprae* using the latter approach [314; 315]. Other techniques such as reverse transcription (RT)-PCR and nucleic acid sequence-based amplification (NASBA) targeting 16S rRNA are reportedly useful in determining viability of *M. leprae* [316; 317].

**Cytokines as biomarkers**

Several studies have been carried out to assess the validity of measuring serum cytokines for detection and monitoring the leprosy spectrum and reactions. Many studies have presented contradictory results with respect to the predominant cytokines involved, which may be related to the different assay conditions, samples and populations examined [318; 319]. Moubasher *et.al.* [254] observed that while leprosy patients irrespective of the spectrum showed elevated levels of IL-1β and TNF-α as compared to healthy controls [254]
some degree of differential expression was noted with IFN-γ and TNF-α being elevated in TT as compared to LL sera whereas the opposite response was seen with respect to IL-10 and IL-1β respectively. Interestingly, although no significant difference was observed in IL-2 levels between the patients and controls, IL-2R was found to be elevated in LL as compared to TT patients [254]. Type I reactional patients showed elevated levels of IFN-γ, IL-2R and IL-1β as compared to non-reactional patients liable to such reaction [254; 320; 321; 322] whereas in Type II reactional patients, in addition to the above mentioned cytokines, IL-10 levels were also elevated [155; 223; 254]. Moreover, patients who developed reactions had significantly higher IL-1β levels as compared to those who did not, suggesting a prognostic value of IL-1b measurement in serum in predicting reactions [323].

**Markers of cellular activation**

Besides T cell cytokines, other indicators of cellular activation such as neopterin [324; 325], soluble CD27 (sCD27) [255], chitotriosidase [in this thesis] and acute phase proteins [69] have been used as a marker for cell mediated immune activity in leprosy and other inflammatory diseases. Neopterin belongs to the class of pteridines derived from guanosine triphosphate (GTP) due to the activity of GTP cyclohydrolase I coupled with a relative deficiency of 6-pyruvoyl tetrahydropterin synthase in human and primate macrophages [325]. Interferon-γ is the central stimulus for GTP cyclohydrolase I mediated production of neopterin in macrophages which may also be super-induced by LPS, TNF-α or IL-2 induced IFN-γ production or by GM-CSF-mediated monocyte/macrophage accumulation. Thus the presence of neopterin in body fluids was suggested to be evidence for the activation of the CMI response [325]. Neopterin production in pulmonary tuberculosis correlated with the extent and activity of the disease [326] and was useful in the monitoring of treatment [327]. Elevated levels of serum neopterin were previously reported in 75% of leprosy patients including lepromatous (LL-BL) patients [255] and in particular in reactions [318; 324] as compared to healthy controls. Furthermore, levels of neopterin were found to be significantly elevated in reactional as compared to non-reactional leprosy patients [255; 318]. It is paradoxical that the elevated neopterin, associated with increased IFN-γ production, is also associated with lepromatous forms of leprosy suggesting that the CMI response may not be completely defective in these patients.

Recent studies have shown that human phagocyte-specific chitotriosidase, the first discovered mammalian chitinase, is an important component of the innate immune response [328]. Chitotriosidase, an endoglucosaminidase belonging to family 18 of glycosylhydrolases, cleaves chitin [329]. Chitotriosidase was discovered in sera of Gaucher patients, unable to degrade the glycosphingolipid glucosylerceramide within the lysosomes due to a lack of the
lysosomal enzyme acid β-glucocerebrosidase [329; 330]. Consequently, glucosylceramide predominantly accumulates in lysosomes of macrophages; the Gaucher cells [331]. Chitotriosidase serves as a crucial macrophage-derived biomarker to monitor disease onset, progression and therapeutic response in Gaucher disease [332]. Increased chitotriosidase activity has been found within atherosclerotic lesions, in which cholesterol-laden foam cells reside [333]. Elevated serum chitotriosidase activity has been reported in malaria [334] and sarcoidosis [335; 336] as well, but not in pulmonary tuberculosis, a mycobacterial disease [336]. However, chitotriosidase activity has been reported in tuberculous pleural effusions [337], suggesting that chitotriosidase production may be local in these patients. Since the macrophage plays a central role in the control of *M.leprae* infection, evaluation of markers of macrophage activation in body fluids may contribute significantly to the diagnosis and classification of leprosy patients.

Acute phase proteins (APP), which are systemic markers of inflammation, have been evaluated in diagnosis, classification and monitoring of leprosy and reactions. The most frequently assessed APPs in relation to leprosy include serum amyloid A (SAA) and C-reactive protein (CRP). Various studies have shown the limited value of CRP in identification or classification of non-reactional leprosy patients [338; 339; 340; 341]. With respect to SAA there are contradictory results with elevated levels reported in LL as compared to TT patients by Scheinberg *et.al.* [338], whereas other studies did not show a significant difference [340; 341]. However, ENL patients were unanimously shown to have elevated levels of SAA and CRP as compared to non-reactional LL/BL patients and controls suggesting their utility as bio-markers [338; 339; 340; 341]. An important limitation of the use of biomarkers such as CRP, SAA, neopterin, chitotriosidase along with cytokines and other soluble/cellular markers is that they reflect the general inflammatory response and would be expected to change in all immune-mediated conditions, thus lacking disease specificity. Hence, the use of such markers needs to be combined with other markers more specific for the disease such as the anti-PGL-1 assay and with clinical observations in order to obtain a more accurate and global view of the progression of the disease.
Outline of the thesis

Leprosy is a chronic mycobacterial disease which displays a clinical and immunological spectrum determined by the host immunological response against *M. leprae* and its antigenic components. The spectral pathogenesis of leprosy is associated with the differential activation of the immune system. The accepted dogma associates lepromatous leprosy with a predominantly Th2 cytokine profile, favouring a strong humoral immune response, tuberculoid leprosy shows a potent cell mediated immune response associated with a Th1 cytokine profile. Furthermore, both leprosy reactions, RR and ENL, are reportedly associated with changes in cytokine activity whereas, *M. leprae*-specific T-cell clones isolated from RR lesions showed a polarized Th1-like cytokine profile. The association of discrete cytokine profiles with the spectral forms of leprosy suggests that identification of cytokine profiles associated with spectral leprosy and reactions might be useful in their detection and monitoring of treatment. Chapters 2, 3, 4 and 5 deal with the assessment of various cytokines, cellular activation markers and acute phase proteins in early diagnosis, classification and monitoring of treatment in leprosy and reactions. Chapter 6 deals with an aspect of immunology which is often neglected in leprosy i.e. the presence of B cells in lesional skin across the leprosy spectrum especially in tuberculoid leprosy. This is on account of the prevailing thought that immunity to intracellular infections is almost exclusively a T-cell mediated event. The present study used immunohistochemistry to demonstrate the presence of B cells in leprosy lesions, including BT lesions, together with a human organotypic skin explant model to demonstrate the secretion of cytokines and *M. leprae*-specific antibodies in the lesional skin. The possible implications of the presence of intralesional B-cells and the role of *in situ* antibody secretion in leprosy pathology are speculated upon in Chapter 6. Antigen presenting cells such as dendritic cells (DC) are central to antigen presentation and in polarizing and regulating the types of T-cell mediated immune response to infectious agents and in the induction of peripheral immunological tolerance. The diverse functions of DCs in immune regulation depend not only on the diversity of DC subsets and lineages but also on the functional plasticity of DCs at the immature stage. Chapter 7 pursues the hypothesis that modulation of maturation of dendritic cells, by *M. leprae* components may influence T-cell responses thus determining the spectral pathology of leprosy. Finally, Chapter 8 is a summary of the results presented in the thesis along with a discussion of some of the salient observations in the context of leprosy pathology.
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