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Original Article

Distribution of CD4+RORg-T Th17 and CD25+ FOXP3+ Treg in leprosy patients with reversal reaction

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Abstract

Objective To compare the distribution of CD4+ Th17 and CD25+ FOXP3 T regulatory cell (Treg) between type I reversal reaction (RR) and type II reversal reaction i.e. erythema nodosum leprosum (ENL) patient groups.

Methods A total of 50 samples, consisted of 27 samples of reversal reaction (RR) and 23 samples of ENL, were collected. Observation of CD4+ RORg-T Th17 and CD25+ FOXP3 Treg were conducted with immunohistochemistry staining technique using anti FOX-P3 and anti RORg-T. Expression of CD4+ ROR-g Th17 and CD25+ FOXP3 Treg in percentage were analyzed using T-test.

Results There is a significant difference in mean CD4+ ROR-g Th17 and IL17 cell distribution for RR patient group (14.96% and 10.72%) compared with ENL (9.12% and 4.28%). No significant difference were found between mean CD25+ FOXP3 Treg and TGF-β cell distribution in RR patient group (6.12% and 5.44%) compared with ENL group (6.16% and 5.96%).

Conclusion There is a significant increment od CD+RORg-T Th17 and IL17 in RR patients group compared with ENL patients group. however, the distribution of CD25+ FOXP3+ Treg and TGF beta in RR has no significant difference compared with ENL.

Key words
CD4+ RORg-T Th17, CD25+ FOXP3+ Treg, IL17, TGF-β, Reversal Reaction, Erythema Nodosum Leprosum.

Introduction

Leprosy is a frightening disease for community, which causes ulceration, mutilation, and deformities to the patients. The disease has variety of manifestations, which depend on patient's immune response towards the infection. Two key purposes for leprosy management are recovery and disability prevention from the disease. WHO recommended a multi-drug therapy (MDT) consisting of rifampicin and dapsone for paucibacillary (PB) leprosy, and rifampicin, dapsone, and clofazamine to treat multibacillary

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(MB) leprosy. Therapy is given for 6 months duration for PB leprosy and 12 months duration for MB leprosy. MDT has been proven effective to eradicate *Mycobacterium leprae* in most of the patients.[1] Even though it has been proven effective, the most difficult issue in leprosy management is to manage the leprosy reaction.

Leprosy reaction is caused by patient's immunological dynamic response to *M. leprae*, which could happen before, during, and after MDT administration.[2] This reaction is an acute episode of the disease with constitutional symptoms, activation, sometimes accompanied with new skin efflorescence. Two types of leprosy reaction are type 1, known as reversal reaction (RR), caused by acute changes in cellular immunity, and type 2 reaction known as erythema nodosum leprosum (ENL), caused by humoral immunity process.[3] Reversal reaction is associated with cell-mediated immunity (CMI) activities, consists of T helper cell-1 (Th1) and 2 (Th2). It is characterized with granuloma expansion, edema, CD4+ recruitment, increasing IL-2, IL-10, and IL-12 receptors, and expression of human leukocyte antigens-D in cellular infiltrate.[4]

Another CD4+ Th subset that shows divergence in leprosy is the Th17 population associated with inflammation.[5] The Th17 subset is highest in healthy contacts exposed to the diseases compared with patients, indicating its importance in innate immunity.[6,7] Th17 cells were found in the leprosy patients who showed non-polarized Th0 subset.[7] Therefore, it appears that Th17 cells play a role in the immune responses to *M. leprae* infection and may be an alternate pathway for bacillary clearance both in the early and later stages of infection. The associated chemokines may help in its migration to lesional sites.

Another distinct lineage of T cells that has exciting implications in dampening inflammatory responses is the T regulatory cell (Treg), which has a CD4+CD25+ nuclear FOXP3+ phenotype and shares a similar differentiation pathway to Th17 cells, although they have opposite effects. Several types of Tregs have been described; some are natural Tregs derived from the thymus and act via contact with target cells.[8] Others are inducible and mediate inhibition through cytokines such as transforming growth factor-β (TGF-β) and IL-10 (induced Treg [iTreg]).[9] Transcription factor FOXP3 is thought to be the primary requirement for the suppressive function, though low and transient expression has been reported in activated human T cells with and without suppressor function. Though Tregs in mice express CD25 constitutively, in humans only those with CD25 show suppressive function.[10]

Leprosy reactions in contrast showed a decrease in Treg cells which paralleled the increase in Th17 population.[11] Moreover, there was downregulation of intracellular TGF-β.[11,12] Most reports showed a reduction in Treg cells in patients with ENL reactions compared with non-reaction lepromatous leprosy counterparts.[11,12] However, in type I leprosy reaction (RR), the distribution of Th17 and Treg has not yet been identified. Thus, this study aimed to compare the distribution between Th17 and FOXP3 Treg in RR and ENL patient groups.

Methods

A total of 50 patients, 27 with RR and 23 with ENL reaction, were enrolled in this study, from outpatient clinic in Donorojo Hospital and Ministry of Health in Jepara from June 2014 to August 2014. Patients were 20-60 years old and had agreed to sign the standard informed consent approved by Ethical Committee of Airlangga University.

Biopsy samples was collected from the lesion area or extensor aspect of lower arm. Then, the area was disinfected with alcohol 70% and local anesthetic lidocaine 0.25 ml was administered subcutaneously near the biopsy area. Punch biopsy was conducted using 3mm-punch. Tissue sample was placed in formalin 10%. The after-biopsy site was cleaned using NaCl 0.9%, then topical antibiotic fusidic acid was applied and the skin was covered with sterile gauze. The sample underwent a fixation and processed in paraffin. Microscopic slide was made using rotary microtome with 4µm slicing. Staining with hematoxylin-eosin was applied to confirm the structure and leprosy type. Observation of CD4+ RORg-T Th17 and C25+ FOXP3+ Treg was done using immunohistochemistry single staining technique with anti-FOX-P3 anti-RORg-T retrieved from Santa Cruz Biotech (USA) and immunohistochemistry kit of D-Bio Sys Immunostaining Kit (Netherland). The staining results were then photomicrographed using Nikon E-100 Microscope with ICLEA7 Sony Camera 400x magnification, then expression analysis was done using ImmunoRatio software (Freeware). Percentage of expression CD4+ RORg-T Th17 and C25+ FOXP3+ Treg were inserted into table and analyzed using t test in IBM-SPSS 21 for Windows.

Results

In this study, there were 27 samples of RR patients and 23 patients of ENL reaction. Each case was classified based hematoxylin-eosin staining microscope examination (**Figure 1** and **2**).

Immunohistochemistry results showed a significant difference in mean CD4+RORg-T Th17 and IL17 cell distribution in RR patient group (14.96% and 10.72%) compared with ENL patient group (9.12% and 4.28%). However, no significant difference in mean CD25+ FOXP3+ Treg and TGF-β cell distribution in RR patient group (6.12% and 5.44%,) compared with ENL patient group (6.16% and 5.96%), **Figure 3**.

Discussion

In this study, expression of FOXP3+ Treg was evaluated from the skin with T lymphocyte infiltrate. We found that Treg were produced in reactive state (either type I or II). Treg has an important role in controlling the overactive immune response towards microbe's antigen, especially pathogens which caused persistent infection in this study. An earlier study explained a significant decrement of FOXP3+ Treg in ENL compared with stabilized leprosy patients, although it was not found in RR patients.[12] In this study, we found FOXP3+ Treg in 100% of all skin specimens of RR and ENL with a low number. From all the cases, positive FOXP3 cell was strongly related with epithelioid cells or macrophages, showing a functional interaction between Treg and histiocyte. We observed no statistically significant differences between FoxP3+ Treg between type I reaction (RR) and type II reaction (ENL), even the number was almost the same.

Other study showed that CD+ FOXP3+ Treg cell produced TGF-β and increased in stabilized lepromatous patients and also could explain issues with this type of leprosy.[11] Our study showed that TGFb1 occurred in type I leprosy reaction (RR) and type II (ENL), where the presentation tends to be lower in RR than in ENL, but this was not statistically significant.

This study emphasized an inhibition of FOXP3 cell expression and also TGF-β in RR condition compared with ENL, which related to an unresponsiveness of T cell observed in RR patients. One interesting finding in this study

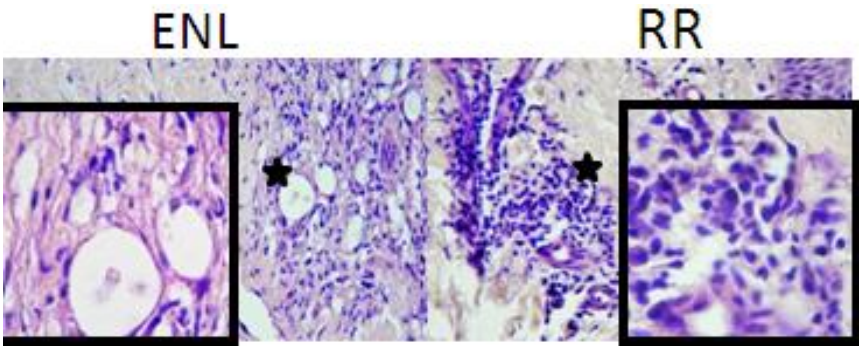


Figure 1 Erythema nodosum leprosum (ENL): Granulomatous infiltrate of vacuolated macrophages and neutrophils in the dermis. Reversal reaction (RR): Influx of mononuclear phagocytes with epithelioid differentiation. Hematoxylin– eosin staining; original magnification x200 and x1000 inserted

Expression of FOXP3, RORg-T, TGFb1 and IL-17 are described below:

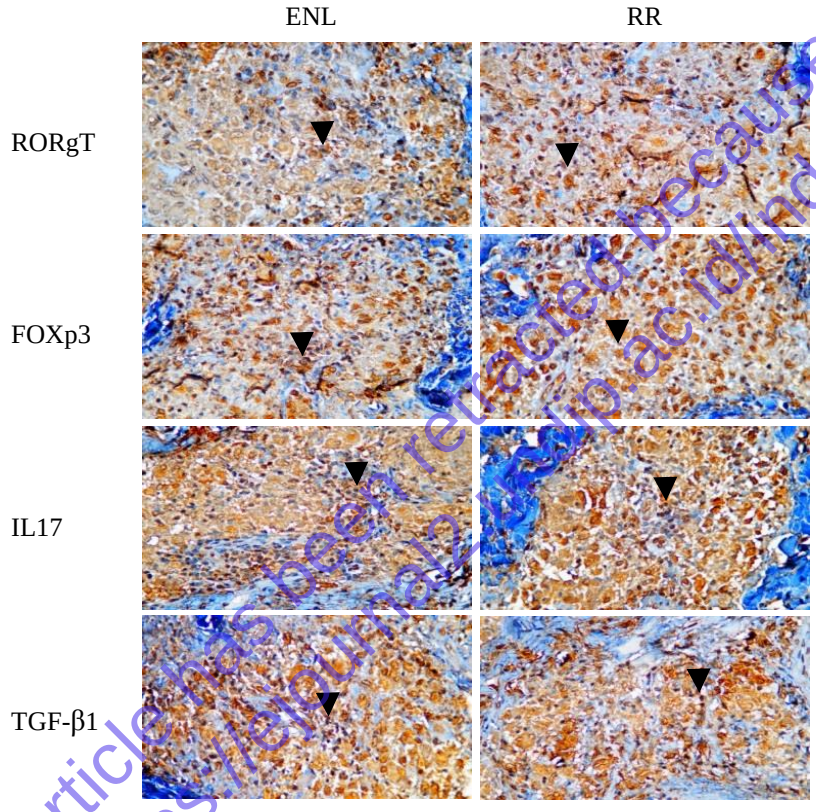
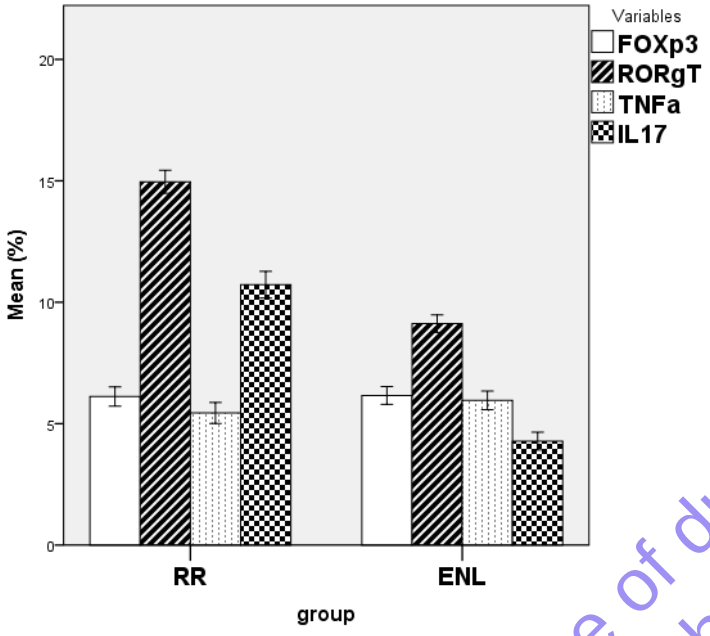


Figure 2 Immunohistochemistry staining for Foxp3, RORgT, TGFb1 and IL17A from biopsy samples of reversal reaction (RR) and erythema nodosum leprosum (ENL) patients. Photomicrograph showing (original magnification:400×) RORgT and FOXp3 cells positive in brown color (arrow).



Graphic 1. Mean FOXP3, RORgT, TNFα dan IL17 percentage in reversal reaction (RR) and erythema nodosum leprosum (ENL) patient group. We found a significant difference in CD4+RORgT Th17 and IL17 in RR patient group (14.96% and 10.72%) compared with ENL patient group (9.12% and 4.28%).

was an increasing RORgT Th17 positive cells found in RR leprosy compared with ENL patients, and it was statistically significant. Also, RR patients showed a higher IL-17A expression than ENL patients.

The present study was undertaken with a view to understanding the inflammation and/or immunopathology seen in patients undergoing episodes of leprosy reactions, which are a cause of severe morbidity and nerve damage. Previous studies had shown that Th17 cells formed a third subset in leprosy and were seen in stable leprosy patients in the absence of Th1 and Th2 polarization.[11] Our study showed a disturbed equilibrium between Th17 cells and Treg in leprosy reaction, especially increased Th17 cells and decreased Treg cells number. This imbalance was mediated by cytokines, showed by decreasing TGF-β number in the same time. Increasing Th17 activities with IL-17A expression would explain the inflammatory and immunopathology process caused by leprosy reaction.

Overall, our results provide some evidence to the hypothesis that, in type 1 reaction, downmodulation of Tregs would favor the development of Th-17 responses that characterize this type of reaction. We thus believe that better understanding of the role played by Tregs in reaction episodes can possibly provide a new target for the treatment of this still-challenging complication of leprosy.

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