ORIGINAL ARTICLE

CD5+ B Cells Ratio In Lepromatous Leprosy

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ABSTRACT

Objective  Leprosy is a unique infectious disease, which is chronic and related to cellular immunity. It was known that who have a defect on cellular immunity, they are susceptible to M. leprae infection. CD19+CD5+ B cells are currently defined as B1 cells and they produce polyreactive antibodies. The aim of this study was to investigate the presence and the possible effect of natural antibody producing CD19+CD5+ B cells in leprosy disease patients.

Materials and Methods  We investigated B lymphocyte subset and total B and B1 cells by flow cytometry in 40 patients with lepromatous leprosy and 40 healthy controls.

Results  Compared to the healthy controls, both CD19+CD5- conventional B cells and CD19+CD5+ B1 cells subsets were found to be higher in leprosy patients group.

Conclusion  The observed significant increases in CD19+CD5- and CD19+CD5+ B1 cells subsets in patients with lepromatous leprosy suggests that B1 lymphocytes known predisposed to autoimmunity may have a role on disorganized protective immune response in Lepromatous leprosy.

Key words  lepromatous leprosy, CD19+CD5+ B cells, Mycobacterium leprae

Introduction  Leprosy is a chronic infectious disease caused by Mycobacterium leprae, an acid-fast bacillus with high infectivity, low pathogenicity and high virulence. Leprosy is one of the major infectious disease of concern defined by the World Health Organization. In 2003, a total of 451,325 cases were detected worldwide[1].

This disease has a broad spectrum of clinical manifestations, with tuberculoid leprosy (TL) at one end of the spectrum and lepromatous leprosy (LL) at the other. LL is characterized by the virtual absence of T-cell responses to M. leprae and advanced clinical disease [2],[3],[4],[5],[6],[7],[8]. It is frequently associated with the presence of autoantibodies which might be related to B1 lymphocyte percentages, such as rheumatoid factor (RF), antinuclear antibodies (ANA), antinuclear antibodies (ANA) [9],[10],[11],[12]. CD5+ B cells (B1 lymphocytes) constitute 15-25% of B cell population in secondary lymphoid tissues in
adults. Especially peritoneal cavity contains B1 lymphocytes populations whose antigen receptors are immunoglobulin molecules and have limited diversity. Many of these cells are specific for polysaccharide and lipid antigens on bacterial wall. B1 lymphocytes produce IgM antibodies which called natural antibodies. Although they are found in fetus intensely, a decrease in cell counts is obtained by increasing age. CD5+ B cells differ from T dependent antigens by producing low-affinity polyreactive natural antibodies in response to polyclonal activators [13],[14],[15],[16]. In autoimmune diseases such as rheumatoid arthritis, Sjogren’s syndrome and systemic lupus erythematosus, the relationship between the increase of autoantibodies and CD5+ B cells is established [13],[14].

Patients And Methods
Organization
Ethical consent was supplied by the Local Ethics Committee at Firat University Medical Faculty. Informed consent was obtained from all study participants. A total of 40 patients (29 males, 11 females) of Lepromatous leprosy with mean age of 60.00 ± 14.43 and mean years of diagnosis 30.66 ± 17.42 were included. Forty healthy individuals (25 males, 15 females) who have no leprosy contact with mean age of 55.00 ± 8.89 were included as controls. All the patients included in this study were free of other infectious diseases. Those post diagnosis received multidrug therapy (MDT) according to the recommendations of the World Health Organization. Patients undergoing an erythema nodosum leprosum episode and those taking thalidomide or corticoids were excluded.

Flow cytometric analysis of peripheral blood: Venous bloods (2 milliliters) from the participants were taken into the tubes with EDTA in order to examine T cell receptors by flow cytometry. All blood samples were researched within 2 hours. Peripheral blood samples were analyzed at Coulter EPICS XL-MCL (Beckman Coulter U.S.A). Immunotech (Marseille, France) monoclonal antibodies were used. Cell surface expression of lymphocyte antigens were examined by mAb staining of peripheral blood samples with two color flow cytometry. CD45/CD14, isotype control, CD3, CD19/CD5, CD20 mAb tubes were prepared and analyzed according to the instructions of manufacturer. Results were counted at the same equipment using Expo-32 analysis software. The quality criteria involved the frequency above 95% of total lymphocytes in the analysis gate and homogenous CD45+ lymphocyte population (minimum of 2000 events in the gate; CD45>95%). Statistical analysis was done by using SPSS 11.0 and related sample T test. For the statistical significance threshold, p<0.05 value was used.

Results
The mean age of patients with lepromatous leprosy were 60 ± 14 years (range 25–80), of healthy control group was 59 ± 9 years (range 34 – 79). The mean duration of disease was 29 ± 17 years in patient with Lepromatous leprosy [Table/Fig 1].

<table>
<thead>
<tr>
<th>Gender (M/F)</th>
<th>Patient (28/12)</th>
<th>Control (26/14)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60±14</td>
<td>59±9</td>
<td></td>
</tr>
<tr>
<td>Duration of the disease</td>
<td>29±17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD19+CD5− B cell %</td>
<td>15.08±4.83</td>
<td>12.14±2.10</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>CD19−CD5+ B1 cell %</td>
<td>4.92±3.57</td>
<td>1.10±0.59</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

*This results presented as (mean ± SD)

Demographic characteristics and laboratory values of patients with Lepromatous leprosy and control subjects

CD19+CD5+ B1 lymphocytes were significantly higher in patients with Lepromatous leprosy compared to healthy controls (p = 0.001). CD19+CD5− B lymphocytes were significantly higher in patients with Lepromatous leprosy compared to healthy controls (p<0.05) [Table/Fig 1], [Table/Fig 2], [Table/Fig 3].
Table/Fig 2

CD5+ B lymphocytes in leprosy patients

Table/Fig 3

CD5+ B lymphocytes in healthy controls
There was a significant correlation between CD19+ B lymphocytes (total B lymphocyte) and CD19+CD5+ B1 lymphocytes (r = 0.54) however no significant correlation were found between CD19 and CD19+CD5+ B1 lymphocytes percentage and disease duration.

Discussion
Leprosy is a chronic infectious disease caused by Mycobacterium leprae. Its broad spectrum of manifestations depends on distinct M. leprae-responsive T-cell subsets. Type 1 T helper (Th1) cells are associated with tuberculoid leprosy (TT), which is characterized by a protective response against the bacillus with effective control of the dissemination of the disease. In contrast, T helper 2 (Th2) cells are primarily associated with lepromatous leprosy (LL), which is characterized by intense polyclonal activation of B lymphocytes and absence of an M. Leprae specific T-cell response, resulting in multibacillary and disseminated disease, as well as development of hypergammaglobulinemia and autoantibodies. Between these extremes are immunologically unstable patients with intermediate signs and symptoms corresponding to the borderline tuberculoid (BT), mid-borderline (BB) and borderline lepromatous (BL) forms [2],[3],[4],[5],[6],[7],[8].

Although the CD5+ B1 cells account for a small percentage of B cells in healthy adult individuals, their study gathers importance in view of their involvement in autoimmune states, in early lymphoid recovery after bone marrow (BM) transplantation and in malignancies such as the B-CLL and mantle cell lymphoma [3]. Additional studies have also shown that normal CD5+ B cells may be expanded in autoimmune diseases, such as rheumatoid arthritis (RA) or primary Sjogren’s syndrome (SS) [13],[14]. Both splenic CD5+ and CD5- B lymphocytes produce platelet GP-specific auto antibodies in chronic idiopathic thrombocytopenic purpura (ITP) and may play a role in the autoimmune pathogenesis of ITP[5]. Lepromatous leprosy patients have a defect on Th1 type cellular response. It is known that leprosy patients have high autoimmune disease risk than healthy population. Compared to the healthy controls, both CD5- conventional B cells and CD5+ B1 cells subsets were found to be higher in leprosy patients. Observed significant increases in CD19+CD5- and CD19+CD5+ B1 cell subsets in patients of Lepromatous leprosy suggests that B1 lymphocytes with known predisposition to autoimmunity may have a role in disorganized protective immune response in Lepromatous leprosy. About 33% of the B cells in the peripheral blood of the Lepromatous leprosy patients expressed markers for autoreactive features while less than 12% of the circulating B cells in the healthy group exhibited such markers. The Lepromatous leprosy patients contained a substantial number of B cells out of which about 30% demonstrated autoreactive features.

Conclusion
It is suggested that circulating B cells in Lepromatous leprosy patients have a higher propensity to autoreactive properties than B cells of healthy populations.

Conflict of Interest: None declared

References


Type 1- and type 2-like lesional skin-derived Mycobacterium leprae-responsive T cell clones are characterized by coexpression of IFN-gamma/TNF-alpha and IL-4/IL-5/IL-13, respectively. J Immunol 1998; 160: 2380-7.


