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Anti-tumour necrosis factor-α antibodies and B cell homeostasis in human inflammatory bowel diseases

Caterina Defendenti[a,*], Fabiola Atzeni[b], Sergio Malandrin[c], Sandro Ardizzone[d], Piero Luigi Almasio[e], Simone Saibeni[f], Cristina Bezzio[f], Simona Bollani[d], Raffaele Salerno[d], Paolo Declich[g], Zoe Sarno[a], Savino Bruno[h], Rossella Talotta[i], Piercarlo Sarzi-Puttini[i]

[a] Laboratory, ASST Fatebenefratelli Sacco Buzzi, Milan, Italy
[b] IRCCS Galeazzi Orthopedic Institute, Milan, Italy
[c] Microbiology and Virology Unit, ASST Ospedale San Gerardo, Monza, Italy
[d] Division of Gastroenterology, ASST Fatebenefratelli Sacco Buzzi, Milan, Italy
[e] Biomedical Department of Internal and Specialized Medicine (DI.BI.M.I.S.), University of Palermo, Palermo, Italy
[f] Division of Gastroenterology, Guido Salvini Hospital, Rho, Italy
[g] Division of Pathology, ASST della Valtellina e dell'Alto Lario, Sondrio, Italy
[h] Humanitas Institute, Milan, Italy
[i] Rheumatology Unit, ASST Fatebenefratelli Sacco Buzzi, Milan, Italy

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ABSTRACT

Background: The expression of CD70 on T cells is greatly enhanced by antigen-presenting cell (APC)-associated signals, such as tumour necrosis factor(TNF)-α, which is constitutionally high in patients with inflammatory bowel disease (IBD). Experimentally, the chronic activation of CD27 as a result of the constitutive expression of CD70 leads to the demise of B cells in bone marrow (BM) and the secondary lymphoid organs. The aim of this study was to assess the number and phenotype of circulating B cell in untreated IBD patients and their counterparts treated with biological anti-TNF drugs.

Methods: The study involved 13 untreated IBD patients, 36 IBD patients treated with biological drugs, and 10 healthy controls. The B cell phenotypes were assessed by means of flow cytometry using monoclonal antibodies specific for CD20, CD19, CD3, CD27 and CD43. In order to evaluate B cell development in bone marrow and peripheral B cell activation, we identified four B cell subsets: hematogones (HBs: $CD20^+19^+3^-27^-43^+$), memory B cells (MBs: $CD20^+19^+3^-27^+43^-$), pre-plasmablasts (PPBs: $CD20^+19^+3^-27^+43^+$), and plasmablasts (PBs: $CD20^-19^+3^-27^+43^+$).

Results: The total number of B cells in the untreated patients was three times lower than that in the patients treated with biological drug ($p < 0.001$), and half that in the healthy controls ($p = 0.03$). The between-group differences (including the healthy donors) were statistically significant in the case of HBs and MBs, but not in the case of PPBs and PBs. Only one treated patient showed a transiently large increase in PPBs. There were statistically significant differences in all of the parameters between the untreated patients and those receiving biological therapy, and in some cases between the untreated patients and healthy controls, but never between the controls and the treated patients. Four non-responders to anti-TNF therapy had a smaller number of total circulating B cells than the untreated patients.

Conclusions: Anti-TNF drugs disinhibit B cell production in IBD patients, but maintain the constant homeostasis of circulating B cells. The presence of individual variations may allow the activity of anti-TNF drugs to be monitored by studying B cell subgroups.

Abbreviations: APC, antigen-presenting cell; BM, bone marrow; GC, germinal centre; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; IFNγ, interferon-gamma; Th, T-helper; IPC, immunoglobulin-producing cell; mAbs, monoclonal antibodies; MBs, memory B cells; HBs, hematogones; PBs, plasmablasts; PPBs, pre-plasmablasts; PBS, phosphate-buffered saline; PCs, plasma cells; PE, phycoerythrin; PerCP-Cy5.5, Peridin-chlorophyll protein; APC-H7, allophycocyanin; FITC, fluorescein isothiocyanate; APE, allophycoerythrin; PTPN2, tyrosine phosphatase non-receptor type 2; SP1, sphingosine-1-phosphate; TNF-α, tumour necrosis factor-alpha; CDAI, Crohn's Disease Activity Index; CAI, Colitis Activity Index

* Corresponding author at: Laboratorio Analisi Chimico Cliniche, ASST Fatebenefratelli Sacco Buzzi, Piazzale Clotilde 3, 20133 Milano, Italy.
E-mail address: caterina.defendenti@asst-fbf-sacco.it (C. Defendenti).

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1. Introduction

Tyrosine phosphatase non-receptor type 2 (*PTPN2*) gene variants are associated with susceptibility to ulcerative colitis (UC) and Crohn's disease (CD), and regulate interferon-gamma(IFNγ)-induced signalling and barrier function in intestinal epithelial cells [1,2]. CD70, the only CD27 ligand, is specifically expressed on differentiated T-helper (Th1) but not Th2 cells [3], and its T cell expression is greatly enhanced by antigen-presenting cell(APC)-associated signals such as tumour necrosis factor-alpha (TNF-α) [4]. Experimentally, the chronic activation of CD27 as a result of the constitutive expression of CD70 leads to the demise of B cells in bone marrow (BM) and the secondary lymphoid organs [5].

CD27 is highly expressed by progenitor lymphoid cells in mouse BM, and stimulation of $CD27^+$ progenitor cells with CD70 inhibits their colony-forming potential *in vitro* and lymphocyte outgrowth *in vivo* [6]. Experimentally, defective germinal centre (GC) B cell differentiation critically depends on CD70-mediated CD27 signalling in T cells, and involves the Fas-dependent impairment of germinal centre B cell differentiation. The T cell FasL induced by CD70 instructs T cells to kill GC B cells actively through a FasL and Fas mechanism that is involved in the apoptosis of GC B cells [7,8].

This could reflect something that occurs in human acute and chronic infections because CD70 is transiently expressed in acute infection, and constitutively expressed in chronic infection or chronic inflammatory conditions such as inflammatory bowel disease (IBD). After removing the BM and GC inhibitory stimulation induced by CD70 (for example, by using anti-TNF antibodies in the case of human IBD), an increase in circulating B cells can be expected, but the chronic stimulation of B cells potentially leads to autoimmunity and lymphoproliferative disorders.

On the basis of these data, we have investigated quantitative variations in total B cell levels in IBD patients receiving biological therapy or not, and the behaviour of the B cell subgroups that show combinations of unusual markers such as CD43 and CD27. CD43 is consistently expressed in hematogones (HBs) in a reproducible pattern that is similar to that of CD10 when combined with CD20. The combinations CD19/CD10, CD19/CD43 and CD43/CD10 closely correlate with each other, which is consistent with the hypothesis of a single population of cells expressing all three markers [9]. Very small numbers of HBs have recently been identified in the peripheral blood of the majority of adult patients, a finding that has implications for the peripheral blood analysis of minimal residual disease in cases of acute lymphoblastic leukemia and follicular lymphoma [10]. In this study, we considered HBs as an index of B cells that have just been released by bone marrow.

CD27 is a marker of memory B (MB) cells [11] and the co-expression of CD43 and CD27 on $CD20^+19^+$ or $CD20^-19^+$ B cells respectively identifies a pre-plasmablast (PPB) and plasmablast (PB) phenotype [12]. In the GC, the centrocyte stage may represent a common precursor of MBs, PPBs and PBs [13]. MBs gradually increase with age, and approximately 40% of adult peripheral blood B cells are $CD27^+$ [14]; in the case of secondary immunisation, memory B cells can be re-stimulated to proliferate and differentiate into PBs and plasma cells (PCs) [15].

$CD20^+19^+3^-27^+43^+$ B cells can also be detected in peripheral blood and may account for between < 1% and > 9% of all circulating B cells regardless of age [16]. Originally described as the human counterpart of murine B-1 cells, their phenotype is closer to $CD20^-$ PBs than to MB cells and they can be differentiated into CD20- PBs and PCs *in vitro*, thus supporting the existence of a PPB phenotype [12]. PPBs secrete small amounts of antibodies, and this stage is followed by the up-regulated expression of a number of gene products required for the generation of PBs [13]. The presence of PBs in the blood is transient because, under physiological conditions, most PBs exit from lymph nodes into lymphatic vessels through a sphingosine-1-phosphate(SP1)-dependent gradient [17] and then survive in the circulation only for a short time unless they find a survival niche in the secondary lymphoid organs, inflamed tissue or BM and further differentiate into long-lived PCs and lose their migratory potential [18].

On the basis of the discoveries made by experimental research and in the absence of data concerning B cell differentiation and proliferation during the course of IBD, the aim of this study was to analyse B cell phenotypes in IBD patients treated with anti TNF-α drugs in order to gain insights into B cell behaviour under these pharmacological conditions.

2. Materials and methods

2.1. Study subjects

This is an observational, not interventional and not in double-blinded study in which blood samples were randomly obtained from 49 IBD patients attending the Gastroenterology Department of Fatebenefratelli Hospital in Milan, Italy: 13 who were untreated IBD patients, 19 treated with subcutaneously administered monoclonal human anti-TNFα antibodies (40 mg every 2 weeks), and 17 patients intravenously treated with chimeric anti-TNFα antibodies (5 mg/kg every 8 weeks), out of which 10 were treated with Remsima and 7 with Remicade. This study is original and the methods were not previously reported or designed by author's own institute. The diagnosis of IBD was confirmed by means of standard endoscopic and histological criteria (the additional hematoxylin and eosin staining of each sample). The patients' clinical and immunological data were obtained from their medical records. Twenty-one (43%) patients had ulcerative colitis (UC) and 28 (57%) had Crohn's disease (CD). The Montreal classification [19] was used for CD, based on the age at diagnosis (A), location (L), and disease behaviour (B). In patients with UC, the disease location was categorized according to the Montreal classification, by distinguishing ulcerative proctitis (1), left-side colitis (E2), and extensive colitis (E3). Patients with indeterminate colitis and pediatric patients were excluded from the study. In all patients, the following clinical features were recorded: family history, age at diagnosis, duration of follow-up, medication for IBD and other medication, presence of perianal fistulae, extraintestinal manifestations (presence or absence of any extraintestinal manifestation), previous abdominal surgery (either colectomy in UC or bowel resection in CD), and smoking and dietary habits (Table 1). A CAI score [20] of > 4 in UC and a CDAI score [21] of > 150 in CD corresponded to active disease. All of the treated patients except for non-responders had been treated for more than six months.

Ten controls were randomly selected from blood donors with a negative history for IBD or immune-mediated diseases.

The study was approved by the hospital's Ethics Committee, and the patients were enrolled after signing an informed consent form in accordance with the Declaration of Helsinki.

2.2. Sample collection and management

The 10 mL venous blood samples for cytokine analysis were collected in pyrogen-free tubes between 8.00 am and 10.00 am.

2.3. Flow cytometry

A five-parameter flow cytometric analysis of fresh whole blood cells was made using a three-laser FACSCanto II instrument equipped with FACSDiva software (BD Biosciences, Erembodegem, Belgium). One hundred microlitres of whole blood were directly stained with 1 $\mu g/10^6$ of different combinations of the following monoclonal antibodies (mAbs): anti-CD20 conjugated with Peridin-chlorophyll protein, PerCP-Cy5.5 (BD Biosciences); anti-CD19 conjugated with allophycoerythrin (APE) (BD Biosciences); anti-CD3 conjugated with allophycocyanin APC-H7 (BD Biosciences); anti-CD27 conjugated with fluorescein isothiocyanate (FITC) (BD Biosciences); anti-CD43 conjugated with phycoerythrin (PE) (BD Biosciences); or isotype-matched mAbs of