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Efficient long-term depletion of CD20⁺ B cells by rituximab does not affect gut-resident plasma cells

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Abstract

The vast majority of antibody-producing B cells are located within the gastrointestinal tract and are key players in maintaining homeostasis. The failure of rituximab, a potent B cell-depleting agent, to ameliorate ulcerative colitis in a single clinical trial has dampened enthusiasm to study B cells in patients with inflammatory bowel disease (IBD). However, several lines of evidence suggest that intestinal B cells may be affected in IBD. Additionally, the pathophysiological mechanisms underlying rituximab's lack of efficacy in IBD remain unclear. Here, on the basis of detailed immunophenotyping of a patient who underwent a colonoscopy 6 months after the end of rituximab-based therapy, we observed that rituximab did not deplete colon-resident plasma cells (PCs) while ablating all CD20⁺ B cells in tissues and in the circulation. On the basis of these observations, we propose that one factor underlying the lack of efficacy of rituximab relates to the fact that it does not affect the entire B cell compartment in tissues, sparing the intestinal-resident PCs while effectively depleting CD20⁺ B cell populations. Thus, we contend that, despite the results of the Rituximab study, there is a need for more intensive B cell-oriented research in inflammatory disorders, including IBD.

Keywords

intestines; B cells; plasma cells; rituximab; inflammation

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Supporting Information

Additional supporting information may be found in the online version of this article.

Competing interests

The authors declare no competing interests.

Background

Since a lymphoplasmacytic infiltrate is the hallmark of active inflammatory bowel disease (IBD), particularly in patients with ulcerative colitis (UC), a clinical trial compared the efficacy of rituximab to treat UC versus placebo and reported negative results.¹ The failure of rituximab, one of the most potent anti-B cell-directed therapies, has resulted in a general lack of interest in exploring the B cell population in patients with IBD. However, multiple lines of evidence implicate B cells in IBD² and support further investigation of the humoral immune system in IBD despite the failure of rituximab therapy.

Case presentation

We present the case of a 25-year-old female who was referred for a colonoscopy in our tertiary care center. Her past medical history was marked by familial dilated cardiomyopathy culminating in a cardiac transplant in 1995 (at the age of 4). A second cardiac transplantation was needed in 2003 for chronic graft failure. In 2001, she was diagnosed with a large B cell lymphoma/postransplant lymphoproliferative disease (PTLD) presenting as an abdominal mass along with general symptoms (fever and sweating). She underwent a surgical resection of the mass that also included a small bowel segment and was treated successfully with chemotherapy based on rituximab and cyclophosphamide. Five years later, she presented with pulmonary nodules, an increased serum Epstein–Barr virus (EBV) viral load and general symptoms leading to the diagnosis of a relapse of the PTLT, confirmed by pathology assessment. Once again, a rituximab-based regimen led to complete remission. In January 2016, 10 years after this treatment, a second relapse was diagnosed based on the occurrence of new masses in the neck, chest, and abdomen along with night sweats and fever. Pathological examination of the abdominal mass confirmed the diagnosis of EBV-related monomorphic PTLT. She then received six cycles of R-EPOCH (rituximab–etoposide–prednisone–Oncovin–cyclophosphamide–hydroxydaunorubicin) from January to June 2016, with a good response according to a positron emission tomography/computed tomography evaluation.

At the time of the colonoscopy (December 2016), she presented with chronic epigastric pain for several months. Her immunosuppressive regimen comprised Prograf (tacrolimus) monotherapy with a recent trough level at 6.4 ng/mL. Gallstones and hepatic vascular thrombosis were ruled out initially. Subsequent work-up of this atypical abdominal pain included a colonoscopic examination. The colonic mucosa appeared normal on gross and pathologic examinations (Fig. 1A). Specifically, there was no evidence of a monoclonal neoplastic process. The abdominal pain was deemed to be of hepatic origin owing to hepatomegaly and congestive hepatopathy. Part of the patient's evaluation included collection of blood and colonic biopsies for detailed immunophenotypic analyses. Peripheral blood mononuclear cells (PBMCs) as well as single-cell suspension of lamina propria cells examined using multiparameter flow cytometry revealed unremarkable T cell phenotypes in the patient. However, there was a near absence of CD20⁺ B cells in the peripheral blood and in the colon (Fig. 2). Remarkably, a significant population of CD19⁺ B cells were found in the colon that were almost exclusively CD38^{high}CD27⁺ (Fig. 2) and indicated the presence of gut-resident plasma cells (PCs). Of note, most of these cells were IgA⁺ (Fig. S1, online

only) and had morphological characteristics of PC (Fig. S2, online only). Since Landsverk *et al.* recently described that the subsets of intestinal PCs that do not express CD19 have a longer life span and a smaller rate of dynamic exchange, we further investigated whether the remaining PCs of the patient were mostly CD19⁻ PCs. We thus compared the CD19⁻/CD19⁺ PC ratio with five healthy controls and, surprisingly, found that this ratio was lower in our patient, while the frequency of colon PCs among live cells was similar (Fig. 3).

Additionally, a very rare subset of circulating CD19⁺CD38^{high}CD27⁺ cells representing less than 0.01% of live PBMCs was also seen in the patient (Fig. 2B). The vast majority of these cells were also IgA⁺.

Within the colonic tissue, immunohistochemistry confirmed the absence of CD20⁺ cells, whereas MUM1⁺ PCs were numerous in the colonic lamina propria (Fig. 1B–D; positive control for CD20 staining is shown in Fig. S4, online only).

Discussion

Rituximab is an anti-CD20 monoclonal antibody. It has been used with success in many autoimmune diseases, mainly those with an autoantibody-mediated mechanism, as well as in B cell malignancies.^{3,4} Our observation underlines the persistence of a substantial number of intestinal PCs after intensive rituximab therapy. One might have thought that intestinal PC precursors (which are mainly CD20⁺ cells) would be depleted by this therapy, limiting the number of intestinal PCs over time. However, we observed that, 6 months after the end of a rituximab-based therapy, while all CD20⁺ B cells were still absent from the circulation and from the intestinal tissue, a substantial number of PCs were located in the colonic lamina propria. Furthermore, one could have expected that the shorter lived CD19⁺ PC subset would have been more significantly affected by the rituximab therapy, as these cells need CD20⁺ precursors for their quicker renewal. Surprisingly, that was not the case, and the CD19⁻/CD19⁺ PC ratio in our patient was even lower compared with healthy controls, suggesting that, somehow, despite the effective depletion of almost all CD20⁺ B cells, there is still an ongoing repletion of the relatively short-lived CD19⁺ intestinal PCs. Although speculative, this could be supported by the presence of a small fraction of circulating CD20⁻CD19⁺ plasmablasts, overall evoking the presence of rituximab-resistant PC precursors.

IBD is thought to result from dysregulation of the cross talk between the host and the microbiota.⁵ Mucosal antibodies, mainly produced by intestinal-resident PCs, are one of the key players in immune–microbial cross talk. A number of lines of evidence show dysfunction in the mucosal antibodies in patients with IBD. For example, in an elegant series of experiments, Macpherson *et al.* demonstrated that the mucosal immunoglobulin repertoire was quantitatively and qualitatively altered in IBD. While there was an increase in the level of mucosal IgG in the context of active IBD, qualitatively, mucosal IgG from IBD patients binds a higher range of nonpathogenic fecal bacteria compared with mucosal IgG from healthy controls, suggesting a humoral response to commensal microflora in IBD.⁶ More recently, using gnotobiotic mice, Palm *et al.* described that the type of IgA coating could define a subset of colitogenic bacteria in IBD patients.⁷ Together, these data suggest that the immunoglobulin response at the mucosal level is altered in IBD. Therefore, the source of

mucosal immunoglobulins(i.e., the intestinal-resident PCs) was pursued as a therapeutic strategy. More direct evidence of PC involvement in the inflammatory loop in the intestine of IBD patients exists. Notably, Uo *et al.* showed that mucosal CXCR4⁺ IgG PCs promote intestinal inflammation via the activation of proinflammatory macrophages in a fragment crystallizable region (Fc)-dependent manner.⁸ Additionally, Cupi *et al.* described intestinal granzyme B–expressing intestinal PCs with cytotoxic activity in patients with IBD, which were resistant to rituximab *in vitro*.⁹

Rituximab, a monoclonal antibody directed against CD20, depletes B cells. A clinical trial evaluating the efficacy of two doses of rituximab given 2 weeks apart was conducted in patients with UC. Strikingly, the rate of remission at week 4 was comparable to placebo.¹ Furthermore, anecdotal evidence suggested that patients might have had disease flares while on treatment with rituximab.^{10–12} Although the mechanisms underlying lack of efficacy of rituximab were not well explored, the abject failure of this clinical trial has resulted in diminished interest in B cells in IBD. Notably, no drugs are currently being developed to directly target the B cell system in IBD.

Here, we highlight the fact that rituximab has little effect on the B cell effectors (i.e., the PCs in gastrointestinal tissue). Our findings are also supported by previous evidence demonstrating that rituximab may have a limited impact on tissue-resident PCs.¹³ However, it was assumed that it affected the generation of new plasmablasts, short-lived PCs, or other precursors, which could explain the efficacy of this therapy in antibody-mediated diseases.¹⁴ Not only does rituximab not affect the survival of tissue-resident PCs, it may even facilitate the emergence of autoreactive clones. For example, in the context of immune thrombocytopenia, rituximab therapy has been thought to promote the emergence, maturation, and longevity of splenic autoreactive, long-lived PCs by providing a suitable environment.¹⁵ In yet another example, Mei *et al.* demonstrated the presence of circulatory plasmablasts with a mucosal phenotype (IgA⁺ integrin β_7^+) in rheumatoid arthritis patients during rituximab therapy despite ablation of CD20⁺ B cells.¹³ Of note, beyond the failure to deplete intestinal PCs, the lack of rituximab efficacy could also be explained by the depletion of the regulatory B cells that express CD20.

In conclusion, our observation shows that, despite efficient depletion of almost all CD20⁺ B cells from the circulation and intestinal tissue, high numbers of intestinal PCs, both CD19[–] and CD19⁺, persist even 6 months after rituximab therapy. They may be newly produced from rituximab-resistant precursors or may be long-lived residents.¹⁶ Persistence of these B cell effectors is likely one reason underlying the failure of rituximab therapy in IBD. Using these data, we would like to reignite interest in not only the mechanisms of persistence of long-lived intestinal PCs, but also in the overall role of the B cell compartment in IBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Leiper K, Martin K, Ellis A, et al. 2011 Randomised placebo-controlled trial of rituximab (anti-CD20) in active ulcerative colitis. *Gut* 60: 1520–1526. [PubMed: 21471566]
2. Brandtzaeg P, Carlsen HS & Halstensen TS. 2006 The B-cell system in inflammatory bowel disease. *Adv. Exp. Med. Biol* 579: 149–167. [PubMed: 16620017]
3. Plosker GL & Figgitt DP. 2003 Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. *Drugs* 63: 803–843. [PubMed: 12662126]
4. Gürcan HM, Keskin DB, Stern JNH, et al. 2009 A review of the current use of rituximab in autoimmune diseases. *Int. Immunopharmacol* 9: 10–25. [PubMed: 19000786]
5. Ungaro R, Mehandru S, Allen PB, et al. 2017 Ulcerative colitis. *Lancet* 389: 1756–1770. [PubMed: 27914657]
6. Macpherson A, Khoo UY, Forgacs I, et al. 1996 Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. *Gut* 38: 365–375. [PubMed: 8675088]
7. Palm NW, de Zoete MR, Cullen TW, et al. 2014 Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 158: 1000–1010. [PubMed: 25171403]
8. Uo M, Hisamatsu T, Miyoshi J, et al. 2013 Mucosal CXCR4⁺ IgG plasma cells contribute to the pathogenesis of human ulcerative colitis through FcγR-mediated CD14 macrophage activation. *Gut* 62: 1734–1744. [PubMed: 23013725]
9. Cupi ML, Sarra M, Marafini I, et al. 2014 Plasma cells in the mucosa of patients with inflammatory bowel disease produce granzyme B and possess cytotoxic activities. *J. Immunol* 192: 6083–6091. [PubMed: 24835396]
10. Bhalme M, Hayes S, Norton A, et al. 2013 Rituximab-associated colitis. *Inflamm. Bowel Dis* 19: E41–E43. [PubMed: 22488947]
11. Varma P, Falconer J, Aga A, et al. 2017 Rituximab-induced Crohn's disease. *Scand. J. Gastroenterol* 52: 606–608. [PubMed: 28129697]
12. Lipka S, Katz S & Crawford JM. 2016 Fulminant colitis following rituximab therapy. *Gastroenterol. Hepatol* 12: 58–60.
13. Mei HE, Frolich D, Giesecke C, et al. 2010 Steady-state generation of mucosal IgA⁺ plasmablasts is not abrogated by B-cell depletion therapy with rituximab. *Blood* 116: 5181–5190. [PubMed: 20829370]
14. Mei HE, Schmidt S & Dörner T. 2012 Rationale of anti-CD19 immunotherapy: an option to target autore-active plasma cells in autoimmunity. *Arthritis Res. Ther* 14: S1.
15. Mahévas M, Patin P, Huetz F, et al. 2013 B cell depletion in immune thrombocytopenia reveals splenic long-lived plasma cells. *J. Clin. Invest* 123: 432–442. [PubMed: 23241960]
16. Landsverk OJB, Snir O, Casado RB, et al. 2017 Antibody-secreting plasma cells persist for decades in human intestine. *J. Exp. Med* 214: 309–317. [PubMed: 28104812]

Figure 1.

Characterization of colonic biopsies by histology and immunohistochemistry. (A) Hematoxylin and eosin staining demonstrates normal-appearing colonic mucosa (100 $\times$). Additional stains included (B) CD20, (C) MUM1, and (D) CD3. Immunostaining revealed an absence of CD20⁺ B cells despite the presence of plasma cells (MUM1⁺ cells) and T cells in the colon.

Figure 2.

CD19⁺ and CD20⁺ cell frequencies in the blood and in the lamina propria. Peripheral blood mononuclear cells (PBMCs) were obtained from the blood of the patient using a density gradient (Lymphocyte Separation Medium™, MP Biomedicals), and a single-cell suspension of lamina propria cells was obtained after a process including an enzymatic digestion of colonic biopsies with collagenase (Sigma–Aldrich). The cells were then stained with anti-CD19–PE.Cy7, anti-CD38–APC, anti-CD27–PerCp.Cy5.5, anti-IgD–Pacific blue, and anti-IgA–FITC antibodies (Biolegend and Miltenyi Biotec) and further analyzed by flow cytometry using the Fortessa-LSRII (BD Biosciences). The data generated were analyzed with FlowJo®. (A) The analysis of the PBMCs revealed an almost total absence of CD19⁺ as well as CD20⁺ circulating B cells, whereas the lamina propria cell analysis showed the presence of numerous CD19⁺ cells. (B) Further phenotyping of these CD19⁺ colonic cells identified them as plasma cells (i.e., CD38^{high} CD27⁺, upper panel). Similar analysis of the CD19⁺ circulating cells revealed that 80% of them were also CD38^{high}CD27⁺ (lower panel).

Figure 3.

Characterization of CD19⁻ and CD19⁺ colonic plasma cell subsets. Colonic plasma cells in the index patient and in five healthy controls were defined based on their CD19 expression. (A) Representative flow plots are shown for a healthy control (upper panel) and the patient (lower panel). (B) Plots show a similar frequency of colonic PCs in the patient compared with controls (upper panel), whereas the CD19⁻/CD19⁺ PC ratio appears lower in the patient (lower panel).