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Serum levels of interferon-γ, tumour necrosis factor-α, soluble interleukin-6R and soluble cell activation markers for monitoring response to treatment of leprosy reactions

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Summary

Identifying pathogen and host-related laboratory parameters are essential for the early diagnosis of leprosy reactions. The present study aimed to clarify the validity of measuring the profiles of serum cytokines [interleukin (IL)-4, IL-6, IL-10, interferon (IFN)-γ and tumour necrosis factor (TNF)-α], the soluble IL-6 receptor (sIL-6R), soluble T cell (sCD27) and macrophage (neopterin) activation markers and *Mycobacterium leprae*-specific anti-PGL-I IgM antibodies in relation to the leprosy spectrum and reactions. Serum samples from 131 Indonesian leprosy patients (82 non-reactional leprosy patients and 49 reactional) and 112 healthy controls (HC) from the same endemic region were investigated. Forty-four (89·8%) of the reactional patients had erythema nodosum leprosum (ENL) while only five (10·2%) had reversal reaction (RR). Follow-up serum samples after corticosteroid treatment were also obtained from 17 of the patients with ENL and one with RR. A wide variability in cytokine levels was observed in the patient groups. However, IFN-γ and sIL-6R were elevated significantly in ENL compared to non-ENL patients. Levels of IFN-γ, TNF-α and sIL-6R declined significantly upon corticosteroid treatment of ENL. Thus, although the present study suggests limited applicability of serial measurement of IFN-γ, TNF-α and sIL-6R in monitoring treatment efficacy of ENL, reactions it recommends a search for a wider panel of more disease-specific markers in future studies.

Keywords: cytokine measurement, leprosy, neopterin, reactions, soluble receptors

Introduction

Leprosy is a chronic disease displaying an immunological spectrum ranging from tuberculoid (TT) leprosy, with strong cell-mediated immunity (CMI) against *Mycobacterium leprae*, to lepromatous (LL) leprosy, showing a complete absence of *M. leprae*-specific CMI [1]. This spectral pathology of leprosy is associated with differential activation of immune cells in parallel with the production of cytokines, which are the signals between the immune and the resident cells [2]. Previous studies suggested that the T cell unresponsiveness to *M. leprae* in LL was caused by defective interferon (IFN)-γ activity in these patients [3,4]. In addition, increased expression of mRNA for T helper 2 (Th2) cytokines interleukin (IL)-4, IL-5 and IL-10 has been shown in skin lesions from LL patients. In contrast, 'protective' Th1 cytokines IL-2, IFN-γ and tumour necrosis factor (TNF)-α are associated with TT leprosy [5,6], suggesting that differential cytokine profiles are associated with the leprosy spectrum.

Furthermore, leprosy reactions, namely type 1 or reversal reaction (RR) and type 2 or erythema nodosum leprosum (ENL), are associated reportedly with changes in cytokine activity [2,5]. *M. leprae*-specific T cell clones generated from RR lesions showed a polarized Th1-like profile [7]. In contrast, a predominant Th2 cytokine profile was observed in LL patients [8]. This suggests that identifying cytokine profiles associated with reactions may help in their early diagnosis and eventual monitoring of treatment efficacy. Many studies have presented contradictory results with respect to the predominant cytokines associated with reactions which may be related to the different assay conditions, samples and populations examined [9–12]. Moreover, in view of the difficulty in obtaining lesional biopsies and peripheral blood mononuclear cells (PBMC) under field conditions

and the relative ease of serum immunoassays, measurement of cytokines and other soluble cellular products in circulation has been used as an alternative in various studies [9–12].

Due to the uncertainty created by the often contradictory previous studies [9–12], the primary aim of the present study was to assess the measurement of a broad panel of soluble 'biomarkers' in serum in relation to the leprosy spectrum and reactions. The soluble markers studied included the cytokines (IL-4, IL-6, IL-10, IFN-γ and TNF-α), the soluble IL-6 receptor (sIL-6R), a soluble T cell activation marker (sCD27), a macrophage activation marker (neopterin) and the *M. leprae*-specific anti-PGL-I IgM antibodies.

Materials and methods

Patients and controls

The study included 131 leprosy patients and 112 normal healthy controls (HC) attending the leprosy clinic at the Hasanuddin University hospital in Makassar, Indonesia. The study was approved by the ethical committee of Hasanuddin University and informed consent was obtained from the patients included. The median age of the patients was 31 years (range: 9–88 years) and included 90 males and 41 females. The median age of the HC was 31 years (range: 19–41 years) and included 91 males and 21 females.

Every patient was assessed clinically by detailed history, medical and dermatological examinations. Bacteriological examination of slit-skin smears was carried out to determine the bacteriological index (BI). The patients were classified according to Ridley and Jopling's five subgroup classification [13] as 34 lepromatous (LL), 78 borderline lepromatous (BL), three mid-borderline (BB), six borderline tuberculoid (BT) and 10 tuberculoid (TT) patients. Forty-nine of the aforementioned patients were diagnosed with reactions, of whom 44 were ENL and five RR. ENL was diagnosed by the acute appearance of nodular skin lesions, accompanied by fever with or without peripheral nerve pain and nerve dysfunction. RR reactions presented typically as an acute inflammation of pre-existing lesions and onset of new erythematous skin lesions. For the purpose of comparisons the BL and LL patients without ENL reactions were grouped together as NE (non-ENL BL/LL, $n = 68$), as this group of patients is prone to ENL. Similarly, BL, BB and BT patients without RR were grouped as NRB (non-reactional borderline, $n = 82$), as this group is prone to RR. Thus the BL patients were common to both NE and NRB groups, as potentially they might develop either ENL or RR. The serum profiles of these groups were compared with ENL and RR patients, respectively.

Leprosy was treated with multi-drug treatment (MDT) according to World Health Organization (WHO) guidelines [14]. Reactions were treated using prednisolone, starting at 40 mg/day and gradually tapering off over a period of 12 weeks [15]. Clinical improvement of reactions was defined as complete subsidence of all reactional symptoms. In the absence of biopsy samples, the clinical assessment was performed by the clinician-in-charge, although no elaborate scoring system was used to grade improvement of reactions. Follow-up samples at the end of corticosteroid treatment were obtained from 17 patients with ENL and one patient with RR only.

After informed consent, blood samples were collected by venipuncture, the serum was separated, aliquoted and stored in liquid nitrogen at Makassar until transported to the Netherlands for analysis.

Cytokine assays

The cytokines (IL-6, IL-10, IFN-γ, TNF-α), sIL-6R and sCD27 were estimated according to the manufacturer's instructions using PeliKine enzyme-linked immunosorbent assay (ELISA) kits (Sanquin Reagents, Amsterdam, the Netherlands). Neopterin was estimated using the Brahms ELISA kit also according to the manufacturer's instructions (Brahms, Henningsdorf, Germany) [16].

Anti-PGL-I IgM assay

Anti-PGL-I IgM antibodies were detected as described in Brett *et al.* [17] using natural trisaccharide linked to bovine serum albumin via a phenolic ring (NT-P-BSA) as a semi-synthetic analogue of PGL-I.

Statistical analysis

The differences in cytokine levels were compared within the leprosy spectrum, reactional patients and HC. Because the data did not follow Gaussian distribution, the Kruskal–Wallis test was performed to test the differences in cytokine levels across the leprosy spectrum, reactions and HC. Dunn's *post-hoc* rank test was used to compare each group of the leprosy spectrum and reactions with HC. As NE and NRB patient groups are prone to ENL and RR, respectively, ENL was compared with NE while RR was compared with NRB and the differences in cytokine levels were analysed using the Mann–Whitney *U*-test. Correlations between different cytokines were analysed using Spearman's rank correlation coefficient. A correlation was assumed when $r \geq 0{\cdot}3$ with $P \leq 0{\cdot}05$. The paired *t*-test was used to compare cytokine levels before and after corticosteroid treatment.

Results

Preliminary statistical analyses suggested no statistically significant effect of MDT status on the cytokine levels in the patients. Hence the patients were grouped into NE, NRB,

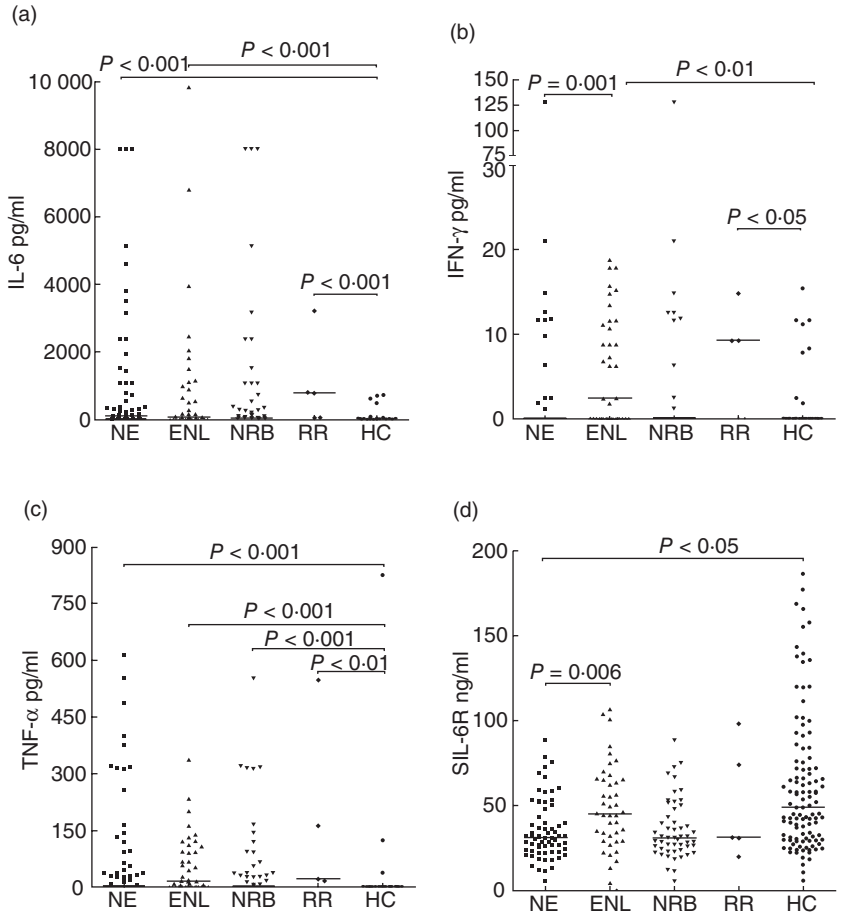

Fig. 1. Levels of interleukin (IL)-6 (a), interferon (IFN)-γ (b), tumour necrosis factor (TNF)-α (c) and soluble IL-6 receptor (sIL-6R) (d) in sera of NE (■) [non-erythema nodosum leprosum (ENL)], NRB (▼) (non-reactional borderline), ENL (▲) and RR (◆) (reversal reaction) leprosy patients and HC (●) (healthy controls). Median value indicated by line (–).

ENL and RR, irrespective of their MDT status to increase the number of patients within each group.

Cross-sectional study of cytokine profiles in the various disease groups

Proinflammatory cytokines (IL-6, IFN-γ, TNF-α)

Significant difference was seen with respect to the overall levels of cytokines IL-6 ($P < 0{\cdot}0001$), IFN-γ ($P < 0{\cdot}0001$) and TNF-α ($P < 0{\cdot}0001$) across the patient groups by the Kruskal–Wallis test (Fig. 1–c). Dunn's *post-hoc* test showed significantly higher IL-6 and TNF-α compared to HC in NE ($P < 0{\cdot}001$, $P < 0{\cdot}001$, respectively), ENL ($P < 0{\cdot}001$; $P < 0{\cdot}001$, respectively) and RR ($P < 0{\cdot}001$; $P < 0{\cdot}01$, respectively) patients. IFN-γ was higher compared to HC in only ENL ($P < 0{\cdot}01$) and RR ($P < 0{\cdot}05$) patients. No significant difference in IL-6 levels was observed either between NE and ENL ($P = 0{\cdot}91$) or NRB and RR patients ($P = 0{\cdot}15$), respectively. Similarly, no significant difference in TNF-α levels was observed between either NE and ENL ($P = 0{\cdot}35$) or NRB and RR patients ($P = 0{\cdot}19$), respectively. A significant difference was observed, however, between IFN-γ levels in NE and ENL patients ($P = 0{\cdot}001$) but not between NRB and RR patients ($P = 0{\cdot}13$).

Cytokine receptor (IL-6R)

A significant difference was seen with respect to overall levels of soluble IL-6R ($P = 0{\cdot}0011$) across the patient groups by the Kruskal–Wallis test. Dunn's *post-hoc* test showed significant differences between HC and NE ($P < 0{\cdot}05$), but not the other patient groups. ENL patients showed significantly higher levels of sIL-6R compared to NE ($P = 0{\cdot}006$), while no significant difference was observed between NRB and RR patients ($P = 0{\cdot}46$) (Fig. 1d).

Cytokines IL-10 and IL-4

IL-10 levels showed significant difference across the patient groups ($P < 0{\cdot}001$) by the Kruskal–Wallis test. Dunn's *post-hoc* test showed significantly higher IL-10 levels in ENL patients compared to HC ($P < 0{\cdot}05$) (Fig. 2a). However, no significant difference was observed in IL-10 levels between either NE and ENL ($P = 0{\cdot}43$) or NRB and RR ($P = 0{\cdot}37$) patients, respectively. A significant difference was found with respect to IL-4 levels across the patient groups ($P < 0{\cdot}0001$) by the Kruskal–Wallis, test which could be attributed to a significant difference between BL patients (within the NE group) and HC. However, no difference with respect to IL-4 levels was seen in NE compared to ENL patients ($P = 0{\cdot}53$)

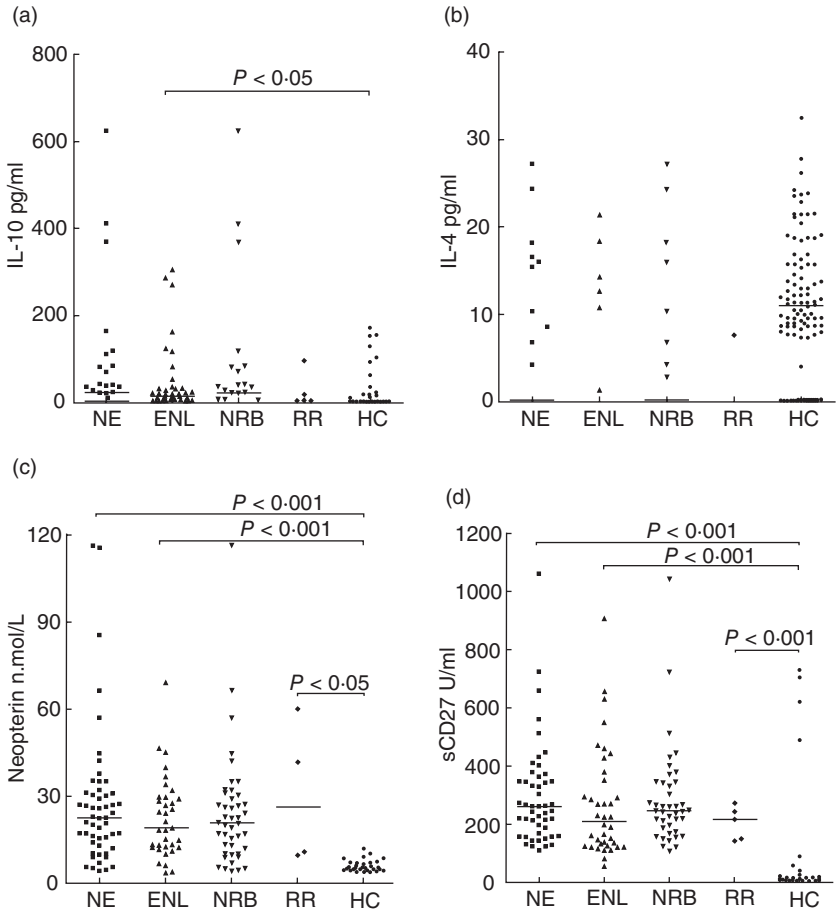

Fig. 2. Levels of interleukin (IL)-10 (a), IL-4 (b), neopterin (c) and sCD27 (soluble T cell activation marker) (d) in sera of NE (■) (non-erythema nodosum leprosum (ENL), NRB (▼) (non-reactional borderline), ENL (▲) and RR (◆) (reversal reaction) leprosy patients and HC (●) (healthy controls). Median value indicated by line (–).

(Fig. 2b). The number of RR samples ($n = 2$) was not sufficient for a comparison of RR with NRB patients.

Cellular activation markers

Neopterin levels showed a significant difference across the patient groups ($P < 0{\cdot}0001$) by the Kruskal–Wallis test and was higher in NE ($P < 0{\cdot}001$), ENL ($P < 0{\cdot}001$) and RR patients ($P < 0{\cdot}05$) compared to HC (Fig. 2c) by Dunn's *post-hoc* test. However, no significant differences were found in neopterin levels between either NE and ENL ($P = 0{\cdot}56$) or NRB and RR ($P = 0{\cdot}67$) patients, respectively. Similarly, sCD27 levels showed a significant difference across the study groups ($P < 0{\cdot}0001$) and was higher in all patient groups compared to HC by Dunn's *post-hoc* test (Fig. 2d). No significant difference, however, was observed in sCD27 levels between NE and ENL ($P = 0{\cdot}14$) or NRB and RR ($P = 0{\cdot}22$) patients, respectively.

Anti-PGL-I antibodies

Anti-PGL-I IgM antibodies were assayed in sera from 72 patients and eight HC. A significant difference was seen in anti-PGL-I antibody levels across the patient group ($P = 0{\cdot}008$) by the Kruskal–Wallis test with Dunn's *post-hoc* test showing higher levels in ENL ($P < 0{\cdot}01$) patients compared to HC. On the other hand, no difference was observed between NE and ENL ($P = 0{\cdot}46$) or NRB and RR ($P = 0{\cdot}82$) patients, respectively.

Correlations between different cytokine levels

Correlation between proinflammatory cytokines

A positive correlation was noted between the levels of IL-6 and TNF-α in NE ($r = 0{\cdot}561$, $P < 0{\cdot}0001$) and in ENL ($r = 0{\cdot}534$, $P = 0{\cdot}0002$) patients. On the other hand, IL-6 correlated with IFN-γ in NE ($r = 0{\cdot}503$, $P < 0{\cdot}0001$) but not in the ENL patients ($r = 0{\cdot}04$, $P = 0{\cdot}78$). A correlation was also observed between IFN-γ and TNF-α in NE ($r = 0{\cdot}472$, $P < 0{\cdot}0001$) but not in ENL patients ($r = -0{\cdot}012$, $P = 0{\cdot}94$).

Correlation between IL-6 and its soluble receptor (sIL-6R)

Although IL-6 levels correlated with sIL-6R levels in NE patients ($r = 0{\cdot}393$, $P = 0{\cdot}002$), no such correlation was observed among ENL patients ($r = 0{\cdot}272$, $P = 0{\cdot}07$).

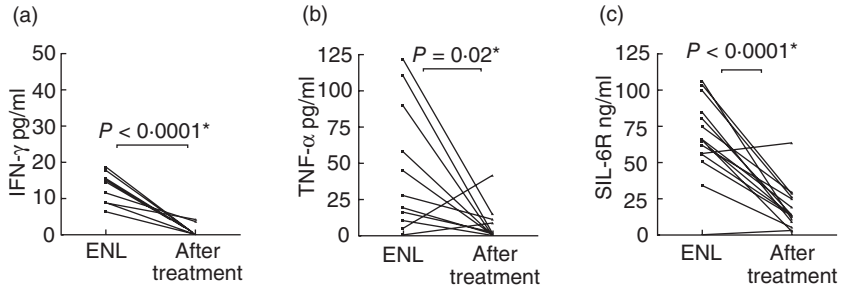

Fig. 3. Levels of interferon (IFN)-γ (a), tumour necrosis factor (TNF)-α (b) and soluble IL-6 receptor (sIL-6R) (c) in follow-up sera of leprosy patients at onset of erythema nodosum leprosum (ENL) (■) and after its treatment with prednisolone (▲). *Statistical significance.

Correlation between IL-10, IL-4 and proinflammatory cytokines

IL-10 correlated significantly with IL-6 in both NE ($r = 0{\cdot}537$, $P = 0{\cdot}0005$) and ENL patients ($r = 0{\cdot}552$, $P = 0{\cdot}0001$). However, while a weak correlation was seen between IL-10 and TNF-α in ENL patients ($r = 0{\cdot}343$, $P = 0{\cdot}026$), no correlation was seen in NE patients ($r = 0{\cdot}261$, $P = 0{\cdot}07$). IFN-γ levels did not correlate with IL-10 in either patient group (data not shown). None of the proinflammatory cytokines correlated with IL-4 (data not shown).

Correlation between T cell cytokines and soluble T cell activation product (sCD27)

Interestingly, a negative correlation was seen between the T cell cytokine IFN-γ and the sCD27 in both NE ($r = -0{\cdot}5126$, $P = 0{\cdot}0002$) and ENL ($r = -0{\cdot}696$, $P < 0{\cdot}0001$) patients. No correlation was noted between IL-10 and sCD27 serum levels for either patient group (data not shown).

Correlation between the macrophage activation product neopterin and macrophage cytokines

Neopterin levels correlated with IL-6 ($r = 0{\cdot}337$, $P = 0{\cdot}047$) as well as TNF-α ($r = 0{\cdot}398$, $P = 0{\cdot}019$) in ENL patients only, although the correlation was not highly significant.

Follow-up of ENL patients during corticosteroid treatment

Cytokine levels were compared at the onset of ENL and at the completion of corticosteroid treatment in 17 patients. Levels of IFN-γ ($P < 0{\cdot}0001$), TNF-α ($P = 0{\cdot}02$) and sIL-6R ($P < 0{\cdot}0001$) (Fig. 3a–c), but not anti-PGL-I antibodies ($P = 0{\cdot}14$) (data not shown), declined significantly with corticosteroid treatment and paralleled the clinical improvement of the patients as assessed by the clinician-in-charge.

Discussion

The present study was undertaken to assess the validity of measuring serum cytokines for detection and monitoring the leprosy spectrum and reactions in a field setting. A wide variability was seen in serum cytokine levels within the patient groups and HC. Nevertheless, IFN-γ was associated significantly with reactions and was higher in ENL compared to NE patients. These results are consistent with previous observations in serum [9], as well as those in culture supernatants and mRNA from *M. leprae*-stimulated and freshly isolated PBMCs from ENL patients [8,18,19]. Concurrently, however, another study reported contradictory results showing low serum IFN-γ levels at the onset of ENL [20]. On the other hand, in contrast to the undetectable levels of IFN-γ reported in our previous study [10], a majority of the RR (three of five) patients in the present study showed detectable serum IFN-γ levels. Such variable results could be attributed to the different patient populations involved in the studies. However, no significant difference was observed in IFN-γ levels between RR and NRB, as was observed in the previous report [10]. It must be stressed that not too much significance can be attached to the results in RR patients in the present study, due to the low sample size. Despite the above-mentioned conflicting results, elevated levels of IFN-γ in ENL, encountered in this study, suggest involvement of the CMI response in ENL pathology which is compatible with the findings of previous reports [19]. The association of IFN-γ with ENL pathology is supported further by studies demonstrating development of ENL in LL patients on administration of IFN-γ [21].

The serum levels of IL-6, TNF-α and the macrophage activation product neopterin were higher in leprosy patients compared to HC, which is consistent with increased immune activity in patients. However, in the present study no significant difference in IL-6, TNF-α and neopterin levels was observed between reactional (ENL and RR) and the non-reactional patients (NE and NRB, respectively), which is in contrast to previous reports [11,18,22,23]. Soluble IL-6R levels, although lower in patients compared to HC, were elevated in ENL compared to NE. It could be speculated that the lower levels of sIL-6R in patients compared to HC may be caused by formation of complexes with IL-6 in these patients, as has been suggested previously in a study in patients with systemic juvenile rheumatoid arthritis [24].

The absence of a significant difference in IL-10 levels between ENL and NE patients is in contrast to previous reports [8,9]. Moreover, unlike the negative correlation observed previously between levels of IL-10 and IFN-γ in

leprosy sera [9], no such correlation was observed in the present study. Interestingly, the positive correlation between IL-10 and TNF-α in ENL patients is similar to the previous observation of simultaneous expression of IL-10 and TNF-α in skin lesions of patients with RR [25]. This was suggested to be indicative of simultaneous activation of proinflammatory and regulatory pathways through suppressive/regulatory cytokines such as IL-10 [25] and probably reflects a control mechanism to prevent the excessive tissue-damaging effects of the proinflammatory cytokines.

A negative correlation was found between sCD27 and IFN-γ within the patient groups, which is surprising given the fact that both IFN-γ and sCD27 [26,27] are secreted by activated T cells. On the other hand, functional studies have shown loss of CD27 in $CD8^+$ T cells as a result of differentiation to effector cell populations [28,29]. Other studies have reported down-regulation of CD27 expression with repeated antigenic stimulation of T cells [30]. Similar situations could be envisaged in a chronic disease such as leprosy, where T cells are likely to be exposed repeatedly to antigenic stimulation, although in the context of the present study this is merely speculative.

Serum levels of sIL-6R, IFN-γ and TNF-α declined with corticosteroid treatment and paralleled clinical improvement in ENL patients. The one RR patient with a follow-up serum sample also showed a decline in IFN-γ, TNF-α and neopterin with corticosteroid treatment (data not shown). Corticosteroids, the primary treatment modality for reactions [31], cause a decrease in the number of circulating lymphocytes and monocytes and decrease production of cytokines such as IFN-γ [32,33], IL-1 [34], TNF-α [32,35] and IL-2R [34] and neopterin expression [10,23]. However, in our previous study, TNF-α appeared to persist even after the completion of corticosteroid treatment in the majority of RR patients [10]. In contrast, in the present study, TNF-α declined significantly in all but two of 17 ENL patients, where an increase with treatment was seen. In this regard, an ongoing study in our laboratory suggested the association of persistent high serum levels of TNF-α at the end of corticosteroid treatment with a probable risk for development of subsequent episodes of RR (unpublished observations), although this observation needs further validation. The increase in TNF-α at the end of corticosteroid treatment in the two exceptional patients is also similar to observations of a previous study, where TNF-α was observed to decline with treatment in RR while in ENL patients it was found paradoxically to increase at the end of steroid treatment [17]. No decline in serum neopterin levels was seen at the end of corticosteroid therapy in ENL patients in the present study, in contrast to previous observations in RR [10,23] and ENL patients [23].

Taken together, the variable results from different studies suggest that the regulation of cytokine secretion is more complex than commonly recognized and can be influenced by the method of analysis. A major limitation of serum analysis is that serum measurements may not reflect adequately the tissue immune response [25]. This may be addressed by alternative approaches, such as human organotypic culture of lesions where biopsies are available [36]. The presence of soluble receptors and other inhibitors could influence the detection of soluble markers which can be assessed using special ELISAs to detect complex formation [24]. The serum biomarkers used in the present study could be supplemented with more conventional markers of inflammation, such as C-reactive protein (CRP) [37]. However, it appears from our ongoing study that serum CRP, despite being associated with ENL, showed no decline during treatment (unpublished observations), suggesting that CRP may not be valuable for monitoring leprosy patients as is usually anticipated with other inflammatory diseases. Moreover, CRP, along with cytokines and other soluble markers used in the present study, reflect the general inflammatory response and would be expected to change in all immune-mediated conditions, thus lacking disease specificity. In this regard, the patients in the present study were examined medically for signs of other concomitant infections and skin inflammations and controls from the same area were used to generate baseline values for cytokines and other soluble markers.

In conclusion, the study suggests limited applicability of serial measurement of the cytokines IFN-γ, TNF-α and sIL-6R in monitoring therapeutic efficacy of ENL patients. However, a cautious approach to interpreting serum cytokine profiles and a further search for a wider panel of more disease-specific markers is recommended in future studies.

Acknowledgements

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References

1 Britton WJ, Lockwood DN. Leprosy. Lancet 2004; **363**:1209–19.
2 Barnes PF, Chatterjee D, Brennan PJ, Rea TH, Modlin RL. Tumor necrosis factor production in patients with leprosy. Infect Immun 1992; **60**:1441–6.
3 Volc-Platzer B, Stemberger H, Luger T, Radaszkiewicz T, Wiedermann G. Defective intralesional interferon-gamma activity in patients with lepromatous leprosy. Clin Exp Immunol 1988; **71**:235–40.

4 Arnoldi J, Gerdes J, Flad HD. Immunohistologic assessment of cytokine production of infiltrating cells in various forms of leprosy. Am J Pathol 1990; **137**:749–53.

5 Yamamura M. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science 1992; **255**:12.

6 Salgame P, Yamamura M, Bloom BR, Modlin RL. Evidence for functional subsets of CD4+ and CD8+ T cells in human disease: lymphokine patterns in leprosy. Chem Immunol 1992; **54**:44–59.

7 Verhagen CE, Wierenga EA, Buffing AA, Chand MA, Faber WR, Das PK. Reversal reaction in borderline leprosy is associated with a polarized shift to type 1-like *Mycobacterium leprae* T cell reactivity in lesional skin: a follow-up study. J Immunol 1997; **159**:4474–83.

8 Sreenivasan P, Misra RS, Wilfred D, Nath I. Lepromatous leprosy patients show T helper 1-like cytokine profile with differential expression of interleukin-10 during type 1 and 2 reactions. Immunology 1998; **95**:529–36.

9 Moubasher AD, Kamel NA, Zedan H, Raheem DD. Cytokines in leprosy, I. Serum cytokine profile in leprosy. Int J Dermatol 1998; **37**:733–40.

10 Faber WR, Iyer AM, Fajardo TT *et al.* Serial measurement of serum cytokines, cytokine receptors and neopterin in leprosy patients with reversal reactions. Lepr Rev 2004; **75**:274–81.

11 Sarno EN, Grau GE, Vieira LM, Nery JA. Serum levels of tumour necrosis factor-alpha and interleukin-1 beta during leprosy reactional states. Clin Exp Immunol 1991; **84**:103–8.

12 Sehgal VN, Bhattacharya SN, Chattopadhaya D, Saha K. Tumour necrosis factor: status in reactions in leprosy before and after treatment. Int J Dermatol 1993; **32**:436–9.

13 Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. Int J Lepr Other Mycobact Dis 1966; **34**:255–73.

14 Saunderson P. The epidemiology of reactions and nerve damage. Lepr Rev 2000; **71** (Suppl.):S106–10.

15 Marlowe SN, Hawksworth RA, Butlin CR, Nicholls PG, Lockwood DN. Clinical outcomes in a randomized controlled study comparing azathioprine and prednisolone versus prednisolone alone in the treatment of severe leprosy type 1 reactions in Nepal. Trans R Soc Trop Med Hyg 2004; **98**:602–9.

16 Mayersbach P, Augustin R, Schennach H *et al.* Commercial enzyme-linked immunosorbent assay for neopterin detection in blood donations compared with RIA and HPLC. Clin Chem 1994; **40**:265–6.

17 Brett SJ, Payne SN, Gigg J, Burgess P, Gigg R. Use of synthetic glycoconjugates containing the *Mycobacterium leprae* specific and immunodominant epitope of phenolic glycolipid I in the serology of leprosy. Clin Exp Immunol 1986; **64**:476–83.

18 Moraes MO, Sarno EN, Almeida AS *et al.* Cytokine mRNA expression in leprosy. a possible role for interferon-gamma and interleukin-12 in reactions (RR and ENL). Scand J Immunol 1999; **50**:541–9.

19 Nath I, Vemuri N, Reddi AL *et al.* The effect of antigen presenting cells on the cytokine profiles of stable and reactional lepromatous leprosy patients. Immunol Lett 2000; **75**:69–76.

20 Partida-Sanchez S, Favila-Castillo L, Pedraza-Sanchez S *et al.* IgG antibody subclasses, tumor necrosis factor and IFN-gamma levels in patients with type II lepra reaction on thalidomide treatment. Int Arch Allergy Immunol 1998; **116**:60–6.

21 Sampaio EP, Moreira AL, Sarno EN, Malta AM, Kaplan G. Prolonged treatment with recombinant interferon gamma induces erythema nodosum leprosum in lepromatous leprosy patients. J Exp Med 1992; **175**:1729–37.

22 Teles RM, Moraes MO, Geraldo NT, Salles AM, Sarno EN, Sampaio EP. Differential TNF-alpha mRNA regulation detected in the epidermis of leprosy patients. Arch Dermatol Res 2002; **294**:355–62.

23 Hamerlinck FF, Klatser PR, Walsh DS, Bos JD, Walsh GP, Faber WR. Serum neopterin as a marker for reactional states in leprosy. FEMS Immunol Med Microbiol 1999; **24**:405–9.

24 de Benedetti F, Massa M, Pignatti P, Albani S, Novick D, Martini A. Serum soluble interleukin 6 (IL-6) receptor and IL-6/soluble IL-6 receptor complex in systemic juvenile rheumatoid arthritis. J Clin Invest 1994; **93**:2114–9.

25 Andersson AK, Chaduvula M, Atkinson SE *et al.* Effects of prednisolone treatment on cytokine expression in patients with leprosy type 1 reactions. Infect Immun 2005; **73**:3725–33.

26 Loenen WA. CD27 and (TNFR) relatives in the immune system. their role in health and disease. Semin Immunol 1998; **10**:417–22.

27 Lens SM, Tesselaar K, van Oers MH, van Lier RA. Control of lymphocyte function through CD27–CD70 interactions. Semin Immunol 1998; **10**:491–9.

28 Hamann D, Roos MTL, van Lier RAW. Faces and phases of human CD8+ T-cell development. Immunol Today 1999; **20**:177–80.

29 Hintzen RQ, De Jong R, Lens SMA, Brouwer M, Baars P, van Lier RAW. Regulation of CD27 expression on subsets of mature T-lymphocytes. J Immunol 1993; **151**:2426–35.

30 van Baarle D, Tsegaye A, Miedema F, Akbar A. Significance of senescence for virus-specific memory T cell responses: rapid ageing during chronic stimulation of the immune system. Immunol Lett 2005; **97**:19–29.

31 Naafs B. Treatment of reactions and nerve damage. Int J Lepr Other Mycobact Dis 1996; **64** (Suppl.):S21–8.

32 Manandhar R, Shrestha N, Butlin CR, Roche PW. High levels of inflammatory cytokines are associated with poor clinical response to steroid treatment and recurrent episodes of type 1 reactions in leprosy. Clin Exp Immunol 2002; **128**:333–8.

33 Arya SK, Wong-Staal F, Gallo RC. Dexamethasone-mediated inhibition of human T cell growth factor and gamma-interferon messenger RNA. J Immunol 1984; **133**:273–6.

34 Moubasher AD, Kamel NA, Zedan H, Raheem DD. Cytokines in leprosy, II. Effect of treatment on serum cytokines in leprosy. Int J Dermatol 1998; **37**:741–6.

35 Chao CC, Hu S, Close K *et al.* Cytokine release from microglia. differential inhibition by pentoxifylline and dexamethasone. J Infect Dis 1992; **166**:847–53.

36 Iyer AM, Mohanty KK, van Egmond D *et al.* Leprosy-specific B-cells within cellular infiltrates in active leprosy lesions. Hum Pathol 2007; **38**:1065–73.

37 Foss NT, de Oliveira EB, Silva CL. 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 1993; **61**:218–26.