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Evaluation of Antibody Response to Polysaccharide Vaccine and Switched Memory B Cells in Pediatric Patients with Inflammatory Bowel Disease

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Background/Aims: Inflammatory bowel disease (IBD) is a chronic disease of the gastrointestinal tract, whose etiologies are still unknown. This study was performed to evaluate the humoral immune response in terms of B cell functions in selected IBD patients. **Methods:** Eighteen pediatric patients with IBD, including 12 cases of ulcerative colitis (UC) and six with Crohn disease (CD), were enrolled in this study. The pneumococcal vaccine was injected in all patients, and the IgG antibody level to the polysaccharide antigen was measured before and 4 weeks after injection. The B cell switch-recombination process was evaluated. **Results:** Five patients with IBD (three CD and two UC) had defects in B cell switching, which was significantly higher than in controls (p=0.05). Ten patients had a specific antibody deficiency and exhibited a higher frequency of bacterial infection than the healthy group. The mean increased level of IgG after vaccination was lower in IBD patients (82.9±32.5 μg/mL vs 219.8±59.0 μg/mL; p=0.001). Among the patients who had an insufficient response, no significant difference in the number of switched memory B-cell was observed. **Conclusions:** A defect in B lymphocyte switching was observed in pediatric IBD patients, and especially in those patients with CD. Owing to an increased risk of bacterial infections in those patients with antibody production defects, pneumococcal vaccination could be recommended. However, not all patients can benefit from the vaccination, and several may require other prophylactic methods. **(Gut Liver 2014;8:24-28)**

Key Words: Inflammatory bowel diseases; Crohn disease; Colitis, ulcerative; Polysaccharide vaccine; Switched memory B cells

INTRODUCTION

Dysregulation of the immune system has been suggested as a pathogenesis of inflammatory bowel disease (IBD). Several components of the immune system such as antibody production, T cells, cytokines and growth factors have been explained to be involved in IBD pathogenesis.[1] IBD patients are at increased risk of developing infections due to different causes[1-3] and immunization with several vaccines can be effective for infection prevention in these patients.[4,5]

The immune response to vaccines containing polysaccharide antigens has been well described in some immune-mediated diseases.[6-9] However, determination of this response is a matter of controversy in IBD. Melmed *et al*.[10] reported that patients receiving immunosuppressive therapy have an impaired antibody response to this vaccine, while another study by Dotan *et al*.[11] showed normal immunization in comparison with healthy controls.

Memory B cells are characterized by cell surface expression of CD27 and rapidly generate immunoglobulins (Ig) of each special isotypes during secondary immune responses.[12] IgD^- $CD27^+$ switched memory B cells are a group of memory B cells expressing IgG, IgA, or IgE and are originated from germinal centers and may have an important role in antibody response against various antigens.[13] Two studies done by Di Sabatino *et al*.[14,15] showed no significant difference in the level of switched memory B cells in IBD patients in comparison with controls.

The aim of this study was to determine the frequency of

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switched memory B cells in IBD patients and to assess their possible causative role in disease pathogenesis and also to investigate the specific antibody response of IBD patients against pneumococcal polysaccharide vaccination.

MATERIALS AND METHODS

1. Subjects

All available IBD patients who were referred to the Children's Medical Center in Tehran, Iran were enrolled in this study. Patients with active infection, primary or secondary immunodeficiency, and history of previous pneumococcal vaccination within the past 5 years were excluded from the study and results of remaining 18 patients were compared with 20 healthy individuals as a control group.

The process of this evaluation was explained to the individuals and/or their legal guardians; then informed consents were taken. Diagnosis of IBD was made based on standard criteria using clinical, endoscopic, pathologic, and radiologic findings.[16] A questionnaire was designed to collect demographic information and medical histories for each patient by reviewing the patient's records. The number of infections which needed clinical admission was considered as number of infection episodes. Unfortunately, since the exact type and the cause of the infection could not be determined in all episodes of infections, to avoid a recall bias, only the number of serious infections which needed admission was included in this study.

The process of this study was approved by the medical ethics committee of Tehran University of Medical Sciences.

2. Immunosuppressive therapy

Of 18 studied patients, 15 individuals received immunosuppressive drugs (6-mercaptopurine, azathioprinem, and/or corticosteroids) and three patients received only anti-inflammatory drug (mesalamine).

3. Vaccination

Patients and their healthy controls received single dose of 0.5 mL unconjugated pneumococcus polyvalent vaccine (PCP, PNEUMO 23; Aventis, Pasteur, France), and blood samples were taken before and 28 days after vaccination. In both patient and control groups, specific antibodies against whole pneumococcal antigens were measured using the protocol of the third-generation enzyme-linked immunosorbent assay format.[17] Results were reported as end point titer determined by the highest dilution giving an optical density of 0.2 or higher. There are no universal criteria for adequate antibody response to pneumococcal specific antibody levels, and each laboratory consider the response in normal healthy controls to generate normal range and to define hyporesponsive persons to vaccine. All subjects showing an increased specific antibody titers equal to or greater than lower limit of the 2-tailed 90% probability interval of postimmunization specific IgG of the healthy adults were defined as responders.[18] The median titers for before and after vaccination in the control group were 70 and 450 U/mL, respectively. The lower limit of the 2-tailed 90% probability interval of postimmunization specific IgG was 129 U/mL, which is used as the minimum significant increase for adequate response in the patient group.

4. Cell preparation and flow cytometric analysis

Five to 10 mL of blood anticoagulated with ethylene diamine tetra acetic acid was drawn from IBD patients and healthy controls. The fraction of peripheral blood mononuclear cells (PBMCs) was isolated by Ficoll-Hypaque density gradient centrifugation and washed twice with phosphate-buffered saline. PBMCs were centrifuged through a layer of 100% heat-inactivated fetal calf serum (Gibco, Grand Island, NY, USA) to reduce cell-bound IgG and resuspended in RPMI 1640 (Sigma, Munich, Germany) supplemented with 10% fetal calf serum.

PBMCs (2.5×10^6/50 μL in RPMI 1640 medium plus 10% fetal calf serum) were stained for 20 minute at 4°C with 10 μL of a mixture of the following antibodies at optimal concentrations: three-color direct immunofluorescent staining with monoclonal antibodies (mAbs) were performed on aliquots of heparinized peripheral blood collected from each patient group using FlowCytomix (Partec, Münster, Germany) and samples were processed within 12 hours of collection. Antibody staining was performed on 2 mL of whole blood as described by the manufacturers. Whole blood samples were stained with a mixture of the following antibodies at optimal concentrations: anti-CD19 phycoerythrin Cy5-conjugated, anti-CD27 phycoerythrin-conjugated, and anti-IgD fluorescein isothiocyanate-conjugated were used to maximize the sensitivity of detecting $CD19^+$, $CD27^+$ and IgD^+ cells. Analysis of the data was performed by Flomax program (Partec). All mAbs used in this study were purchased from Dako (Glostrup, Denmark).

By using Paris classification (Table 1), impairment in class switching of memory B cells was considered if the percentage of IgD^- $CD27^+$ cells was lower than 8% of total B cells (MB1 group).[19]

Table 1. Paris Classification Systems for Common Variable Immunodeficiency Based on Staining of Peripheral Blood Lymphocytes with Anti-CD27/IgM/IgD[19]

CVID patient B cell subsets		
MB0	MB1	MB2
% $CD27^+$ B cells <11%	% $CD27^+$ B cells >11% % $CD27^+$ IgM^- IgD^- <8%	Neither MB0 nor MB1

CVID, common variable immunodeficiency; CD, cluster of differentiation; Ig, immunoglobulin; MB, memory B cells.

5. Statistical analysis

Specific antibody titers were expressed as the geometric mean. Comparisons between groups were performed using Student t-test; Mann-Whitney U test were used when the distribution was not normal for the selected variable. Chi-square test was performed in order to evaluate the difference between categorical variables. A p-value of 0.05 or less was considered statistically significant in our study.

RESULTS

1. Patients' characteristics

Eighteen IBD patients (10 male and eight female) were included in this study (mean age, 10.7±4.2 years). A group of 20 healthy donors (11 male and nine female) were also enrolled as controls (mean age, 23.9±3.9 years). In patient group, the mean age at the time of onset of disease was 7.6±3.6 years. At the time of diagnosis, mean age of the subjects was 8.9±3.2 years. The mean diagnostic delay was 9.9±11.3 months. Twelve out of 18 subjects (66.7%) had ulcerative colitis (UC) (mean age, 9.0±4.2 years) and six patients (33.3%) were affected with Crohn disease (CD) (mean age, 13.7±2.4 years).

2. Response to vaccination

The mean increased level of total IgG after vaccination was significantly lower in IBD patients than controls (82.9±32.5 μg/mL vs 219.8±59.0 μg/mL; p=0.001). Ten out of 18 patients (55.5%) were found to be hyporesponsive to the vaccine and had specific antibody deficiency (SAD) against PCP antigen. We divided patients into hyporesponsive and normal patients and their immunological and clinical characteristics were compared, which are presented in Table 2. In hyporesponsive patients, mean values of IgG levels after immunization (p=0.018), ab-

Table 2. Characteristics of Patients with Inflammatory Bowel Disease according to Antibody Deficiency

Characteristic	Specific antibody deficiency (n=10)	Normal specific antibody production (n=8)	p-value
Current age, yr	9.6 (3.2-16.1)	11.9 (2.4-15.2)	0.27
Male/Female	5/5	5/3	0.59
Age at the time of onset, yr	6.8 (0.4-12.3)	8.5 (1.5-11.6)	0.34
Age at the time of diagnosis, yr	8.9 (2.2-13.17)	8.9 (1.6-11.8)	0.99
Diagnostic delay, mo	14.89 (2-39)	4.3 (2-12)	0.05
Duration of disease, mo	33.2 (12-99)	40.3 (14-84)	0.57
Ulcerative colitis/Crohn disease	8/2	4/4	0.18
Episodes of infection	5.8 (1-15)	1 (0-3)	0.013*
Total IgG preimmunization, μg/mL	67.8 (5.9-218)	31.5 (7-49)	0.17
Total IgG postimmunization, μg/mL	180.7 (37-298)	251.3 (160-388)	0.018*
Increase in total IgG, μg/mL	82.9 (20-120)	219.8 (131-345)	<0.001*
Fold increase mean ratio of postimmunization to preimmunization	4.2 (1.1-8.7)	10.8 (3.6-28.5)	0.001*
Immunosuppressive therapy, y/n	9/1	6/2	0.39
Switched memory B cells (% of total B cells)	12.57 (2.2-29.5)	7.3 (1.4-18.3)	0.11
Paris classification switching, impaired/normal	6/4	7/1	0.31

Data are presented as number or median (range).
*Significant difference.

Table 3. Number of Inflammatory Bowel Disease Patients with Impaired Switched Memory B Cells Compared with Controls

Group	Paris classification		OR (CI)	p-value
	Normal	Impaired		
Inflammatory bowel disease	13	5	7.3 (0.7-70.0)	0.05
Ulcerative colitis	10	2	3.8 (0.3-47.2)	0.27
Crohn disease	3	3	19.0 (1.45-248.3)	0.007*
Controls	19	1	References	-

OR, odds ratio; CI, confidence interval.
*Significant difference.

solute increase in the level of antipneumococcal IgG (p<0.001) and its fold increase ratio (p=0.001) were significantly lower than in normal-response group. The rate of hyporesponses was statistically similar between CD and UC patients (p=0.18) (Table 2).

3. Switched memory B cells

In the patients group, the median percentage of switched memory B cells (IgD^- $CD27^+$ $CD19^+$) in total B cells was 9.1±4.0 (range, 1.4 to 29.5) and significantly lower than its value in healthy controls which was 13.7±3.8 (range, 10.4 to 20.7; p=0.007). By using Paris classification, five out of 18 IBD patients (27.7%) showed impairment in class switching. In contrast, in control group, only one out of 20 individuals (5%) had switched memory B cells lower than 8% of total B cells which was almost significant (p=0.05). The number of patients with impaired switched memory B cells was significantly higher in CD in comparison with healthy individuals (p=0.007) (Table 3).

The frequency of switched memory B cells was also compared in both groups of hyporesponse and normal response to PCP vaccine, which had no significant difference (Table 2).

DISCUSSION

This study was performed on selected IBD patients who were referred to the main referral center for pediatric gastroenterology in Iran.[16,20]

Impaired class switching and SAD were found in 27.8% and 55.5% of these patients respectively; which indicated both features were more frequent in pediatric IBD cases rather than controls. A few studies have been done to investigate immune responses to various vaccines in IBD patients.[21] A study by Stevens *et al*.[22] showed a defective humoral response to booster immunization with tetanus toxoid in the majority of their patients. In another study, Brogan *et al*.[23] explained that impaired immunization in IBD cases could be because of defects in development of IgG precursors. Mamula *et al*.[24] in a study on 50 IBD patients, in influenza seasons of 2002 to 2004, reported a generally significant low immune response to one influenza vaccine antigen in patients in comparison with healthy subjects. Also in current study, IBD patients with SAD are significantly more susceptible to episodes of infections. It shows that measurement of post pneumococcal vaccination antibody levels in IBD patients can be used to screen the poor responders who most likely are at increased risk of infectious complications.

We found that 27% of IBD patients showed impairment in class switching process that is defined by levels of switched memory B cells lower than 8%. Also the percentages of switched memory B cells in total B cells of patients with CD are significantly lower than controls. However, in the studies by Di Sabatino *et al*.,[14,15] no defect in switching recombination process was seen in IBD patients which our study only shows a non-significant difference between levels of switched memory cells in subjects with UC rather than controls. The $CD27^+$ memory B cells consist of two subgroups. One of them is IgD^+ $CD27^+$ memory B cells (nonswitched) that express IgM. This type of memory B cells has an identified role in immune response against encapsulated bacteria that need spleen for their generation and/or survival.[25,26] The other one is IgD^- $CD27^+$ memory B cells (switched) that express IgG, IgA or IgE and are originated from germinal centers.[13] It can show that a defect in B cell differentiation to switched memory B cells, and impaired maturation can be considered as a pathogenesis in a selected group of IBD cases specially CD patients.

This study also showed that the immune response to polysaccharide vaccination has no significant correlation with the levels of switched memory B cells in IBD patients. This irrelevancy could be interpreted with different mechanism. Whereas this study focused on number of switched memory B cells, probable functional defects of this subset of lymphocytes due to secondary hyposplenism still is remained to be investigated in IBD cases in future study. Moreover it was documented previously that patients who received immunomodulatory therapy had a significant lower response to vaccine antigens as compared with controls.[24] This effect on specific antibody production may be upstream of class switching recombination process. A study done by Melmed *et al*.,[10] on 45 IBD subjects and 19 normal controls, showed an impaired response to PCP antigens in patients with CD receiving an immunosuppressive therapy combined with tumor necrosis factor-blockers. In contrast, Dotan *et al*.[11] reported immunosuppression in IBD has no effect on the normal rates of responses to PCP vaccine. In our study one out of three patients without receiving immunosuppressive drugs showed SAD. It could suggest that all IBD patients, despite receiving nonimmunosuppressive treatment, are better to be screened for possible impaired response to pneumococcal vaccination before initial administrations. Finally defects of different component of humoral immune system especially complement system may have an intermediate role for SAD regardless of class switching recombination.

Whereas the cause and type of the infection has not been provided in this study, therefore future investigations are required for an accurate analysis for this issue and its relation with the quality of response to pneumococcal vaccination in IBD patients.

In conclusion, the results of our study show that significant numbers of IBD patients are hyporesponsive to PCP vaccine and have SAD against PCP antigen. Vaccination in this group of patients may not be sufficient for their protection against pneumococcal infections. As a result, IBD patients may benefit from other prophylactic methods such as administration of pneumococcal conjugate vaccine and long term antibiotics therapy.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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