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Role of *Bradyrhizobium enterica* in gastrointestinal graft-versus-host disease

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Bradyrhizobium enterica in cord colitis syndrome was first described as an agent by Herrera et al. Cord colitis syndrome is defined as chronic active colitis and granulomatous inflammation that responds to antibiotics, with late-onset diarrhea after umbilical cord blood transplantation without known infectious agents or graft-versus-host disease (GvHD) [1]. No known infectious agent has been detected in cord colitis syndrome. However, since the colitis clinic responded to antibiotherapy such as metronidazole, alone or in combination with a fluoroquinolone, DNA samples of the newly discovered bacterium *Bradyrhizobium enterica* were detected in the DNA determinations made from samples taken from the intestines of all patients diagnosed with cord colitis.

Bradyrhizobium is a gram-negative, aerobic, slow-growing, non-spore-forming bacillus, a motile bacterial genus with a single subpolar flagella. As a result of comparative genomic analyses and algorithms performed by global alignment of amino acid sequences, the gene structure of *Bradyrhizobium enterica* has been found to be almost identical to that of *Bradyrhizobium japonicum*. The association of the detected sequences with cord colitis suggests that *Bradyrhizobium enterica* may be an opportunistic human pathogen [2, 3]. *Bradyrhizobium enterica* has not been investigated in patients who previously developed gastrointestinal (GIS) GvHD after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

In our study, we investigated *Bradyrhizobium enterica* as a factor in patients who developed GIS GvHD after allo-HSCT.

In our study, 16 patients who were proven with tissue biopsy samples taken from the colon, where GIS GvHD develops according to the Glucksberg criteria [4] after allo-HSCT, were included. Thirteen of the patients were male and three were female. Their mean age was 45 ± 5 years. Eight of the patients were diagnosed with acute myeloid leukemia, three were diagnosed with acute lymphoblastic leukemia, two patients were diagnosed with aplastic anemia, and one patient was diagnosed with each of the following: plasma cell leukemia, mantle cell lymphoma, and chronic lymphocytic leukemia. CMV DNA was found positive in some of the patients, but none of these patients were found to have CMV in their intestinal biopsy, which was evaluated as CMV reactivation. Table I summarizes other information about these patients.

Bacterial DNA needed to be obtained from tissue biopsy samples taken from the colon. In order to obtain DNA, for DNA isolation from tissue samples in the paraffin block, NucleoSpin DNA FFPE XS (Macherey-Nagel), a commercial DNA isolation kit, was used. The obtained DNA samples were stored at –20°C until use. Polymerase chain reaction for detection of *Bradyrhizobium enterica* bacteria, forward for *Bradyrhizobium enterica* search, 5'-TCGAGGGCTACGGCTTGAAGATTT-3' and reverse 5'-ACAACGTGTTGCCGCCAATATGAG-3, a target site was attempted to amplify a 367 base pair. As a control, primers belonging to the human actin gene and the 16S ribosomal RNA gene region, which is a common gene in bacteria, were used. Forward 5'-GCGAGAAGATGACCCAGATC-3' targeting the 102 base pair gene region for the human actin gene; reverse 5'-CCAGTGGTACGGCCAGAGG-3' primers and forward 5'-GTGCAATATTCCCCACTGCT-3 targeting 93 base pairs gene region for 16S RNA; reverse 5′-CGATCCCTA GCTGGTCTGAG-3' primers were used. The following thermal cycling conditions were applied for all PCR tests: denaturation at 95°C for 2 minutes, 35 cycles: 30 seconds at 95°C, 30 seconds at 62.1°C, 40 seconds at 68°C, and final elongation at 68°C for 5 minutes [5]. In order to show

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Table I. Information on patients participating in study

Case number	Age	Gender	GIS GvHD stage	GvHD	allo-HSCT regimen	CMV DNA	Donor*
1	57	F	3	GIS	Nonmyeloablative	–	1
2	34	M	3	GIS, liver, skin	Nonmyeloablative	+	3
3	23	M	3	GIS, liver, skin	Nonmyeloablative	+	3
4	55	F	3	GIS, skin	Myeloablative	+	2
5	40	F	4	GIS, liver, skin	Myeloablative	+	1
6	62	M	3	GIS, liver, skin	Nonmyeloablative	–	1
7	39	M	4	GIS, liver, skin	Myeloablative	+	3
8	24	M	2	GIS, skin	Myeloablative	–	1
9	60	M	2	GIS, skin	Myeloablative	–	1
10	54	M	3	GIS, liver, skin	Nonmyeloablative	+	1
11	58	M	3	GIS, liver, skin	Nonmyeloablative	–	1
12	50	M	2	GIS, skin	Myeloablative	+	1
13	34	M	4	GIS, liver, skin	Myeloablative	+	1
14	44	M	3	GIS, liver, skin	Nonmyeloablative	+	3
15	44	M	3	GIS, skin	Nonmyeloablative	+	3
16	53	M	3	GIS, liver, skin	Myeloablative	+	1

*Donor scoring: 1 — human leukocyte antigen (HLA) identical sibling donor; 2 — haploidentical; 3 — HLA 9–10/10 matched unrelated donor; GIS — gastrointestinal; GvHD — graft-versus-host disease; allo-HSCT — allogeneic hematopoietic stem cell transplantation; CMV; F — female; M — male

the amplified gene regions, the amplification products were observed in UV light by running at 90 V for 45 minutes in gel electrophoresis.

As a result of the PCR experiment, gene region of *Bradyrhizobium enterica* could not be determined in any of the DNA samples isolated from patients' tissues. Control genes were found in all samples. For this study, approval was obtained from the Ethics Committee for Clinical Studies at the Adnan Menderes University School of Medicine (date: June 12, 2021; No: 2020/1368).

The intestinal mucosa is the innermost layer of the four histological layers of the major intestinal tract, followed by the submucosa, muscularis externa and serosa. The epithelium is a single-cell layer lining of the interior lumen of the gastrointestinal tract. Immediately adjacent to the epithelial layer is the lamina propria, an interstitial tissue with a rich vascular and lymphatic network and abundant leukocytes. There are various cell types within the epithelium, such as intestinal epithelial cells, goblet cells, paneth cells, intestinal stem cells and tuft cells, each with their own specific functions, including nutrient absorption and barrier function, mucus production, production of antimicrobial molecules, production of growth factors, and cellular regeneration. In human and animal studies, it has been shown that these cells have been decreased in acute GvHD [6]. In addition, the human intestinal tract contains an estimated 10 trillion bacteria from about 1,000 species. Approximately 15,000 different bacterial species such as *Gemella, Staphylococcus, Enterococcus, Lactobacillus, Streptococcus, Blautia, Eubacterium, Erysipelatoclostridium, Acidaminococcus, and Bacteroides* genus have been identified in human populations [7].

Even though GvHD is an iatrogenic illness, its pathogenesis is not completely understood, and deaths from GvHD are a continuing obstacle to successful transplantation [8]. Although the impact of bacteria on acute GIS GvHD is not fully understood, the loss of enteric flora diversity correlates with the risk of developing acute GvHD. Patients who have lost *Clostridiale* bacteria from the gut and have a significant increase in *Lactobacillales* develop acute GvHD rapidly [9]. Reduced intestinal microbial diversity represents an independent risk for post-transplant mortality [10]. To date, the *Bradyrhizobium* species has not been associated with human disease.

The detection of *Bradyrhizobium enterica* in all patients with cord colitis in our study suggests that *Bradyrhizobium enterica* may be a pathogenic bacterium for cord colitis. In our study, *Bradyrhizobium enterica* was not detected in the tissue samples taken from the colons of patients with acute GIS GvHD. Consequently, we conclude that the bacterium *Bradyrhizobium enterica* has no role in GIS GvHD after allo-HSCT.

Authors' contributions

CS — collected the data, conceived and designed the analysis, wrote the paper,contributed data tools. MT — perform

the analysis microbiology samples review. AZB — performed the analysis. İY — conceived and designed the analysis, performed the analysis ,contributed data tools.

Conflict of interest and financial support

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; uniform requirements for manuscripts submitted to biomedical journals.

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