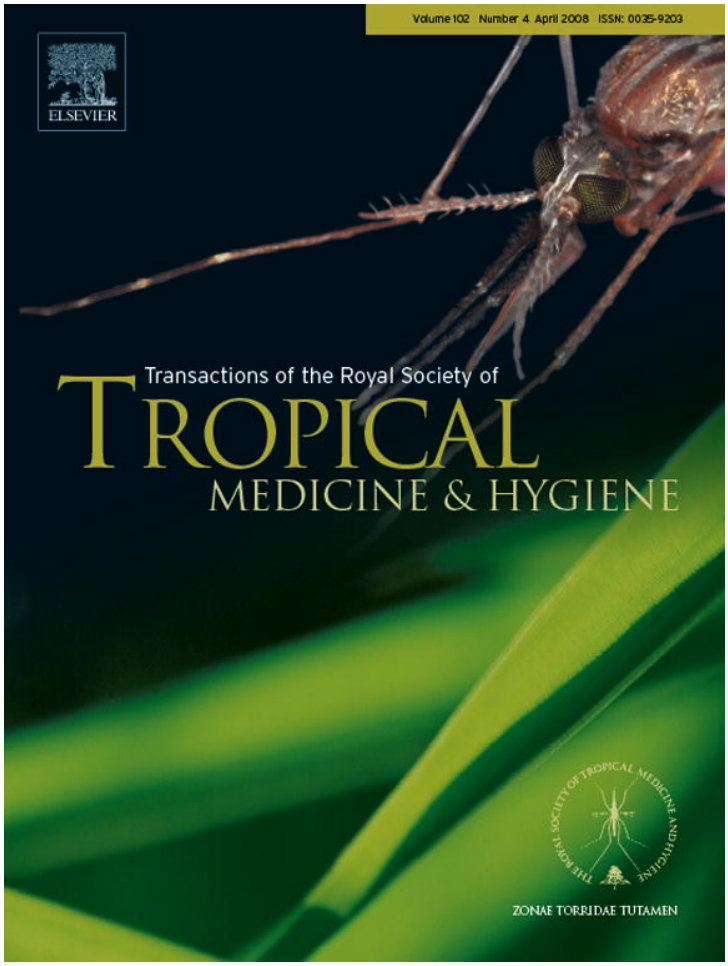

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REVIEW

Towards understanding the pathology of erythema nodosum leprosum

I.P. Kahawita*, D.N.J. Lockwood

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

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Summary Erythema nodosum leprosum (ENL) is an immune-mediated complication of leprosy presenting with inflammatory skin nodules and involvement of multiple organ systems, often running a protracted course. Immune complex production and deposition as well as complement activation have long been regarded as the principal aetiology of ENL. However, new data show that cell-mediated immunity is also important. We have performed a critical analysis of studies on the pathology of ENL. Our main findings are as follows. ENL is characterised by an inflammatory infiltrate of neutrophils with vasculitis and/or panniculitis. There is deposition of immune complexes and complement together with *Mycobacterium leprae* antigens in the skin. Changes in serum levels of Igs indicate a transient, localised immune response. The major T-cell subtype in ENL is the CD4 cell, in contrast to lepromatous leprosy where CD8 cells predominate. The cytokines TNFα and IL-6 are consistently found whilst IL-4 is low or absent in ENL lesions, indicating a T_H1 type response. Keratinocyte 1a and intercellular adhesion molecule-1 (ICAM-1) have been shown to be present in the epidermis in ENL, which is evidence of a cell-mediated immune response. Co-stimulatory molecules such as B7-1 have also been studied but further work is needed to draw strong conclusions. We also highlight potential areas for future research.

1. Introduction

Erythema nodosum leprosum (ENL) is a serious, difficult to manage inflammatory complication of lepromatous (LL) or borderline lepromatous (BL) leprosy, manifesting as crops of painful, erythematous nodules with fever, malaise and inflammation elsewhere producing iritis, arthritis, neuritis and lymphadenitis. ENL may occur before, during or after treatment with multidrug therapy but in most patients ENL occurs during the first year of treatment (Becx-Bleumink and Berhe, 1992; Manandhar et al., 1999; Pocaterra et al., 2006). It often has a protracted course with episodes occurring over 7 or more years, although the majority last 12–24 months (Kumar et al., 2004; Pocaterra et al., 2006). Although the number of leprosy cases has decreased worldwide, the reac-

* Corresponding author. Tel.: +44 20 7612 7863; fax: +44 20 7637 4314.
E-mail address: indira.kahawita@gmail.com (I.P. Kahawita)

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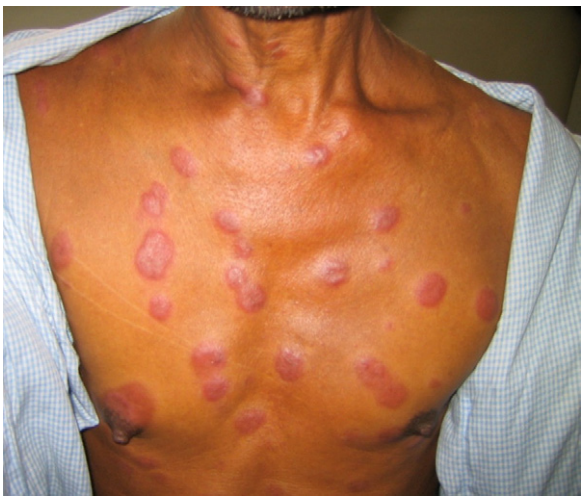

Figure 1 Skin lesions of erythema nodosum leprosum.

tions that complicate leprosy remain an important clinical problem. Patients with ENL may now present to general physicians and it is important that doctors in a wide range of specialties can recognise this entity.

Immune-mediated reactions in mycobacterial diseases are now being recognised to a wide range of mycobacteria, not just *Mycobacterium leprae*. Furthermore, the immune-mediated reactions in mycobacterial diseases in patients with HIV and the immune reconstitution inflammatory syndrome (IRIS) illustrate the broad implications of these phenomena (Lipman and Breen, 2006; Ustianowski et al., 2006). It is therefore useful to review the pathology of ENL since there are aspects that will be applicable to other diseases.

ENL is classically seen as an immune complex-mediated phenomenon but recent data suggest that cell-mediated immune responses play an important role. Understanding the pathogenesis will promote further research into the development of better, safer and more effective treatment.

We have critically reviewed the current data on the pathology of ENL and also highlight where further research is needed.

The search strategy used was a PubMed search with the terms 'leprosy', 'ENL', 'histopathology', 'immunohistochemistry', 'immune complexes', 'cytokines', 'B lymphocytes', 'MMP' and 'risk factors' in varying combinations. Only English language articles were selected. Some cross-references from articles retrieved from the PubMed search were also used. All searches were complete to the end of September 2007.

2. Erythema nodosum leprosum (type 2 reactions)

ENL (or type 2 leprosy reactions) is an immune-mediated phenomenon occurring in patients with LL or BL leprosy. The reaction causes acute inflammation in any organ or tissue invaded by the leprosy bacillus (Pfaltzgraff and Ramu, 1994).

The skin lesions present as erythematous, tender papules or nodules that may be superficial or deep seated (Figure 1). The lesions differ clinically from erythema nodosum by their evanescent nature, large number of lesions and widespread distribution beyond the lower legs (Pfaltzgraff and Ramu, 1994). In severe reactions, skin lesions may become vesicular, bullous or necrotic (Jopling and McDougall, 1988).

Table 1 Clinical features of erythema nodosum leprosum

- Painful, tender, erythematous skin nodules appearing in crops
- Generalised illness with fever and malaise
- Neuritis, less severe than type 1 reactions
- Iritis, episcleritis or conjunctivitis
- Orchitis
- Tender, generalised lymphadenopathy
- Arthritis or arthralgia
- Bone pain and tenderness, especially tibial tenderness
- Dactylitis
- Oedema of the extremities
- Transient proteinuria
- Exacerbation of upper respiratory symptoms

ENL reaction usually produces a generalised illness with high fever, systemic upset, oedema of the face, hands and feet, and proteinuria (Table 1). Other manifestations include iritis, episcleritis, arthritis, arthralgia, dactylitis, lymphadenopathy, organomegaly and orchitis (Figure 2) (Pfaltzgraff and Ramu, 1994). Neuritis may be part of ENL but is often milder than that seen in type 1 reactions.

There are no good quality contemporary studies on the effect of ENL on the liver (Cook and Corachan, 1982; Kumar et al., 1987). The only significant abnormality in renal function found in patients with ENL compared with LL patients without ENL is impaired creatinine clearance (Bajaj et al., 1981).

The majority of patients with ENL experience multiple acute episodes or chronic ENL lasting more than 6 months (Pocaterra et al., 2006). ENL can have a protracted course lasting several years (Kumar et al., 2004). It is usually diagnosed clinically but a skin biopsy can be helpful. Corticosteroids are the mainstay of treatment (Girdhar et al., 2002; WHO, 1998) but many other alternatives are used (Burte et al., 1983; Helmy et al., 1971; Moreira et al., 1998; Sales et al., 2007; Villahermosa et al., 2005). Thalidomide

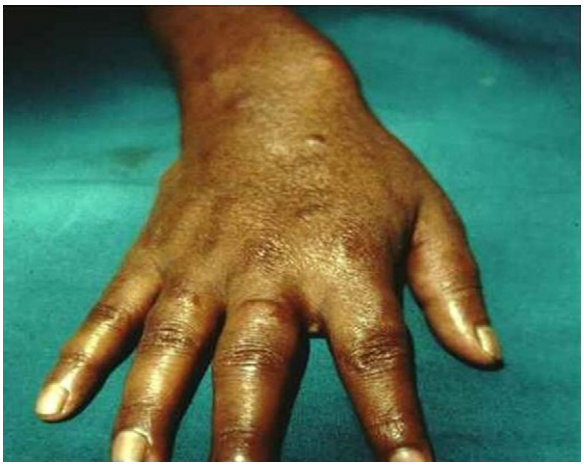

Figure 2 Hand showing dactylitis and nodules.

is very effective in the treatment of ENL, but cost and the risk of teratogenicity limit its use in many patients (Walker et al., 2007).

3. Risk factors

LL leprosy and a bacterial index (BI) $\geq$4 are significant risk factors for the development of ENL (Manandhar et al., 1999; Pocaterra et al., 2006; Saunderson et al., 2000). Manandhar et al. (1999) showed that skin infiltration is a risk factor for ENL. An Ethiopian study has shown that HIV is a risk factor for ENL but there were only two patients with co-infection and this has not been replicated elsewhere (Saunderson et al., 2000).

Pregnancy, lactation, puberty, intercurrent infection, vaccination and psychological stress have been proposed as risk factors for ENL (Pfaltzgraff and Ramu, 1994), but these have not been confirmed in prospective studies. It has been shown that 30—40% of women are at risk to develop ENL during pregnancy and lactation, however these studies lacked appropriate controls for definitive conclusions (Duncan and Pearson, 1984; Maurus, 1978).

4. Histopathology of ENL

A comprehensive account of the histopathology of ENL is given by Mabalay et al. (1965) in their work carried out in the Philippines. The classical changes described in the histopathology of acute ENL include a polymorphonuclear leukocyte (PMNL) inflammatory infiltrate in the deeper layers of the dermis and subcutis, within pre-existing lepromatous lesions, often associated with vasculitis (Mabalay et al., 1965). Oedema of the dermis is another frequent finding (Job et al., 1964).

5. Inflammatory infiltrate in ENL

The inflammatory infiltrate in ENL is usually situated in the deeper layers of the dermis and subcutis. The constituent cells in the infiltrate vary as the lesion evolves (Mabalay et al., 1965).

In acute lesions where skin biopsy is performed within 72 h of development, the predominant cell type is PMNLs, sometimes even leading to microabscess formation. Varying numbers of eosinophils and mast cells have also been detected (Job et al., 1964; Mabalay et al., 1965). In a study in Pakistani patients, PMNLs have been detected in only 64% of biopsies but the biopsies were done within 7 days of appearance of lesions (Hussain et al., 1995).

In biopsies performed within 72—96 h, the subacute infiltrate consists of approximately equal numbers of PMNLs, lymphocytes and plasma cells with persistence of mast cells (Mabalay et al., 1965).

Chronic lesions >9 days old have significantly fewer neutrophils and eosinophils with more lymphocytes, plasma cells and histiocytes. This indicates the transition from the acute phase to the regressing phase of the reaction and highlights the importance of the timing of the skin biopsy in ENL, as late biopsies may not demonstrate the typical cellular infiltrate.

6. Lepromatous infiltrate and staining for acid fast bacilli

The infiltrate due to LL leprosy is usually seen in the upper and mid dermis and consists of large numbers of macrophages with granular or foamy cytoplasm and fewer numbers of lymphocytes arranged in sheets without obvious granuloma formation.

Live *M. leprae* bacilli stain solid with Wade—Fite stain for acid fast bacilli (AFB), whilst dead organisms show granular staining. Early ENL lesions show the presence of granular and fragmented forms of bacilli (Job et al., 1964; Mabalay et al., 1965; Sehgal et al., 1986a). Ridley (1960) showed that there is a preponderance of granular over solid forms in ENL lesions compared with LL lesions without reaction. He also reported that bacilli became granular more rapidly before the onset of ENL (Ridley, 1960).

7. Changes in the dermis and subcutaneous tissue

Vascular changes are common in ENL and it has been hypothesised that vasculitis is the major pathological event in ENL (Sehgal et al., 1986a). Classical features of vasculitis, necrotizing changes (Job et al., 1964) and thrombus formation (Sehgal et al., 1986a) have been demonstrated. Late lesions may show proliferative or obliterative changes (Mabalay et al., 1965).

Interstitial oedema is frequently seen (Job et al., 1964; Sehgal et al., 1986a) and necrosis of the connective tissue fibres has been reported (Walcott, 1947).

The inflammatory infiltrate of ENL can involve the subcutaneous fat similar to the adjacent deep dermis. In the acute phase there may be panniculitis (Mabalay et al., 1965). Necrosis and abscess formation in the subcutis (Job et al., 1964) as well as vasculitis have been reported (Walcott, 1947).

8. Lucio's phenomenon

Lucio's phenomenon is a necrotizing skin reaction in non-nodular LL leprosy commonly found in Mexico and Central America. Histopathologically there is a dermal vasculitis (Latapi and Zamora, 1948). In a study comparing Mexican patients with Lucio's phenomenon and ENL, Lucio's phenomenon was shown to consist of a necrotizing process in the subpapillary plexus with necrosis of the underlying epidermis, whereas the infiltrate in ENL involved the deeper dermis (Rea and Ridley, 1979). Lucio's phenomenon showed less heavy neutrophil infiltration than ENL and showed the colonisation of endothelial cells with solid-staining AFB (Rea and Ridley, 1979).

9. Mucosal changes

There are only a few reports describing the changes in mucosal surfaces in ENL. Job and Chacko (1988) described 20 patients with gross and microscopic changes in the nasal mucosa during reactional states. Approximately two-thirds of the patients showed microscopic evidence of ENL, with

infiltration of PMNLs and mucosal oedema being the most common findings (Job and Chacko, 1988). Vasculitis was seen in 20% of the biopsies. A study describing the ultrastructural changes in the face and palatal mucosa in LL patients reported the presence of increased lysosomal activity in active ENL cases (Reichart et al., 1985).

10. Ethnic variation

There is geographic variation in the clinical frequency of ENL. Contemporary estimates show that ENL is more frequent in Southeast Asia and Brazil (25—49%) (Nery et al., 1998; Pocaterra et al., 2006; Schreuder, 1998), whereas figures as low as 5% are reported in Africa (Becx-Bleumink and Berhe, 1992; Saunderson et al., 2000). Ridley et al. (1981) hypothesised that there is a variation in the pathological changes in ENL among different ethnic groups, based mainly on their work on New Guinean patients.

The ethnic variation in ENL may be due to genetic variation. de Messias et al. (1993) have shown a significant association between the non-expressed C4B allele (C4B*Q0) and the occurrence of ENL in Brazilian patients. In this cohort, all the patients who were homozygously C4B-deficient had ENL. They hypothesised that the absence of the C4B allele may lead to the lack of immune complex clearance in ENL (de Messias et al., 1993).

11. ENL and serum markers of inflammation

The relationship between ENL and serum markers of inflammation has been studied as it is a disorder with systemic manifestations and thought to be mediated by immune complexes.

C-reactive protein (CRP) is significantly elevated in ENL. Some studies show that 90% of patients have an elevated CRP level (Bhatia et al., 1983; Foss et al., 1993; Hussain et al., 1995; Sehgal et al., 1992; Sengupta et al., 1979). Hussain et al. (1995) considered CRP to be a predictor of severity of recurrence in view of elevated CRP being associated with the presence of PMNLs in the skin and a higher chance of recurrences. Foss et al. (1993) showed a strong positive correlation between TNFα and CRP levels in ENL.

Levels of serum amyloid A protein and alpha-1 antitrypsin have also been reported to be elevated in ENL (Hussain et al., 1995; Memon et al., 1996).

The host response to infection may also be accompanied by alterations in lipid metabolism. Memon et al. (1996) have reported significantly low levels of triglycerides as well as total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol in patients with ENL compared with those with LL/BL leprosy.

12. ENL and autoantibodies

Rheumatoid factor has been reported to be present in varying amounts ranging from 3.4% to 48% in LL patients (Bhatia et al., 1983; Ochieng et al., 1994; Sharma et al., 1982). Anti-nuclear antibody, anti-smooth muscle antibody, anti-neutrophil cytoplasmic antibody (ANCA) and anti-thyroid antibody levels have also been studied (Freire et al., 1998; Ochieng et al., 1994; Sharma et al., 1982) but do not show a significant correlation with ENL.

13. Immunopathology of ENL

There is long-standing evidence that ENL is an immune complex-mediated phenomenon, however recent work has shown evidence of a cell-mediated immune response in ENL. Here we critically analyse evidence supporting both theories.

13.1. Immune-mediated mechanism

13.1.1. Immune complex and complement deposition in skin lesions

A key piece of research was done by Wemambu et al. in 1969 that showed the presence of Ig and complement in the skin in 59% of patients with ENL, which was not seen in any of those with LL disease without ENL (Wemambu et al., 1969). It was reported that 70% of the patients with Ig deposition showed the presence of *M. leprae* antigens within the immune complexes. The granular deposits in the skin were similar to those seen in Arthus type reaction. Later, the same group showed that Igs were likely to be absent in lesions where there were fewer PMNLs, indicating that the lesions were likely to be older than 24 h by which time Arthus type reaction may be absent from the skin (Turk, 1970; Waters et al., 1971).

The presence of *M. leprae* antigen, IgG, IgM, and complement C3, C1q and C3d at the same sites both in extracellular locations (exudates) and intracellularly within PMNLs has been reported, further strengthening the immune complex theory (Ridley and Ridley, 1983). Later work showed the presence of immune complexes, IgM to phenolic glycolipid-1 (PGL-1) and Tac peptide in suction blisters over ENL lesions (Bhoopat et al., 1991; Scollard et al., 1992). Scollard et al. (1992) reported that even though immune complexes were increased, there was no elevation in total IgG, IgM and IgA levels or an increase in intracutaneous levels of anti-*M. leprae* antibody.

Earlier work has shown that patients with ENL have significant immune complex deposition in their skin but serum Ig levels similar to those without ENL (Wemambu et al., 1969). This led to the hypothesis that ENL is caused by the deposition of immune complexes locally.

Parallels for the pathogenesis of ENL can be drawn from rheumatoid arthritis (RA). Contemporary data suggest that RA is initiated by immune complexes and complement activation, perpetuated by cytokines and effected by metalloproteinases (Weissmann, 2006). Recent work on RA has shown a correlation between rheumatoid factor, polyethylene glycol (PEG)-precipitated IgG levels and the induction of TNFα by PEG-precipitated synovial fluid immune complexes (Mathsson et al., 2006). No such association was demonstrated for serum immune complexes. Furthermore, there is an association between the levels of TNFα induced by synovial fluid precipitates and the number of swollen joints, which does not exist for serum immune complexes, supporting the hypothesis of a localised immune mechanism.

13.1.2. Igs in the vascular compartment

High levels of Igs are well documented in LL leprosy. Bjorvatn et al. (1976) reported an increase in complement and a significant elevation of C3d, a product of complement catabolism, in ENL. They also found that C1q binding activity, a measure of immune complexes, was correlated with C3d levels, indicating an increase in immune complex formation (Bjorvatn et al., 1976).

Many studies have shown an increase in serum levels of anti-PGL IgM across the spectrum from tuberculoid to LL leprosy (Levis et al., 1986; Schwerer et al., 1984), with a positive linear correlation with the BI (Levis et al., 1986). Patients with ENL have significantly lower levels of anti-PGL IgM compared with those patients with LL and the same BI but without ENL (Levis et al., 1986; Schwerer et al., 1984). Other studies have shown significantly lower levels of IgG and IgM in ENL compared with patients with LL leprosy and post-ENL patients (Rao and Rao, 1988; Sharma et al., 1982). Serum C3 levels were significantly elevated in patients with ENL, whilst C4 levels remained unchanged (Rao and Rao, 1988; Wemambu et al., 1969). The levels of IgG, IgM, IgA, C3 and C4 in immune complexes were significantly lower in ENL than in LL disease (Rao and Rao, 1988). The lower serum Ig level in ENL may be due to them being used up in immune complexes.

IgG and IgM levels were shown to be increased in patients following an episode of ENL (Sharma et al., 1982). The composition of PEG precipitates and autoantibodies in the sera from patients with ENL is similar to that reported in rheumatological disorders, and the immune complexes are not cleared even after apparent clinical cure (Saha et al., 1984). It has been suggested that circulating immune complexes (CIC) may impair the macrophage function of bacterial clearance by attaching to T-cells by Fc receptors. It was later shown that CICs from patients with LL and BL leprosy and ENL have the ability to suppress lymphocyte proliferation induced by *M. leprae* antigens (Tyagi et al., 1992).

Further studies into the subclasses of IgG showed upregulation of polyclonal IgG_1 antibody synthesis during ENL (Kifayet et al., 1996). Post-ENL patients showed IgG characteristics similar to those with LL leprosy, indicating that the changes in the Igs observed during ENL are transient.

13.2. Cell-mediated immune response

Rea et al. (1972) postulated that cell-mediated immunity (CMI) plays a role in the pathogenesis of ENL. They noted that ENL can occur in the absence of treatment and that patients with ENL have apparently normal CMI compared with those with LL leprosy (Rea et al., 1972). In 1982, Mshana hypothesised that ENL is precipitated by an imbalance of T-lymphocytes. He hypothesised that ENL has two phases: initiation, due to an imbalance in T-cell subpopulations with decreased suppressor cells; and perpetuation (Mshana, 1982). A reduction in suppressor T-cells in ENL was later found.

In 1985, Laal et al. provided evidence of the natural emergence of a transient T-cell reactivity in ENL by showing the presence of a significantly strong leukocyte migration inhibition and antigen-induced in vitro lymphoproliferation in sera from patients with ENL (Laal et al., 1985).

13.2.1. T-cells in ENL

There are several pieces of evidence for increased T-cell activity in LL patients with ENL in comparison with those with LL alone. Patients with LL leprosy without reaction have a CD4:CD8 ratio of approximately 1:2 with a predominance of T suppressor cells (Modlin et al., 1983; Wallach et al., 1984). A reversed $CD4^+$:$CD8^+$ ratio of 2:1 in lesions of ENL has been reported (Modlin et al., 1983, 1985, 1986; Narayanan et al., 1984; Rao and Rao, 1986). Modlin et al. (1986) and Narayanan et al. (1984) showed that ENL lesions contained $Leu3a^+$ lymphocytes, whereas LL lesions without reaction contained a predominantly $Leu2a^+$ response. However, Shen et al. (1987) found no difference in the expression of Ta1 antigen, a measure of T-cell activation, on T-lymphocytes from ENL and LL lesions.

13.2.2. B-cells in ENL

There is surprisingly little evidence of an increase in B-lymphocytes in ENL lesions. There is only one report documenting an increased percentage and absolute count of B-cells in the sera from patients with ENL (Sehgal et al., 1986b), but normal numbers of circulating B-cells have also been reported (Rao and Rao, 1986). A study looking at T-cell phenotypes in reactional lesions showed that there is no increase in B-cells (Narayanan et al., 1984).

Singh et al. (1994a,b) showed that sera from patients with ENL produce antibodies against B-cell epitopes of a specific fusion protein (LSR) in *M. leprae*. They found that the B-cell epitopes, overlapping peptides 2 and 3, were recognised by >95% of ENL sera and that peptide 2 selectively reacted with sera from active disease, suggesting that antibody reactivity to these peptides may be candidate markers for the development of ENL (Singh et al., 1994b). Even within the 19 amino acid sequence that comprised these two overlapping peptides there were multiple sites involved in antibody reactivity and they varied in their specificity to different immunopathological disease variants (Singh et al., 1994a).

B-cells are precursors for antibody-secreting plasma cells. However, B-cells may also act as antigen-presenting cells (APC) and play a role in the initiation and regulation of T- and B-cell responses (Martin and Chan, 2006; Youinou et al., 2006). The role of B-cells in the pathogenesis of autoimmune disorders such as RA and systemic lupus erythematosus (SLE) is now being re-examined (Martin and Chan, 2006; Martinez-Gamboa et al., 2006). It would therefore be interesting to examine the role of B-cells in the pathogenesis of ENL.

13.3. Cytokines in ENL

There is evidence of both T_H1- and T_H2-type involvement in ENL in skin lesions and serum (Moraes et al., 1999, 2000; Nath et al., 2000; Sreenivasan et al., 1998; Teles et al., 2002). TNFα and IL-6 were shown to be present in almost all reactional lesions by many authors (Moraes et al., 1999, 2000; Sreenivasan et al., 1998; Teles et al., 2002). Peripheral blood mononuclear cells (PBMC) from patients with ENL show the highest release of TNFα compared with inactive ENL, reversal reaction (RR) and LL lesions (Barnes et al., 1992). This is reduced by >90% with the administration of thalidomide. Sreenivasan et al. (1998) found a T_H1-like cytokine

pattern in 64% of ENL sera and 85% of RR sera with basal expression of IFNγ, which was mirrored in the skin lesions. This was further confirmed by Moraes et al. (1999) who detected IFNγ in 84% of ENL and all RR lesions. Cooper et al. (1989) showed that the highest expression of IFNγ mRNA was seen in RR lesions and that only a few cells were positive for IFNγ mRNA in ENL lesions. However, this small number of positive cells was five times that seen in LL leprosy lesions without ENL (Cooper et al., 1989). The other cytokines found in varying amounts in ENL lesions are IL-12, IL-10 and IL-12p40. Granulocyte—macrophage colony-stimulating factor (GM-CSF) and perforin were also reported to be secreted by PBMCs of patients with ENL (Singh et al., 1994b). IL-4 was found to be absent or present in low amounts in ENL lesions (Moraes et al., 1999, 2000; Nath et al., 2000). This is strong evidence that ENL is associated with a T_H1-type reaction, as the major cytokines expressed in a T_H2-type response are IL-4, IL-5 and IL-10.

In contrast to the above findings, Goulart et al. (2000) reported that ENL elicited a T_H2-type response with production of IL-6, IL-8, IL-10 and TNFα, whilst RR showed a predominantly T_H1 response with the release of IL-1b, TNFα, IL-12 and IFNγ. They also found significant amounts of transforming growth factor-beta (TGFβ) in sera of ENL patients, both in the unstimulated and stimulated states, compared with RR and LL patients (Goulart et al., 2000).

14. Role of co-stimulatory molecules and matrix metalloproteinases

Optimum activation of T-helper cells requires not only occupancy of the T-cell receptor by antigen—MHC II complex but also a set of co-stimulatory signals complementary to each other, which include intercellular adhesion molecule-1 (ICAM-1) and B7-1 or 2 on APCs, and lymphocyte function-associated antigen-1 (LFA-1) and CD28 on T-helper cells. It has been shown that *M. leprae* impedes the cell-mediated immune response by reducing the co-stimulatory activity of host cells and that there is a significant reduction of B7-1 and CD28 in untreated LL and BL patients (Agrewala et al., 1998). Expression of B7-1 in ENL lesions was reported recently, although the strongest expression of B7-1 was found in RR lesions (Santos et al., 2007). The number of patients included was small and the expression of B7-1 in the skin lesions was not quantified so it is difficult to draw conclusions from this study.

Matrix metalloproteinases (MMP) are a family of proteolytic enzymes that play an important role in the normal immune response to infection. Excess MMP activity following infection may lead to tissue damage and this has been shown to occur in tuberculosis (Elkington et al., 2005; Harris et al., 2007; Taylor et al., 2006). The presence of MMPs in nerves affected by leprosy has been reported but to our knowledge the role of MMPs in leprosy reactions has not been studied (Teles et al., 2007).

15. Changes in the epidermis

The thickness of the nucleated epidermis is increased in ENL (Rea, 2000), with increased numbers of Langerhans cells in the epidermis both in ENL and RR lesions (Modlin et al., 1983; Narayanan et al., 1984; Rea et al., 1986; Thangaraj et al., 1988). ENL lesions show a strong but patchy keratinocyte 1a expression (Rea et al., 1986; Thangaraj et al., 1988). Keratinocyte 1a expression in the human epidermis is a sign of delayed type hypersensitivity reaction or cell-mediated immune responses. In addition, the epidermis in ENL has been demonstrated to strongly express ICAM-1 on keratinocytes and LFA-1 on epidermal lymphocytes compared with RR and non-reactional LL lesions (Sullivan et al., 1991). ICAM-1 and LFA-1 act as co-stimulatory molecules to enhance the cell-mediated immune response. The changes observed in the epidermis in ENL suggest that the epidermis plays an immunological role in the pathogenesis of ENL.

16. Role of neutrophils

Influx of PMNLs is a feature of early ENL lesions. A recent study showed that apoptosis of PMNLs is significantly increased in ENL. It was also shown that polymorphs can be stimulated to secrete TNFα and IL-8 by *M. leprae* and lipoarabinomannan from *M. leprae* (Oliveira et al., 1999). It has been hypothesised that PMNLs release cytokines that in turn promote further recruitment of leukocytes and tissue damage.

17. Conclusions

We propose that ENL is initiated by the release of mycobacterial antigens, which leads to the formation of immune complexes and complement activation. This in turn leads to the activation of mononuclear cells to release cytokines that act as mediators of tissue damage. The molecules acting as the link between the immune complexes and the mononuclear cells are as yet unknown. We hypothesise that B-cells act as APCs and/or cytokine-secreting cells to bring about changes in the cell-mediated immune response.

There are recent unpublished data showing that pure disrupted *M. leprae* fixes significantly more complement than intact *M. leprae*, leading to a hypothesis that cleavage products from the bacillus initiates complement activation and in turn ENL (Lahiri et al., unpublished data). This work has to be substantiated by in vivo studies to draw conclusions. Work is also needed to study the factors that perpetuate the reactions leading to chronicity, as ENL may continue even in the absence of bacteria in the skin.

The role of B-cells in the pathogenesis of ENL must be studied further. In the model proposed for the pathogenesis of RA it is suggested that lipid mediators and kinase/phosphate signalling act as mediators between immune complexes and cytokines. The presence of a similar link in ENL should also be explored.

The role played by co-stimulatory molecules and MMPs in the pathogenesis of ENL is another important area of research. Some work has been done on B7-1 and ICAM-1 in ENL but larger studies are warranted.

Current data support the involvement both of immune complexes and cell-mediated immune responses in the pathogenesis of ENL but there are many grey areas in our knowledge of ENL. Further work that could shed light on these aspects will help immensely to improve the management of this difficult to treat condition.

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References

Agrewala, J.N., Kumar, B., Vohra, H., 1998. Potential role of B7-1 and CD28 molecules in immunosuppression in leprosy. Clin. Exp. Immunol. 111, 56—63.

Bajaj, A.K., Gupta, S.C., Sinha, S.N., Govil, D.C., Gaur, U.C., Kumar, R., 1981. Renal functional status in lepromatous leprosy. Int. J. Lepr. Other Mycobact. Dis. 49, 37—41.

Barnes, P.F., Chatterjee, D., Brennan, P.J., Rea, T.H., Modlin, R.L., 1992. Tumor necrosis factor production in patients with leprosy. Infect. Immun. 60, 1441—1446.

Becx-Bleumink, M., Berhe, D., 1992. Occurrence of reactions, their diagnosis and management in leprosy patients treated with multidrug therapy; experience in the leprosy control program of the All Africa Leprosy and Rehabilitation Training Center (ALERT) in Ethiopia. Int. J. Lepr. Other Mycobact. Dis. 60, 173—184.

Bhatia, V.N., Balakrishnan, S., Harikrishnan, S., 1983. Serological study for presence of C-reactive protein, rheumatoid factor, anti streptolysin O in leprosy cases. Lepr. India 55, 86—90.

Bhoopat, L., Scollard, D.M., Theetranont, C., Chiewchanvit, S., Nelson, D.L., Utaipat, U., 1991. Studies of human leprosy lesions in situ using suction-induced blisters: cell changes with IgM antibody to PGL-1 and interleukin-2 receptor in clinical subgroups of erythema nodosum leprosum. Asian Pac. J. Allergy Immunol. 9, 107—119.

Bjorvatn, B., Barnetson, R.S., Kronvall, G., Zubler, R.H., Lambert, P.H., 1976. Immune complexes and complement hypercatabolism in patients with leprosy. Clin. Exp. Immunol. 26, 388—396.

Burte, N.P., Chandorkar, A.G., Muley, M.P., Balsara, J.J., Bulakh, P.M., 1983. Clofazimine in lepra (ENL) reaction, one year clinical trial. Lepr. India 55, 265—277.

Cook, G.C., Corachan, M., 1982. Hepatic structure and function in Papua New Guineans with leprosy. Trans. R. Soc. Trop. Med. Hyg. 76, 721—727.

Cooper, C.L., Mueller, C., Sinchaisri, T.A., Pirmez, C., Chan, J., Kaplan, G., Young, S.M., Weissman, I.L., Bloom, B.R., Rea, T.H., et al., 1989. Analysis of naturally occurring delayed-type hypersensitivity reactions in leprosy by in situ hybridization. J. Exp. Med. 169, 1565—1581.

de Messias, I.J., Santamaria, J., Brenden, M., Reis, A., Mauff, G., 1993. Association of C4B deficiency (C4B*Q0) with erythema nodosum in leprosy. Clin. Exp. Immunol. 92, 284—287.

Duncan, M.E., Pearson, J.M., 1984. The association of pregnancy and leprosy—III. Erythema nodosum leprosum in pregnancy and lactation. Lepr. Rev. 55, 129—142.

Elkington, P.T., Emerson, J.E., Lopez-Pascua, L.D., O'Kane, C.M., Horncastle, D.E., Boyle, J.J., Friedland, J.S., 2005. *Mycobacterium tuberculosis* up-regulates matrix metalloproteinase-1 secretion from human airway epithelial cells via a p38 MAPK switch. J. Immunol. 175, 5333—5340.

Foss, N.T., de Oliveira, E.B., Silva, C.L., 1993. Correlation between TNF production, increase of plasma C-reactive protein level and suppression of T lymphocyte response to concanavalin A during erythema nodosum leprosum. Int. J. Lepr. Other Mycobact. Dis. 61, 218—226.

Freire, B.F., Ferraz, A.A., Nakayama, E., Ura, S., Queluz, T.T., 1998. Anti-neutrophil cytoplasmic antibodies (ANCA) in the clinical forms of leprosy. Int. J. Lepr. Other Mycobact. Dis. 66, 475—482.

Girdhar, A., Chakma, J.K., Girdhar, B.K., 2002. Pulsed corticosteroid therapy in patients with chronic recurrent ENL: a pilot study. Indian J. Lepr. 74, 233—236.

Goulart, I.M., Mineo, J.R., Foss, N.T., 2000. Production of transforming growth factor-beta 1 (TGF-β1) by blood monocytes from patients with different clinical forms of leprosy. Clin. Exp. Immunol. 122, 330—334.

Harris, J.E., Nuttall, R.K., Elkington, P.T., Green, J.A., Horncastle, D.E., Graeber, M.B., Edwards, D.R., Friedland, J.S., 2007. Monocyte—astrocyte networks regulate matrix metalloproteinase gene expression and secretion in central nervous system tuberculosis in vitro and in vivo. J. Immunol. 178, 1199—1207.

Helmy, H.S., Pearson, J.M., Waters, M.F., 1971. Treatment of moderately severe erythema nodosum leprosum with clofazimine—a controlled trial. Lepr. Rev. 42, 167—177.

Hussain, R., Lucas, S.B., Kifayet, A., Jamil, S., Raynes, J., Uqaili, Z., Dockrell, H.M., Chiang, T.J., McAdam, K.P., 1995. Clinical and histological discrepancies in diagnosis of ENL reactions classified by assessment of acute phase proteins SAA and CRP. Int. J. Lepr. Other Mycobact. Dis. 63, 222—230.

Job, A., Chacko, C.J., 1988. Reactional states in the nasal mucosa: a clinical and histopathological study. Int. J. Lepr. Other Mycobact. Dis. 56, 523—526.

Job, C.K., Gude, S., Macaden, V.P., 1964. Erythema nodosum leprosum. A clinico-pathologic study. Int. J. Lepr. 32, 177—184.

Jopling, W.H., McDougall, A.C., 1988. Leprosy reactions (reactional states), in: Handbook of Leprosy. Heinemann Medical, London, pp. 85—88.

Kifayet, A., Shahid, F., Lucas, S., Hussain, R., 1996. Erythema nodosum leprosum is associated with up-regulation of polyclonal IgG1 antibody synthesis. Clin. Exp. Immunol. 106, 447—453.

Kumar, B., Koshy, A., Kaur, S., Kaur, I., Rajwanshi, A., 1987. Leprosy, liver and jaundice. Indian J. Lepr. 59, 194—202.

Kumar, B., Dogra, S., Kaur, I., 2004. Epidemiological characteristics of leprosy reactions: 15 years experience from north India. Int. J. Lepr. Other Mycobact. Dis. 72, 125—133.

Laal, S., Bhutani, L.K., Nath, I., 1985. Natural emergence of antigen-reactive T cells in lepromatous leprosy patients during erythema nodosum leprosum. Infect. Immun. 50, 887—892.

Latapi, F., Zamora, A.C., 1948. The 'spotted' leprosy of Lucio (La lepra 'manchada' de Lucio). An introduction to its clinical and histological study. Int. J. Lepr. 16, 421—429.

Levis, W.R., Meeker, H.C., Schuller-Levis, G., Sersen, E., Schwerer, B., 1986. IgM and IgG antibodies to phenolic glycolipid I from *Mycobacterium leprae* in leprosy: insight into patient monitoring, erythema nodosum leprosum, and bacillary persistence. J. Invest. Dermatol. 86, 529—534.

Lipman, M., Breen, R., 2006. Immune reconstitution inflammatory syndrome in HIV. Curr. Opin. Infect. Dis. 19, 20—25.

Mabalay, M.C., Helwig, E.B., Tolentino, J.G., Binford, C.H., 1965. The histopathology and histochemistry of erythema nodosum leprosum. Int. J. Lepr. 33, 28—49.

Manandhar, R., LeMaster, J.W., Roche, P.W., 1999. Risk factors for erythema nodosum leprosum. Int. J. Lepr. Other Mycobact. Dis. 67, 270—278.

Martin, F., Chan, A.C., 2006. B cell immunobiology in disease: evolving concepts from the clinic. Annu. Rev. Immunol. 24, 467—496.

Martinez-Gamboa, L., Brezinschek, H.P., Burmester, G.R., Dorner, T., 2006. Immunopathologic role of B lymphocytes in rheumatoid arthritis: rationale of B cell-directed therapy. Autoimmun. Rev. 5, 437—442.

Mathsson, L., Lampa, J., Mullazehi, M., Ronnelid, J., 2006. Immune complexes from rheumatoid arthritis synovial fluid induce FcγRIIa dependent and rheumatoid factor correlated production of tumour necrosis factor-α by peripheral blood mononuclear cells. Arthritis Res. Ther. 8, R64.

Maurus, J.N., 1978. Hansen's disease in pregnancy. Obstet. Gynecol. 52, 22–25.

Memon, R.A., Hussain, R., Raynes, J.G., Lateff, A., Chiang, T.J., 1996. Alterations in serum lipids in lepromatous leprosy patients with and without ENL reactions and their relationship to acute phase proteins. Int. J. Lepr. Other Mycobact. Dis. 64, 115–122.

Modlin, R.L., Gebhard, J.F., Taylor, C.R., Rea, T.H., 1983. In situ characterization of T lymphocyte subsets in the reactional states of leprosy. Clin. Exp. Immunol. 53, 17–24.

Modlin, R.L., Bakke, A.C., Vaccaro, S.A., Horwitz, D.A., Taylor, C.R., Rea, T.H., 1985. Tissue and blood T-lymphocyte subpopulations in erythema nodosum leprosum. Arch. Dermatol. 121, 216–219.

Modlin, R.L., Mehra, V., Jordan, R., Bloom, B.R., Rea, T.H., 1986. In situ and in vitro characterization of the cellular immune response in erythema nodosum leprosum. J. Immunol. 136, 883–886.

Moraes, M.O., Sarno, E.N., Almeida, A.S., Saraiva, B.C., Nery, J.A., Martins, R.C., Sampaio, E.P., 1999. Cytokine mRNA expression in leprosy: a possible role for interferon-gamma and interleukin-12 in reactions (RR and ENL). Scand. J. Immunol. 50, 541–549.

Moraes, M.O., Sarno, E.N., Teles, R.M., Almeida, A.S., Saraiva, B.C., Nery, J.A., Sampaio, E.P., 2000. Anti-inflammatory drugs block cytokine mRNA accumulation in the skin and improve the clinical condition of reactional leprosy patients. J. Invest. Dermatol. 115, 935–941.

Moreira, A.L., Kaplan, G., Villahermosa, L.G., Fajardo, T.J., Abalos, R.M., Cellona, R.V., Balagon, M.V., Tan, E.V., Walsh, G.P., 1998. Comparison of pentoxifylline, thalidomide and prednisone in the treatment of ENL. Int. J. Lepr. Other Mycobact. Dis. 66, 61–65.

Mshana, R.N., 1982. Hypothesis: erythema nodosum leprosum is precipitated by an imbalance of T lymphocytes. Lepr. Rev. 53, 1–7.

Narayanan, R.B., Laal, S., Sharma, A.K., Bhutani, L.K., Nath, I., 1984. Differences in predominant T cell phenotypes and distribution pattern in reactional lesions of tuberculoid and lepromatous leprosy. Clin. Exp. Immunol. 55, 623–628.

Nath, I., Vemuri, N., Reddi, A.L., Bharadwaj, M., Brooks, P., Colston, M.J., Misra, R.S., Ramesh, V., 2000. Dysregulation of IL-4 expression in lepromatous leprosy patients with and without erythema nodosum leprosum. Lepr. Rev. 71 (Suppl.), S130–S137.

Nery, J.A., Vieira, L.M., de Matos, H.J., Gallo, M.E., Sarno, E.N., 1998. Reactional states in multibacillary Hansen disease patients during multidrug therapy. Rev. Inst. Med. Trop. Sao Paulo 40, 363–370.

Ochieng, N.A., Bwire, M.S., Orege, P.A., Nyawalo, J.O., K'Omollo, J.W., 1994. Autoantibodies in lepromatous leprosy. Afr. J. Health Sci. 1, 79–83.

Oliveira, R.B., Moraes, M.O., Oliveira, E.B., Sarno, E.N., Nery, J.A., Sampaio, E.P., 1999. Neutrophils isolated from leprosy patients release TNF-alpha and exhibit accelerated apoptosis in vitro. J. Leukoc. Biol. 65, 364–371.

Pfaltzgraff, R.E., Ramu, G., 1994. Clinical leprosy, in: Hastings, R.C. (Ed), Leprosy. Churchill Livingstone, Edinburgh, pp. 237–290.

Pocaterra, L., Jain, S., Reddy, R., Muzaffarullah, S., Torres, O., Suneetha, S., Lockwood, D.N., 2006. Clinical course of erythema nodosum leprosum: an 11-year cohort study in Hyderabad, India. Am. J. Trop. Med. Hyg. 74, 868–879.

Rao, T.D., Rao, P.R., 1986. Tr, T mu and B lymphocytes in erythema nodosum leprosum reactions of leprosy. Indian J. Lepr. 58, 601–608.

Rao, T.D., Rao, P.R., 1988. Serum immune complexes in erythema nodosum leprosum reactions of leprosy. Indian J. Lepr. 60, 189–195.

Rea, T.H., 2000. Frequency and extent of thickening of the nucleated epidermis in leprosy lesions. Int. J. Lepr. Other Mycobact. Dis. 68, 410–416.

Rea, T.H., Ridley, D.S., 1979. Lucio's phenomenon: a comparative histological study. Int. J. Lepr. Other Mycobact. Dis. 47, 161–166.

Rea, T.H., Levan, N.E., Schweitzer, R.E., 1972. Erythema nodosum leprosum in the absence of chemotherapy: a role for cell-mediated immunity. Lancet 2, 1252.

Rea, T.H., Shen, J.Y., Modlin, R.L., 1986. Epidermal keratinocyte Ia expression. Langerhans cell hyperplasia and lymphocytic infiltration in skin lesions of leprosy. Clin. Exp. Immunol. 65, 253–259.

Reichart, P.A., Metah, D., Althoff, J., 1985. Ultrastructural aspects of oral and facial lepromatous lesions. Int. J. Oral Surg. 14, 55–60.

Ridley, D.S., 1960. A bacteriologic study of erythema nodosum leprosum. Int. J. Lepr. 28, 254–266.

Ridley, M.J., Ridley, D.S., 1983. The immunopathology of erythema nodosum leprosum: the role of extravascular complexes. Lepr. Rev. 54, 95–107.

Ridley, D.S., Rea, T.H., McAdam, K.P., 1981. The histology of erythema nodosum leprosum. Variant forms in New Guineans and other ethnic groups. Lepr. Rev. 52, 65–78.

Saha, K., Chakrabarty, A.K., Sharma, V.K., Sehgal, V.N., 1984. Polyethylene glycol precipitates in serum during and after erythema nodosum leprosum—study of their composition and anticomplementary activity. Int. J. Lepr. Other Mycobact. Dis. 52, 44–48.

Sales, A.M., de Matos, H.J., Nery, J.A., Duppre, N.C., Sampaio, E.P., Sarno, E.N., 2007. Double-blind trial of the efficacy of pentoxifylline vs thalidomide for the treatment of type II reaction in leprosy. Braz. J. Med. Biol. Res. 40, 243–248.

Santos, D.O., Castro, H.C., Bourguignon, S.C., Bastos, O.M., Rodrigues, C.R., Van Heuverswyn, H., Nery, J.A., Miranda, A., 2007. Expression of B7-1 costimulatory molecules in patients with multibacillary leprosy and reactional states. Clin. Exp. Dermatol. 32, 75–80.

Saunderson, P., Gebre, S., Byass, P., 2000. ENL reactions in the multibacillary cases of the AMFES cohort in central Ethiopia: incidence and risk factors. Lepr. Rev. 71, 318–324.

Schreuder, P.A., 1998. The occurrence of reactions and impairments in leprosy: experience in the leprosy control program of three provinces in northeastern Thailand, 1987–1995 [correction of 1978–1995]. II. Reactions. Int. J. Lepr. Other Mycobact. Dis. 66, 159–169.

Schwerer, B., Meeker, H.C., Sersen, G., Levis, W.R., 1984. IgM antibodies against phenolic glycolipid I from *Mycobacterium leprae* in leprosy sera: relationship to bacterial index and erythema nodosum leprosum. Acta Leprol. 2, 394–402.

Scollard, D.M., Bhoopat, L., Kestens, L., Vanham, G., Douglas, J.T., Moad, J., 1992. Immune complexes and antibody levels in blisters over human leprosy skin lesions with or without erythema nodosum leprosum. Clin. Immunol. Immunopathol. 63, 230–236.

Sehgal, V.N., Gautam, R.K., Koranne, R.V., Beohar, P.C., 1986a. The histopathology of type I (lepra) and type II (ENL) reactions in leprosy. Indian J. Lepr. 58, 240–243.

Sehgal, V.N., Gautam, R.K., Sharma, V.K., 1986b. Immunoprofile of reactions in leprosy. Int. J. Dermatol. 25, 240–244.

Sehgal, V.N., Bhattacharya, S.N., Shah, Y., Sharma, V.K., Gupta, C.K., 1992. Reaction in leprosy: acute phase reactant response during and after remission. Int. J. Dermatol. 31, 632–634.

Sengupta, U., Sinha, S., Ramu, G., 1979. Immunological assessment of sera of leprosy patients. Lepr. India 51, 43–48.

Sharma, V.K., Saha, K., Sehgal, V.N., 1982. Serum immunoglobulins and autoantibodies during and after erythema nodosum leprosum (ENL). Int. J. Lepr. Other Mycobact. Dis. 50, 159–163.

Shen, J.Y., Hofman, F.M., Gunter, J.R., Modlin, R.L., Rea, T.H., 1987. In situ identification of activated Ta1+ T lymphocytes in human leprosy skin lesions. Int. J. Lepr. Other Mycobact. Dis. 55, 494–498.

Singh, S., Jenner, P.J., Narayan, N.P., Ramu, G., Colston, M.J., Prasad, H.K., Nath, I., 1994a. Critical residues of the *Mycobacterium leprae* LSR recombinant protein discriminate clinical activity in erythema nodosum leprosum reactions. Infect. Immun. 62, 5702—5705.

Singh, S., Narayanan, N.P., Jenner, P.J., Ramu, G., Colston, M.J., Prasad, H.K., Nath, I., 1994b. Sera of leprosy patients with type 2 reactions recognize selective sequences in *Mycobacterium leprae* recombinant LSR protein. Infect. Immun. 62, 86—90.

Sreenivasan, P., Misra, R.S., Wilfred, D., Nath, I., 1998. Lepromatous leprosy patients show T helper 1-like cytokine profile with differential expression of interleukin-10 during type 1 and 2 reactions. Immunology 95, 529—536.

Sullivan, L., Sano, S., Pirmez, C., Salgame, P., Mueller, C., Hofman, F., Uyemura, K., Rea, T.H., Bloom, B.R., Modlin, R.L., 1991. Expression of adhesion molecules in leprosy lesions. Infect. Immun. 59, 4154—4160.

Taylor, J.L., Hattle, J.M., Dreitz, S.A., Troudt, J.M., Izzo, L.S., Basaraba, R.J., Orme, I.M., Matrisian, L.M., Izzo, A.A., 2006. Role for matrix metalloproteinase 9 in granuloma formation during pulmonary *Mycobacterium tuberculosis* infection. Infect. Immun. 74, 6135—6144.

Teles, R.M., Moraes, M.O., Geraldo, N.T., Salles, A.M., Sarno, E.N., Sampaio, E.P., 2002. Differential TNFα mRNA regulation detected in the epidermis of leprosy patients. Arch. Dermatol. Res. 294, 355—362.

Teles, R.M., Antunes, S.L., Jardim, M.R., Oliveira, A.L., Nery, J.A., Sales, A.M., Sampaio, E.P., Shubayev, V., Sarno, E.N., 2007. Expression of metalloproteinases (MMP-2, MMP-9, and TACE) and TNF-alpha in the nerves of leprosy patients. J. Peripher. Nerv. Syst. 12, 195—204.

Thangaraj, H., Laal, S., Thangaraj, I., Nath, I., 1988. Epidermal changes in reactional leprosy: keratinocyte Ia expression as an indicator of cell-mediated immune responses. Int. J. Lepr. Other Mycobact. Dis. 56, 401—407.

Turk, J., 1970. Immunological aspects of clinical leprosy. Proc. R. Soc. Med. 63, 1053—1056.

Tyagi, P., Patil, S.A., Girdhar, B.K., Katoch, K., Sengupta, U., 1992. Suppressive effect of circulating immune complexes from leprosy patients on the lymphocyte proliferation induced by *M. leprae* antigens in healthy responders. Int. J. Lepr. Other Mycobact. Dis. 60, 562—569.

Ustianowski, A.P., Lawn, S.D., Lockwood, D.N., 2006. Interactions between HIV infection and leprosy: a paradox. Lancet Infect. Dis. 6, 350—360.

Villahermosa, L.G., Fajardo Jr, T.T., Abalos, R.M., Balagon, M.V., Tan, E.V., Cellona, R.V., Palmer, J.P., Wittes, J., Thomas, S.D., Kook, K.A., Walsh, G.P., Walsh, D.S., 2005. A randomized, double-blind, double-dummy, controlled dose comparison of thalidomide for treatment of erythema nodosum leprosum. Am. J. Trop. Med. Hyg. 72, 518—526.

Walcott, R.R., 1947. Erythema nodosum in leprosy. Int. J. Lepr. 15, 380—388.

Walker, S.L., Waters, M.F., Lockwood, D.N.J., 2007. The role of thalidomide in the management of erythema nodosum leprosum. Lepr. Rev. 78, 197—215.

Wallach, D., Flageul, B., Bach, M.A., Cottenot, F., 1984. The cellular content of dermal leprous granulomas: an immuno-histological approach. Int. J. Lepr. Other Mycobact. Dis. 52, 318—326.

Waters, M.F.R., Turk, J.L., Wemambu, S.N.C., 1971. Mechanisms of reactions in leprosy. Int. J. Lepr. 39, 417—428.

Weissmann, G., 2006. The pathogenesis of rheumatoid arthritis. Bull. NYU Hosp. Jt. Dis. 64, 12—15.

Wemambu, S.N., Turk, J.L., Waters, M.F., Rees, R.J., 1969. Erythema nodosum leprosum: a clinical manifestation of the arthus phenomenon. Lancet 2, 933—935.

WHO, 1998. WHO Expert Committee on Leprosy. World Health Organization, Geneva, Technical Report Series No. 874.

Youinou, P., Hillion, S., Jamin, C., Pers, J.O., Saraux, A., Renaudineau, Y., 2006. B lymphocytes on the front line of autoimmunity. Autoimmun. Rev. 5, 215—221.