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Increased CD5+ B-cells are associated with autoimmune phenomena in lepromatous leprosy patients

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ABSTRACT

Background and objective: Leprosy is a chronic slowly progressive infection caused by *Mycobacterium leprae* that primarily affects the skin and peripheral nerves. Lepromatous leprosy is characterized by absence of T-cell responses to *M. leprae* and advanced clinical disease. It is frequently associated with the presence of autoantibodies, which might be related to $CD19^+CD5^+$ and $CD19^+CD5^-$ B lymphocyte percentages. Therefore, the aim of this study was to evaluate the percentages of $CD19^+CD5^+$ and $CD19^+CD5^-$ B cell subsets as well as the total B cells in lepromatous leprosy patients.
Materials and methods: Twenty lepromatous leprosy patients and ten healthy subjects served as control were included in this study. Venous blood samples were analyzed by flow cytometry to determine the B cell subsets and total B cell percentages.
Results: Compared to healthy controls, the percentages of $CD19^+CD5^+$ B cell subset and total B cells were found to be significantly higher in the patient group. While, the percentage of $CD19^+CD5^-$ B cell subset was found to be higher in the patient group than the control without any significantly difference. Regarding the eye affection, the percentage of total B cells was observed to be significantly higher in affected patients compared to the non-affected group.
Conclusion: The observed significant increases in $CD19^+CD5^+$ and total B cell percentages in patients with lepromatous leprosy suggests a possible role of these cells in the disorganized protective immune response as well as the development of eye complications in these patients.

Introduction

Leprosy is a chronic infectious granulomatous disease caused by *Mycobacterium leprae*, an acid fast bacillus with high infectivity, low pathogenicity and high virulence. In addition, it has a certain predilection to peripheral nerves and skin [1]. The disease has a wide granulomatous spectrum ranging from tuberculoid leprosy (TT) at one end to lepromatous leprosy (LL) and at the other end of the spectrum [2]. It was first described by Ridley and Jopling [3] as a six-member spectrum based on clinical and histological changes ranging from high to low resistance; including polar tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), subpolar lepromatous (LLs), and polar lepromatous (LLp). It was found that, the disease has a long incubation period ranging from 2 to 5 years for tuberculoid cases and 8–12 years for lepromatous cases [4]. Lepromatous leprosy is characterized by virtual absence of T cell responses to *M. leprae* and advanced clinical disease [5]. It is frequently associated with a range of autoantibodies, both organ-specific (directed against thyroid, nerve, testis and gastric mucosa), and non-specific such as rheumatoid factor, anticardiolipin, antineutrophil cytoplasmic autoantibodies, antinuclear and anti-DNA antibodies as well as cryoglobulins [6]. Moreover, it is associated with a biological false positive serologic test for syphilis [7]. Antiphospholipid antibodies are present in 50% of lepromatous leprosy patients [8] and may give rise to a lupus anticoagulant or agglutination of sheep erythrocytes. These autoantibodies might be related to B1a lymphocyte ($CD19^+CD5^+$) percentage in the blood of

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Table 1
Comparison between patientsand controls regarding the mean percentages (%) of B cell subsets.

B cell subsets	Groups	Percentage (%)		Statistical analysis	
		Range	Mean ± SD	t-test	P-value
$CD5^+(CD19^+CD5^+)$ B cells	Patients	0.50–15	4.82 ± 3.75	2.933	0.007*
	Controls	1.00–1.7	1.30 ± 0.24		
$CD5^-(CD19^+CD5^-)$ B cells	Patients	0.05–12	6.19 ± 3.32	0.329	0.745
	Controls	2.20–7.7	5.80 ± 2.28		
$CD19^+$(Total) B cells	Patients	0.55–22	11.00 ± 5.40	2.162	0.039*
	Controls	3.20–9.1	7.10 ± 2.44		

* Significant $P < 0.05$.

these patients [9]. The CD19 is a member of immunoglobulin (Ig) superfamily expressed only on B cells and follicular dendritic cells, and is considered a pan B cell marker [10]. The B1a ($CD5^+$) cells constitute 15–25% of B cell population in secondary lymphoid tissues and up to 47% of total B cells in normal peripheral blood in adults [1]. They are specific for polysaccharide and lipid antigens on bacterial cell wall and produce IgM (natural) antibodies [11]. In autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis and Sjogren syndrome, the relationship between the increase of autoantibodies and $CD5^+$B cells is established [12,13]. In this study, we found that the percentages of $CD19^+CD5^+$ and total ($CD19^+$) B cells is significantly higher in lepromatous leprosy patients than in healthy controls, which may have a role in disorganized immune response in lepromatous leprosy patients.

Materials and methods

Patients and controls

The current study comprised 20 lepromatous leprosy patients and 10 healthy individuals in the same age and sex served as control. Cases were collected from the Outpatient Clinic of Dermatology and Venereology Department at Tanta University Hospitals, Tanta, Egypt. All lepromatous leprosy patients included in this study were free from other infectious diseases and they were not undergoing an episode of erythema nodosum leprosum (ENL). As well as, they were not taking thalidomide or corticosteroids. A written consent was obtained from all study participants.

History and examination

Full personal history has taking including, present and past history of diseases and drugs used. Detailed dermatological examination has recorded including skin, mucous membrane and nail examination to detect any lesions or abnormalities. Lesions were examined according to the methodology described by Coates et al. [14]. Nerve and eye investigation was also carried out by method described by Coates et al. [14].

Flow cytometry analysis (FCM)

Venous blood samples were withdrawn from the antecubital vein using 18 G needle. Blood was put in glass tubes with EDTA in order to examine B cell markers by flow cytometry. All blood samples were researched within 24 h. Peripheral blood samples were analyzed using BD FACS (BD USA), BD monoclonal antibodies were used. A 100 μl of whole blood was incubated with 10 μl of anti-Human CD19-FITC (Clone: HIB19) and anti-Human CD5-PE (Clone: BC96) or their isotype control for 25 min at 4° in the dark. 2 ml of red blood cells lysis-buffer was added after staining to each tube, vertexed well and incubated for 10 min at room temperature in the dark. Then washed twice with phosphate buffer saline (PBS) and the supernatant was discarded. The cell pellet was dissolved in 300 μl of 2% paraformaldehyde (PFD). Lastly, 20,000 cells were acquired by using FACS (BD USA) and analyzed using Cellquest-Pro analysis software to determine subpopulations count.

Data analysis

Statistical analysis was performed using the mean, standard error, standard deviation, Student *t-test*, and chi-square by SPSS-V17 software. The $P < 0.05$ was considered as statistically significant.

Results

Mean percentages of B cell subsets in lepromatous leprosy patients

All study participants were subjected to blood analysis using FCM to determine the percentages of $CD19^+CD5^+$, $CD19^+CD5^-$ as well as $CD19^+$ (total) B cells. Flow cytometric results showed 3 dot plots; the 1st one is the isotype control, which is used to determine the non-specific binding. The 2nd one represents a healthy control subject while the 3rd dot plot represents a lepromatous leprosy case (Fig. 1). In the 2nd and 3rd dot plots, the lower left quadrant represents cells that are negative for both CD19 & CD5 cell markers, while the upper left quadrant shows the cells that are CD5 positive but CD19 negative. The lower right quadrant represents cells that are CD19 positive but CD5 negative (conventional, $CD19^+CD5^-$ or B2 cells), while the upper right quadrant shows cells that are double positive; $CD19^+CD5^+$ (B1a cells) (Fig. 1). The percentages of $CD5^+$ and $CD19^+$ (total) B cells showed significant difference between patients and control groups (Table 1), while the percentage of $CD5^-$ B cells showed no significant difference between patient and control groups (Table 2).

The relation between B cell subsets and disease complications

By comparing males to females in the patient group, the mean percentages of $CD5^+$, $CD5^-$ and $CD19^+$ (total) B cells revealed no significant difference between male and female patients (Data not shown). Regarding to the disease complications, the current data showed that the mean percentages of B cell subsets in patients with previous history of ENL and in patients with neurological complications showed no significant difference compared with patients without previous history of ENL and those without neurological complications, respectively (Table 2). For eye complications, data showed that there was no significant difference between patients with eye complications group and patients without eye affection group regarding the mean percentages of $CD5^+$ and $CD5^-$ B cells. However, the $CD19^+$ (total) B cell percentage showed significant difference between patients with eye complications and patients without eye complications (Table 2).

Table 2
Comparison of the mean percentages (%) among the B cell subsets regarding the disease complications.

B Cell subsets	Disease complications											
	History of reactions (ENL)				Neurological complications				Eye complications			
	Negative No. (12)	Positive No. (8)	Statistical analysis		Negative No. (7)	Positive No. (13)	Statistical analysis		Negative No. (15)	Positive No. (5)	Statistical analysis	
	Mean (±SD)	Mean (±SD)	t-test	P-value	Mean (±SD)	Mean (±SD)	t-test	P-value	Mean (±SD)	Mean (±SD)	t-test	P-value
$CD5^+$	3.58 (2.60)	7.83 (6.50)	−1.945	0.072	2.90 (1.91)	5.85 (4.15)	−1.766	0.094	4.77 (4.22)	4.94 (2.16)	−0.115	0.910
$CD5^-$	6.07 (3.48)	5.10 (3.73)	0.473	0.643	5.53 (3.42)	6.54 (3.35)	−0.639	0.531	6.12 (3.08)	8.38 (1.46)	−1.560	0.135
$CD19^+$ (Total)	9.65 (4.82)	12.93 (7.79)	−1.011	0.328	8.43 (5.25)	12.39 (5.16)	−1.630	0.120	9.90 (5.72)	14.32 (2.43)	−2.414	0.028*

* Significant $P < 0.05$; No. number of LL patients associated with positive or negative disease complications; the number between the brackets represent the standard deviation (SD).

Fig. 1. Dot plots of A; isotype control, B; health control and C; lepromatous leprosy patients.

Discussion

In autoimmune diseases, the B-lymphocyte cell compartment is characterized by expression of the CD19 surface antigen [15]. They were distinguished into a minor B1 ($CD5^+$) subpopulation, and a major B2 ($CD5^-$) subpopulation [16]. Both B cell subsets (mainly the $CD5^+$ subset) are implicated as a source of autoantibodies in autoimmune diseases and may have a role in their immune pathogenesis [17]. Therefore, the current study evaluated the mean percentages of total as well as B cell subsets and its relation to autoimmunity in LL patients using FACS. The percentage of $CD19^+$ cells (total B "sum of $CD5^+$ & $CD5^-$ cells") was significantly higher in LL patients than the control. This was confirmed by the previous results that reported high percentages and absolute numbers of B lymphocytes in both clinically active and inactive LL disease [18]. The high number of B lymphocytes may represent an over-compensation or stimulation of B cells when the T-cell population and its functions are deficient [18]. Therefore, the increased production of autoantibodies, both organ-specific and non-specific, in LL patients could be considered as normal sequlae. In addition, LL

patients produce specific anti *M. leprae* antibodies against LAM, PGL 1 and protein antigens of *M. leprae* and their levels correlate with the bacillary load measured by slit skin smear. This also could be attributed to the increased $CD19^+$ (total B) cell percentage [2]. Comparison between the percentages of $CD19^+$ (total) B cells in the patients suffered from eye affection and those without eye affection showed a significant difference. So, in addition to the damaging effect of *M. leprae* on the nerve and eye tissues, this could be partly considered a sequlae of the higher amounts of autoantibodies, which are attributed to the higher percentage of $CD19^+$ (total) B cells in the blood of this patient group. By contrast, a significant decrease in the percentages of peripheral blood B lymphocytes in LL patients as compared to controls had been reported. However, as in experimentally infected mice, the humoral immune response in lepromatous patients seems to be normal in spite of B cell depletion in the peripheral blood [19]. Results of this study denoted a significant difference of $CD5^+$ B cells in patient group than the healthy individuals. This agreed with the results of Ilhan et al. [1]. Similarly, other studies reported significant percentages of $CD5^+$ B cells in autoimmune diseases such as RA, SLE, Grave's disease, SS, MS as well as early phases of T1D [17]. In case of $CD5^-$ B cells, data showed the percentages of these cells were higher in patient group than the control subjects without any significantly difference. On the other hand, it had reported the presence of significant percentages of $CD5^-$ B cells in LL patients compared to healthy controls [1]. This difference may be due to many reasons, including the wide differences in the epitope density on the B cell membrane, methodological differences such as the type of assay used and heat inactivation of the sera, patient selection, drug regimen, as well as ethnic and geographical variations in the patients groups [20,21]. Although $CD5^+$ B cells represent a small subset of B cells in healthy population, they are involved in autoimmune diseases where the relationship between the increase of autoantibodies and $CD5^+$ B cell percentages is established [22]. In autoimmune diseases the autoreactive B cells are selected and stimulated where the $CD5^+$ B cells are induced to produce natural autoantibodies forming immune complexes with their antigen [23,24]. These immune complexes could then select autoreactive and pathogenic B cells, capable of maintaining the self-perpetuating chronic inflammatory disease [17].

Conclusion

The current study demonstrated that, lepromatous leprosy patients have high autoimmune disease risk than healthy population. Observed significant increases in the percentages of $CD5^+$ B cell subset and $CD19^+$ (total) B cells in LL patients suggest that these B lymphocytes with known predisposition to autoimmunity may have a role in disorganized protective immune response in LL patients. Moreover, significant percentage of $CD19^+$ (total) B cells may have a role in the development of eye complications in LL patients.

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Competing interests

None declared.

Ethical approval

Ethical approval of this study was obtained from the Ethics Committee, Ministry of Health, Cairo, Egypt. A written consent was obtained from all study participants after counseling and prior to blood sampling.

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