

ANTINUCLEAR ANTIBODIES (ANA) IN LEPROSY

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1. INTRODUCTION

Antinuclear antibodies (ANA) not only presented in autoimmune diseases but also in some chronic infectious diseases like tuberculosis, leprosy, malaria, syphilis... The detection of ANA is very important in diagnosis, follow up of treatment, especially in systemic diseases [4,5].

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (M. leprae). The course of the disease is long-term and can affect many organs. It has long been confirmed that the immune response plays an important role in the course of the disease. Many studies on humoral immune response, cell-mediated immunity, the role of immune complex were conducted for the investigation of pathogenesis of lepra reactions. Some author also detected ANA in lepromatous leprosy with a relatively high rate [1,2,3]. However, the role of ANA in leprosy, especially in the pathogenesis of erythema nodosum Leprosum (ENL), has not had any in-depth research.

The objective of study was to determine the role of ANA in leprosy and especially its relationship with the mechanism of ENL reaction (Erythema Nodosum Leprosum).

2. MATERIALS AND METHODS

2.1. Patients

57 patients in the leprosy department of the National Hospital of Dermatology and Venereology (NHDV) were enrolled for study. All

patients were classified according to the clinical and histopathological criteria of the Ridley-Jopling classification. They consisted:

- 12 PB (paucibacillary) leprosy patients.
- 18 MB (multibacillary) leprosy patients with no ENL reaction.
- 27 MB (multibacillary) leprosy patients with ENL reaction.

All patients were being treated with multidrug therapy (MDT) at the leprosy department of NHDV.

The duration of the disease at the time of the study:

- PB group: 8 patients less than 1 year, 4 patients 1 to 2 years.
- MB group: 18 patients less than 1 year, 27 patients 1 to 2 years.

2.2. Methods

ANA was detected by the immunofluorescence reaction with the substrate is.

Hep2 cell (cultured at Dutch Institute of Hematology and Blood Transfusion).

- Principles:

Antibodies (ANA - if presented) in the patient's serum combine with antigens (hep2 cell nucleus). This complex is detected by combining with a fluorescent bound immunoglobulin antibody.

- Procedure:

- + Preparing Hep2 cell.
- + Diluting serum at different concentrations.
- + Add 30 microlit of each concentration into each slides with Hep2 cell.

1: National Hospital of Dermatology and Venereology

2: National Institute of Hygiene and



+ Incubating 30 minutes in room temperature (RT). Washing 3 times with PBS.

+ Add 30 microlit of fluorescent-bound human immunoglobulin sheep serum into each slide.

+ Incubating 30 minutes in room temperature (RT). Washing 3 times with PBS.

+ Evans blue stain.

+ Reading the results on a fluorescence microscope.

a) Results

- Positive: On a red cell background, the cell nucleus is large and round with green glow. (only at titer > 1:40).

- Negative: The entire cell is red, the nucleus is darker with no green glow.

3. RESULTS

- 12 PB leprosy patients: no ANA-positive.

- 18 MB leprosy patients with no ENL reaction: 8 patients ANA-positive (44%).

- 27 MB leprosy patients with ENL reaction: 11 patients ANA-positive (41%).

Positive reaction at titres:

Groups	1:40	1:80	1:160
MB with no ENL	5	2	1
MB with ENL	5	6	0

In 19 patients with ANA positive, 17 had the disease for 1 - 2 years, 2 had the disease for less than 1 year.

For patients with ENL: Taking blood before, during, and after the reaction shown no change in result, except for 4 cases where the post-reaction titer decreased from 1:80 to 1:40

4. DISCUSSION

All patients of PB group typed as I (intermediate), TT (tuberculoid), and BT (borderline tuberculoid) are characterized by the presence of few than five skin lesions and/or negative bacterial index. In this group, there is heightened cell-mediated immunity (CMI) with a largely Th1 type immune response whereas response of humoral immunity is very weak. The type one reaction (reversal reaction) is a delayed - type hypersensitivity reaction that develops commonly in this group patients. All patients of this group had negative antinuclear antibody. MB group of leprosy consist of BB (borderline), BL (borderline lepromatous) and LL (lepromatous) are characterized by very low cell-mediated immunity with a humoral Th2 response. Intracellular pathogens are recognized by the innate immune system.

The course of this group of the disease is very prolonged and it affects many organs such as the skin, nerves, eyes, bone... As a result, some cells are destroyed and transformed. For some reasons, the body cannot recognize them and treat them as antigens (autoantigen) and produce antibodies (ANA).

The ratio of ANA-positive in this group was 42% (19/45). And it was worth noting that the ratio of ANA in the 2 groups (with ENL and no ENL) was insignificantly different (44% and 41%).

Kuzina et al. [1] with another detection method, found that the ratio of ANA-positive is 51% at 1:20 titer and 10,5% at titer 1:160 - 1:320 in lepromatous leprosy patients.

Another thing that was also worth noting is that in 19 ANA-positive patients, 17 had duration

of the disease for longer time (1 - 2 years) while only 2 had the time of the disease for less than a year. Thus, the longer the illness, the more likely the occurrence of ANA.

Erythema Nodosum Leprosum (ENL) is considered an allergic immune response type III (immune complex: IC). Some authors argued that ANA has a role in creating the IC to induce the ENL. But some other authors confirmed that IC causing ENL is due to specific antibodies combined with decomposed leprosy bacillus antigens [2,3].

In a previous study, with the gelatin granulation reaction, we found that during the ENL, the specific antibody level decreased, indicating that it formed IC to induce ENL. But does ANA produce IC to co-form the ENL? If yes, during the reaction, the ANA titer must have been dropped. But in this study, before, during

or after the reaction, the ANA titer did not change. However, after ENL, 4 cases had ANA titer decreased from 1:80 to 1:40. This may explain that ANA did not play a role in the induction of the ENL reaction [4,5].

5. CONCLUSION

Antinuclear Antibody (ANA) were investigated in the sera of 57 leprosy patients immunofluorescence, the results shown that:

- PB leprosy patients had ANA negativity.
- 42% patients of the MB leprosy group had ANA with titres range from 1:40 to 1:160.
- The longer the time the patients had the disease, the higher the chance of ANA- positive.
- ANA doesn't play a role in the pathogenesis of ENL reaction.

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