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Immunoglobulin M memory B cell decrease in inflammatory bowel disease

*A. DI SABATINO, R. CARSETTI**, M.M. ROSADO**, R. CICCOCIOPPO, P. CAZZOLA, R. MORERA, F.P. TINOZZI*, S. TINOZZI*, G.R. CORAZZA*

*Gastroenterology Unit and *Department of Surgery, IRCCS Policlinico S. Matteo, University of Pavia – Pavia (Italy)*
***Research Center Ospedale Bambino Gesù – Rome (Italy)*

Abstract. – ***Background & Objectives:*** **Memory B cells represent 30-60% of the B cell pool and can be subdivided in IgM memory and switched memory. IgM memory B cells differ from switched because they express IgM and their frequency may vary from 20-50% of the total memory pool. Switched memory express IgG, IgA or IgE and lack surface expression of IgM and IgD. Switched memory B cells derive from the germinal centres, whereas IgM memory B cells, which require the spleen for their survival and/or generation, are involved in the immune response to encapsulated bacteria. Since infections are one of the most frequent comorbid conditions in inflammatory bowel disease, we aimed to verify whether IgM memory B cell pool was decreased in Crohn's disease and ulcerative colitis patients.**

Patients & Methods: **Peripheral blood samples were obtained from 22 Crohn's disease patients, 20 ulcerative colitis patients, 22 healthy controls and 18 splenectomized patients. To analyse peripheral blood lymphocytes, flow cytometry was performed using anti-CD19, anti-CD22, anti-CD27, anti-IgM, anti-IgD and anti-CD38 monoclonal antibodies.**

Results: **Circulating IgM memory B cells were significantly lower in Crohn's disease (median 7.1%, range 1.8-20.7) and ulcerative colitis patients (median 8.1%, range 2.1-18.8) in comparison to control subjects (median 14.0%, range 6.8-31.1). As expected, there was a highly significant difference in the proportion of IgM memory B cells between splenectomized patients (median 2.4%, range 0.9-6.9) and healthy controls. Crohn's disease patients with abscesses showed the lowest frequency of IgM memory B cells.**

Discussion: **Our findings show that peripheral IgM memory B cells are reduced in inflammatory bowel disease patients. Further studies are necessary to answer the question of whether high risk of infection (abscess development) is promoted by the reduction/depletion of IgM memory B-cell pool in inflammatory bowel disease.**

Key Words:

Crohn's disease, IgM memory B cell, Ulcerative colitis.

Abbreviation list

CAI = Clinical activity index
CDAI = Crohn's disease activity index
Ig = Immunoglobulin

Introduction

Several studies have focused on the mechanisms that regulate T cell survival, differentiation and activation in inflammatory bowel disease[1,2], but very little is known about B cells and their function. MacDermott et al. have shown that immunoglobulin (Ig) synthesis and secretion by peripheral blood and intestinal mononuclear cells are changed in patients with Crohn's disease and ulcerative colitis[3], and experimental mouse models suggest a protective role of B cells in chronic colitis[4,5].

In human peripheral blood, B cell subsets can be identified using surface markers such as CD22, CD24 and CD27. The latter discriminates peripheral naïve ($CD27^-$) from memory ($CD27^+$) B cells[6]. Memory B cells represent 30-60% of the B cell pool[7] and can be subdivided in IgM memory and switched memory. IgM memory B cells differ from switched because they express IgM and their frequency may vary from 20-50% of the total memory pool. Switched memory express IgG, IgA or IgE and lack surface expression of IgM and IgD[8]. It has recently been shown that these two cell subsets have different origins and functions. Switched memory B cells, which are only transiently depleted after splenectomy and are present at normal frequency in asplenic patients, derive from the

germinal centres, whereas IgM memory B cells, which are involved in the immune response to encapsulated bacteria, are reduced/undetectable in splenectomized patients and thus require the spleen for their survival and/or generation[9,10].

As it is known that infections are one of the most frequent comorbid conditions in inflammatory bowel disease[11], the aim of our study was to verify whether in Crohn's disease and ulcerative colitis patients the IgM memory B cells pools was decreased.

Patients and Methods

Patients

Peripheral blood samples were obtained from 22 patients with Crohn's disease (mean age 36.1, range 24-69), 20 patients with ulcerative colitis (mean age 38.2 yrs, range 20-68), 22 healthy controls (mean age 38.3 yrs, range 22-68) and 18 splenectomized patients (mean age 40.3 yrs, range 24-69). Diagnosis of Crohn's disease and ulcerative colitis was established according to the usual clinical criteria, and the site and extent of the disease were confirmed by endoscopy and histology in all patients. Disease activity in Crohn's disease patients was assessed by Crohn's disease activity index (CDAI). Patients with scores below 150 were classified as being in remission, whereas those with scores over 450 had severe disease[12]. Disease activity in ulcerative colitis patients was assessed according to the clinical activity index (CAI) of Rachmilewitz[13]. Clinical remission was defined as a CAI score below 6.

Among the 22 Crohn's disease patients, 10 were untreated at the time of inclusion in the study, 8 were treated with mesalazine and two with antibiotics and had suspended the treatment with steroids or other immunosuppressive agents at least three months earlier, two were treated with steroids. Among the 20 ulcerative colitis patients, 10 were untreated, 8 were treated with mesalazine and had suspended the treatment with steroids or other immunosuppressive agents at least three months before the analysis and two were treated with steroids.

We also analysed 18 splenectomized patients. In 10 individuals splenectomy was performed after traumatic rupture of the spleen caused by external injury. The remaining 8 patients had had an elective procedure for staging of Hodgkin's disease (4 cases), or non-malignant disease, such as hypersplenism due to cirrhosis (1 case), immune thrombocytopenia (1 case), splenic artery aneurism (1 case) and splenic cysts (1 case).

Each subject gave informed consent to the study.

Flow Cytometric Analysis

Peripheral blood mononuclear cells isolated from heparinized peripheral blood by Lymphoprep gradient centrifugation (Nicamed, Oslo, Norway) were stained with the appropriate antibody combinations of fluorescein, phycoerythrin, APC, cychrome, or biotin-labelled antibodies followed by streptavidin-cychrome and streptavidin-APC (Caltag Laboratories Inc., Burlingame, CA), as previously described[9]. Monoclonal antibodies HIB19 (anti-CD19), HIB22 (anti-CD22), M-T271 (anti-CD27), G20-127 (anti-IgM), IA6-2 (anti-IgD) and HIT2 (anti-CD38) were obtained from BD Biosciences (San Jose, CA) and anti-IgM Fc5µ fragment specific was obtained from Jackson Immuno-Research Laboratories, Inc. (West Grove, PA). Dead cells were excluded from analysis by side/forward scatter gating. All analyses were performed on a FACSCalibur (Becton Dickinson Co.) interfaced to a Macintosh CellQuest™ computer program.

Statistical Analysis

Data were analyzed in the GraphPad Prism statistical PC program (GraphPad Software, San Diego, CA) by means of the non-parametric Mann-Whitney U-test. A level of $p < 0.05$ was considered statistically significant.

Results

Mature and memory B cells can be identified using a combination of the B cell marker CD22[14] and CD27, which is expressed on memory B cells[6]. Based on the relative expression of IgM and IgD, memory B cells were subdivided into IgM memory (IgM^{bright} IgD^{dull}) and switched memory B cells (IgM^{-} IgD^{-}). Figure 1 shows the frequency of B cells and the distribution of their subsets in all pa-

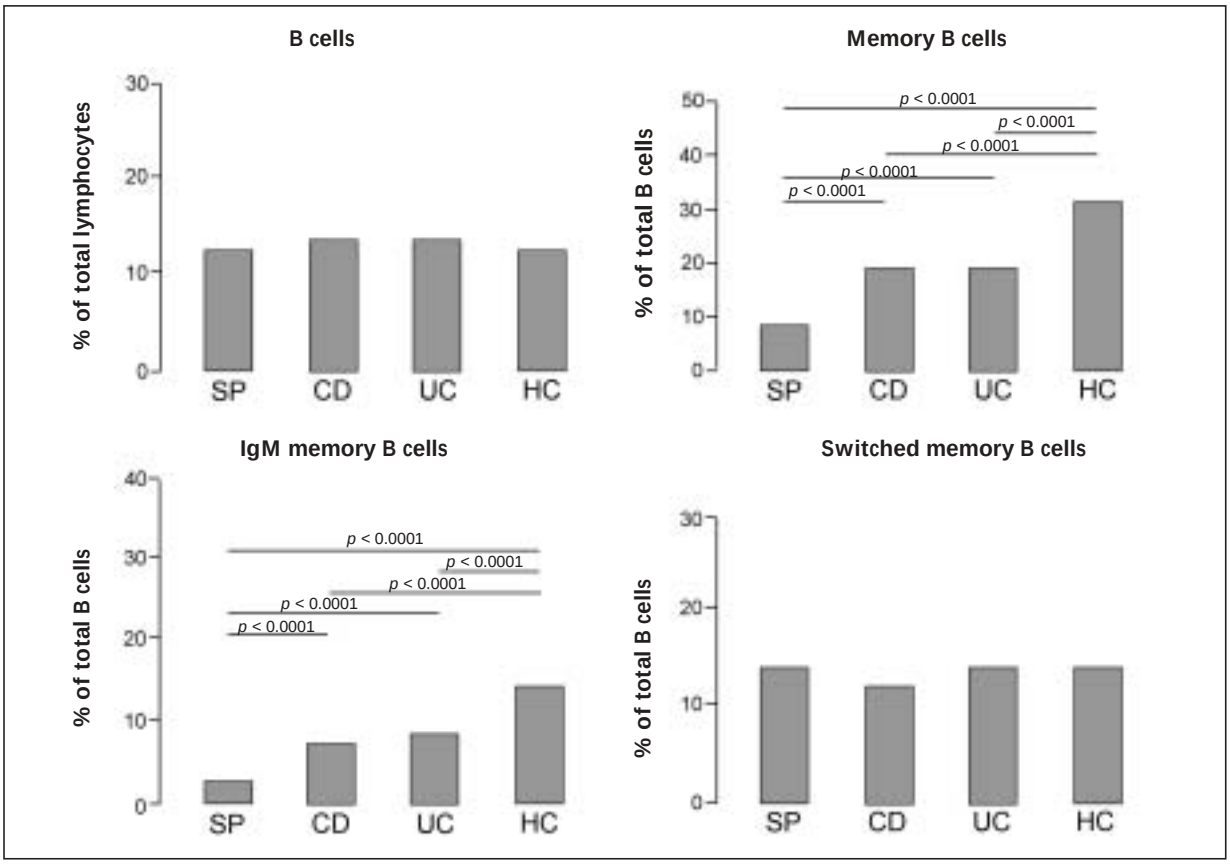

Figure 1. Frequency of B cells, calculated as percentage of total lymphocytes, and frequency of memory, IgM memory and switched memory B cells, calculated as a percentage of total B cells, in 18 splenectomized patients (SP), 22 Crohn's disease patients (CD), 20 ulcerative colitis patients (UC) and 22 healthy controls (HC).

tients and controls. The frequency of circulating memory B cells was significantly lower in Crohn's disease (median 18.0%, range 4.1-31.4) and ulcerative colitis patients (median 18.9%, range 6.1-40.9) than in controls (median 29.8%, range 21.1-44.2), although it remained significantly higher than in splenectomized patients (median 9.5%, range 3.7-18.7). There was no significant difference in the frequency of memory B cells between ulcerative colitis and Crohn's disease patients.

Further analysis demonstrated that only IgM memory B cells were significantly lower in Crohn's disease (median 7.1%, range 1.8-20.7) and ulcerative colitis patients (median 8.1%, range 2.1-18.8) in comparison to controls (median 14.0%, range 6.8-31.1). As expected, there was a highly significant difference in the proportion of IgM memory B cells between splenectomized patients (median 2.4%, range 0.9-6.9) and healthy controls. In the Crohn's disease group, the four patients who had abscesses showed the lowest frequency of IgM memory B cells. Unlike IgM memory, switched memory B cells did not significantly differ in splenectomized patients (median 13.1%, range 2.2-24.6), Crohn's disease (median 12.3%, range 2.8-30.2), ulcerative colitis (median 13.7%, range 4.6-27.5) and the control group (median 13.4%, range 6.0-24.7). The percentages of circulating memory, IgM memory and switched memory B cells did not correlate with disease activity and duration, either in Crohn's disease or ulcerative colitis.

Discussion

We studied B-cell subsets in the peripheral blood of patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis) and compared the data with those ob-

tained in controls and in splenectomized patients. We found that unlike total B cells, which did not differ in the peripheral blood of all the four groups studied, memory B cells were significantly decreased in Crohn's disease and ulcerative colitis patients in comparison to healthy controls.

The memory B cell population is composed of two subsets, switched memory B cells and IgM memory B cells. We have recently shown that IgM memory B cells are reduced in the blood of splenectomized and asplenic patients and suggested that IgM memory B cells require the spleen for their generation and/or survival, whereas switched memory B cells can develop in other lymphoid tissues[9]. IgM memory B cells produce natural antibodies, mostly of IgM type, including those recognizing polysaccharides of encapsulated bacteria. Natural antibodies, by opsonizing invading pathogens, play a crucial role in the activation of the complement cascade and amplification of early innate defence mechanisms[10]. It has been recently shown that localized infections are more frequent and often develop into invasive diseases in subjects lacking IgM memory B cells, such as splenectomized and asplenic individuals, infants and a group of patients with common variable immunodeficiency[9]. It is noteworthy that infections are one of the most frequent comorbid conditions in inflammatory bowel disease[11]. We did observe a severe depletion of IgM memory B cells in Crohn's disease patients with abscesses, but further studies are necessary to answer the question of whether high risk of infection (abscess development) is promoted by the reduction/depletion of IgM memory B-cell pool in inflammatory bowel disease. This is a very important point on the context of new therapies for Crohn's disease using monoclonal antibodies, such as anti-human tumour necrosis factor-α, infliximab[15]. In mice, blocking tumour necrosis factor-α results in an impaired clearance of *Streptococcus pneumoniae*[16]; in Crohn's disease patients, which are already at high risk for bacterial infection due to low frequency of IgM memory B-cell, infliximab treatment might further enhance the susceptibility to infections by encapsulated bacteria. In agreement with this possibility it has been recently described a case report of severe pneumococcal pneumonia following treatment with infliximab[17].

In conclusion, our findings show that peripheral IgM memory B cells are reduced in inflammatory bowel disease patients. As inflammatory bowel disease may be complicated by hyposplenism[18-20], it would be interesting to verify whether there was a correlation between the size of functional splenic tissue and the frequency of IgM memory B cells in the blood of Crohn's disease and ulcerative colitis patients.

Our results together with the recent demonstration that mice with null mutation of G protein α inhibitory subunit, which are characterized by an impaired development of marginal zone and B-1a B cells[21], develop colonic inflammation with features similar to those of human inflammatory bowel disease, suggest a putative protective role for IgM memory B cells in colitis.

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