Journal of Crohn's and Colitis, 2018, 499–502
doi:10.1093/ecco-jcc/jjx170
Advance Access publication December 14, 2017
Short report

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Induction of Complete Remission by Azacitidine in a Patient with Myelodysplastic Syndrome-Associated Inflammatory Bowel Disease

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Abstract

Myelodysplastic syndrome [MDS] is a clonal disorder of bone marrow [BM] cells, caused by acquired chromosomal abnormalities and gene mutations. Pro-inflammatory antigen-presenting cells [APCs] originating from BM cells bearing chromosomal abnormalities and gene mutations can cause immune-mediated disorders including inflammatory bowel disease [IBD]. Here, we report the first case with MDS-associated IBD that was successfully treated with the DNA methyltransferase inhibitor, azacitidine [AZA]. A 75-year-old man with a 5-year history of MDS was admitted for examination of diarrhoea and high fever. Blood examination revealed pancytopenia and a marked elevation of C-reactive protein. Colonoscopy revealed multiple round ulcers from the terminal ileum to the sigmoid colon. Pathological examination of the endoscopic biopsy specimens showed destruction of crypt architecture and infiltration of $CD3^+$ T cells and $CD68^+$ macrophages. Surprisingly, administration of AZA, which has been approved for the treatment of high-risk MDS, improved the symptoms, and the multiple round ulcers disappeared. AZA treatment markedly decreased the expressions of tumour necrosis factor-α, interleukin-12 (IL-12)/23p40 and IL-17 in colonic biopsy samples, as assessed by quantitative reverse transcription polymerase chain reaction. In contrast, AZA treatment did not change the expression of forkhead box P3, a master regulator of regulatory T cells. These data suggest that AZA treatment led to complete remission in MDS-associated IBD through suppression of pro-inflammatory cytokine responses.

Key Words: Azacitidine, inflammatory bowel disease, myelodysplastic syndrome

1. Introduction

Excessive immune responses towards intestinal microflora underlie the immunopathogenesis of inflammatory bowel diseases [IBD] such as Crohn's disease [CD] and ulcerative colitis [UC].[1] Pro-inflammatory cytokines produced by T cells and bone marrow [BM]-derived antigen-presenting cells [APCs] such as macrophages and dendritic cells play pivotal roles in the development of IBD.[1,2] Thus, BM-derived myeloid APCs producing pro-inflammatory cytokines are colitogenic populations that cause persistent intestinal inflammation. This idea has been fully supported by clinical trials in which the blockade of

tumour necrosis factor α (TNF-α) and interleukin-12 (IL-12)/23 p40 expressed in APCs results in remarkable success in patients with IBD.[3] Moreover, resetting of BM-derived immunological microenvironments by autologous haematopoietic stem cell transplantation [HSCT] has been implicated as an alternative treatment option for patients with severe and treatment-resistant CD.[4,5] Thus, it is clear that BM-derived APCs with the ability to produce pro-inflammatory cytokines contribute to the development of IBD.

Myelodysplastic syndrome [MDS] is a clonal disorder of haematopoietic cells characterized by impaired haematopoiesis, peripheral blood cytopaenia and a pre-condition of acute myeloid leukaemia.[6] MDS is caused by acquired chromosomal abnormalities and gene mutations that have significant influence on the sequence and function of oncogenes and tumour suppressor genes.[6,7] These chromosomal abnormalities and gene mutations often alter the function and properties of BM-derived APCs, and pro-inflammatory APCs originating from abnormal BM environments in MDS can cause immune-mediated disorders including IBD.[8] In fact, several cases with concurrent IBD and MDS have been reported,[9,10] and trisomy 8, a frequent chromosomal abnormality in patients with MDS, has been identified as a risk factor for intestinal Behcet's disease [BD].[11,12] Although some cases of MDS-associated IBD have been successfully treated with HSCT,[13,14] no effective treatment for this condition has yet been established. Azacitidine [AZA], a DNA methyltransferase inhibitor, has been shown to be safe and effective for the treatment of patients with MDS.[15] However, the therapeutic efficacy of AZA on MDS-associated IBD has not been reported. Here, we report a case of a patient with MDS-associated IBD who was successfully treated with AZA.

2. Case Report

A 75-year-old Japanese man with no past history of gastrointestinal diseases was admitted to our hospital for evaluation of diarrhoea and high fever that had persisted for 3 months. He had been diagnosed with MDS [refractory anaemia with excess blast type 2, RAEB 2] at the age of 70 years. No oral or genital ulceration, uveitis, or skin lesions were observed. Blood tests showed pancytopaenia [leukocyte count, 1.55 × 10^3/μl; red blood cell count, 2.59 × 10^6/μl; haemoglobin, 7.5 g/dl; haematocrit, 22.9%; platelet count, 2.3 × 10^4/μl] and a marked elevation of serum C-reactive protein [CRP, 12.3 mg/dl]. Both the human leukocyte antigen B51 and anti-neutrophil cytoplasmic antibody tests showed negative results.

Colonoscopy performed to investigate the cause of diarrhoea revealed multiple round ulcers in the terminal ileum and the entire colon [Figure 1A]. Colonoscopic examination showed no

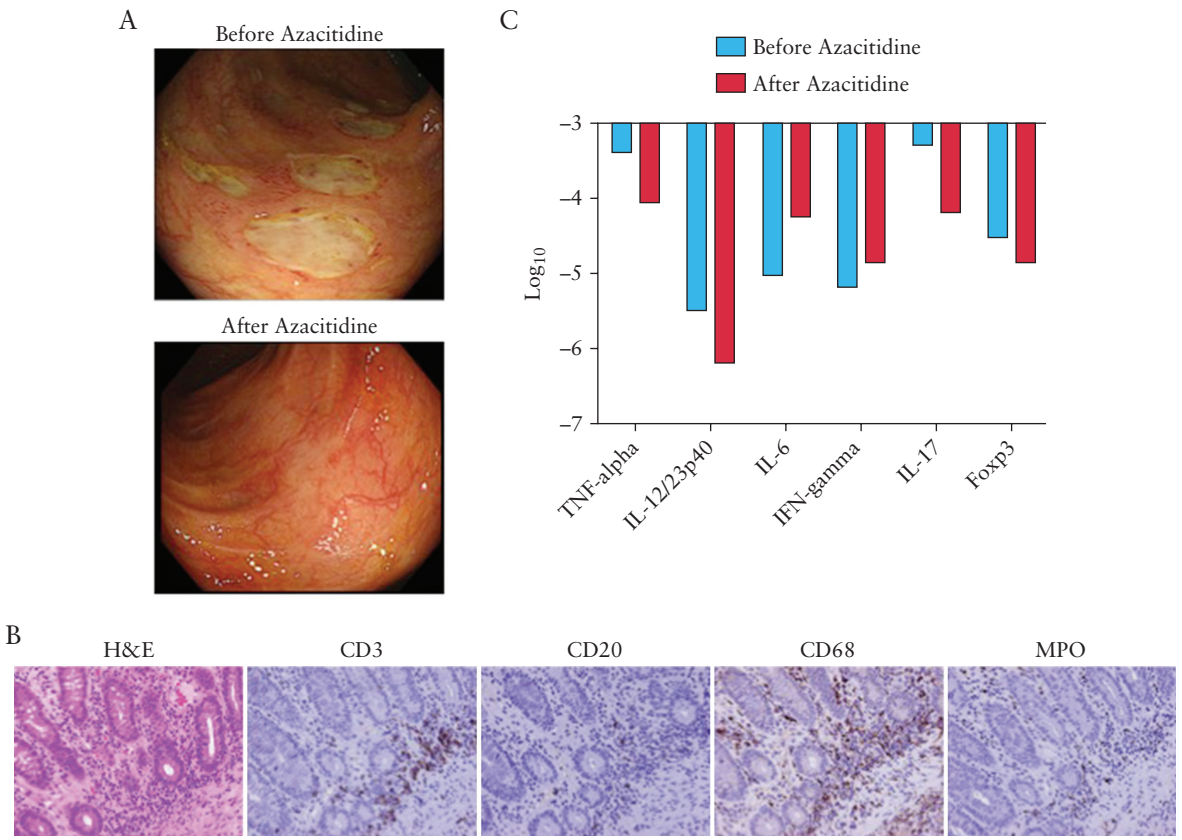

Figure 1. Endoscopic and pathological findings and cytokine profiles of a patient with myelodysplastic syndrome-associated inflammatory bowel disease. [A] Endoscopic images of the patient. Multiple round ulcers were observed in the transverse colon before azacitidine [AZA] treatment, and they disappeared after AZA treatment. [B] Pathological images of the patient. Endoscopic biopsy samples obtained from the transverse colon before AZA treatment were fixed in 10% formalin, followed by haematoxylin & eosin [H&E] staining. H&E staining revealed massive infiltration of immune cells and destruction of crypt architecture. Biopsy samples were also subjected to immunohistochemical analysis by using anti-CD3 antibody [Ab], anti-CD20 Ab, anti-CD68 Ab and anti-myeloperoxidase [MPO] Ab. Infiltration of $CD3^+$ T cells and $CD68^+$ macrophages was observed in the transverse colon [×200]. [C] Cytokine profiles of the patient. mRNA was isolated from endoscopic biopsy specimens of the transverse colon before and after AZA and subjected to quantitative reverse transcription polymerase chain reaction to determine the expression of TNF-α, IL-12/23p40, IL-6, IFN-γ, IL-17 and forkhead box p3 [Foxp3]. Expression of these molecules was normalized by the expression of β-actin.

longitudinal ulcers/cobble stone appearance, continuous lesions, diffuse mucosal oedema or erosion. These findings suggested BD rather than CD or UC. Polymerase chain reaction [PCR] for cytomegalovirus and tuberculosis using colon biopsy specimens showed negative results. No small bowel lesions were detected in contrast-enhanced abdominal computed tomography scans although endoscopic examinations of the small bowel were not performed. Stool and urine cultures for pathogenic micro-organisms were negative and serum level of β-d-glucan was within normal limits. Thus, microbe infection was less likely to cause colitis in this case.

Pathological analysis of the colon biopsy specimen revealed a marked infiltration of inflammatory cells and destruction of crypt architecture [Figure 1B]. Immunohistochemical analysis was performed to determine the type of immune cells accumulated into the lesions, as previously described.[10] Immunohistochemical analysis showed that inflammatory cells were mainly composed of $CD68^+$ macrophages and $CD3^+$ T cells [Figure 1B]. Infiltration of $myeloperoxidase\ [MPO]^+$ neutrophils or $CD20^+$ B cells was barely observed in the intestinal lesions [Figure 1B]. Thus, these pathological examinations suggest that macrophages and T cells, but not neutrophils, played a pathogenic role in this case; these findings were not consistent with those of BD.

BM examination showed that the nucleated cell count was 27 000/μl and the megakaryocyte count was 27/μl, with dysplastic features of megakaryocyte and myeloid cells. The karyotype of BM cells was 44, XY, add(4)(q11), del(5)(q?), -6, -7, add(11)(q13), add(13)(p11.2)(2 cells)/45, idem, add(3)(q11, 2), add(5)(q13), add(8)(q11, 2), -add(11), +add(11)(q13), -12, -13, -add(13), +mar1, +mar2, +mar3, +mar4(2 cells)/46, XY(12 cells). Trisomy 8, a strong risk factor for BD,[11,12] was absent in the BM. The patient was again diagnosed with high-risk RAEB2 according to the international prognostic system of MDS.[6,7]

Because the diagnosis of CD, UC or BD was not supported by physical, endoscopic or pathological examinations, we considered this case as MDS-associated IBD. Administration of colchicine and antibiotics did not improve the symptoms of the patient. AZA [75 mg/m^2/day for 5 days of the week] was also started for the treatment of high-risk MDS. Administration of ceftriaxone [2 g/day] was continued during the AZA treatment to prevent systemic bacterial infection due to bacterial translocation. Surprisingly, the diarrhoea and fever of the patient disappeared after administration of AZA. A marked reduction of serum CRP level and increase of blood leukocyte, red blood cell and platelet counts were observed. More importantly, multiple round ulcers observed before AZA treatment became scars and mucosal healing was achieved [Figure 1A]. Thus, AZA treatment successfully induced complete remission of MDS-associated IBD in this case.

We then tried to determine the molecular mechanisms underlying the AZA-induced complete remission in MDS-associated IBD. For this, isolated mRNA from colon biopsy specimens before and after AZA treatment was subjected to quantitative reverse transcription PCR [qPCR] analysis, as previously described.[16] Ethical permission for this study was obtained by the Review Boards of Kindai University Faculty of Medicine. As shown in Figure 1C, expression of pro-inflammatory cytokines such as TNF-α, IL-12/23p40 and IL-17 was markedly reduced after AZA treatment, whereas expression of IL-6 and interferon-γ [IFN-γ] was unchanged. Thus, induction of complete remission by AZA treatment was accompanied by suppression of pro-inflammatory cytokine responses [TNF-α, IL-12/23p40 and IL-17]. One possible mechanism accounting for the suppression of pro-inflammatory cytokine responses is expansion of regulatory T cells [Tregs] expressing forkhead box p3 [Foxp3].[17,18] Lal and colleagues have reported that demethylation of CpG sites of the Foxp3 non-intronic upstream enhancer by AZA results in strong and stable expression of Foxp3 and, thereby, differentiation of Tregs.[19,20] We then assessed the expression of Foxp3 before and after AZA treatment. As shown in Figure 1C, Foxp3 expression was unchanged after AZA treatment. Taken together, these qPCR data suggest strongly that AZA treatment suppressed IBD through down-regulation of pro-inflammatory cytokine responses rather than expansion of Tregs.

3. Discussion

BM-derived APCs are the cellular source of pro-inflammatory cytokines such as TNF-α, IL-12, IL-23 and IL-6, all of which have been implicated as pathogenic mediators in IBD.[1,2] MDS is caused by acquired chromosomal abnormalities and gene mutations that may have significant influence on the function of BM-derived APCs.[6,7] Therefore, it is not surprising that pro-inflammatory APCs originating from abnormal BM environments in patients with MDS are involved in the development of immune disorders including IBD.[8–10] Although the immunopathogenesis of MDS-associated IBD has not been fully clarified, Nakamura *et al*. have provided evidence that chromosomal abnormalities of BM cells set the stage for the development of colitogenic APCs, producing a large amount of pro-inflammatory cytokines and exhibiting resistance to apoptosis upon stimulation with microbial antigens.[10] In line with this finding, a marked elevation of the APC-derived cytokines, TNF-α and IL-12/23p40, was observed in this patient at active phase. Thus, our data support the idea that chromosomal abnormalities and gene mutations in BM cells lead to the development of pathogenic APCs with the ability to cause MDS-associated IBD through the production of pro-inflammatory cytokines.

To our knowledge, this is the first case of MDS-associated IBD successfully treated with AZA. Regarding the molecular mechanisms, treatment with AZA markedly reduced the expression of TNF-α, IL-12/23p40,and IL-17, whereas the expression of IL-6 and IFN-γ remained unchanged. Given that IL-12/23p40 and TNF-α are potent inducers for T helper 17 cells producing IL-17,[1,2] these cytokine data suggest that the TNF-α–IL-12/23p40–IL-17 axis contributes to the development of MDS-associated IBD. Impaired function of Foxp3-expressing Tregs mediates intestinal inflammation.[18] Although AZA induces the differentiation of naïve $CD4^+$ T cells into Foxp3-expressing Tregs,[19,20] no significant change was observed in Foxp3 expression before and after AZA treatment. Therefore, we speculate that AZA treatment led to complete remission through suppression of pro-inflammatory cytokine responses, but not expansion of Tregs. Consistent with this observation, Sánchez-Abarca *et al*. reported that AZA inhibits the production of pro-inflammatory cytokines including TNF-α.[21] Collectively, our findings, together with previous reports, support the idea that AZA might suppress MDS-associated IBD through immunomodulatory action against pro-inflammatory cytokine responses.

To the best of our knowledge, this is the first case of MDS-associated IBD successfully treated with AZA. AZA treatment might be recommended for MDS-associated IBD. Confirmation of this idea awaits future studies addressing both the efficacy of AZA treatment and pro-inflammatory cytokine responses in a large number of patients with MDS-associated IBD.

Funding

This work was supported by the Naito Foundation, SENSHIN Medical Research Foundation, Yakult Bio-Science Foundation, Smoking Research Foundation, Kobayashi Foundation for Cancer Research and Takeda Science Foundation.

Conflict of Interest

None to declare.

Author Contributions

M. Kono, YK, TS and HI took care of the patient. M. Kono, TW, YK, AO, KM, KK and SH wrote the manuscript. EE performed pathological examinations. M. Kono and TW performed the experiments. IM and M. Kudo supervised the research.

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