Chapter 6

Demyelination in Leprosy

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Additional information is available at the end of the chapter

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Abstract

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that has a predilection for peripheral nerves, especially Schwann cells (SCs). Leprosy medications may only eradicate the bacteria without preventing or recovering peripheral nerve damage. Early nerve damage detection is necessary. The expression of Krox-20 in Schwann cells will be examined immunohistochemically, and the level of neuron growth factor (NGF), neuregulin 1 (NRG1), protein 0 (P0), and peripheral myelin protein 22 (PMP22) will be examined in the blood plasmas. A significant decrease was noticed in Krox-20 and NGF, NRG1, P0, and PMP22 level ($p < 0.05$) in disability degree 1 compared to degree 0. Studies proved that markers have shown promising results; Krox-20, NGF, NRG1, P0, and PMP22 could be useful diagnostic tools for early peripheral nerve damage detection in leprosy.

Keywords: leprosy, disability, nerve damage detection, marker

1. Introduction

1.1. Background

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* and has predilection for the skin and peripheral nerves, especially in Schwann cells (SCs) [1–4]. During infection, *M. leprae* causes peripheral nerve damage and even causes disability and deformity in patients. Although the anti-leprosy drug treatment can already eradicate the bacteria, disability and deformity that occur cannot be restored and neither can the function of the nerve that has been lost. By understanding the mechanism of nerve damage caused by *M. leprae*, it is expected that nerve damage can be prevented [5].

Until now leprosy treatment is only for eradication of bacteria, but it cannot prevent or cure peripheral nerve damage and its components, so early detection to determine the presence of bacteria in Schwann cells is important [6, 7]. Several studies in early detection of nerve damage by invasion of *M. leprae* have been conducted, namely, using ultrasound to detect peripheral nerve damage [8], vasomotor reflex and sympathetic skin responses [9], electrophysiology of the peripheral nerves involved [10], and examining axonal markers on skin lesions [11]. There is Ridley-Jopling classification of leprosy based on clinical, bacteriological, histological, and immunological symptoms. Based on bacteriological examination, there are two types of leprosy: paucibacillary (PB) and multibacillary (MB). PB types include tuberculoid type (TT) and borderline tuberculoid (BT) in the Ridley-Jopling classification, whereas MB types include borderline-borderline (BB), borderline-lepromatous (BL) leprosy, and lepromatous leprosy (LL) types [12].

According to the WHO, the levels of disability in leprosy patients are divided into three degrees, ranging from the absence of symptoms to apparent damage or disability [13]. In general, according to Seddon, peripheral nerve damage is divided into three, namely, neuropraxia, axonotmesis, and neurotmesis, whereas in leprosy nerve damage occurs as a result of demyelination of peripheral nerves [14].

In the central nervous system (CNS), myelin is formed by oligodendrocyte. Myelin in the CNS has a spiral structure such as peripheral nerve myelin which has an inner mesaxon and an outer mesaxon that ends in a loop or cytoplasmic junction. The cytoplasm of glia in the CNS is confined to only a portion of the myelin sheath. The glia junction continues with the oligodendrocyte plasma membrane through a layered process. One oligodendrocyte can eliminate about 40 or more axons, whereas in the peripheral nervous system (PNS), the myelin deposition of an axon can reach up to 100 layers of myelin [15].

Schwann cell differentiation is governed by the expression of certain transcription factors. After receiving signals from axons, immature Schwann cells including NRG1 will increase the expression of some transcription factors such as NFκB, Oct-6, and Brn2. These factors will stimulate initiation of the promyelination stage whereby the Schwann cell will interact with the axon and begin to express the initial myelination marker. An increase in the Krox-20 gene requires Schwann cells to initiate the myelination process and express the specific protein of myelin. In mature nerves, Schwann cells that do not express Krox-20 will remain nonmyelinated cells. In the injury condition, c-Jun and Sox-2 will increase rapidly. This will lead to a decrease in Krox-20 and Schwann cell differentiation. Cross-resistance Krox-20 and c-Jun will stimulate the switch of complex transcription. Promyelination signals from axons such as neuregulin will result in Krox-20 expression via the phosphatidylinositol-3 kinase (PI3K) pathway. The activation of the Janus kinase (JNK) pathway during the injury period will stimulate c-Jun expression. However, the signals that activate these pathways in Schwann cells are still not known [15].

The study evaluated the Krox-20 expression on Schwann cells in the skin biopsy of leprosy patients, and it was observed that there was a significant difference of Krox-20 expression among patients with degree of disability 0 and 1. (T-tailed test shows $F = 8.881$ with $p = 0.000$ ($p < 0.05$)). Findings summarized a significant decline in Krox-20 expression in degree of

disability 1 compared to degree of disability 0 (T-test, F = 8.881, p = 0.000 (<0.005)). Sensitivity and specificity can be 100%. It means that if the Krox-20 expression is more than 8, the degree of disability will be 0 and vice versa. ROC curve showed that area under the curve is 1.0 (100%) with p = 0.000. On the other hand, in patients with degree of disability 1, it can be seen that the expression of Krox-20 is minimal [16].

The function of Schwann cells is to synthesize the myelin sheath. When these cells are infected by *M. leprae*, the consequence is a demyelination resulting from neuritis. It is suspected that *M. leprae* infection in Schwann cells is a direct cause of Schwann cell dysfunction that can cause demyelination in leprosy patients [2].

Leprosy is one of the diseases that can cause nontraumatic nervous system disorder and is most commonly found in the world. Diagnosis of this disease can be established relatively easily because it does not require sophisticated equipment. The problem is the number of disabilities even though *M. leprae* has been eradicated in accordance with the applicable protocol. Early identification of the occurrence of disability is also the constraint in establishing diagnosis of disability as a sequel of leprosy. Nerve cell damage by leprosy is the result of the demyelination of peripheral nerve cells. Demyelination is caused by the entry of *M. leprae* into Schwann cells as the main target. The entry of these bacteria can cause the demyelination of Schwann cells suspected through the activation of the c-Jun pathway. When damage occurs in Schwann cells, automatically as a form of defense, Schwann cells will repair the damage that occurs, namely, by remyelination. The process of remyelination is influenced by NRG1 and NGF as a neurotrophic factor, as well as the availability of PMP22 and P0 as specific basic materials of myelin in peripheral nerves. Until now, based on the literature review we have read, the factors used to determine early disability in leprosy patients are unknown. Meanwhile, the use of WHO criteria to determine the degree of disability is still rough because it involves only three sensory organs: feet, hands, and eyes. This study is aimed to determine the early markers of nerve damage, namely, demyelination and remyelination in leprosy patients with degrees of damage 0 and 1 based on WHO criteria.

2. Literature review

2.1. Leprosy in general

2.1.1. Definitions

Leprosy is a chronic granulomatic infection caused by *M. leprae.* This disease attacks the skin, nasal mucous membranes, and peripheral nerves [17].

2.1.2. Etiology

The cause of leprosy is *M. leprae* which is transmitted by droplet from nasal secretions and received by the nasal mucosa and other respiratory tracts. This bacterium mainly attacks

Schwann cells in the peripheral nervous system and can cause peripheral nerve functional disabilities as well as disability [18].

2.1.3. Epidemiology

Multidrug therapy (MDT) program has greatly reduced the prevalence of leprosy to less than 1 case per 10,000 people in 90% of endemic countries where leprosy is considered as a public health problem. However, the leprosy case detection rate is still high [19].

2.1.4. Diagnosis

Based on physical examination, according to the WHO, there are three special physical signs for leprosy which can already be used to make the diagnosis. The three special signs are:

1. Redness or hypopigmented skin lesions with loss of sensation (especially sensation of touch and temperature)
2. Peripheral nerve involvement, such as peripheral nerve thickening with loss of sensation (especially touch and temperature)
3. Acid-resistant bacteria in the skin smear of the patient in a certain place

The diagnosis of the degree of disability in leprosy patients is based on the criteria published by the WHO as shown in **Table 1**.

The main classification according to WHO leprosy disease consists of paucibacillary (PB) and multibacillary (MB). If five hypopigmented patches can be found in the patient's skin and there is no BTA in the skin smear, it can be classified as PB type. However, if more than five leprosy hypopigmentations are spotted, and/or BTA are found in skin smear, it may be considered as MB leprosy patients [19].

	Symptoms
Hands and feet	
Grade 0	No anesthesia, deformity, or structure damage
Grade 1	There is anesthesia, no deformity
Grade 2	There is anesthesia and deformity
Eyes	
Grade 0	No problems with the eyes
Grade 1	Eye problems due to leprosy, visus is not worse than 6/60 or finger count on 6 m
Grade 2	Some severe disorders (visus < 6/60, unable to count fingers, and lagophthalmos, iridocyclitis, and opacity of the cornea)

Table 1. Assessment of the degree of disability and deformity index (hands, feet, and eyes) according to the WHO [20].

2.1.4.1. Monofilament test

One of the techniques which is used to assess neuronal function in leprosy patients is monofilament test (MFT). The test is performed using five different single filaments, i.e., 200 mg, 2 g, 4 g, 10 g, and 300 g. Each site is then assessed by the perceived filament, which is the heaviest monofilament obtained and the highest value with a total value of 15 for the ulnar, median, and radial (four filaments) nerves and a total value of 12 for the foot (three filaments). The normal threshold value is 200 mg for the hand and 2 g for the foot (other than the heel) [10, 11]. This test is considered positive if the MFT value is 3 for each nerve. "Fixed" or "unchanged" test result is when the test score has one- or two-point difference from the previous test score (basal value). If the value increases by three points or more, it is said to be "damaged" or "broken." If the value decreases three points or more, it is said to be "improved." If the patient's examination value improves by three points or more, and the total value of the neural examination is reduced by two points or less when compared to the current patient condition, it is said to be "cured."

For the ulnar, median, and posterior tibial nerves, the same examination as the INFIR study is performed, except for the median nerve on the tip of middle finger rather than the little finger (**Figure 1**).

2.1.5. Therapy

Patients with PB type can be given two types of drugs which are already available in the package. The first is for 6 months, i.e. In PB case, in patients over 15 years old, the drug is given in multidrug treatment (MDT) form with rifampicin 600 mg and dapsone 100 mg on the first day, the second day, and until the 28th day and so on for up to 6 months. The second is for 1 year, i.e., MB case. In patients over 15 years, rifampicin 600 mg, clofazimine 300 mg, and dapsone 100 mg on the first day of the first month can be given. In the second and subsequent days, they are given dapsone 100 mg and clofazimine 50 mg [19].

2.1.6. Prognosis

In general, the prognosis of leprosy after getting the correct treatment is good. Healing in complications of neurological disorders is very limited, which means they are difficult to heal. Lesions on the skin can usually be healed in the first year of treatment, while color disorder of the skin and skin damage usually persist. Physical therapy, reconstructive surgery, nerve and tendon transplantation, and surgical operations to correct contractures can improve the patient's quality of life [20].

2.1.7. Complications

Good care and attention to the possibility of reversal reactions due to leprosy treatment will minimize long-term neurological sequelae. Here are some possible leprosy complications:

2.1.7.1. Reaction type 1:

Slow hypersensitivity reaction occurs when BL leprosy shifts into LL leprosy during treatment. This reaction is a description of good immune response and formation of IFN-γ and

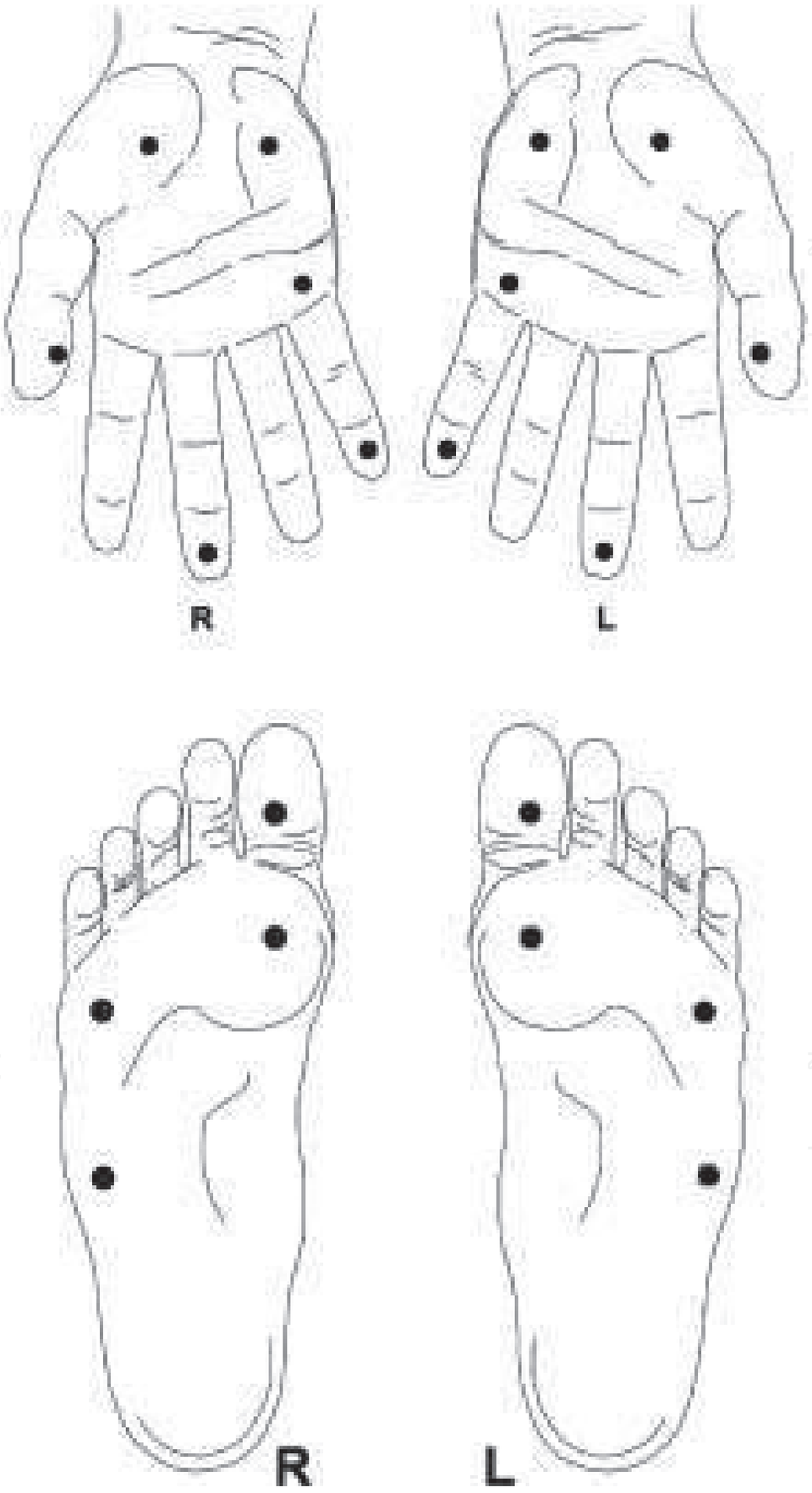

Figure 1. Semmes-Weinstein monofilament testing points [21].

TNF-α locally. This reaction is characterized by edema and erythema in skin lesions, the formation of skin lesions, neuritis, and loss of sensation and motor. The incidence of type 1 reactions in leprosy patients with BL is about 30%. Nonsteroidal anti-inflammatory drugs (NSAIDs) and high-dose steroids can be given in the treatment of type 1 reactions.

2.1.7.2. Reaction type 2:

It is often referred to as erythema nodosum leprosum (ENL), which is the complication of LL type. This reaction is characterized by the formation of subcutaneous inflammatory nodules with fever, lymphadenopathy, and arthralgia. High levels of TNF-α and deposition of the immune complex are suspected to be closely related to ENL.

In addition, a complication that may also occur is Lucio phenomenon, which is a severe complication in MB-type leprosy characterized by bluish bleeding plaque and necrotic ulceration. In this phenomenon, leprosy bacteria may have spread to endothelial cells along with the appearance of necrotic and vasculitic epidermis with thrombus formation and endothelial proliferation [6, 20].

2.1.8. Immunoprophylaxis and chemoprophylaxis

Bacille Calmette-Guerin (BCG) vaccine has been widely used in leprosy endemic countries to provide protection against leprosy. The first dose of BCG provides protection between 14 and 80% against leprosy, while the second dose provides protection between 0 and 50%. Single dose of rifampicin administered to close contacts from newly diagnosed leprosy patients shows an efficacy of 57% in reducing new cases of leprosy [22]. Other studies show the protective effect of immunoprophylaxis and chemoprophylaxis in controlling leprosy. This is done by giving BCG vaccine at infancy in combination with rifampicin given to leprosy contacts. However, the effect of this combination is more useful for PB form contacts rather than MB form [23].

2.2. The peripheral nerve as a target of *M. leprae*

The Schwann cell (SC) of the peripheral nervous system (PNS) is essential for the survival and function of neurons, that is, to enclose axons. SCs were discovered by Theodore Schwann who investigated peripheral nerves. The function of this SC is to assist the myelination of axons and to direct the neurons. SC develops after birth from immature cells to become myelin or without myelin. The first immature cells go through the myelination path at birth and then go through the non-myelin path later in its development [24].

Both SCs which are myelin and without myelin produce an extracellular matrix that forms basal lamina containing collagen around axons. These neurons consist of units of axon cells—Schwann and some neurons (fascicles) surrounded by solid fibrous tissues enveloping the perineurium. Furthermore, these fascicles are also grouped and create neural stems surrounded by other tissues called the epineurium.

Myelinated SCs form myelin that surrounds large axons to increase the conductivity of neurons. The unmyelinated SC surrounds several small axons separated by the cytoplasm. Myelin is formed by the differentiation of the plasma membrane of SC. Myelin consists of multilayered membrane that encloses the axon in both the CNS and PNS. This helps in increasing the

speed of nerve impulses along the axon. Myelin contains 80% fat and 20% different proteins between the PNS and CNS [25, 26]. The major lipid classes found in other membranes are also found in myelin such as neutral lipids, phosphoglycerides, and sphingolipids. However, myelinated PNS has more sphingomyelin (10–35%); higher content of monogalactosylsphingolipid, cerebroside [Gal-C] (14–26%), and sulfatide [SGal-C] (2–7%); and less galactolipid and cholesterol than CNS myelin. The main fatty acid in myelinated PNS is oleic acid [C18: 1 (n − 9)] (30–40% of total fatty acids). Myelin is also characterized by a very high long-chain fatty acid (>18 carbon). The long-chain fatty acids contained in sphingolipids are mostly saturated fatty acids. Myelinated PNS proteins are enriched in glycoproteins and basic proteins [25, 27].

The main proteins of the PNS are 28 kD myelin Protein-0 (P0) 50–60% and 100 kD myelin associated glycoprotein (MAG); both proteins presented only with myelinated SC to maintain the myelin solid structure and the integrity of the axons-myelin. Peripheral myelin protein-22 (PMP-22 kD), myelin basic protein (MBP, 15%), and myelin P2 (10%). Both MBP and P2 myelin are located in the cytoplasm. Other heavy molecular mass of glycoproteins such as 170 kD periaxin are present in small amounts. Myelin P0, an immunoglobulin-like immune cell protein, is immutable among species, with various posttranslational modifications such as phosphorylation, acylation in amino acid regions 110–119, and glycosylation with single, N-linked nine asparagine sugar chains 93 [25]. MBP in the PNS has four polypeptide bands ranging from 14 to 21 kD. This structure has various posttranslational modifications such as phosphorylation and methylation, and this is one of the major autoantigens in multiple sclerosis disease. Myelin P2 or fatty acid-binding protein 8 is a small protein (14 kD) with a high positive charge and is concentrated mainly in thick myelin sheaths. This structure is a member of the family of fatty acid-binding proteins with a high affinity for oleic acid, retinoic acid, and retinol. This structure function is to assemble and maintain myelin lipids. P2 is also an autoantigen in autoimmune peripheral neuropathy, Guillain-Barre syndrome (GBS). Its function is related to stabilizing the dynamics of myelin membranes and transport to and from lipid membranes [26, 27].

Myelin is only produced if the cell is in contact with several types of axons. Axons send signals that are important to identify the SC. Examples of axon signals are beta-neuregulin-1 (NRG1) and glial growth factor (GGF). SC can be activated to enter proliferation via axonal signaling NRG1 to bind and activate the ErbB2/ErbB3 receptor complex on SC to activate MAPK for cell proliferation. The factors governing myelination of SC include transcriptional factors Krox-20, Oct-6, and Sox-10; they also inhibit cell death and proliferation. Special myelin protein, P0 for diminished myelinated SC if immature cells are not associated with axons. If SC loses interaction with an axon, there can be dedifferentiation into an immature SC. If the cells associate with their axon again, they will become myelinated or nonmyelinated depending on the stimulating signal. SC has the ability to block apoptosis through the effects of growth factors such as insulin-like growth factors (IGFs), platelet-derived growth factor-BB (PDGF-BB), and neurotrophin-3 (NT-3) on autocrine circuit [24].

One of the events associated with SC pathogenesis is nerve injury and demyelination. This can be due to axonal damage and axon-SC signal interference. Another cause is the immune stimulation (autoimmunity) that targets myelin as in multiple sclerosis and Guillain-Barre syndrome (GBS). As with autoimmune disorders, *M. leprae* causes peripheral nerve demyelination that begins with damage to the myelin sheath and decreases the speed of the action-potential conduction [28].

2.3. Nerve damage in leprosy

Nerve damage is a major characteristic of leprosy pathogenesis. The first stage of nerve damage in leprosy is the localization of *M. leprae* in peripheral nerves. A recent research about leprosy in experimental animal armadillos successfully revealed that after *M. leprae* successfully penetrate the skin barrier, these bacteria gather in epineurial lymph and blood vessels around the nerve and then go into the endoneurium compartment through the blood supply. However, if *M. leprae* can enter SC freely through the only way through the Schwann cells exposed to the dermis; the only way to prevent transmission of leprosy is to prevent the attachment of leprosy to Schwann cells [2].

In a study by Harboe et al., it is declared that *M. leprae* binds to the G domain of the α2-laminin chain (LN-α2) expressed by the Schwann cell axons. In addition, it is shown that $\alpha\beta$-dystroglycan (DG) in the basal lamina acts as complex receptor of LN-α2/*M. leprae* bonds. Presumably, there are other receptors that play a role in Schwann-*M. leprae* cell interactions because blocking of these receptors has not been successful in completely preventing the attachment of *M. leprae*. The third stage is the role of LBP-21 protein (LPS-binding protein, 21 kDa) which is surface antigen of *M. leprae* which acts as adhesin molecules to interact with Schwann cells. In addition, other *M. leprae* surface antigens sphingoglycolipid-1 (PGL-1) are also shown to bind to laminin-2. Therefore, PGL-1 is also involved in the Schwann cell invasion via the basal lamina via laminin-2-dependent pathway. It is suspected that PGL-1 serves as a second receptor to *M. leprae* where the combination of PGL-1 and LBP-21 provides sufficient energy in binding to Schwann cells so that *M. leprae* can enter Schwann cells safely [6].

The reversal reaction in leprosy is closely related to the increase of immense cellular immune reactions against mycobacterial antigens. Histologically, the lesions are invaded by mononuclear cells and result in edema and hyperemia. These events are the basis of immunosuppressant administration in leprosy reactions, while antimycobacterial drugs should be continued as well. In the study, TNF-α and TNF-α mRNA levels were very high. This suggests a strong immunological reaction to *M. leprae* antigens.

Contact-dependent demyelination induced by *M. leprae* in nerve culture in the absence of immune cells also shows the role of nonimmune mechanisms during early infection and nerve involvement in leprosy infection. During the development of acute ENL with type 2 cytokine pattern in leprosy patients, there is an increase in IL-6, IL-, and IL-10 as well as the persistent expression of IL-4 and IL-5 mRNAs in the lesions. Chronic ENL often leads to nerve damage, which is possible due to induction of local immune complex deposition with granulocytes that cause tissue damage and complement activation.

The c-Jun molecule is a major component of complex transcription of transcription factors and forms JunB and JunD in the Jun mammalian protein family. The c-Jun molecule is involved in cellular functioning and dependent on N-terminal phosphorylation performed by the Jun N-terminal kinase (JNK) enzyme. Thus, JNK can affect protein content of c-Jun. The levels of c-Jun in Schwann cell culture are high despite the simple culture medium. The c-Jun proteins are present in immature Schwann cells on embryonic and neonate nerves, but their presence is suppressed in individual cells as transcription factors of Krox-20 premyelination are activated and the myelinating begins. In Schwann cell culture, the addition of Krox-20 expression is sufficient to suppress the expression of c-Jun protein. Krox-20 is also involved

in c-Jun suppression in vivo, as c-Jun levels remained high in the Krox-20 null nerve where myelination is discontinued. In vitro experiments indicate that c-Jun suppression is absolutely necessary for myelination processes since Schwann cells with high c-Jun expression will be inhibited in the myelination process of the axon in which the induction of Krox-20 or cAMP myelin genes is inhibited. In contrast, in Schwann c-Jun null cells, it has increased myelinated gene expression [29].

c-Jun will be upregulated rapidly after nerve injury. This is a procedure that triggers the dedifferentiation of Schwann cells. To determine the function of c-Jun under these conditions, Arthur-Farraj et al. made the Schwann cell without c-Jun. The process of myelination at the stage of development is not so affected on the specimen. However, it turns out that c-Jun is normally suppressed as the myelination process begins. However, after the process of injury passed, there is a delayed degradation of the myelin sheath. This presumably occurs because of decreased ability of c-Jun null cells in digesting myelin. In addition, there is a delay in the inactivation of myelin genes and the failure to activate the necessary molecules of demarcated cells including L1, p75NTR, and N-cadherin. All of these molecules are important because after the injury, c-Jun protein required Schwann cells to differentiate and adjust the molecular phenotype as it is immature. One of the properties that can be known when the cell is deficient or when there is disruption of c-Jun in Schwann cells is the loss of regeneration ability dramatically and the loss of recovery ability after injury [30].

The key role of axon integrity in controlling switches from c-Jun negative, positive Krox-20 which functions in maintaining myelin differentiation, to positive c-Jun; negative Krox-20 in dedifferentiated cells can be clearly observed in experiments using Wild mice. In these mice, axonal degeneration and myelin degradation that occur after neural cuts are delayed for up to 2–3 weeks. It is presumed that during the period of maintenance of myelin after an axotomy, the expression of Krox-20 is maintained and c-Jun is inhibited. At the time when axons degenerate and the myelin sheath begins to break down, c-Jun is expressed, while Krox-20 is no longer expressed [31].

2.4. Immunopathogenesis nerve damage in leprosy

2.4.1. Immune-mediated damage

2.4.1.1. The role of cellular immunity

SC can take, process, and present the specific antigen of *M. leprae* to T cells resulting in the production of Th1 modulatory immune cytokines such as TNF-α and INF-γ [32]. SC can present mycobacterial antigen to MHC class I, CD8+ cytotoxic T cell, and also can present mycobacterial antigen to class II MHC, CD4+ CTLs. SC was found to express co-stimulatory molecules and adhesions to T cells. As a result of this stimulation, SC will be killed by cytotoxic granules (granulysin, granzyme, and perforins) produced by CTLs [33]. SC can express TLRs (TLR1 and TLR2) which are activated by LAM *M. leprae*, a lipoprotein such as 19 and 33 kD. SC activation will lead to cytokine production (TNF-α, IL-12) and apoptosis [34]. TLR expression was found to be greater in TT patients than LL patients [35]. The large amount of cytokines released by Th1 cells, especially IL-12, IL-2, and TNF-α, will cause apoptosis of infected cells and decrease in bacterial load and increase granuloma formation such as lesions in TT type [36].

Macrophages also play role in the stimulation of immunity in leprosy. Infected macrophages by *M. leprae* can present its antigens to T and B cells and release cytokines including TNF-α [1]. Both of these cells can produce reactive oxygen intermediate (ROI) which results in further nerve damage at the site of granuloma [26]. *M. leprae* stimulates macrophages to produce TGF-β which is responsible for decreased nerve regeneration [26]. Other cells may also present antigens which are dendritic cells, also found to effectively present the *M. leprae* antigen and stimulate CD4+ and CD8+ cytotoxic T cells. Th1 cells are thought to be involved in CMI-DTH reactions (cell-mediated immunity-delayed-type hypersensitivity) and are important in response to intracellular pathogens. In contrast, Th2 cytokines stimulate the production of antibodies. C-type lectin DC-SIGN shows binding to LAM and triggers the production of IL-10 and TGF-β and inhibits the production of IL-12 and TNF-α [37].

Scollard proposes that *M. leprae* interacts with the vascular endothelial cells and perineurium before successfully infecting the SC. This leads to the possibility that *M. leprae* infects the peripheral nerve tissue through the bloodstream [2]. This mechanism is thought to play an important role in SC immunopathogenesis and peripheral nerve damage to leprosy.

2.4.1.2. The role of humoral immunity

High levels of antibodies (IgM and IgG) are usually found in LL-type leprosy patients. Th2 (IL-4, IL-5, and IL-10) cytokines found in LL patients decrease TLR2 expression and stimulate activation of B cells. Activated B cells can make IgM, IgA, and IgG antibodies ineffective in killing intracellular bacteria. Therefore, *M. leprae* is able to survive and spread, causing various nerve damages [38]. After chemotherapy, there is usually a decrease in antibody levels. There is a strong correlation between bacterial load and humoral immune response. Analysis of antibody response has also been proposed as a tool for leprosy classification [39].

The antibodies produced against *M. leprae* play a role in the uptake of *M. leprae* by phagocyte cells and initiate the pathogenesis of the disease. For example, the discovered antibodies are binding to complement, which then binds PGL-I and binds C3 complement to *M. leprae*. The binding of this complement will mediate the uptake of bacilli through complement receptors in phagocytes [40]. In addition, secreted antibodies can form immune complexes with *M. leprae* antigen or with cross-reactive host molecules. This complex can then be recognized by antigen-presenting cells through specific receptors and delivered to T cells. In LL patients an excess of immune complex antibodies is found. However, if the antibodies are taken by APC cells (macrophages), they fail to activate T cells. Specific mature B-cell markers (CD20, CD79, CD138) that produce antibodies are found to be higher in skin lesions of BL/LL patients than BT patients [41]. In addition, when compared to gene expression of leprosy skin lesions of LL and TT types, it showed upregulation of B-cell-specific gene. Immunohistology of LL and TT skin lesions showed that IgM and IgA are more common in LL-type leprosy lesions, correlated with Th2 immunity and increased IL-5. LL is associated with an increase in systemic humoral response [42].

Another proposed mechanism which can cause neuropathy in leprosy and is also associated with stimulation of the immune response during infection is autoimmunity. The concept and general criteria of autoimmunity in leprosy have been established since 1969. It was found that in leprosy, an increase in immune complex (1gG-IgM, IgG-IgA, and complement components) is similar to other autoimmune diseases such as systemic lupus erythematosus (SLE),

Guillain-Barre syndrome (GBS), and rheumatoid arthritis. The formation of antigen-antibody complexes and complement stimulation as well as the recruitment of PMN cells can cause tissue damage and injury to the vessel wall as seen in autoimmune and leprosy diseases [42].

T cells in leprosy lesions may be produced either against specific antigens of *M. leprae* or autoantigens such as HSP-66. This may cause other mechanisms of nerve damage caused by autoimmune damage such as from tissue (neuropathy). In LL-type leprosy patients, there was a decrease in the specific antigen level of T cells against *M. leprae* antigen 65 kD, but at the same time, there was an increase in anti-antibody levels of 65 kD IgG. The similarity between bacterial proteins and host components or the presence of molecular mimicry is an important aspect for host-pathogen interactions. By using this mechanism, pathogen can avoid detection by the immune system or may cause autoimmunity. The monoclonal antibodies that arise against *M. leprae* antigens such as 65 kD antigen react with host antigens such as peripheral axons found in the skin.

In addition, the protein sequence of myelin PNS P0 is compared to the *M. leprae* protein sequence (leproma) and also other genomic databases for protein sequences and structural equations in other pathogens involved in neurodegeneration. This resulted in 11 hits with the right pair ranging from six to seven P0 myelin residues in the *M. leprae* genome, but not in other genomes of mycobacteria. Among these, two suitable on *M. leprae* are special proteins including ferredoxin NADP reductase (62%) and conserved membrane protein (36%) (ML2453, ML1504). Comparisons to other pathogen databases show that P0 has similar sequences with polio virus receptors (23.4%) and herpes virus (4%). In addition, searching for the myelin P0 sequence on the whole genomic database revealed that it has similar sequence to the immunoglobulin superfamily. This family plays an important role in the interactions of proteins-proteins and protein-ligands. The similarity between bacteria and host is that they can cause autoimmunity and neurodegeneration (demyelination) as can be seen in leprosy transmission. For example, anti-neural antibodies from serum of leprosy patients were found to bind the myelin protein P0.

Some autoantibodies found to be important in leprosy patients from western India with 50% detected in LL, 44.4% in BL, and 54.8% in BT. These autoantibodies are antinuclear antibodies (ANA), anti-double-stranded DNA (dsDNA) and anti-single-stranded anti-DNA (ssDNA), and antinuclear antigens (anti-ribonucleoprotein (nRNP), anti-Smith and anti-histone antigen (AHA)).

Since leprosy is one of the differential diagnoses of rheumatic diseases, some autoantibodies can be examined in leprosy serum using ELISA techniques and correlated with joint involvement. For example, in Brazil, most leprosy patients have no active reaction. Therefore, the frequency of IgM-rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibody, antinuclear antibody (ANA), and antineutrophil cytoplasmic antibody (ANCA) is low in leprosy patients. However, the prevalence of anticardiolipin (aCL) antibodies and anti-β2 glycoprotein I (β2GPI) antibodies is significantly higher in leprosy patients than in the control group.

Glycolipids and glycosphingolipids expressed as determinants of myelin surfaces in SST are important for the function and stability of myelin itself. The resistance to these molecules (glycolipids) by autoantibodies can cause demyelination and nerve damage as found in leprosy patients. Therefore, many scientists study the autoantibody and its relationship to nerve

damage found in leprosy. Glycolipid or glycosphingolipid neural antibodies such as ceramide have been studied in leprosy patients in India. The anti-ceramide antibody IgM titers were found to be significantly higher in MB-type leprosy patients than both control and PB leprosy patients (96% of MB-type and 60% of PB-type patients). Groups in Brazil found elevated levels of anticardiolipin (aCL) and anti-β2-glycoprotein I (anti-β2-GPI) in leprosy patients, especially in LL-type patients with IgM-isotype dominant. The percentage of antiphospholipids in leprosy can be due to exposure to phospholipid antigen by tissue damage during the infection process. Another explanation is that the homology between PL bacteria and host results in the production of antibodies against cross-reactive heterological sequences.

Other anti-glycolipid antibodies are anti-sulfatide (cerebroside) which has also been reported in various cases of demyelinated peripheral polyneuropathy. Sulfatide, a glycolipid with single sulfate saccharide, is associated with the myelin membrane of nerve cells. Antibodies inhibit sulfatide synthesis expressed on myelin as a surface determinant, resulting in demyelination. IgM-subtype antibodies against sulfatide were increase in MB- or LL-type leprosy compared to PB and control. IgM anti-sulfatide is positively correlated with the patient's bacterial index. The similarity between mycobacterial sulfolipid with trehalose sulfate and host tissue (sulfatide) can stimulate autoantibodies against host sulfatide with galactose sulfate.

Variations in the number of autoantibodies found in leprosy patients can be attributed to the genetic background of the study population, the presence of infectious diseases, and the techniques used to detect autoantibodies. The hypothesis proposed for the development of such antibodies during infection is due to adaptive immune responses and activation of polyclonal B cells. Bacterial or viral antigens, with homologous sequences to tissue hosts, will be presented to T lymphocytes that stimulate B lymphocytes to produce antibodies against heterologous sequences.

In addition, autoantibodies to the antigenic epitope of myelin protein are reported in various chronic demyelinating diseases. For example, in chronic inflammatory polyradiculoneuropathy (CIPD), in which both humoral and cellular immunities are involved, there are antibodies to the myelin protein. The major antigenic components of myelin are myelin P0, P2 myelin, and peripheral myelin protein 22 (PMP22). These proteins are also associated with induced experimental autoimmune neuritis (EAN). Such autoantibodies may be produced during tissue damage and then continue to exacerbate further tissue damage during the disease process.

2.4.2. Demyelination without immunological process

Beside inflammatory process, axon demyelination can be caused by the presence of *M. leprae*. Rambukkana et al. have proved this both in vitro (in schwannn cell coculture) and in vivo (in $Rag1^{-/-}$ mice) had low levels of T cells and B lymphocytes. In this model *M. leprae* is able to induce demyelination within 24 hours postinfection without apoptosis or toxic effects on cells. The *M. leprae* component such as PGL-1 cell wall is also associated with the demyelination of SC in the model. Therefore, Rambukkana concluded that the survival of *M. leprae* was unnecessary in nerve demyelination induction in vitro and in vivo [5]. In addition, in his study *M. leprae* was found to be capable of inducing extracellular signals regulated by the Erk1/Erk2

kinase signal via the MAPK-MEK-dependent pathway. This activation caused by contact dependent between M. leprae and primary SC without apoptosis or cell death in SC. In this scenario, *M. leprae* is found to bind and induce the phosphorylation of ErbB2 receptors in SC other than laminin receptors located closely to ErbB2. As a result of this signal activation, *M. leprae* succeeds in inducing SC proliferation and demyelination [5]. Rambukkana stated that the mechanism of nerve damage in the absence of an immunological process played a role in the early stages of the disease. However, it is an immune system-mediated element that eventually causes nerve damage. When the *M. leprae* antigen is presented by myelinated and unmyelinated SC, both types of cells are subjected to attack by macrophages, T cells, and cytokines that are released as a result of the cell's inflammatory response. This inflammatory process will produce two SC phenotypes and sensoric-motoric damage subsequently [5].

Another mechanism that is suspected to cause nerve damage can be grouped in the form of nonimmune mediated, and it is the biochemical and metabolic changes in the nerve compartment. The examples of these mechanisms are axonal atrophy due to hypophosphorylation of myelin proteins and axonal neurofilament. Many proteins in PNS are phosphorylated such as myelin P0, MAP, and neurofilament proteins. An experiment which studied the phosphorylation of PNS proteins in leprosy nerves compared to normal nerves found that decrease in protein phosphorylation protein levels of 25 kD in leprosy patients' nerves. The phosphorylated (25 kD) protein is thought to be the myelin glycoprotein P0 [43]. Later studies by the same group also found that *M. leprae* could bind these myelin P0 (25 kD) glycoproteins and inhibit phosphorylation in vitro. The outer binding of the myelin can help *M. leprae* to reach the SCs target for invasion [43].

The neurofilament protein belongs to the filament intermediate (IF) found together with the microtubules and microfilaments in the cytoskeleton structure. Other proteins that make up IF in vimentin, peripherin, internexin, and nestin neuron. The NFS protein (neurofilament) in the axon consists of triplet proteins, namely, the molecular weight of NF-H (high), NF-M (medium), and NF-L (low) neurofilament proteins. The neurofilament protein contains an amino-terminal head domain, which is central-helical domain, and terminal-carboxyl-tailed domain in various lengths. Increasing the total number of NF proteins in axon results in an increase of axonal diameter. In addition, the phosphorylation of NF proteins is important to determine the axonal caliber. NF-M and NF-H proteins are highly phosphorylated in the C-terminal tail domain on the replication of KSP (lysine-serine-proline) in myelinated axons (Chung-Ho Liang, 1996). Several studies have shown that NF-H migrates more rapidly in SDS-PAGE after extensive dephosphorylation by alkaline phosphatase. Several studies have demonstrated the important role of NFS in the growth of myelinated radial axon using NFS protein knockout gene.

In leprosy patients, a decrease in axonal diameter was found to be associated with the loss of sensoric and motoric function. Therefore, the relationship between neuropathy and phosphorylation of NF protein was then investigated. The technique used was Western blot and immunohistochemistry to examine the phosphorylated NF epitope (SMI 31) on leprosy patients' nerves. In addition, Shetty et al. found the decrease or loss of SMI 31 in staining of infected nerve fibers. The NF protein band migrates faster (lower) than expected and decreases the

levels of NF protein content in the infected nerve. These results indicate the presence of hypophosphorylated NF subunits during leprosy infection, which are thought to lead to increasing susceptibility to proteolytic degradation of NFS. This result is consistent with the previous research, where phosphorylation was found to protect NFS against nonspecific proteolysis by calpain. Several studies by Shetty et al. found that lipoarabinomannan (LAM) *M. leprae* could inhibit the protein kinase C (PKC) enzyme which is responsible for the phosphorylation of the neurofilament protein. Recently, Save et al. also found that hypophosphorylation of NFS proteins by measuring the activity of enzyme kinases is responsible for NFS phosphorylation in the nerves of infected mice. The authors point out that as long as NFS loses its reactivity to specific NF-phosphate (SMI 31) antibodies, there is a decrease in KSPXK kinase activity from cyclin-dependent kinase (CDK) and MAPK in *M. leprae*-infected nerves. Decreases in NFS phosphorylation and NFS degradation may subsequently result in decreasing in interfilamentary distances that affect axonal growth and result in axonal atrophy.

In addition, *M. leprae* was found to induce upregulation of metalloproteinase matrix (MMP-2 and MMP-9) in SC which causes demyelination and damage to the blood-nerve barrier (Teles, 2010). The MMP protein family consists of proteolytic enzymes that participate in remodeling the extracellular matrix and the regulation of leucocyte migration. The function of MMP-2 is to degrade the type I collagen (gelatin), and the function of MMP-9 is to degrade the type IV collagen, which is a major component of the basal membrane. Increased MMP secretion is associated with tissue damage and can be used as a biomarker in many inflammatory disorders. During mycobacterial infection there was an increase in MMP-9 secretion that correlated with TNF-α production. It was found that during tuberculoid leprosy and type I (RR) lesion reactions, MMPs increased in the central region of granuloma, where dominant macrophages and epithelioid cells were obtained.

Nerve damage in leprosy infection is divided into two stages: (1) early stage which has no inflammation cells. This phase is initiated by contact between *M. leprae* with SC in SST and causes nerve damage. This phase often occurs on the entire spectrum of leprosy. It is characterized by sub-perineural edema, axonal atrophy, and demyelination with loss of myelinated nerve fibers. (2) The second phase is the phase which is mediated by inflammation with lymphatic cells in the form of tuberculoid and macrophage cells in lepromatous leprosy lesions. In this stage, the presence of autoantibodies against nerve components is reported in leprosy as another mechanism of nerve damage. The presence of common antigenic determinant between *M. leprae*, the skin, and nerves such as heat-shock proteins leads to the production of autoantibodies [26].

The presence of *M. leprae* in the nerves can also cause leprosy neuritis which has no skin manifestation, but nerve damage can be detected. Nerve damage can be caused by a full inflammatory inflammation of macrophages that produce foam cells in granulomas. This inflammatory process causes the stimulation of cytotoxic T-cell activity, axonal degeneration after SC death, and demyelination. Using different SC and axonal markers on the immunohistochemical slide of leprosy neuritic nerve, there was a decrease in immunoreactivity of NF200 as a result of the loss of myelinated fibers. In addition, the S-100 protein staining of the myelinated fibers was reduced due to loss fiber after the onset of demyelination. NGFr staining of the neuritic

nerve is also reduced in SC and/or small fiber axons. A decrease in myelinated fibers results in a decrease in MBP [44].

2.5. Schwann cells and their interactions with NGF, NRG, P0, and PMP22

2.5.1. Nerve growth factor (NGF)

Nerve growth factor (NGF) is firstly discovered in neurotrophin family. NGF is essential in the development and maintenance of phenotypic peripheral nerve cells and for the functional integrity of the cholinergic nerves in the central nervous system. The mature form of NGF (from ProNGF precursors) has an important role in development and in adult life and also has proapoptotic and neurotropic properties.

A study by Chan et al. about NGF in controlling axon receptivity on myelination by Schwann cells revealed that NGF is an axonal signal regulator that controls the myelination of nerve cells in the dorsal ganglia that expresses TrkA. NGF triggers the myelination by Schwann cells, but it inhibits myelination by oligodendrocyte cells. This reinforces that NGF plays a role in the Schwann cell myelination in the PNS.

2.5.2. Neuregulin 1 (NRG1)

In early life, Schwann cells are made of cells in neural plates and undergo massive migration, proliferation, and maturation before finally undergoing differentiation. During this period, Schwann cells are constantly in contact with axons and axonal signals, particularly neuregulin-1 (NRG1). In fact, NRG is a signaling protein that mediates the interactions of cells in the nervous system, heart, breast, and other organ systems. Neuregulin is also ligand for the ErbB family of tyrosine kinase receptors. Neuregulin itself has four families, namely, NRG1, NRG2, NRG3, and NRG4. However, until now, the biological functions of NRG2, NRG3, and NRG4 are still not widely known [45]. NRG1 is a growth factor that is very influential on the development of cells in the neural plate during the early stages of embryonic development. NRG1 is also involved in migration, axon growth, and synapse formation [46, 47]. In addition, NRG1 is a strength for Schwann cells to differentiate. This is proven in cell cultures where many aspects of Schwann cells are administered by NRG1, which are:

1. NRG1 serves to suppress the neuronal differentiation of the neural stem cell plate but to stimulate the differentiation of glial cells.
2. NRG1 is required for Schwann cell progenitor survival.
3. NRG1 also serves to stimulate proliferation and migration of Schwann cell precursors.
4. NRG1 gives an important signal to myelinate.

In vivo, the early stages of Schwann cell development depend on NRG1/ErbB signaling, as in mouse-fed animals without Schwann cell progenitors in peripheral neuronal development such as ErbB2, ErbB3, or NRG1.

2.5.3. Protein 0 (P0)

P0 or MPZ is one of the major protein components of myelin nervous system. P0 protein is transmembrane glycoprotein belonging to the immunoglobulin superfamily. It is the largest part of protein in SST myelin and is thought to be responsible for the adhesion of the outer surface of the cell and the myelin plasma membrane [48].

2.5.4. Peripheral myelin protein 22 (PMP22)

Peripheral myelin protein 22 (PMP22) is a major component of myelin (Snipes et al., 1992). This is evidenced by the discovery of PMP22 mRNA in Schwann cells and the PMP22 protein in the solid part of the myelin sheath. Assessment of the presence of PMP22 as a peripheral nerve myelin protein is also supported by the finding that the regulation of PMP22 expression during neural development and after neuronal trauma is identical [16].

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