contribute to anti-inflammation, in part, through their ability of IL-10 production. However, various functions of Bregs including an IL-10–independent pathway (direct contact with T cells) have also been reported. Further investigations are necessary to more fully understand the functions of Bregs in intestinal immune regulation.

There are several limitations in the present study that must be considered. First, the colitis model of SCID mice created by transfer of SAMP1CD4$^+$ T cells does not contain other subsets of T cells, thus limiting a full evaluation of the regulatory roles of B cells associated with the function of CD8$^+$ T cells. Furthermore, we did not fully investigate IL-10 production and its function in intestinal B cells (without CpG DNA stimulation) in vivo. Although we found IL-10–dependent anti-inflammatory effect of Bregs in vitro studies, it remains unknown regarding the specific mechanisms of Breg in intestinal inflammation. Moreover, we found a higher level of IL-6 production in CpG DNA-stimulated CD19^{hi}CD1d^{hi} B cells and CD19^{hi}CD1d^{hi}-depleted B cells. However, we could not determine the role of IL-6 production in the regulatory subset of B cells from our findings. Yanaba et al[42] showed that the expression level of IL-6 in IL-10–secreting B cells was similar to that in nonsecreting B cells and also that IL-6 produced by B cells contributes to wound healing.[43] Although IL-6 has been reported to have an anti-inflammatory role under certain conditions,[44–47] its expression and role in the regulatory subset of B cells remain unknown. Additional studies are needed to clarify this point.

In this study, we demonstrated the immunoregulatory role of CD19^{hi}CD1d^{hi} B cells in experimental colitis mouse models. Although we also found decreased production of IL-10 in CpG DNA-stimulated peripheral blood B cells in patients with CD, it remains unknown whether Bregs dysfunction is directly correlated with the development and pathogenesis of CD. Moreover, recent reports have shown that total depletion of mature B cells with rituximab in patients with autoimmune disease induced the development of UC,[48–50] suggesting that Bregs may also be associated with the pathogenesis of UC. To confirm these points, it will be important to address the various mechanisms related to the maturation and differentiation, and CpG DNA-dependent cellular signaling in Bregs, and the roles of intestinal microbial flora in Bregs functions. Such findings may lead to a novel therapeutic strategy targeting Bregs for IBD.

REFERENCES

1. Ishihara S, Aziz MM, Yuki T, et al. Inflammatory bowel disease: review from the aspect of genetics. *J Gastroenterol.* 2009;44:1097–1108.
2. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature.* 2001;411: 603–606.
3. Saitoh T, Fujita N, Jang MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature.* 2008;456:264–268.
4. Katz SI, Parker D, Turk JL. B-cell suppression of delayed hypersensitivity reactions. *Nature.* 1974;251:550–551.
5. Wolf SD, Dittel BN, Hardardottir F, et al. Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J Exp Med.* 1996;184:2271–2278.
6. Mizoguchi A, Mizoguchi E, Smith RN, et al. Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. *J Exp Med.* 1997; 186:1749–1756.
7. Mizoguchi E, Mizoguchi A, Preffer FI, et al. Regulatory role of mature B cells in a murine model of inflammatory bowel disease. *Int Immunol.* 2000;12:597–605.
8. Fillatreau S, Sweenie CH, McGeachy MJ, et al. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol.* 2002;3:944–950.
9. Mizoguchi A, Mizoguchi E, Takedatsu H, et al. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity.* 2002;16:219–230.
10. Mauri C, Gray D, Mushtaq N, et al. Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med.* 2003;197:489–501.
11. Wei B, Velazquez P, Turovskaya O, et al. Mesenteric B cells centrally inhibit CD4+ T cell colitis through interaction with regulatory T cell subsets. *Proc Natl Acad Sci U S A.* 2005;102:2010–2015.
12. Lenert P, Brummel R, Field EH, et al. TLR-9 activation of marginal zone B cells in lupus mice regulates immunity through increased IL-10 production. *J Clin Immunol.* 2005;25:29–40.
13. Brummel R, Lenert P. Activation of marginal zone B cells from lupus mice with type A(D) CpG-oligodeoxynucleotides. *J Immunol.* 2005;174: 2429–2434.
14. Mizoguchi A, Bhan AK. A case for regulatory B cells. *J Immunol.* 2006; 176:705–710.
15. Evans JG, Chavez-Rueda KA, Eddaoudi A, et al. Novel suppressive function of transitional 2 B cells in experimental arthritis. *J Immunol.* 2007;178:7868–7878.
16. Amu S, Saunders SP, Kronenberg M, et al. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. *J Allergy Clin Immunol.* 2010;125:1114–1124.
17. Matsushita T, Horikawa M, Iwata Y, et al. Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. *J Immunol.* 2010;185:2240–2252.
18. Blair PA, Noreña LY, Flores-Borja F, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity.* 2010;32: 129–140.
19. Carter NA, Vasconcellos R, Rosser EC, et al. Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. *J Immunol.* 2011;186:5569–5579.
20. Hirotani M, Niino M, Fukazawa T, et al. Decreased IL-10 production mediated by Toll-like receptor 9 in B cells in multiple sclerosis. *J Neuroimmunol.* 2010;221:95–100.
21. Knippenberg S, Peelen E, Smolders J, et al. Reduction in IL-10 producing B cells (Breg) in multiple sclerosis is accompanied by a reduced naïve/ memory Breg ratio during a relapse but not in remission. *J Neuroimmunol.* 2011;239:80–86.
22. Matsumoto S, Okabe Y, Setoyama H, et al. Inflammatory bowel disease-like enteritis and caecitis in a senescence accelerated mouse P1/Yit strain. *Gut.* 1998;43:71–78.
23. Mishima Y, Ishihara S, Aziz MM, et al. Decreased production of interleukin-10 and transforming growth factor-β in Toll-like receptor-activated intestinal B cells in SAMP1/Yit mice. *Immunology.* 2010;131:473–487.
24. Kosiewicz MM, Nast CC, Krishnan A, et al. Th1-type responses mediate spontaneous ileitis in a novel murine model of Crohn's disease. *J Clin Invest.* 2001;107:695–702.
25. Burns RC, Rivera-Nieves J, Moskaluk CA, et al. Antibody blockade of ICAM-1 and VCAM-1 ameliorates inflammation in the SAMP-1/Yit adoptive transfer model of Crohn's disease in mice. *Gastroenterology.* 2001;121:1428–1436.
26. Schmidt EG, Larsen HL, Kristensen NN, et al. B cells exposed to enterobacterial components suppress development of experimental colitis. *Inflamm Bowel Dis.* 2012;18:284–293.
27. Duddy M, Niino M, Adatia F, et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J Immunol.* 2007;178:6092–6099.
28. Correale J, Farez M, Razzitte G. Helminth infections associated with multiple sclerosis induce regulatory B cells. *Ann Neurol.* 2008;64: 187–199.

29. Scapini P, Lamagna C, Hu Y, et al. B cell-derived IL-10 suppresses inflammatory disease in Lyn-deficient mice. *Proc Natl Acad Sci U S A.* 2011;108:E823–E832.
30. Yanaba K, Bouaziz JD, Haas KM, et al. A regulatory B cell subset with a unique $CD1d^{hi}CD5^{+}$ phenotype controls T cell-dependent inflammatory responses. *Immunity.* 2008;28:639–650.
31. Matsushita T, Yanaba K, Bouaziz JD, et al. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest.* 2008;118:3420–3430.
32. Matsumoto M, Fujii Y, Baba A, et al. The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. *Immunity.* 2011;34:703–714.
33. Dalwadi H, Wei B, Schrage M, et al. B cell developmental requirement for the G alpha i2 gene. *J Immunol.* 2003;170:1707–1715.
34. Okkenhaug K, Bilancio A, Farjot G, et al. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science.* 2002; 297:1031–1034.
35. Sun CM, Deriaud E, Leclerc C, et al. Upon TLR9 signaling, $CD5^{+}$ B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs. *Immunity.* 2005;22:467–477.
36. Ronet C, Hauyon-La Torre Y, Revaz-Breton M, et al. Regulatory B cells shape the development of Th2 immune responses in BALB/c mice infected with Leishmania major through IL-10 production. *J Immunol.* 2010;184:886–894.
37. Bouaziz JD, Calbo S, Maho-Vaillant M, et al. IL-10 produced by activated human B cells regulates CD4(+) T-cell activation in vitro. *Eur J Immunol.* 2010;40:2686–2691.
38. Iwata Y, Matsushita T, Horikawa M, et al. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood.* 2011;117:530–541.
39. Ding Q, Yeung M, Camirand G, et al. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J Clin Invest.* 2011;121:3645–3656.
40. Yanaba K, Yoshizaki A, Asano Y, et al. IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model. *Am J Pathol.* 2011;178: 735–743.
41. Goetz M, Atreya R, Ghalibafian M, et al. Exacerbation of ulcerative colitis after rituximab salvage therapy. *Inflamm Bowel Dis.* 2007;13: 1365–1368.
42. Yanaba K, Bouaziz JD, Matsushita T, et al. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. *J Immunol.* 2009;182: 7459–7472.
43. Yanaba K, Bouaziz JD, Matsushita T, et al. B-lymphocyte contributions to human autoimmune disease. *Immunol Rev.* 2008;223: 284–299.
44. Cuzzocrea S, Sautebin L, De Sarro G, et al. Role of IL-6 in the pleurisy and lung injury caused by carrageenan. *J Immunol.* 1999;163: 5094–5104.
45. Cuzzocrea S, Mazzon E, Dugo L, et al. Absence of endogenous interleukin-6 enhances the inflammatory response during acute pancreatitis induced by cerulein in mice. *Cytokine.* 2002;18:274–285.
46. Balto K, Sasaki H, Stashenko P. Interleukin-6 deficiency increases inflammatory bone destruction. *Infect Immun.* 2001;69:744–750.
47. Alonzi T, Fattori E, Lazzaro D, et al. Interleukin 6 is required for the development of collagen-induced arthritis. *J Exp Med.* 1998;187: 461–468.
48. El Fassi D, Nielsen CH, Kjeldsen J, et al. Ulcerative colitis following B lymphocyte depletion with rituximab in a patient with Graves' disease. *Gut.* 2008;57:714–715.
49. Ardelean DS, Gonska T, Wires S, et al. Severe ulcerative colitis after rituximab therapy. *Pediatrics.* 2010;126:e243–e246.
50. Olson TS, Bamias G, Naganuma M, et al. Expanded B cell population blocks regulatory T cells and exacerbates ileitis in a murine model of Crohn disease. *J Clin Invest.* 2004;114:389–398.

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ORIGINAL ARTICLE

Prevalence of irritable bowel syndrome-like symptoms in ulcerative colitis patients with clinical and endoscopic evidence of remission: prospective multicenter study

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Abstract

Objective. Irritable bowel syndrome (IBS)-like symptoms are often found in ulcerative colitis (UC) patients in remission. However, the prevalence of those symptoms in UC patients with endoscopic evidence of remission shown by mucosal healing remains unknown. ***Material and methods.*** IBS diagnosis was evaluated by questionnaire results according to the Rome III criteria. Clinical remission was assessed by clinical activity index (CAI), whereas endoscopic remission was evaluated by endoscopic index (Matts grade). ***Results.*** We enrolled 172 patients in clinical remission (CAI ≤ 4), after excluding 36 for incomplete questionnaire results or nonremission findings, as well as 330 control subjects. Of the 172 UC patients, 46 (26.7%) met the Rome III criteria, which was a significantly higher rate as compared with the controls (4.8%). The prevalence rate of IBS-like symptoms in UC patients with endoscopic remission findings (Matts grade ≤2) was 25.6%, which was similar to that of those with clinical remission. When endoscopic remission was defined as Matts grade 1, the prevalence rate of IBS-like symptoms was decreased to 15.4%, although the prevalence rate remained higher than that of the control subjects. ***Conclusions.*** The prevalence of IBS-like symptoms in UC patients with clinical and endoscopic remission findings was significantly higher than that of control subjects. Furthermore, the prevalence rate in patients with complete endoscopic remission was decreased. These findings suggest that residual low-grade inflammation may influence the presence of IBS-like symptoms in UC patients in remission.

Key Words: *clinical remission, endoscopic remission, IBS-like symptoms, ulcerative colitis*

Introduction

Irritable bowel syndrome (IBS) is a chronic functional disorder of the intestinal tract in the absence of organic abnormalities and characterized by clinical symptoms such as abdominal pain and discomfort, along with alterations in bowel habits [1–5]. IBS represents a highly prevalent intestinal disease that affects around 10–15% of adults in most countries, and a number of studies have demonstrated that psychological distress, disturbances of intestinal motility and visceral hypersensitivity are closely associated with its pathogenesis [6–8]. In recent years, the role of gastrointestinal infection has been investigated as a new aspect in the etiology of IBS, with low-grade gut mucosal inflammation and immune activation following an episode of acute gastroenteritis currently recognized as important

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factors underlying the pathogenesis of the disorder [6,9–14].

Ulcerative colitis (UC) and Crohn's disease (CD) are two major forms of inflammatory bowel disease (IBD) and characterized by chronic immune-mediated intestinal disorders of unknown etiology. Although the disease entity IBD is essentially distinguished from IBS based on the presence or absence of intestinal organic lesions, IBS-like symptoms are often reported by IBD patients [15]. Recent epidemiological studies have revealed that an episode of gastroenteritis and the presence of IBS are risk factors for development of IBD [16,17]. In addition, etiological factors regarding immune regulation, genetic polymorphisms and responses to luminal microbial pathogens partially overlap between IBS and IBD [18]. Those findings suggest that overlapping etiological factors may influence the presence of IBS-like symptoms in IBD patients.

Numerous studies have investigated the presence of IBS-like symptoms in UC patients without evidence of ongoing disease activity [19–23]. A recent meta-analysis indicated that IBS-like symptoms occurred in approximately 30% of UC patients in remission, with remission in the studies reviewed mainly defined by evaluating clinical symptoms [15]. However, the prevalence of IBS-like symptoms in UC patients with endoscopic evidence of remission shown by mucosal healing remains unknown. In the present study, we investigated UC patients with clinical as well as endoscopic remissions, and evaluated the presence of IBS-like symptoms.

Materials and methods

Patients and control subjects

This study was prospectively conducted from May 2011 to February 2012 at one university hospital and three general hospitals in Japan. Diagnoses of UC were based on standard clinical, endoscopic and histological criteria. At each hospital, all UC patients (≥18 years old) defined by their attending physician as in good condition were assessed, whereas patients with hematochezia, history of colectomy or C-reactive protein >0.5 mg/dl were excluded. Thus, 172 patients were enrolled in this study. In addition, consecutive individuals who underwent a general medical checkup at Matsue Seikyo Hospital were enrolled as healthy control subjects. The study protocol was approved by the institutional ethics committee of each hospital.

Evaluation of IBS- and FD-like symptoms

After obtaining written consent from each patient and control subject, the presence of IBS-like symptoms was evaluated by questionnaire results according to the Rome III criteria. The Japanese version of the questionnaire used in this study was validated in our previous study [2].

Definition of clinical and endoscopic remissions

Disease activity was assessed using clinical activity index (CAI) (Rachmilewitz index [24]); and Matts grading [25]. The Matts grading system is as follows: 1. normal; 2. mild granularity of the mucosa with mild contact bleeding; 3. marked granularity and edema of the mucosa, contact bleeding, and spontaneous bleeding; and 4. severe ulceration of mucosa with hemorrhage. Clinical remission was defined as a CAI score of ≤4 for at least 6 months, whereas endoscopic remission was defined as Matts grade ≤2. Colonoscopy examinations for most patients in clinical remission were conducted at the same time as the questionnaire. However, when that coordinated timing was not possible, colonoscopy findings obtained from patients in remission within 3 months before answering the questionnaire were used for analysis. Questionnaire results and endoscopic findings for the patients were carefully evaluated by two IBD experts (SI and KK).

Statistical analysis

Differences for prevalence of IBS-like symptoms between the UC and control groups were evaluated using a chi-squared test. A p-Value of <0.05 was considered to be significant. All calculations were performed with SPSS version 19.0 for Windows.

Results

Study subjects

A total of 172 UC patients with clinical evidence of remission were enrolled in the present study by their physicians and then carefully screened by the two IBD experts prior to the present analysis. Of them, 43 agreed to undergo a colonoscopy examination, with those results showing that 39 had endoscopic evidence of remission (Matts grade ≤2). In addition, 330 healthy subjects were enrolled during the study period as a control group. A flow chart of the present study patients is presented in Figure 1, while the baseline characteristics of the patients and controls are shown in Tables I and II. Thirty four patients in clinical remission (including eight in endoscopic remission) who were undergoing tapering of steroid administration were also enrolled. There were no

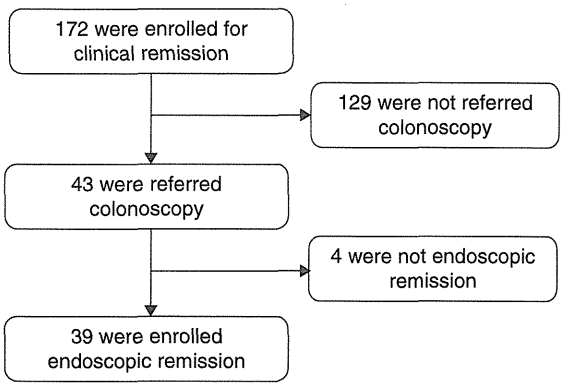

Figure 1. Flow chart of patients in the present study.

differences in regard to gender and average age between the groups.

Prevalence of IBS-like symptoms in UC patients with clinical evidence of remission

The prevalence rate of IBS-like symptoms in the UC patients with clinical evidence of remission (CAI ≤ 4) was 26.7% (46/172, 95% confident interval (CI): 21.0–33.8%) and in the control subjects was 4.8% (95% CI: 3.0–7.7%) (26.7% vs. 4.8%, OR: 7.17, 95% CI: 3.94–13.0, $p < 0.01$) (Figure 2A). IBS can be subclassified into several categories using the Rome III criteria based on the predominant bowel symptom of the patient [1]. The prevalence rate of each IBS type (IBS-D, diarrhea predominant; IBS-C, constipation predominant; IBS-M, mixed, IBS-U, unspecified) in the patients and controls are shown in Figure 2B. A high rate of prevalence of IBS-U (41.3%) was found in the UC patients with IBS-like symptoms as compared with the control group.

Table II. Characteristics of UC patients in remission with or without IBS-like symptoms.

		IBS-	IBS+	*p*-Value
Number		126	46	-
Gender (M/F)		69/57	24/22	0.99
Age		52 ± 18	46 ± 16	0.04#
Disease duration (y)		9.8	8.4	0.22
Classification of lesion	Extensive	41	19	0.75
	Left side	34	11	
	Proctitis	46	14	
	Others	5	2	
5-ASA/SASP		89% (112)	93% (43)	0.91
Steroid		19% (24)	22% (10)	0.99
Immune modulators		13% (16)	26% (12)	0.11
Biologics		0.8% (1)	8.7% (4)	0.02*

#Chi-square test; *Fisher's exact test.

Prevalence of IBS-like symptoms in UC patients with endoscopic evidence of remission

Of the 39 UC patients with endoscopic evidence of remission (Matts grade ≤2), 10 met the Rome III criteria (25.6%, 95%CI: 14.6–41.1%), which was similar to that found in UC patients with clinical evidence of remission (26.7%). The prevalence rate of IBS-like symptoms was significantly higher than that in the control subjects (25.6% vs. 4.8%, OR 6.77, 95% CI: 2.87–16.0, $p < 0.01$, Figure 3). When endoscopic remission was strictly defined as Matts grade 1, the prevalence rate decreased to 15.4% (95%CI: 4.3–42.2%), and there was no statistical difference as compared with the control group (15.4% vs. 4.8%, OR 3.57, 95% CI 0.83–15.8, $p = 0.14$, Figure 3). In addition, we did not find a statistical difference between Matts grade ≤2 and grade 1 in regard to the prevalence rate of IBS-like symptoms (25.6% vs. 15.4%, OR 1.9, 95% CI: 0.39–8.80).

Table I. Baseline characteristics of each group.

		UC with clinical remission	UC with endoscopic remission	Controls
Number		172	39	330
Gender (M/F)		93/79	25/14	159/171
Age		50.0 ± 17 (19–86)	50.2 ± 16 (21–76)	47.4 ± 13 (21–83)
Disease duration (y)		9.4 ± 7.5(0–44)	7.7 ± 5.1(2–21)	-
Classification of lesion	Extensive	60	13	-
	Left side	45	15	-
	Proctitis	60	11	-
	Others	7	0	-
5-ASA/SASP		145/172	29/39	-
Steroid		34/172	8/39	-
Immune modulators		28/172	6/39	-
Biologics		5/172	0/39	-
Prokinetic drugs		4/172	0/39	-
Antidepressants		10/172	2/39	-

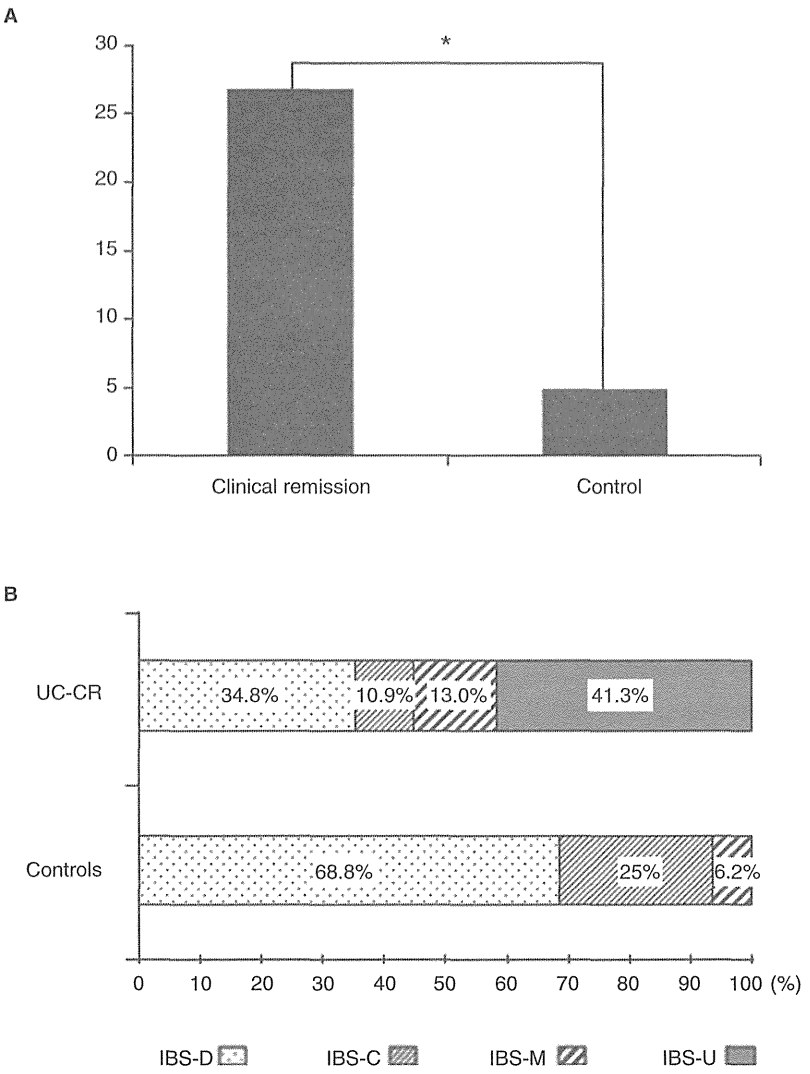

Figure 2. Prevalence (A) and subtypes (B) of IBS-like symptoms in UC patients with clinical remission findings, and healthy control subjects. *$p < 0.01$ vs. control.

Discussion

The present results indicate that the prevalence of IBS-like symptoms in UC patients with clinical and endoscopic evidence of remission is significantly higher than that in healthy individuals. In addition, we precisely analyzed endoscopic findings in UC patients and found that the prevalence rate in those with complete endoscopic remission (Matts grade 1) was lower than that in patients with clinical findings of remission. Our findings suggest that the endoscopic finding of Matts grade 2 does not simply define clinical remission, because a substantial proportion of our patients with Matts grade 2 still had symptoms in parallel with lower mucosal inflammation. Thus, the presence of IBS symptoms may be associated with, at least in part, residual low-grade inflammation.

Previos studies have indicated that IBS symptoms often occur in UC patients in apparent remission [19–23]. Although various criteria were used to define UC remission in those studies, a range of 9.1–46% of patients in remission met the Manning or Rome II criteria for IBS. Halpin et al. recently performed a systematic review and meta-analysis of cross-sectional and case-control investigations, and finally analyzed 13 studies (1703 cases) to evaluate the prevalence of IBS-like symptoms in IBD patients [15]. Their findings showed that the prevalence of IBS-like symptoms was significantly higher in IBD patients in remission (UC, 31%; CD, 41%) as compared to control cases. In the present case-control study, 172 UC patients

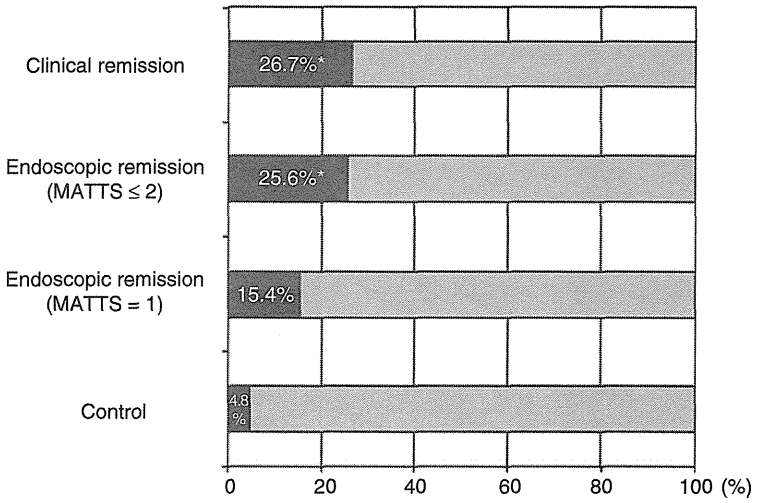

Figure 3. Prevalence of IBS-like symptoms in UC patients with clinical and endoscopic evidence of remission, and healthy control subjects. *$p < 0.01$ vs. control.

and 330 control subjects were analyzed, and the prevalence rate of IBS-like symptoms in UC patients ($n = 172$) with clinical remission findings was 26.7%, which was similar to the rate (31%) identified in that meta-analysis, as compared to 4.8% in the present control group ($n = 330$). Thus, IBS-like symptoms commonly occur in UC patients in remission.

In previous studies, remission in UC patients was defined by various factors, including clinical activity, blood or fecal markers and endoscopic or radiological findings. However, the prevalence of IBS-like symptoms in UC patients with endoscopic evidence of remission shown by mucosal healing has not been thoroughly investigated [26]. Although Isgar et al. and Simren et al. included endoscopic findings in their criteria for UC remission, the definitions used in those studies were not sufficiently validated [19,20]. In the present study, Matts grade ≤2 was incorporated into the criteria for endoscopic remission, since that is widely used as a definition of mucosal healing in UC patients [27]. Our analysis with this definition indicated that the prevalence rate of IBS-like symptoms in UC patients was 25.6%, which was similar to that when the definition of clinical remission was used (26.7%). In the present study, 4 of 43 patients with evidence of clinical remission were excluded from analysis due to the absence of mucosal healing (Matts grade 3), of whom all 4 had IBS-like symptoms. This finding suggests that approximately 10% of patients showing evidence of clinical remission do not have colonic mucosal healing. In such cases, a colonoscopy examination or fecal calprotectin test may be useful for identifying these patients.

On the other hand, when endoscopic remission was strictly defined as Matts grade 1 (complete mucosal healing), the prevalence of IBS-like symptoms was decreased to 15.4%, although that rate remained higher than the rate seen in the control subjects. As Matts grade 2 is defined as an endoscopic evidence of residual mild mucosal damage, our findings suggest that low-grade colonic inflammation may partially influence the presence of IBS-like symptoms in UC patients in remission. Similarly, Ansari et al. used the Mayo scoring system (0 or 1) for definition of endoscopic remission and found that the prevalence of IBS-like symptoms was 46% [21], which was relatively higher than other previous reports. As Mayo 1 includes endoscopic findings of mild mucosal damage similar to Matts grade 2; the prevalence rate might be affected by residual low-grade mucosal inflammation in UC patients. Recently, Keohane et al. reported that an increased level of fecal calprotectin, a surrogate marker of subclinical inflammation, was associated with the frequency of IBS-like symptoms in IBD patients in remission [28]. The finding also suggests that the presence of these symptoms is dependent on low-grade mucosal inflammation. However, we did not find a statistical difference in regard to the prevalence rate of IBS-like symptoms between patients defined as Matts grade 1 and grade ≤2. To confirm this finding, a study with a larger number of subjects who underwent a colonoscopy examination is required. Furthermore, fecal calprotectin testing may also be useful for clarifying the influence of mucosal low-grade inflammation on development of IBS-like symptoms in UC patients with remission evidence. In addition, establishment of consensus among pathologists is needed for classifying intestinal low-grade inflammation to distinguish it from normal mucosa.

IBS is recognized to be a common functional disorder in the absence of organic abnormalities. However, recent studies have demonstrated that low-grade inflammation, as shown by infiltration of various immune cells, occurs in the colonic mucosa of some IBS patients [29,30], even though their endoscopic findings are normal. In the present study, IBS-like symptoms were noted in 15.4% of UC patients without active endoscopic findings (Matts grade 1). In those cases with normal endoscopic findings, it is unclear whether the IBS symptoms are affected by residual occult inflammation or mainly due to other causes, including dysfunction of functional mechanisms and psychological factors. Further studies are necessary to confirm this point.

The main limitation of this study is that the number of patients who underwent a colonoscopy was small, because that examination was only conducted after receiving consent. As the patients in clinical remission were in relatively good health as compared with those in an active stage, it is understandable that they might not agree to undergo a colonoscopic examination after achieving remission. However, the low number of patients analyzed in our study might have affected the results. To confirm the relationship of endoscopic findings with the presence of IBS-like symptoms in UC patients, it is important to analyze a large number of subjects who underwent a colonoscopy. In addition, the prevalence rate of IBS found in the control group was relatively low (4.8%), which was similar to the result of our recent study regarding the prevalence of IBS in Japanese subjects (4.4%) [2]. Epidemiological studies using a validated symptom-based definition, such as that by Manning, or Rome criteria have demonstrated various prevalence rates of IBS ranging from 2.5% to 25% in general populations [4]. Thus, prevalence rates might vary depending on the study population, as well as the methodology (criteria) and sampling techniques utilized. Furthermore, we did not investigate the influence of psychological factors on the presence of IBS-like symptoms. Since those are etiologically important for understanding such symptoms, they should be included in a future analysis.

In summary, we performed a prospective multicenter study to evaluate the presence of IBS-like symptoms in UC patients with clinical and endoscopic remission findings. Although the prevalence of IBS-like symptoms was significantly higher in the patient group as compared with the control group, the rate was decreased after excluding patients with endoscopic findings of Matts grade 2. Our findings suggest that residual low-grade mucosal inflammation has an influence on the presence of IBS-like symptoms in UC patients in remission.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology 2006;130:1480–91.

[2] Ishihara S, Yashima K, Kushiyama Y, Izumi A, Kawashima K, Fujishiro H, et al. Prevalence of organic colonic lesions in patients meeting rome III criteria for diagnosis of IBS: a prospective multi-center study utilizing colonoscopy. J Gastroenterol 2012;47:1084–90.

[3] Grundmann O, Yoon SL. Irritable bowel syndrome: epidemiology, diagnosis and treatment: an update for health care practitioners. J Gastroenterol Hepatol 2010;25:691–9.

[4] Spiller R, Aziz Q, Creed F, Emmanuel A, Houghton L, Hungin P, et al. Guidelines on the irritable bowel syndrome: mechanisms and practical management. Gut 2007;56:1770–98.

[5] Khan S, Chang L. Diagnosis and management of IBS. Nat Rev Gastroenterol Hepatol 2010;7:565–81.

[6] Öhman L, Simrén M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. Nat Rev Gastroenterol Hepatol 2010;7:163–73.

[7] Stasi C, Rosselli M, Bellini M, Laffi G, Milani S. Altered neuro-endocrine–immune pathways in the irritable bowel syndrome: the top-down and the bottom-up model. J Gastroenterol 2012;47:1177–85.

[8] Ahl A, Mikocka-Walus A, Gordon A, Andrews JM. Are self-administered or minimal therapist contact psychotherapies an effective treatment for irritable bowel syndrome (IBS): a systematic review. J Psychosom Res 2013;75:113–20.

[9] Ford AC, Talley NJ. Mucosal inflammation as a potential etiological factor in irritable bowel syndrome: a systematic review. J Gastroenterol 2011;46:421–31.

[10] Ishihara S, Aziz M, Oshima N, Mishima Y, Imaoka H, Moriyama I, et al. Irritable bowel syndrome and inflammatory bowel disease: infectious gastroenteritis-related disorders? Clin J Gastroenterol 2009;2:9–16.

[11] Ishihara S, Tada Y, Fukuba N, Oka A, Kusunoki R, Mishima Y, et al. Pathogenesis of irritable bowel syndrome-review regarding associated infection and immune activation. Digestion 2013;87:204–11.

[12] Spiller RC. Postinfectious irritable bowel syndrome. Gastroenterology 2003;124:1662–71.

[13] Spiller R, Garsed K. Postinfectious irritable bowel syndrome. Gastroenterology 2009;136:1979–88.

[14] Spiller R. Serotonin, inflammation, and IBS: fitting the jigsaw together? J Pediatr Gastroenterol Nutr 2007;45:S115–19.

[15] Halpin SJ, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol 2012;107:1474–82.

[16] Porter CK, Cash BD, Pimentel M, Akinseye A, Riddle MS. Risk of inflammatory bowel disease following a diagnosis of irritable bowel syndrome. BMC Gastroenterol 2012;12:55.

[17] Rodriguez LG, Ruigómez A, Wallander M, Johansson S, Olbe L. Detection of colorectal tumor and inflammatory bowel disease during follow-up of patients with initial

diagnosis of irritable bowel syndrome. Scand J Gastroenterol 2000;35:306–11.
[18] Spiller R, Lam C. The shifting interface between IBS and IBD. Curr Opin Pharmacol 2011;11:586–92.
[19] Isgar B, Harman M, Kaye M, Whorwell P. Symptoms of irritable bowel syndrome in ulcerative colitis in remission. Gut 1983;24:190–2.
[20] Simrén M, Axelsson J, Gillberg R, Abrahamsson H, Svedlund J, Björnsson ES. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. Am J Gastroenterol 2002;97:389–96.
[21] Ansari R, Attari F, Razjouyan H, Etemadi A, Amjadi H, Merat S, et al. Ulcerative colitis and irritable bowel syndrome: relationships with quality of life. Eur J Gastroenterol Hepatol 2008;20:46–50.
[22] Farrokhyar F, Marshall JK, Easterbrook B, Irvine EJ. Functional gastrointestinal disorders and mood disorders in patients with inactive inflammatory bowel disease: prevalence and impact on health. Inflamm Bowel Dis 2006;12: 38–46.
[23] Minderhoud IM, Oldenburg B, Wismeijer JA. Henegouwen, Gerard P Van Berge, Smout AJ. IBS-like symptoms in patients with inflammatory bowel disease in remission; relationships with quality of life and coping behavior. Dig Dis Sci 2004;49:469–74.
[24] Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. BMJ 1989;298:82–6.
[25] Matts SG. The value of rectal biopsy in the diagnosis of ulcerative colitis. Q J Med 1961;30:393–407.
[26] Zallot C, Peyrin-Biroulet L. Deep remission in inflammatory bowel disease: looking beyond symptoms. Curr Gastroenterol Rep 2013;15:315.
[27] Hibi T, Sameshima Y, Sekiguchi Y, Hisatome Y, Maruyama F, Moriwaki K, et al. Treating ulcerative colitis by Adacolumn therapeutic leucocytapheresis: clinical efficacy and safety based on surveillance of 656 patients in 53 centres in Japan. Dig Liver Dis 2009;41:570–7.
[28] Keohane J, O'Mahony C, O'Mahony L, O'Mahony S, Quigley EM, Shanahan F. Irritable bowel syndrome–type symptoms in patients with inflammatory bowel disease: a real association or reflection of occult inflammation? Am J Gastroenterol 2010;105:1789–94.
[29] Guilarte M, Santos J, de Torres I, Alonso C, Vicario M, Ramos L, et al. Diarrhoea-predominant IBS patients show mast cell activation and hyperplasia in the jejunum. Gut 2007;56:203–9.
[30] Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, et al. Activation of the mucosal immune system in irritable bowel syndrome. Gastroenterology 2002;122: 1778–83.

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Reliability of Symptoms and Endoscopic Findings for Diagnosis of Esophageal Eosinophilia in a Japanese Population

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Key Words
Esophageal eosinophilia · Eosinophilic esophagitis · Endoscopy · Diagnostic utility

Abstract
Background/Aims: The clinical characteristics of esophageal eosinophilia (EE), which is essential for diagnosis of eosinophilic esophagitis (EoE), have not been fully clarified in a Japanese population. The aim of this study was to analyze the reliability of symptoms and endoscopic findings for diagnosing EE in Japanese individuals. ***Methods:*** We prospectively enrolled subjects who complained of esophageal symptoms suggesting EoE and/or those with endoscopic findings of suspected EoE at the outpatient clinics of 12 hospitals. Diagnostic utility was compared between the EE and non-EE groups using logistic regression analysis. ***Results:*** A total of 349 patients, including 319 with symptoms and 30 with no symptoms but endoscopic findings suggesting EoE were enrolled. Of those with symptoms, 8 (2.5%) had EE, and 3 were finally diagnosed with EoE. Of those without symptoms but endoscopic findings, 4 had EE. Among 8 symptomatic patients, 7 had abnormal endoscopic findings suspicious of EoE. Although dysphagia was a major symptom in EE, none of the presenting symptoms was useful for diagnosis of EE. Among the endoscopic findings, linear furrow was the most reliable (OR = 41.583). ***Conclusion:*** EE is uncommon among patients with esophageal symptoms in Japanese individuals. The most useful endoscopic finding for diagnosis of EE was linear furrow, whereas subjective symptoms were not supportive.

Introduction

Eosinophilic esophagitis (EoE) is a chronic inflammatory immune-mediated disease characterized by esophageal dysfunction and eosinophil-predominant infiltration in the esophageal epithelium [1, 2]. EoE has become

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increasingly prevalent over the past decade, especially in Western countries [3, 4]. While the epidemiology of EoE has not been fully evaluated, it appears that the incidence and prevalence of EoE and esophageal eosinophilia (EE) are also increasing in Asian countries, including Japan [5–8]. Pathologically, the hallmark of EoE is EE, commonly defined by more than 15 eosinophils per high power field (HPF) in at least 1 esophageal biopsy specimen. EE is predominantly found in patients with the following clinical conditions: gastroesophageal reflux disease (GERD), EoE, and proton-pump inhibitor-responsive EE (PPI-REE). According to the current clinical consensus and guidelines [9, 10], histological suspicion of EoE should be confirmed by unresponsiveness to high-dose PPI therapy. However, the clinical significance of this diagnostic requirement, based on the response to PPI administration, remains controversial [11–14]. PPIs are reported to have immunosuppressive effects and improve the inflammatory process in patients with EoE [15, 16]. Therefore, at present, pathological identification of EE is considered to be the most important and critical step for diagnosis of EoE.

Multiple studies have found that EoE is 3–4 times more common in men than women, and affected individuals are more likely to be Caucasian than other racial groups [17, 18]. Moreover, race may influence the clinical presentation and have a role in the phenotypic expression of EoE. As compared with Caucasian patients, African-Americans are less likely to have typical symptoms, such as food impaction and endoscopic findings (concentric rings, strictures) associated with EoE [19, 20]. Even though the variety of clinical features distinguished by racial differences remains controversial [21, 22], it is important to evaluate such features including symptoms and endoscopic findings in Asian populations for usefulness in diagnosis of EoE. Therefore, we sought to investigate the diagnostic utility of EE, which contains the main histological features of EoE, based on symptoms and endoscopic findings in a Japanese population. A multicenter prospective study was performed to determine the most reliable symptom and endoscopic finding for diagnosis of EE in Japanese individuals.

Materials and Methods

Patients

We prospectively enrolled subjects who complained of chest or epigastric symptoms suggesting EoE, such as heartburn, dysphagia, epigastric pain, chest pain, acid regurgitation, food impaction, and vomiting at least once during the last week when esophagogastroduodenoscopy (EGD) was scheduled, and/or those with endoscopic findings of suspected EoE at the outpatient clinics of 12 hospitals in the western part of Japan between August 2011 and August 2012. We used a specific questionnaire to evaluate the frequency and severity of esophageal symptoms for the enrollment, which comprised of the questions including patient demographics (age, sex), concurrent allergic disease, frequency and severity of esophageal symptoms (number of days with episodes during the last 7 days), and information on medications, including PPIs and steroids, prior to each endoscopic examination. With regard to the severity of the symptoms, those with moderate symptoms, which was defined as discomfort sufficient to cause interference with normal activities, or more severe symptoms were enrolled. Those less than 15 years of age who received glucocorticoid administration and had a high risk of bleeding from a biopsy were excluded. The patients who had organic causes of the symptoms, such as endoscopically proven reflux esophagitis, gastroduodenal ulcers, and upper gastrointestinal malignant tumors, were also excluded. Reflux esophagitis was diagnosed when esophageal mucosal breaks of grade A, B, C, or D (Los Angeles classification) were found [23]. The protocol of this study was evaluated and approved by the ethical committee of Shimane University School of Medicine, and written informed consent was obtained from all subjects prior to enrolment.

Endoscopic Assessment and Biopsy Examination

All subjects underwent EGD performed by experienced endoscopists at each medical center. All examinations were done with high-resolution endoscope (GIF-H260 or GIF-H260Z; Olympus Medical Systems Co, Tokyo, Japan). During the endoscopy procedures, findings were recorded in patient charts. Endoscopic findings suspicious of EoE included longitudinal linear furrows, multiple concentric rings (ringed esophagus, corrugated esophagus), whitish exudates, and reddening, as well as others (edema, pallor, decreased vascularity, and mucosal fragility) as previously described [24, 25]. Representative endoscopic images with each finding suspicious of EoE were shown in figure 1. At least 2–4 biopsy samples were taken from the upper and lower esophagus, as well as the area of EoE shown in endoscopic findings as recommended by current clinical guideline [9]. In addition, biopsy samples were taken from the gastric and duodenal mucosa, irrespective of the mucosal appearance, in all the enrolled cases, to exclude eosinophilic gastroenteritis, as that exclusion is essential for diagnosis of EoE [10].

Histological Assessment

Biopsy specimens were fixed in 10% formalin, and samples were stained with hematoxylin and eosin, and then the numbers of eosinophils that infiltrated the esophageal epithelial layer were counted under an Olympus BX50 microscope. Histological diagnosis of EE was defined as the presence of more than 15 eosinophils per HPF discovered in biopsy samples taken with endoscopy. In addition, degree of inflammatory cell infiltration (mild, moderate, or severe), presence or absence of basal layer hyperplasia and dilated intracellular spaces, were also evaluated in cases with EE, according to the consensus guidelines for the recognition and assessment of microscopic lesion related to GERD [26, 27]. All biopsies were reviewed by an experienced team of pathologists in the Pathology Department of Shimane University Hospital.

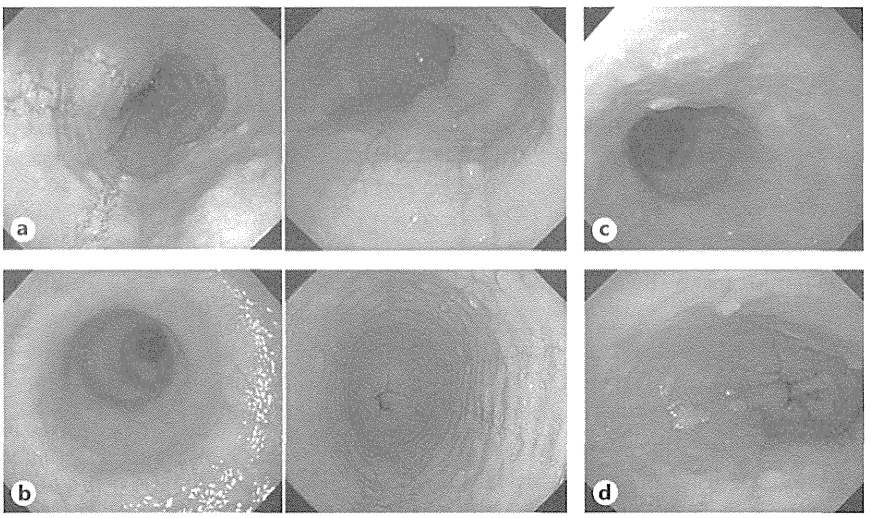

Fig. 1. Representative endoscopic images suspicious of EoE. **a** Linear furrows. **b** Rings including ringed esophagus (left) and corrugated esophagus (right). **c** Whitish exudate. **d** Reddening.

Treatment and Definition of PPI Response

Standard dose of PPI was prescribed for 4–8 weeks to symptomatic patients who were able to take them. Their symptoms, endoscopic findings, and histological abnormalities were reevaluated after the treatment with PPI. Positive response to PPI was defined as a case in which administration of PPI improved symptoms and intraepithelial eosinophilic infiltration (<5/HPF). PPI-resistant cases with EE were defined as cases with EoE.

Statistical Analysis

Fisher's exact probability test was used to compare 2 variables. The diagnostic utility of subjective symptoms and endoscopic findings was compared between the EE and non-EE groups using logistic regression analysis. All tests of significance were two-tailed, and p values less than 0.05 were considered to be significant. All analyses were done using SPSS 18.0 (IBM SPSS Japan Inc., Tokyo, Japan).

Results

Baseline Characteristics of Enrolled Patients

During the study period of 13 months, EGD was performed in 17,324 patients at 12 medical centers, of whom 349 (163 men, 186 women; mean age 60.6 years) were enrolled in this study (fig. 2). Thirty-nine percent of enrolled subjects continued to take PPI when EGD was scheduled. Of the 349 enrolled patients, 319 complained of chest and/or epigastric symptoms suggesting EoE (symptomatic group, group 1), while 30 had no symptoms along with endoscopic findings suggesting EoE (asymptomatic group, group 2). We next subdivided group 1 into 2 groups; group 1a (n = 30), composed of patients with both symptoms and endoscopic findings suggesting EoE, and group 1b (n = 289), who had symptoms but no endoscopic findings suggesting EoE (fig. 1). Twelve patients with EE were identified in this study, and 3 patients (No. 5, 7, 8) were finally diagnosed with EoE after PPI trial (table 1). Of 3 patients with EoE, 2 were treated by fluticasone swallowing and improved their symptoms and endoscopic findings, while 3 patients (No. 1, 4, 12) were responsive to PPI and diagnosed with PPI-REE. Other 6 patients were not treated by PPI because of mild or no symptom, drug allergy, and lactation. Baseline characteristics between EE-positive and -negative subjects are shown in table 2. The mean age of these 12 patients (7 men, 5 women) was 49.3 years, and significantly younger than EE negative patients (mean age; 62.9, $p < 0.05$). All of 3 patients with EoE had dysphagia, while none of 3 patients with PPI-REE had that, though the number was too small to compare statistically. As for histological findings, basal layer hyperplasia, dilated intracellular spaces, and mild to severe inflammatory infiltration (mainly lymphocyte infiltration) were found in all EE cases, and no histological findings independently distinguished EoE from EE patients (table 1). No cases with eosinophilic gastroenteritis were found in the enrolled subjects.

Symptoms Not Useful for Predicting EE

In the symptomatic group (group 1), 8 patients (2.5%) were finally diagnosed with EE. A total of 497 symptoms were reported in 319 patients, as shown in figure 3. Of the 8 patients with EE, 5 complained of dysphagia, 4 of heartburn, and 1 had both symptoms. None of the patients had a history of food impaction. Among the symptoms examined, dysphagia tended to be more common

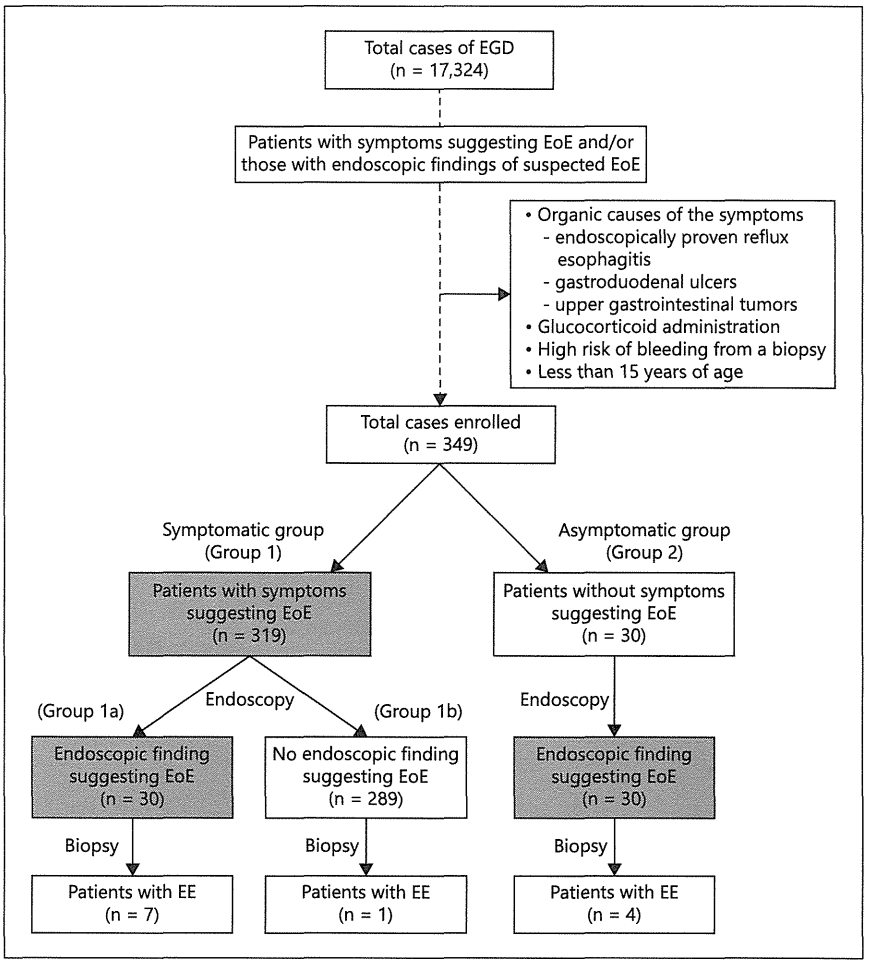

Fig. 2. Flow diagram delineating enrolled patients for diagnosis of EE.

Table 1. Clinical characteristics of 12 patients with EE

No.	Age	Sex	Allergy	Symptoms				Endoscopic findings					Histology		PPI response
				heart-burn	dysphagia	epigastric pain	chest pain	linear furrows	rings	whitish exudates	reddening	others	eosinophil/ HPF	lymphocyte infiltration	
1	33	M	–	+	–	+	–	+	–	+	–	–	48	moderate	Yes
2	26	F	–	+	–	–	–	+	–	+	–	–	95	moderate	NT
3	78	F	–	–	+	–	–	–	–	–	–	+ decreased vascularity	44	moderate	NT
4	51	M	–	+	–	–	–	+	+	–	–	–	38	moderate	Yes
5	78	M	–	–	+	–	–	+	–	–	+	+ edema	46	moderate	No
6	67	M	–	–	+	–	–	+	–	–	+	–	41	mild	NT
7	32	F	+	–	+	–	–	+	–	+	–	–	>20	mild	No
8	24	F	+	+	+	–	+	–	–	–	–	–	18	severe	No
9	42	M	–	–	–	–	–	+	+	+	–	–	168	moderate	NT
10	82	F	–	–	–	–	–	+	–	–	–	–	25	mild	NT
11	29	M	–	–	–	–	–	+	–	–	–	–	25	mild	NT
12	49	M	+	–	–	–	–	+	–	+	–	–	86	mild	Yes

Patients 5, 7, 8 were finally diagnosed with EoE after PPI trial. NT = Not treated.

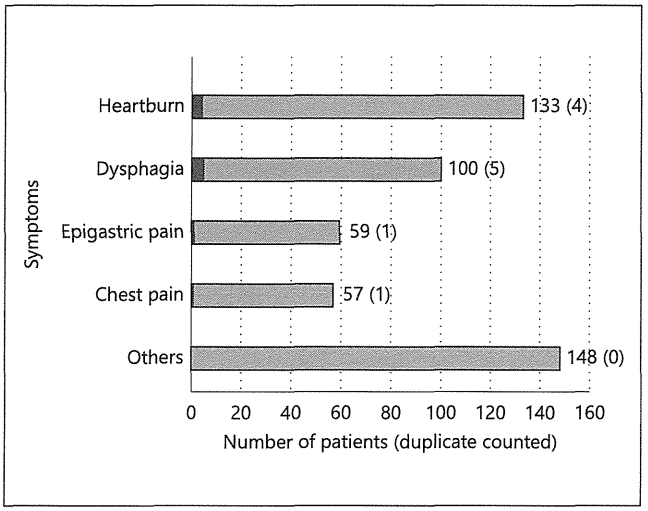

Fig. 3. Summary of symptoms. Symptoms noted in the enrolled patients (symptomatic group; n = 319) are shown as a bar chart (497 symptoms, duplicates counted). Others included acid regurgitation, nausea, and vomiting. Patients with EE are shown as a closed bar in each column.

Table 2. Baseline characteristics between EE-positive and EE-negative subjects

	EE positive	EE negative
Total subjects	12	337
Men:women	7:5	156:181
Age, years		
Mean ± SD	49.3±21.8	62.9±14.9
Range	24–82	22–88
Concurrent allergic disease (duplicates counted)		
Asthma	2	6
Atopic dermatitis	1	8
Hay fever	1	11
Food allergy	1	5
Others	1	11

Table 3. Diagnostic utility for EE by presenting symptoms

Symptoms	b	Exp (b)	Exp (b) 95% CI	p value
Dysphagia	0.384	1.469	8.796–983.727	0.566
Heartburn	−0.317	0.729	0.192–13.509	0.657
Epigastric pain	−0.498	0.608	0.875–289.243	0.641
Chest pain	−17.814	0.000	0.851–28.0	0.997

b = Partial regression coefficient; Exp (b) = odds ratio; CI = confidence interval.

in patients with EE, though the difference was not significant (62.5 vs. 30.4%, p = 0.054). Indeed, the patients with EE accounted for only 5% (5/100) of all patients who complained of dysphagia. Although present in some of the EE patients, the ratios of those with heartburn, epigastric pain, and chest pain were also not significantly different from those among non-EE patients (n = 311). Next, we examined 4 major symptoms (heartburn, dysphagia, epigastric pain, chest pain) for usefulness in diagnosis of EE. The partial regression coefficient value for all of the items was <1, indicating that none of the presenting symptoms was useful for EE diagnosis (table 3).

Endoscopic Findings More Important Than Symptoms to Predict EE

Of the patients with symptoms suggesting EoE (group 1), 30 had abnormal endoscopic findings suspicious of EoE (group 1a), while 289 had no such endoscopic findings (group 1b). Interestingly, 7 patients in group 1a (23.3%; 7/30) were diagnosed with EE, while 1 patient was diagnosed as EE in group 1b (0.35%; 1/289). Therefore, the presence of abnormal endoscopic findings was significantly more important to predict EE in symptomatic patients. In other words, the frequency of EE was quite low in patients with symptoms but no endoscopic findings. Moreover, 4 patients among asymptomatic patients with abnormal endoscopic findings (group 2; 13.3%; 4/30) were diagnosed with EE, suggesting the importance of endoscopic findings to predict EE.

Presence of Linear Furrows Was the Most Reliable for Diagnosis of EE

Among all 349 patients examined, 60 had typical endoscopic findings of EoE including linear furrows (n = 30), whitish exudates (n = 23), multiple concentric rings (n = 13), and reddening (n = 8), with some overlap (fig. 4). Patients with endoscopic findings suspicious of EoE consisted of both symptomatic (n = 30, group 1a) and asymptomatic (n = 30, group 2) patients. Eleven (18.3%) of 60 patients with endoscopic findings were diagnosed as EE, and linear furrows were seen in 10 (90.9%), while other findings were not so frequent (table 1). Overall, 33.3% (10/30) of the patients with linear furrows were histologically diagnosed with EE.

Next, we examined 5 major endoscopic findings (linear furrows, multiple concentric rings, whitish exudates, reddening, others) to examine their diagnostic utility for EE. Linear furrows were the most reliable, as

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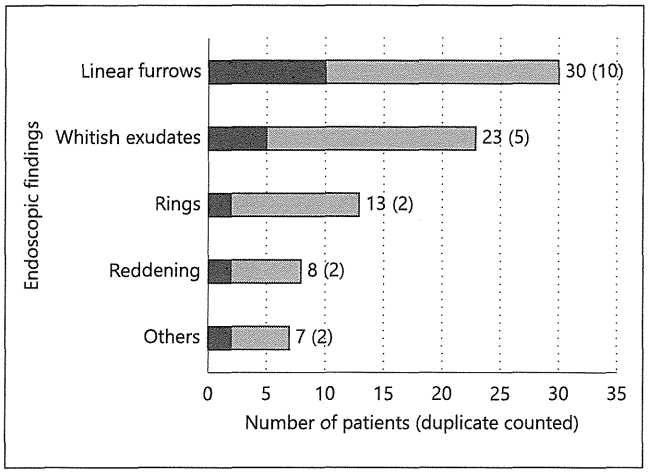

Fig. 4. Summary of endoscopic findings. Endoscopic findings suspicious of EoE in enrolled patients are shown as a bar chart. Others included edema, pallor, and decreased vascularity. Patients with EE are shown as a closed bar in each column.

Table 4. Diagnostic utility for EE by endoscopic findings

Endoscopic findings	b	Exp (b)	Exp (b) 95% CI	p value
Linear furrows	3.728	41.583	2.936–588.879	0.006
Rings	0.076	1.079	0.139–8.360	0.942
Whitish exudates	1.355	3.876	0.704–21.348	0.120
Reddening	1.751	5.763	0.375–88.660	0.209
Others	0.890	2.435	0.224–26.523	0.465

shown by partial regression coefficient analysis (table 4), with an odds ratio of 41.583, which was the only statistically significant finding ($p = 0.006$). The probability of correctly diagnosing EE based on the presence of linear furrows was 87.3%. However, the sensitivity for linear furrows was modest at 83%, whereas specificity was 95%. Furthermore, the positive predictive value was 37% and the negative predictive value was 99% (table 5).

Discussion

This is the first reported investigation comparing the diagnostic utility of symptoms and endoscopic findings for EE in a Japanese population. We conducted the present multicenter prospective study of 349 patients taken from biopsy samples because of suspicious symptoms and/or endoscopic abnormalities. Symptoms suggesting esophageal dysfunction were noted in 319 cases and abnormal endoscopic findings were found in 60. Our findings showed that the prevalence of EE was 2.5% (8/319), and 5% (5/100) for patients with esophageal symptoms, and dysphagia, respectively. Of 8 patients with EE, 3 were finally diagnosed with EoE after PPI trial. The recent study conducted in USA by Dellon et al. [28] has shown that EE was found in 38% (66/173) of patients with dysphagia. In that study, 40 of 66 patients with EE were confirmed to have EoE and 24 had PPI-REE after PPI trial. Consistent with recent findings [29], no clinical or endoscopic feature independently distinguished PPI-REE from EoE before the PPI trial. In addition, there were no differences between the 2 patient groups for histological findings including amount of eosinophil infiltration and degree of inflammatory cell infiltration in this study. The prevalence of EE may be affected by the proportion of GERD patients in enrolled patients. Although patients with endoscopically proven reflux esophagitis were excluded, most of symptomatic GERD patients could be enrolled in this study. Indeed, 38.1% (133/349) of the patients had heartburn and 39.0% continued to take PPI when EGD was scheduled, while only patients with dysphagia were enrolled in the study by Dellon et al. [28]. Nonetheless, our data indicate that both EE and EoE are uncommon among patients with chest or epigastric symptoms in a Japanese population as compared with Western populations.

As for clinical features, the most common symptom among Japanese patients with EoE is dysphagia, and none of the patients in our previous study had a history of food impaction [7], a common symptom associated with EoE in Western individuals [18], especially Caucasians, suggesting racial differences with regard to EoE-related symptoms. Dysphagia is consistently the most common symptom reported by patients with EE. Although the ratio of dysphagia was higher in our patients with EE (62.5%) than in those without EE (30.4%), subjective symptoms including dysphagia, heartburn, and chest pain were not specific enough to make a diagnosis of EE, which was shown by logistic regression analysis.

A strength of this study is that an esophageal biopsy was performed in all of the enrolled patients with symptoms suggesting esophageal dysfunction with or without endoscopic abnormalities ($n = 319$). Interestingly, only a single patient (0.35%) was diagnosed with EE among those with normal endoscopy findings, as compared with 18.3% (11/60) of the patients with abnormal findings.

Table 5. Sensitivity, specificity, and predictive value of endoscopic findings

	Linear furrows	Rings	Whitish exudates	Reddening
Sensitivity	83 (75–91)	17 (4–38)	42 (14–70)	17 (4–38)
Specificity	95 (93–97)	97 (95–99)	95 (93–97)	98 (97–99)
PPV	37 (28–46)	15 (11–19)	22 (6–38)	25 (–5 to 55)
NPV	99 (98–100)	98 (96–100)	98 (96–100)	97 (95–99)

Figures indicate percentages (95% CI). PPV = Positive predictive value; NPV = negative predictive value.

Consistent with our results, Mackenzie et al. [30] prospectively assessed the risk factors and prevalence of EoE in an adult population with dysphagia. Of 261 patients with dysphagia, 31 (12%) met the pathological criteria for EE, while EE was found only in 5 cases (1.9%) without suspicious endoscopic findings. These findings contradict the routine esophageal biopsies for the purpose of detecting EE in patients without abnormal endoscopic findings suggesting EoE. An esophageal biopsy procedure may not be useful or cost-effective to determine EoE in symptomatic patients without abnormal endoscopic findings. However, in patients with abnormal endoscopic findings suspicious of EoE, irrespective of symptoms, biopsy samples should be taken from the esophagus to determine the presence of EE.

Endoscopic abnormalities in patients with EoE can vary within a wide range, including esophageal rings, linear furrows, strictures, and whitish exudates [24, 31]. There may also be racial differences in EoE-related endoscopic findings [19, 20]. In the present study, only 2 (16.7%) of the patients with EE had esophageal rings and none had esophageal strictures. Consistently, we previously confirmed that rings and strictures were not frequent in patients with EE or EoE in a Japanese population, in contrast to Western populations [25]. In addition, the present study revealed that linear furrows were the most frequent endoscopic findings in patients with EE as they were found in 83.3% (10/12), while only 1 (8.3%) of the patients with EE had no characteristic endoscopic finding. In our previous report, approximately 40% of patients with EoE had no specific endoscopic findings [7]. These differences may be related to not only study design but also awareness of the disease among Japanese endoscopists, as it has been widely reported. According to a recent meta-analysis, prospective studies showed that at least 1 abnormality was detected by endoscopy in 93% of EoE patients [25]. Therefore, endoscopic findings suspicious of EE, especially linear furrows, can be detected in most patients with EE by an experienced endoscopist with careful observation using a high-resolution or narrow-band imaging endoscopy [32].

Among the various endoscopic findings noted in the present study, linear furrows were the most useful for diagnosis of EE, as shown by logistic regression analysis. A previous pooled analysis of several studies showed modest sensitivity for EoE, such as 48% for linear furrows, 44% for corrugated rings, and 27% for whitish exudates [25], whereas sensitivities for EE in the present study were found to be 83, 17, and 42%, respectively. These suggest that the endoscopic finding of linear furrows is the most important for detection of EE in Japanese individuals. Recently, Hori et al. [33] investigated the diagnostic utility of endoscopic features for EE. Although the numbers of cases of EE (n = 5) were lower as compared to our study, the diagnostic utility of linear furrows and corrugated rings for EE was found to be superior to white exudates. Importantly, the results of interobserver agreement in a study of endoscopic findings of EoE indicated that gastroenterologists identified rings ($\kappa = 0.56$) and furrows ($\kappa = 0.48$) with fair to good reliability, whereas they did not reliably identify white exudate ($\kappa = 0.29$) by white-light endoscopy and narrow-band imaging endoscopy [34].

Here, we focused on patients with EE, which is essential for diagnosis of EoE. If dense eosinophilic infiltration is found in esophageal epithelium, EoE, GERD, and PPI-REE are the most common clinical possibilities. Recent clinical guidelines strongly recommend a PPI trial for such patients, and patients with persistent eosinophilic infiltration and symptoms after such a trial can be formally diagnosed with EoE [9, 10]. However, the appropriateness of this strategy for diagnosis of EoE remains to be elucidated. Gastric acid might play a role in the pathogenesis of EoE, and PPIs are effective in some cases via decreasing esophageal acid exposure [12, 35, 36]. Moreover,

a number of potential anti-inflammatory effects of PPIs have been described [37], suggesting that those drugs have anti-inflammatory actions independent of their effects on gastric acid secretion [15, 16]. Thus, EoE patients might benefit from PPI therapy regardless of whether they have coexisting GERD. Additional studies are sorely needed to recognize, define, and mechanistically understand PPI-REE [11, 38]. Nonetheless, long-term clinical outcome in patients with EE should be clarified in the future study.

In summary, EE remains a rare condition among Japanese patients with chest and epigastric symptoms. Reported symptoms including dysphagia do not lend support to a diagnosis of EE in Japanese cases. As for endoscopic findings, the presence of linear furrows was the most frequent and useful for EE diagnosis.

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Disclosure Statement

The authors have no conflict of interest to declare.

References

1 Dellon ES: Diagnosis and management of eosinophilic esophagitis. Clin Gastroenterol Hepatol 2012;10:1066–1078.

2 Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME: Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology 2007;133:1342–1363.

3 Prasad GA, Alexander JA, Schleck CD, Zinsmeister AR, Smyrk TC, Elias RM, Locke GR 3rd, Talley NJ: Epidemiology of eosinophilic esophagitis over three decades in Olmsted county, Minnesota. Clin Gastroenterol Hepatol 2009;7:1055–1061.

4 Sealock RJ, Rendon G, El-Serag HB: Systematic review: the epidemiology of eosinophilic oesophagitis in adults. Aliment Pharmacol Ther 2010;32:712–719.

5 Fujishiro H, Amano Y, Kushiyama Y, Ishihara S, Kinoshita Y: Eosinophilic esophagitis investigated by upper gastrointestinal endoscopy in Japanese patients. J Gastroenterol 2011; 46:1142–1144.

6 Joo MK, Park JJ, Kim SH, Kim KH, Jung W, Yun JW, Lee BJ, Kim JH, Yeon JE, Kim JS, Byun KS, Lee SW, Bak YT: Prevalence and endoscopic features of eosinophilic esophagitis in patients with esophageal or upper gastrointestinal symptoms. J Dig Dis 2012;13:296–303.

7 Kinoshita Y, Furuta K, Ishimura N, Ishihara S, Sato S, Maruyama R, Ohara S, Matsumoto T, Sakamoto C, Matsui T, Ishikawa S, Chiba T: Clinical characteristics of Japanese patients with eosinophilic esophagitis and eosinophilic gastroenteritis. J Gastroenterol 2013;48: 333–339.

8 Shi YN, Sun SJ, Xiong LS, Cao QH, Cui Y, Chen MH: Prevalence, clinical manifestations and endoscopic features of eosinophilic esophagitis: a pathological review in China. J Dig Dis 2012;13:304–309.

9 Dellon ES, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA; American College of Gastroenterology: ACG clinical guideline: evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). Am J Gastroenterol 2013;108:679–692.

10 Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, Burks AW, Chehade M, Collins MH, Dellon ES, Dohil R, Falk GW, Gonsalves N, Gupta SK, Katzka DA, Lucendo AJ, Markowitz JE, Noel RJ, Odze RD, Putnam PE, Richter JE, Romero Y, Ruchelli E, Sampson HA, Schoepfer A, Shaheen NJ, Sicherer SH, Spechler S, Spergel JM, Straumann A, Wershil BK, Rothenberg ME, Aceves SS: Eosinophilic esophagitis: updated consensus recommendations for children and adults. J Allergy Clin Immunol 2011;128:3–20.

11 Cheng E: Proton pump inhibitors for eosinophilic oesophagitis. Curr Opin Gastroenterol 2013;29:416–420.

12 Molina-Infante J, Katzka DA, Gisbert JP: Review article: proton pump inhibitor therapy for suspected eosinophilic oesophagitis. Aliment Pharmacol Ther 2013;37:1157–1164.

13 Molina-Infante J, Zamorano J: Distinguishing eosinophilic esophagitis from gastroesophageal reflux disease upon PPI refractoriness: what about PPI-responsive esophageal eosinophilia? Digestion 2012;85:210.

14 von Arnim U, Wex T, Rohl FW, Neumann H, Kuster D, Weigt J, Monkemuller K, Malfertheiner P: Identification of clinical and laboratory markers for predicting eosinophilic esophagitis in adults. Digestion 2011;84:323–327.

15 Cheng E, Zhang X, Huo X, Yu C, Zhang Q, Wang DH, Spechler SJ, Souza RF: Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. Gut 2013;62: 824–832.

16 Zhang X, Cheng E, Huo X, Yu C, Zhang Q, Pham TH, Wang DH, Spechler SJ, Souza RF: Omeprazole blocks STAT6 binding to the eotaxin-3 promoter in eosinophilic esophagitis cells. PLoS One 2012;7:e50037.

17 Spergel JM, Brown-Whitehorn TF, Beausoleil JL, Franciosi J, Shuker M, Verma R, Liacouras CA: 14 years of eosinophilic esophagitis: clinical features and prognosis. J Pediatr Gastroenterol Nutr 2009;48:30–36.

18 Straumann A, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU: Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. Gastroenterology 2003;125:1660–1669.

19 Bohm M, Malik Z, Sebastiano C, Thomas R, Gaughan J, Kelsen S, Richter JE: Mucosal eosinophilia: prevalence and racial/ethnic differences in symptoms and endoscopic findings in adults over 10 years in an urban hospital. J Clin Gastroenterol 2012;46:567–574.

20 Sperry SL, Woosley JT, Shaheen NJ, Dellon ES: Influence of race and gender on the presentation of eosinophilic esophagitis. Am J Gastroenterol 2012;107:215–221.

21 Dellon ES, Gibbs WB, Fritchie KJ, Rubinas TC, Wilson LA, Woosley JT, Shaheen NJ: Clinical, endoscopic, and histologic findings distinguish eosinophilic esophagitis from gastroesophageal reflux disease. Clin Gastroenterol Hepatol 2009;7:1305–1313.

22 Kinoshita Y, Furuta K, Ishimura N, Ishihara S: Elevated plasma cytokines in Japanese patients with eosinophilic esophagitis and gastroenteritis. Digestion 2012;86:238–243.

23 Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, Johnson F, Hongo M, Richter JE, Spechler SJ, Tytgat GN, Wallin L: Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. Gut 1999;45:172–180.

24 Hirano I, Moy N, Heckman MG, Thomas CS, Gonsalves N, Achem SR: Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. Gut 2013;62:489–495.

25 Kim HP, Vance RB, Shaheen NJ, Dellon ES: The prevalence and diagnostic utility of endoscopic features of eosinophilic esophagitis: a meta-analysis. Clin Gastroenterol Hepatol 2012;10:988–996.

26 Yerian L, Fiocca R, Mastracci L, Riddell R, Vieth M, Sharma P, Franzen S, Fernstrom P, Ruth M: Refinement and reproducibility of histologic criteria for the assessment of microscopic lesions in patients with gastroesophageal reflux disease: the Esohisto Project. Dig Dis Sci 2011;56:2656–2665.

27 Fiocca R, Mastracci L, Riddell R, Takubo K, Vieth M, Yerian L, Sharma P, Fernstrom P, Ruth M: Development of consensus guidelines for the histologic recognition of microscopic esophagitis in patients with gastroesophageal reflux disease: the Esohisto Project. Hum Pathol 2010;41:223–231.

28 Dellon ES, Speck O, Woodward K, Gebhart JH, Madanick RD, Levinson S, Fritchie KJ, Woosley JT, Shaheen NJ: Clinical and endoscopic characteristics do not reliably differentiate PPI-responsive esophageal eosinophilia and eosinophilic esophagitis in patients undergoing upper endoscopy: a prospective cohort study. Am J Gastroenterol 2013;108:1854–1860.

29 Moawad FJ, Schoepfer AM, Safroneeva E, Ally MR, Chen YJ, Maydonovitch CL, Wong RK: Eosinophilic oesophagitis and proton pump inhibitor-responsive oesophageal eosinophilia have similar clinical, endoscopic and histological findings. Aliment Pharmacol Ther 2014;39:603–608.

30 Mackenzie SH, Go M, Chadwick B, Thomas K, Fang J, Kuwada S, Lamphier S, Hilden K, Peterson K: Eosinophilic oesophagitis in patients presenting with dysphagia-a prospective analysis. Aliment Pharmacol Ther 2008;28:1140–1146.

31 Muller S, Puhl S, Vieth M, Stolte M: Analysis of symptoms and endoscopic findings in 117 patients with histological diagnoses of eosinophilic esophagitis. Endoscopy 2007;39:339–344.

32 Tanaka K, Rubio CA, Dlugosz A, Truskaite K, Befrits R, Lindberg G, Schmidt PT: Narrow-band imaging magnifying endoscopy in adult patients with eosinophilic esophagitis/esophageal eosinophilia and lymphocytic esophagitis. Gastrointest Endosc 2013;78:659–664.

33 Hori K, Watari J, Fukui H, Tanaka J, Tomita T, Sakurai J, Kondo T, Oshima T, Toyoshima F, Yamasaki T, Okugawa T, Miwa H: Do endoscopic features suggesting eosinophilic esophagitis represent histological eosinophilia? Dig Endosc 2014;26:156–163.

34 Peery AF, Cao H, Dominik R, Shaheen NJ, Dellon ES: Variable reliability of endoscopic findings with white-light and narrow-band imaging for patients with suspected eosinophilic esophagitis. Clin Gastroenterol Hepatol 2011;9:475–480.

35 Blanchard C, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, Buckmeier BK, Jameson SC, Greenberg A, Kaul A, Franciosi JP, Kushner JP, Martin LJ, Putnam PE, Abonia JP, Wells SI, Rothenberg ME: Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. J Immunol 2010;184:4033–4041.

36 Molina-Infante J, Ferrando-Lamana L, Ripoll C, Hernandez-Alonso M, Mateos JM, Fernandez-Bermejo M, Duenas C, Fernandez-Gonzalez N, Quintana EM, Gonzalez-Nunez MA: Esophageal eosinophilic infiltration responds to proton pump inhibition in most adults. Clin Gastroenterol Hepatol 2011;9:110–117.

37 Kedika RR, Souza RF, Spechler SJ: Potential anti-inflammatory effects of proton pump inhibitors: a review and discussion of the clinical implications. Dig Dis Sci 2009;54:2312–2317.

38 Hirano I: Should patients with suspected eosinophilic esophagitis undergo a therapeutic trial of proton pump inhibition? Am J Gastroenterol 2013;108:373–375.

ORIGINAL ARTICLE

Apoptotic Cells Ameliorate Chronic Intestinal Inflammation by Enhancing Regulatory B-cell Function

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Abstract: Apoptosis is a programmed physiological death of unwanted cells, and handling of apoptotic cells (ACs) is thought to have profound effects on immune-mediated disorders. However, there is scant information regarding the role of ACs in intestinal inflammation, in which immune homeostasis is a major concern. To investigate this, we injected ACs into a severe combined immunodeficiency adoptive transfer model of chronic colitis in the presence and absence of cotransferred whole B or regulatory B cell (Breg)-depleted B cells. We also injected syngeneic ACs into AKR/N mice as a control and into milk fat globule–epidermal growth factor 8 knockout mice deficient of phagocytic function. Chronic colitis severity was significantly reduced in the AC as opposed to the phosphate-buffered saline group with cotransferred whole B cells. The AC-mediated effect was lost in the absence of B cells or presence of Breg-depleted B cells. In addition, ACs induced splenic B cells to secrete significantly increased levels of interleukin 10 in AKR/N mice but not milk fat globule–epidermal growth factor 8 knockout mice. Apoptotic leukocytes were induced by reactive oxygen species during granulocyte/monocyte apheresis therapy in rabbits and H_2O_2-induced apoptotic neutrophils ameliorated mice colitis. Our results indicate that ACs are protective only in the presence of B cells and phagocytosis of ACs induced interleukin 10 producing Bregs. Thus, the ameliorative effect seen in this study might have been exerted by AC-induced Bregs through increased production of the immunosuppressive cytokine interleukin 10, whereas an AC-mediated effect may contribute to the anti-inflammatory effect of granulocyte/monocyte apheresis as a novel therapeutic mechanism for inflammatory bowel disease.

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Key Words: apoptotic cells, regulatory B cells, IL-10, phagocytosis, inflammatory bowel disease

Apoptotic cell (AC) death is a highly controlled means of eliminating dangerous, damaged, or unnecessary cells without causing an inflammatory response or tissue damage.[1,2] In recent years, a number of studies have demonstrated that ACs are not inert and can significantly influence the immune system,[3,4] as exposure to ACs can induce suppression of immunity through engulfment of dead cells by dendritic cells.[5–7] However, decreased phagocytosis of ACs contributes significantly to the development of systemic lupus erythematosus in mice and humans.[8] Thus, immune response to ACs is largely dependent on the capability of handling these cells by the host immune system.

AC-dependent immunosuppression is generated by several mechanisms including production of immunosuppressive cytokines by phagocytes,[9] deletion of T cells,[10] induction of regulatory B cells (Bregs),[11] and activation of $CD8^+$ regulatory T cells (Tregs).[5] ACs were shown to protect mice from autoimmune-mediated inflammation[12,13] and induce B cells to adopt an interleukin (IL)-10–secreting Breg phenotype.[11] Transforming growth factor beta and IL-10 are the most notable cytokines among the several soluble effectors reported to be involved in immunosuppression by ACs.[9,14] Despite these findings, the key cellular and molecular mechanisms that promote tolerance have yet to be characterized.

Ulcerative colitis and Crohn's disease, 2 major forms of human inflammatory bowel disease (IBD), are characterized by chronic immune-mediated disorders and affected individuals experience relapsing episodes of abdominal pain, diarrhea, melena, and weight loss.[15] Although there is increasing evidence that genetic, immunological, and environmental factors may be involved in the pathogenesis of IBD, their details remain unclear.[16–20] Current treatment regimens for IBD are based on suppression and control of inflammation using corticosteroids, immune-modulating drugs, and antitumor necrosis factor antibodies.[21] Although such drug therapies targeting inhibition of the inflammatory process may provide better therapeutic options for IBD, numerous studies have been conducted to evaluate innovative approaches. Recently, we

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reported anti-inflammatory roles of Breg in a mouse colitis model,[22] which might lead to a novel therapeutic strategy for IBD. However, methodologies regarding the effective activation or induction of Bregs in vivo remain unknown. Because previous studies revealed the immunosuppressive effects of ACs associated with the function of Bregs, we speculated that ACs may ameliorate intestinal inflammation by controlling that function.

In this study, we initially investigated the immunosuppressive potential of injected apoptotic thymocytes in a colitis model of severe combined immunodeficiency (SCID) mice by adoptive transfer of $CD4^+$ T cells cotransferred with whole or Breg-depleted B cells. Next, we performed milk fat globule-epidermal growth factor 8 knockout (MFG-E8 KO) mice with impaired uptake of ACs[23] and examined whether engulfment of injected ACs regulates the function of Bregs in the anti-inflammatory process. Evaluation of colitis parameters indicated that AC-mediated immunosuppressive effects were generated by induction of an IL-10–producing Breg population, which was dependent on phagocytosis of ACs in the mouse spleen. Finally, we found that apoptosis was induced among circulating leukocytes by granulocyte/monocyte apheresis (GMA) therapy using Adacolumn and confirmed experimentally that this apoptosis-inducing feature might contribute to the anti-inflammatory effects of GMA as a novel therapeutic mechanism for IBD.

MATERIALS AND METHODS

Reagents

We used the following antibodies for flow cytometry: PE-conjugated anti-mouse CD19 (BD Biosciences Pharmingen, San Diego, CA), FITC-conjugated anti-mouse CD1d (BD Biosciences Pharmingen), APC-conjugated anti-mouse CD19 (BD Biosciences Pharmingen), PE-conjugated anti-mouse IL-10 (BD Biosciences Pharmingen), and PE-conjugated anti-mouse CD62L (L-selectin) monoclonal antibody (Beckman Coulter, Brea, CA). We also used anti-mouse CD4 and CD19 microbeads (Miltenyi Biotec Inc., Auburn, CA). For intracellular examinations, GolgiStop (BD Biosciences Pharmingen) was used. Phorbol 12-myristate 13-acetate (PMA) and ionomycin were obtained from Sigma–Aldrich (St Louis, MO). Unmethylated CpG DNA (5′-TGACTGT-GAACGTTCGAGATGA-3′) was synthesized by Hokkaido System Science Co. Ltd. Enzyme immunoassays kits for Mouse IL-10 Immunoassays were obtained from R&D Systems.

Flow Cytometry

The above mouse antibodies were used for flow cytometry analyses as necessary. GolgiStop was added to the medium during the last 5 hours of the culture period for intracellular cytokine staining. Flow cytometry analysis was performed using an FACSAria II (BD Biosciences Pharmingen), FACSCalibur (BD Biosciences Pharmingen), or FACSCan (BD Biosciences Pharmingen).

Mice

SAMP1/Yit (SAMP1) mice were kindly provided by S. Matsumoto (Yakult Central Institute for Microbiological Research, Tokyo, Japan). AKR/N (AKR) mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). AKR mice share a genetic background with SAMP1 mice, and their entire major histocompatibility complex region is identical. SCID mice (CB17/Icr-Prkdc^{scid}/CrlCrlj) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). C57BL/6N mice were purchased from Japan SLC Inc. MFG-E8$^{-/-}$ mice with a C57BL/6 genetic background were obtained from RIKEN BRC. All experiments with animals in this study were approved by the Ethics Committees for Animal Experimentation of Shimane University and JIMRO Co. Ltd., and they were handled according to institutional guidelines.

Generation of Apoptotic Thymocytes

Thymi were removed from 4-week-old AKR mice and teased into single-cell suspensions. They were then cultured at a concentration of 10^7 cells per milliliter in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 10 mM HEPES, 100 U/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen), and 10 μM dexamethasone (Sigma–Aldrich) for 8 hours at 37°C with 5% CO_2. AC death stage was analyzed using a PE Annexin V apoptosis detection kit I (BD Biosciences Pharmingen) with a FACSCalibur (BD Biosciences Pharmingen). After extensive washing, these apoptotic thymocytes were injected in an intravenous manner for in vivo experiments.

Induction of Chronic Colitis in SCID Mice and Apoptotic Cell Coinjection

For this experiment, we used SAMP1 $CD4^+$ mesenteric lymph node (MLN) T cell–mediated chronic colitis model SCID mice previously reported by our group.[22] $CD4^+$ T cells were magnetically isolated from MLNs of SAMP1 mice (30–50 weeks) by positive selection with CD4 microbeads. Isolated $CD4^+$ T cells (5×10^5 cells per mouse) were intraperitoneally injected into SCID mice (8–10 weeks) (day 1) to induce chronic colitis after 6 to 7 weeks. To investigate the protective effects of ACs, dexamethasone induced apoptotic thymocytes (1×10^7 cells per mouse) or phosphate-buffered saline (PBS) (vehicle) were cotransferred intravenously (tail vein) (week 1) into the SAMP1 $CD4^+$ MLN T cell–mediated chronic colitis mouse model.

Sorting of B Cells and Cotransfer Experiments

We recently reported that CD19hiCD1dhi cells, which secrete high levels of IL-10,[22] can be considered as a Breg-rich population. Total splenic $CD19^+$ B cells were magnetically isolated from AKR mice (15–25 weeks) by positive selection with CD19 microbeads. CD19hiCD1dhi-depleted B cells, excluding the Breg population, were sorted from whole splenic $CD19^+$ B cells using an FACS sorting system. To investigate the effects of AC–Bregs interaction in chronic intestinal inflammation, whole or CD19hiCD1dhi-depleted B cells (2×10^6 cells per mouse) were cotransferred intravenously (tail vein) (day 0) into the above T cell–mediated chronic colitis model, followed by intravenous injection of ACs (at week 1). Body weight (BW) changes were monitored weekly using a top loading balance. All mice were