

Letter to the Editor

Impairment of splenic IgM-memory but not switched-memory B cells in a patient with celiac disease and splenic atrophy

To the Editor:

Celiac disease (CD) is a common lifelong autoimmune disorder affecting 0.3% to 1% of the Western world. Splenic hypofunction complicates the CD of between 32% and 75% of adult patients, and mostly when associated with spleen atrophy represents a marker of poor prognosis.¹⁻³ Splenic hypofunction and atrophy predispose to serious complications such as overwhelming septicemia caused by encapsulated bacteria.⁴

We describe here the case of a young woman affected by CD complicated with splenic atrophy and analyze the spleen architecture and function in the light of the role of the spleen in maintaining the IgM-memory B-cell pool. The IgM-memory B-cell pool produces IgM natural antibodies that are essential for the protection from encapsulated bacteria.^{5,6}

A 26-year-old woman was admitted to the hospital with a 2-month history of diarrhea and marked weight loss. The patient, suspected to have CD, was tested for serum antiendomysial and antitissue transglutaminase antibodies, and both tests were positive. HLA class II typing showed DQ2 positivity. The patient underwent gastroduodenoscopy, and histologic examination of duodenal biopsies showed villous atrophy (Marsh IIIc lesion). A gluten-free diet was then introduced, and the patient clinically responded with prompt benefit.

Twelve months later, the patient was readmitted on account of colicky abdominal pain. Routine laboratory tests were normal with the exception of thrombocytosis (platelet count $636 \times 10^9/L$, normal range 140-450) and low serum albumin (2.8 g/dL, normal range 3.5-5.0). Histologic examination of duodenal biopsies again showed villous atrophy (Marsh IIIa lesion) and marked epithelial infiltration by small lymphocytes lacking CD8 and CD4 and containing intracytoplasmic but not surface CD3 ϵ chains, indicative of refractory sprue. Abdominal bowel sonography (Fig 1, A) and computed tomography scanning (Fig 1, B) revealed multiple cysts arising from the mesenteric root and a small spleen. A pitted red cell value of 16.4% (normal $\leq 4\%$) was indicative of functional hyposplenism.²

A few days later, the patient developed severe abdominal pain with signs of acute intestinal obstruction and was treated conservatively for 12 hours. An increase in the severity of the symptoms warranted an urgent exploratory laparotomy. Multiple cysts arising from the small bowel mesentery were carefully dissected free and resected. At histology, cysts revealed a fibrous wall of uniform thickness with areas of ulceration and hemorrhage. The absence of residual foci of lymphoid tissue excluded the diagnosis of mesenteric lymph node cavitation, a rare condition associated with splenic atrophy in CD.⁴

At gross examination, the spleen appeared markedly atrophic (approximate diameters, $5 \times 3 \times 1$ cm). From a macrobiopsy of the spleen, paraffin sections and isolated splenocytes were obtained. Histologic spleen examination (Fig 1, C) and immunohistochemical detection of CD20 (Fig 1, D) and CD45RO (Fig 1, E) revealed severe atrophy of the white pulp B-cell but not T-cell compartment, reduction in the number and size of B-cell nodules, marginal zone atrophy, massive parenchymal (white and red pulp) and capsular fibrosis, as well as hyaline degeneration and thickening of the arteriolar walls, both of which are reported in an autopsic adult patient with CD.⁷ Fig 1, F-H, shows normal spleen architecture with well defined B-cell nodules and periarteriolar T zones, and scattered small germinal centers.

Mature and memory B cells (me) were identified by flow cytometry.^{3,5} On the basis of the relative expression of IgM, memory B cells were subdivided into IgM-memory (IgM^{bright}, IgM me) and switched-memory (IgM^{neg}, sw me; Fig 2, A-D). Spleen cells gated on CD21^{bright}IgM^{bright} identify marginal zone B cells (Fig 2, E and F). IgM-memory B cells were also identified as IgM^{bright}IgD^{dull} in the gate of CD19^{pos}CD27^{pos} cells, whereas switched-memory B cells were IgM^{neg}IgD^{neg} (Fig 2, G and H). Peripheral memory B cells were lower in CD (9.1%) than in the control (41.2%). The reduction was more evident in IgM-memory (3.4% vs 22.2%, respectively) than switched-memory B cells (5.7% vs 19.0%). Splenic memory B cells were lower in CD (25.2%) than in the control (54.6%). IgM-memory B cells showed a bigger reduction (8.4% vs 30.3%, respectively) than switched-memory B cells (17.6% vs 24.3%). Further, the percentage of CD19^{pos}CD21^{bright} marginal zone B cells was decreased in CD (31.4%) in comparison with control (67.1%).

To investigate whether celiac splenic memory B cells were functional, we performed *in vitro* stimulation with cytosine-phosphate-guanosine oligodeoxynucleotides (CpG-ODN), a polyclonal activator that has a strong stimulatory effect on B cells, and measured cell division and antibody production. Spleen cells were labeled with 0.1 $\mu\text{g/mL}$ 5-chloromethylfluorescein diacetate (Molecular Probes, Eugene, Ore) and cultured for 7 days with 2.5 $\mu\text{g/mL}$ CpG-ODN (Hycult Biotechnology, Uden, The Netherlands). Cell proliferation was assessed on day 5 by measuring through flow cytometry CMFDA intensity in gated CD19^{pos} cells cultured in CpG-ODN presence (*black line*) or absence (*dashed line*). Secreted Igs were detected in the supernatants at day 7 by ELISA.⁶ CpG-ODN induced B-cell proliferation in both celiac and control splenocytes (Fig 2, I). Normal spleen B cells secreted IgM, IgA, and IgG, whereas celiac B cells were unable to produce IgA and showed a marked impairment in secreting IgM but were still able to produce IgG (Fig 2, J).

The patient had an uncomplicated recovery from surgery and received pneumococcal-conjugate vaccination (Prevnam; Wyeth Lederle Vaccines, Louvain-la-Neuve, Belgium). She was maintained on a strict gluten-free diet

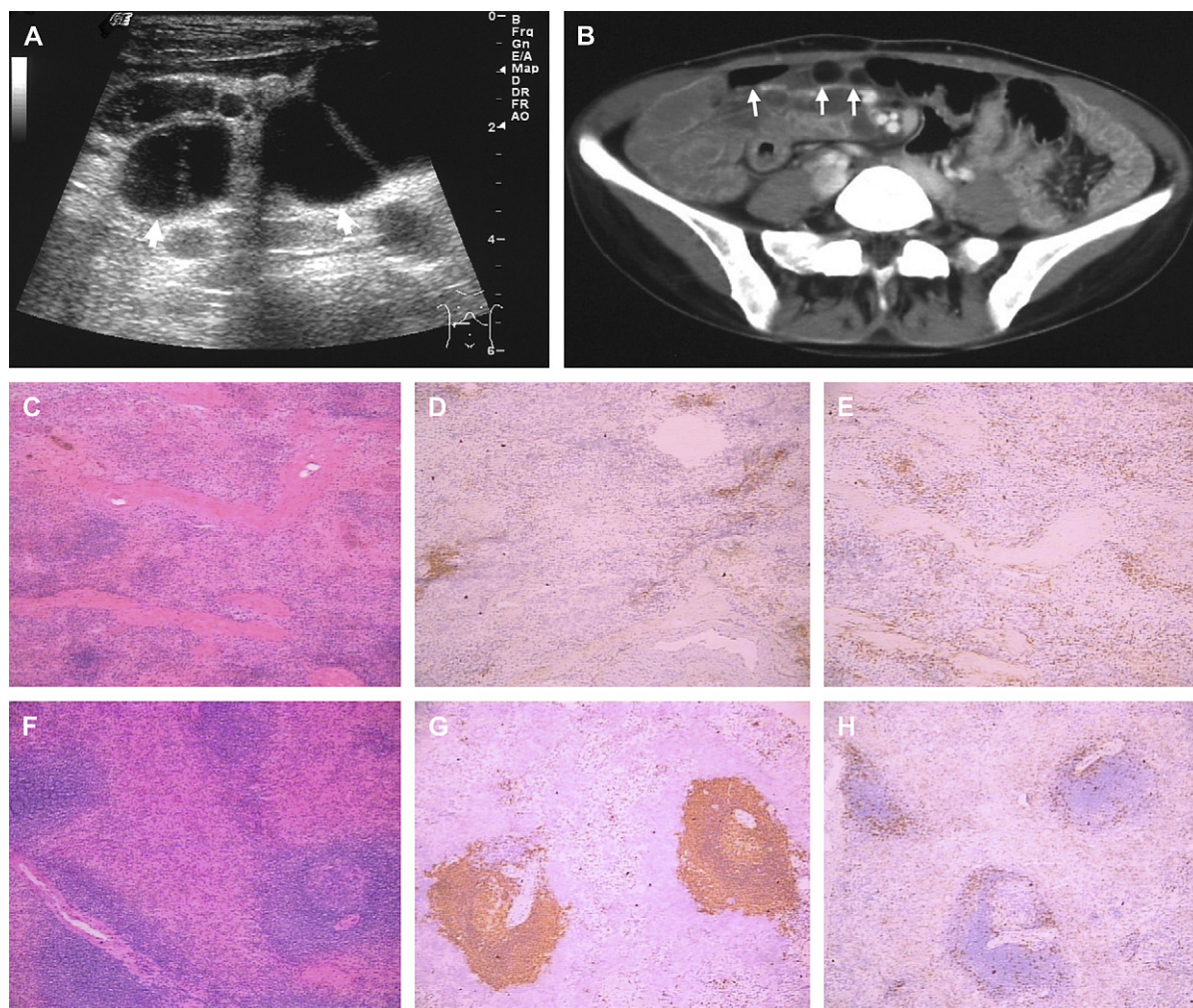


FIG 1. Mesenteric cysts evident at abdominal bowel sonography (**A**, arrows) and computed tomography scan (**B**, arrows) in the patient with CD. Spleen section analysis in the patient with CD (**C-E**) and control subject (**F-H**) by hematoxylin/eosin stain (**C** and **F**) and immunohistochemistry for CD20 (**D** and **G**) and CD45RO (**E** and **H**).

and was in a fairly good clinical condition at the time of the study.

More than 20 years ago, we described at autopsy the histologic changes occurring in the spleen of a patient with CD complicated by splenic atrophy.⁷ However, this is the first time that the phenotype and *in vitro* function of B cells from a celiac atrophic spleen are investigated. Of note, we found in the celiac spleen a considerable depletion of memory and marginal zone B cells. Memory B cells can be subdivided into IgM-memory and switched-memory. The latter, which are only transiently depleted after splenectomy, are present at normal frequency in asplenic patients and are derived from the germinal centers. IgM-memory B cells are reduced/undetectable in splenectomized patients, implying that they require the spleen for their survival and/or generation. Localized infections are more frequent and often develop into invasive diseases in subjects lacking IgM-memory B cells.^{5,6} The frequency of circulating IgM-memory B cells correlates with the size of functional splenic tissue in CD and is often reduced in

hyposplenic celiacs complicated by enteropathy-associated T-cell lymphoma, refractory sprue, and ulcerative jejunoileitis.³

We then explored the ability of splenic B cells to respond to polyclonal activation by testing their proliferation and antibody production in response to CpG-ODN. We found that celiac B cells responded to CpG-ODN in terms of proliferation but did not differentiate into antibody-secreting cells. In contrast, IgG switched-memory B cells were able to differentiate into plasma cells and produce IgG. Therefore, switched-memory B cells found in the celiac spleen are functional. It remains to be determined whether switched-memory B cells were generated in the atrophic spleen or arise in the lymph nodes and accumulate in the white pulp of the atrophic spleen.

In conclusion, splenic atrophy complicating CD affects the size of both the marginal zone and white pulp B-cell compartment and is accompanied by fibrosis. Fibrosis contributes to the disruption of the normal architecture and

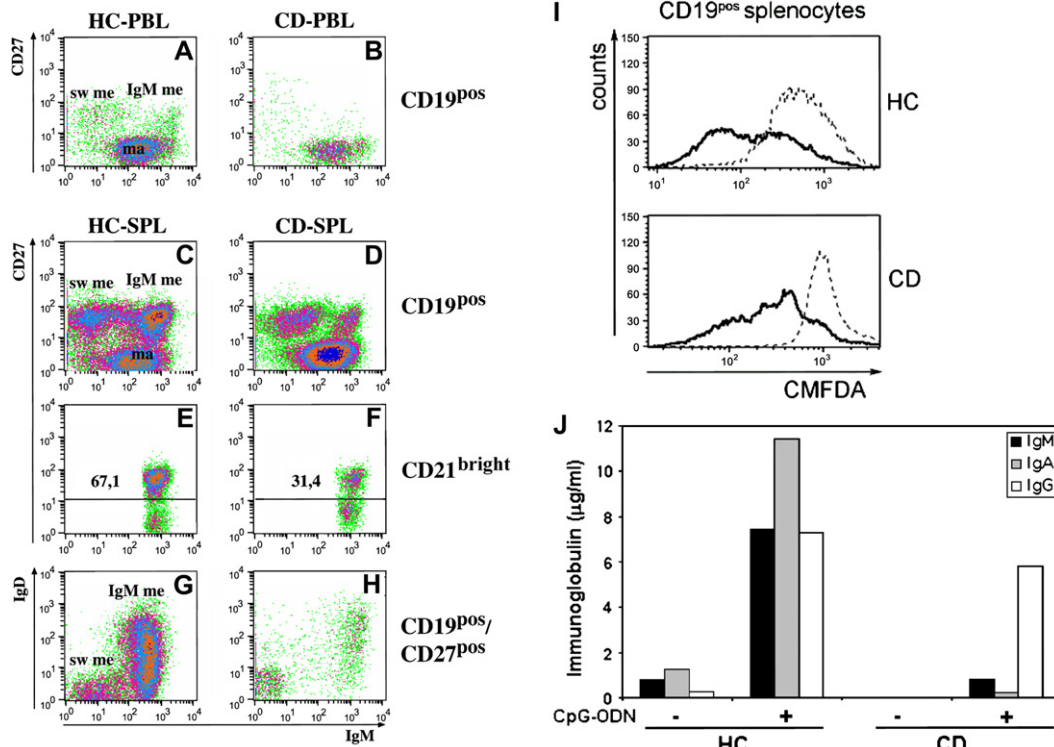


FIG 2. Phenotypal characterization (A-H) of peripheral (PBL) and spleen (SPL) memory B cells in the patient with CD (CD) and control (HC). B-cell proliferation (I) and Ig production (J) by CpG-ODN-stimulated splenocytes.

leads to functional impairment of the spleen. IgM-memory B-cell depletion and defective IgM and IgA antibody production by CpG-ODN-stimulated B cells might explain the higher susceptibility of hyposplenic celiacs to infections by encapsulated bacteria. Because IgG serologic memory is not impaired, hyposplenic celiacs should receive polysaccharide protein-conjugate vaccine instead of traditional 23-valent pneumococcal polysaccharide vaccine, as it has been hitherto proposed.⁸ In the former, polysaccharides are coupled to a protein carrier and the response becomes T-cell-dependent, thus bypassing the immunologic impairment caused by the lack of IgM-memory B cells.^{9,10}

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