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ORIGINAL ARTICLE

Peripheral Blood B and T Cell Profiles in Children with Active Juvenile Idiopathic Arthritis

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Abstract

Juvenile idiopathic arthritis (JIA) is one of the most common autoimmune diseases in children. Our study aimed to evaluate the peripheral blood B and T lymphocyte subpopulations in children with JIA. This case–control study included 20 children with JIA as well as 20 healthy children with matching age and sex as a control group. All patients included in the study were in activity as determined by visual analog scale. In addition to complete clinical evaluation, basic investigations, peripheral blood B and T lymphocyte subpopulations were done to all participants by flow cytometry. JIA patients displayed a significant decrease in IgM memory B lymphocytes, switched memory B lymphocytes, and total memory B lymphocytes when compared to the healthy controls. The percentages of naïve B lymphocytes were significantly increased in JIA patients than in controls. Total T lymphocytes, $CD8^{+}CD28^{null}$ cells, and $CD4^{+}CD28^{null}$ cells were significantly increased in JIA patients as compared to controls. In conclusion; JIA patients have an alteration in both B and T lymphocytes with the predisposition of memory cells which may have a role in sustaining the JIA disease activity.

Keywords Juvenile idiopathic arthritis · B lymphocytes · T lymphocytes · Children

Introduction

Juvenile idiopathic arthritis (JIA) is a common type of immune-mediated chronic arthritis that leads to joint stiffness and chronic inflammation in children (Petty et al. 2004; Prelog et al. 2008). The involvement of B and T lymphocytes in the pathogenesis of JIA has been suggested; these cells may play an essential role in the disease progression (Smoleńska et al. 2012). In the peripheral lymphoid organs, B lymphocytes meet their specific antigen; after that, they migrate to the T cell zone in lymph nodes. B and T cells will then make a germinal center where T and B cells will interconnect through costimulatory molecules (CD40L on T cells and CD40 on B cells), and T cells then give B cells further signals through the production of cytokines for proliferation and starting the process of differentiation (MacLennan et al. 2003; Rehnberg et al. 2009; Saad et al. 2014). Activated B cells will clonally enlarge and, at this stage, genes encoding for different regions of immunoglobulins undertake widespread somatic point mutations resulting in augmented affinity of the antigen binding sites (affinity maturation) (MacLennan et al. 2003; Rehnberg et al. 2009). Furthermore, B cells go through recombination of the constant portion of the immunoglobulin causing replacement of IgM and IgD, by IgA, IgE, or IgG genes. This process is recognized as a B cell isotype switch (Rehnberg et al. 2009). The switch of immunoglobulin classes designates the development of antigen-specific "memory B cells" that have the capability of differentiating into long-lasting memory B cells

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as well as plasma cells (MacLennan et al. 2003; Rehnberg et al. 2009). The principal function of the memory B cells is to quickly proliferate and differentiate into plasma cells after re-stimulation with a specific antigen (Crotty et al. 2003). The role of B cells in JIA pathogenesis is still unclear; however, switched memory B cells have been suggested to have a role in the disease pathogenesis by upregulation of CD80 and CD86 (co-stimulating molecules) and presenting antigen to T cells (Rehnberg et al. 2009; Tangye and Tarlinton 2009). $CD8^+$ T cells represent about 40% of all T lymphocytes infiltrating the synovial compartment in patients with rheumatoid arthritis. Besides, they are found before the development of clinical stages of the active disease (Carvalheiro et al. 2015). The functional subset profiles of $CD8^+$ T cells include the following types: (1) short-lived effector cells that have the tremendous migratory ability and intense manufacturing of cytotoxic molecules and pro-inflammatory cytokines, (2) the effector memory subset, which is apoptosis-resistant T cells and usually collects in the peripheral organs, (3) central-memory cells, which produce a variety of cytokines and induce a robust proliferative response, and (4) suppressor subset, which produces interleukin 10 (Carvalheiro et al. 2015).

$CD4^+CD28^{null}$ cells are a unique type of highly differentiated effector memory $CD4^+$ T cells. They lack the expression of CD28 costimulatory molecule, which is necessary for T cell stimulation (Pieper et al. 2014). $CD4^+CD28^{null}$ cells are different from $CD4^+$ T cells concerning cytotoxic capacity, expression of NK cell receptors, shortened telomeres and resistance to apoptosis (Pieper et al. 2014; Wagner et al. 2003). $CD4^+CD28^{null}$ cells may have a role in the progression of several inflammatory disorders due to their pro-inflammatory features which include cytokine production and cytotoxicity. Significantly high frequencies of $CD4^+CD28^{null}$ cells could be found in the peripheral circulation of many immune disorders, such as rheumatoid arthritis (Pieper et al. 2014; Wagner et al. 2003). There were few studies evaluating B and T cell subpopulations in children with JIA and no studies from our locality, so our study aimed to evaluate the peripheral blood B and T cell subpopulations status in a cohort of Egyptian children with JIA.

Subjects and Methods

Ethical Considerations

Our study was approved by Assiut University Ethical Scientific Committee, Assiut, Egypt. Parents of all children included in the study gave their written informed consent following Assiut University Hospital Ethical Committee guidelines.

Participants

This prospective case–control study included 20 JIA children (11 females). Their ages ranged from 4 to 14 years (average: 10 years) admitted to the Allergy and Immunology unit at Assiut University Children Hospital, and newly diagnosed as JIA from history, clinical examination and investigations, as well as 20 apparently healthy children with matched sex and age, and without history of infections in the previous month served as controls. Patients with other connective tissue or autoimmune diseases were excluded from our study. All patients included in the study were in activity as determined by visual analog scale (Filocamo et al. 2010).

Methods

In addition to complete clinical evaluation, including personal and family history and recent pathologic events, a detailed physical examination was also performed. All laboratory parameters were done at the onset of admission at their first presentation and before the start of any anti-rheumatoid medications to avoid the effects of different lines of treatment on our results. The investigations included complete blood count, C-reactive protein, erythrocyte sedimentation rate, antinuclear antibody, and rheumatoid factor. As regards, controls we assessed ESR, CRP, B- and T-lymphocyte subpopulations for comparison with patients.

Flow Cytometric Detection of Lymphocyte Subsets

T lymphocyte subsets were detected by staining 50 µl of the blood sample with 5 µl of fluoroisothiocyanate (FITC)-conjugated CD4, phycoerythrin (PE)-conjugated CD8, peridinium-chlorophyll-protein (Per-CP)-conjugated CD3 and allophycocyanin-conjugated CD28. B lymphocyte subsets were stained with 5 µl of Per-CP-conjugated CD19, PE-conjugated IgM and FITC-conjugated CD27. All antibodies were from Becton–Dickinson (BD) Biosciences (San Jose, CA, USA). After incubation for 15 min, red blood cells lysis and washing with phosphate buffer saline (PBS) at room temperature were done. The cells were resuspended in PBS and analyzed by FACS Calibur flow cytometry with CellQuest software (BD Biosciences, USA). An isotype-matched negative control was used with each sample. Forward and side scatters were used to define the lymphocytes population. Then, the percentages of $CD19^+$ (total B lymphocytes), $CD19^+CD27^+$ (total memory B cells), $CD19^+CD27^-$ (naive B cells), $CD19^+CD27^+IgM^+$ (IgM memory B cells), $CD19^+CD27^+IgM^-$ (switched memory B cells), $CD3^+$ (T lymphocytes), $CD8^+$ (T-cytotoxic), and $CD4^+$ (T-helper

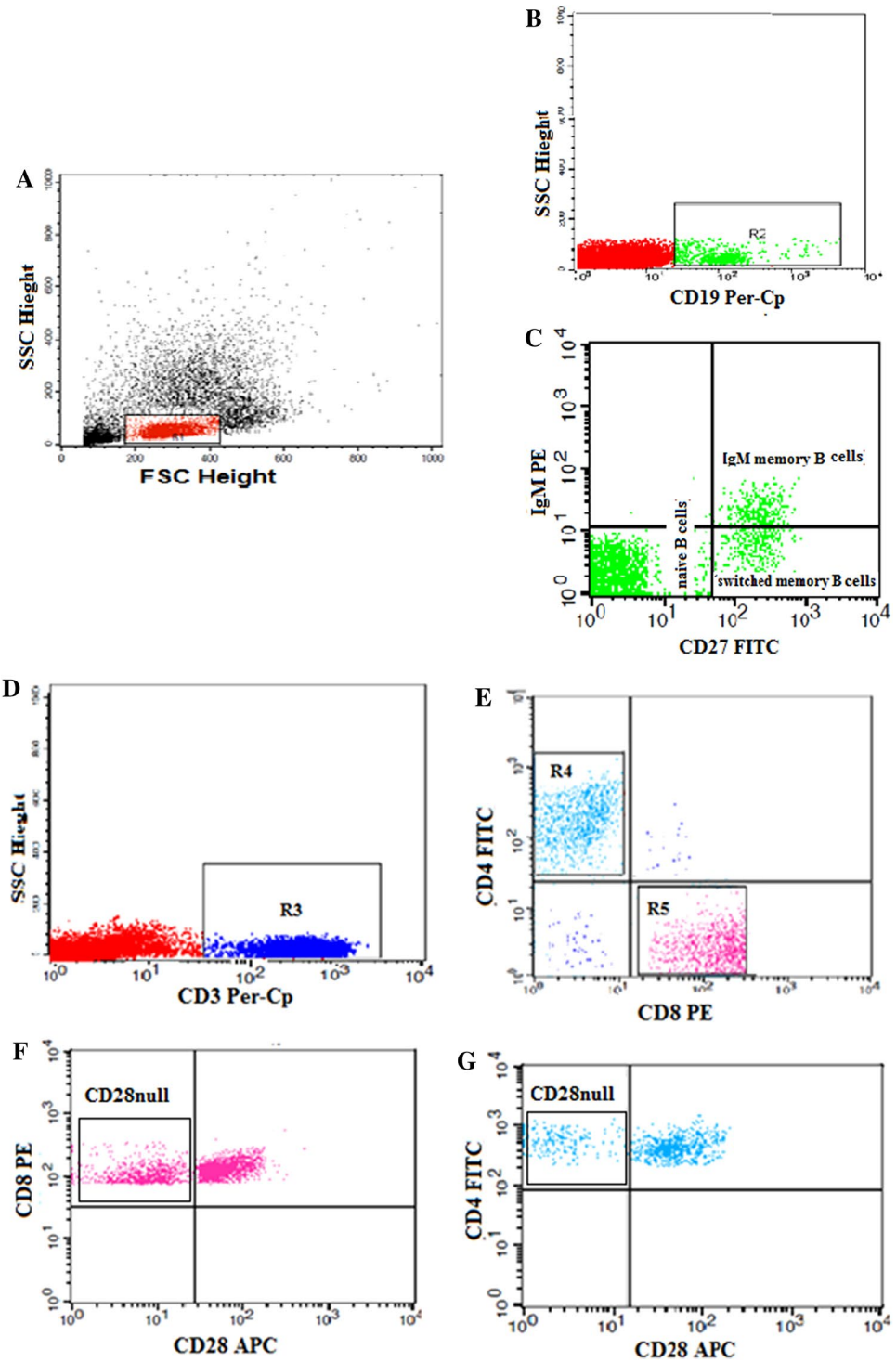

Fig. 1 Flow cytometric detection of memory B lymphocytes, $CD4^+CD28^{null,}$ and $CD8^+CD28^{null}$ lymphocytes: **a** Forward (FSC) and side (SSC) scatters were used to define the lymphocytes population. **b**, **c** $CD19^+$ cells were gated for further analysis of the expression of IgM and CD27 in B cells to detect $CD19^+CD27^-$ (naive B cells), $CD19^+CD27^+$ (total memory B cells), $CD19^+CD27^+IgM^+$ (IgM memory B cells), and $CD19^+CD27^+IgM^-$ (switched memory B cells). **d**, **e** $CD3^+$ cells were gated for further detection of $CD4^+$ and $CD8^+$ T lymphocytes, which were then gated for further detection of the expression of CD28. **f**, **g** The expression of CD28 on $CD4^+$ and $CD8^+$ T lymphocytes to detect $CD8^+CD28^{null}$ and $CD4^+CD28^{null}$. *APC* allophycocyanin, *PE* phycoerythrin, *Per-CP* peridinium-chlorophyll-protein, *FITC* fluoroisothiocyanate

(Th) cells) were assessed on lymphocyte population. Then, the expression of CD28 on $CD4^+$ and $CD8^+$ T lymphocytes was assessed to detect the percentages of $CD8^+CD28^{null}$ and $CD4^+CD28^{null}$ (Fig. 1).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA). Data were represented as mean ± SD. Independent *T* test was applied to find out the significant differences between the two groups.

Table 1 Demographic and clinico-laboratory data of JIA patients and control

	Patients $n=20$	Control $n=20$	p value
Sex: (male/female %)	9/11 (45/55%)	8/12 (40/60%)	NS
Age (year) Mean ± SD	9.31 ± 3.9	9.38 ± 3.1	NS
Antinuclear antibody (ANA): positive negative	3 17	– –	– –
Erythrocyte sedimentation rate (ESR): 1st hour (mean ± SD) 2nd hour (mean ± SD)	62.3 ± 24.3 94.3 ± 36.5	5.6 ± 4.3 7.1 ± 3.1	< 0.0001 < 0.0001
C-reactive protein (CRP) (mean ± SD)	96.4 ± 28.4	2.44 ± 1.4	< 0.0001
Rheumatoid factor: positive negative	4 16	– –	– –
B lymphocytes ($CD19^+$)	12.12 ± 3.48	10.95 ± 3.15	NS
Total memory B cells ($CD19^+CD27^+$)	22.15 ± 7.39	34.17 ± 5.17	< 0.0001
IgM memory B cells ($CD19^+CD27^+IgM^+$)	11.95 ± 1.95	18.94 ± 4.88	< 0.0001
Class switch memory B cells ($CD19^+CD27^+IgM^-$)	10.52 ± 3.03	15.1 ± 4.93	0.001
Naive B lymphocytes ($CD19^+CD27^-$)	76.45 ± 5.292	64.92 ± 6.96	< 0.0001
T lymphocytes ($CD3^+$)	69.38 ± 7.51	62.58 ± 10.74	0.026
T-cytotoxic ($CD8^+$)	15.18 ± 5.91	19.49 ± 8.46	NS
T-helper ($CD4^+$)	50.95 ± 18.34	42.47 ± 11.9	NS
$CD4^+CD28^{null}$	11.49 ± 3.36	6.5 ± 1.9	< 0.0001
$CD8^+CD28^{null}$	46.94 ± 12.62	22.811 ± 7.4	< 0.0001

NS non-significant

Results

The demographic and the clinical characteristics of the JIA patients and the healthy controls are shown in Table 1. With regard to B lymphocyte subpopulations, there was no significant difference in the number of total B lymphocytes between JIA children and the healthy controls ($p=0.35$). On the other hand, JIA patients demonstrated significant decreases in the total memory B lymphocytes ($p<0.0001$), switched memory B lymphocytes ($p<0.001$), and IgM memory B lymphocytes ($p<0.0001$) than the healthy children, whereas the percentage of naïve B lymphocytes was significantly higher in JIA patients when compared to the healthy control group ($p<0.0001$) (Table 1). Regarding T lymphocyte subpopulations; the percentages of total T lymphocytes were significantly increased in patients than controls, Additionally, both $CD8^+CD28^{null}$ cells ($p<0.0001$) and $CD4^+CD28^{null}$ cells ($p<0.0001$) were significantly higher in JIA patients than the healthy control. However, the percentages of T-helper lymphocytes ($CD4^+$ cells) and T-cytotoxic lymphocytes ($CD8^+$ cells) were comparable between patients and controls (Table 1).

Discussion

Many studies have suggested that various immune abnormalities were involved in the pathogenesis of JIA. The immune cells as B and T lymphocytes have an important role in the pathogenesis of JIA as well as in its progression (Smoleńska et al. 2012). Elucidation of the role of these immune cells may provide a valuable basis that could help in the management of JIA (Goldzweig and Hashkes 2011). In our study, we assessed the peripheral blood T and B lymphocyte subpopulations in JIA patients.

Regarding the B lymphocyte subpopulations, the percentage of naïve B lymphocytes was significantly increased in JIA patients than in the healthy controls. In line with our results, previous studies showed that there was a 1- to 1.2-fold elevation in naïve B lymphocytes in JIA patients than healthy controls (Moura et al. 2010; Wang et al. 2013). Our data showed that JIA patients had a significantly lower number of memory B lymphocytes with both subtypes, IgM memory B lymphocytes, and switched memory B lymphocyte than controls. The roles of B lymphocytes in blood and synovial membrane in JIA patients include autoantibody production, activation of T cells, and cytokine production (Bugatti et al. 2014). Fluctuations in the memory B lymphocytes were reported in many studies. Our results were in line with a previous study by Souto-Carneiro et al. (2009) who reported that JIA patients had significantly lower absolute numbers and frequencies of peripheral blood

pre-switch IgD^+CD27^+ memory B lymphocytes when compared with healthy controls (JIA patients: $10.4 \pm 1.3\%$ versus healthy control $15.1 \pm 1.1\%$; $p = 0.003$). Similar results were reported previously that either one or both subtypes of memory B lymphocytes decrease in the JIA patients (Moura et al. 2010; Wang et al. 2013). In JIA patients, the memory B lymphocyte, especially pre-switch memory cells, is used to accumulate in the synovial membrane in these patients due to an elevated level of tumor necrosis factor, and this could explain why the peripheral blood percentages of these cells were decreased (Souto-Carneiro et al. 2009).

Regarding the T lymphocyte subpopulations, the results of the current study were in line with Smoleńska et al. (2012) who found that JIA patients had an elevated number of total $CD3^+$ T lymphocytes than in healthy control, while they did not observe the same elevation in the number of T-cytotoxic lymphocytes ($CD8^+$ cells) (Smoleńska et al. 2012). Similarly, Wouters et al. (2002) showed that all the studied JIA subtypes had comparable percentages of the Th lymphocytes ($CD4^+$ cells) and T-cytotoxic lymphocytes ($CD8^+$ cells) when compared to the healthy controls (Wouters et al. 2002). On the other hand, our results were not in agreement with that of Carvalheiro et al. (2013) who found that T-cytotoxic lymphocytes ($CD8^+$ cells) were elevated in JIA patients than in the healthy control. The studied JIA patients were recently diagnosed, and this could explain the insignificant difference found in the percentages of Th lymphocytes ($CD4^+$ cells) and T-cytotoxic lymphocytes ($CD8^+$ cells) in JIA patients when compared to the healthy control in this study.

Regarding $CD8^+CD28^{null}$ and $CD4^+CD28^{null}$, our results were in agreement with those of Pawlik et al. (2003) and Ceeraz et al. (2013) who reported that all JIA patients had elevated percentage of circulating T lymphocytes that were without CD28. Furthermore, Pawłowska et al. (2009) revealed that JIA patients had a high number of both $CD4^+CD28^-$ and $CD8^+CD28^-$ T lymphocytes when compared with the healthy controls, and they also observed that $CD8^+CD28^-$ T lymphocytes were more elevated than $CD4^+CD28^-$ type. Moreover, it was noticed that the increased percentage of $CD4^+CD28^-$ type of T lymphocytes was more correlated with the JIA activity, suggesting a direct important role of this T lymphocytes as a controller and a regulator of the disease activity more than $CD8^+CD28^-$ type of T lymphocytes (that was supposed to be involved more in the regional joint activity) (Pawłowska et al. 2009). An elevated proportion of $CD4^+CD28^-$ Th lymphocytes in JIA patients was associated with more active disease state, denoting that these types of cells are involved in the progression of a state of inflammation, which in turn could raise the possibility of a cardiovascular disease, as these cells are cytotoxic and are involved in the injury of coronaries (Nakajima et al. 2002).

The current study had some limitations; as missing the qualitative assessment of the lymphocytes through measurement of levels of cytokines, we have only assessed the lymphocyte quantitatively in JIA patients. Also, all our JIA patients were recently diagnosed with early manifestations of the disease, and if they were at different durations from the onset of the diagnosis, the results might be changed.

In conclusion, the current study showed that JIA patients had a significant shift in the percentages of both B and T lymphocytes, especially the memory cells, which decreased significantly in JIA patients than healthy control. From these results, we recommend that significant efforts and further researches must be done to improve old and invent novel therapeutic agents that can antagonize or modify these alterations and help sustain the JIA disease state.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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