CD8+ Lymphocyte Infiltration is a Specific Feature of Colitis Induced By Immune Checkpoint Inhibitors

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Research

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Abstract

Background

Immune checkpoint inhibitors (ICPIs) have revolutionized cancer therapy, although immune-related adverse events (irAEs) remain a severe issue. The clinical characteristics of colitis induced by ICPIs are very similar to inflammatory bowel disease. Recently, CD8+ lymphocyte infiltration into organs has been associated with the onset of irAEs. The present study compared the histological infiltration of CD8+ lymphocytes in irAE colitis with that in other colitis.

Methods

Among 102 newly diagnosed and untreated patients, 12 with irAE colitis, 37 with ulcerative colitis (UC), 22 with Crohn's disease (CD), and 31 with ischemic colitis (IC) were retrospectively enrolled. Biopsy specimens were obtained from endoscopic areas of high inflammation for immunohistochemical analysis of the number of CD4+ and CD8+ lymphocytes in the most inflamed high-powered microscopic field.

Results

In irAE colitis, CD8+ lymphocyte infiltration was significantly greater than that of CD4+ lymphocytes ($p < 0.01$). The amount of CD8+ lymphocyte infiltration was significantly higher in irAE colitis than in UC ($p < 0.05$), CD ($p < 0.05$), and IC ($p < 0.01$). The CD8+/CD4+ ratio was also significantly higher in irAE colitis ($p < 0.01$ vs. UC, CD, and IC, respectively). The optimal cut-off CD8+/CD4+ ratio for diagnosing irAE colitis was 1.17 (sensitivity: 83%, specificity: 84%). The optimal cut-off the number of CD8+ lymphocytes for diagnosing irAE colitis was 102 cells/high-power field (sensitivity: 75%, specificity: 81%).

Conclusions

Greater CD8+ lymphocyte infiltration and a higher CD8+/CD4+ ratio may be simple and useful biomarkers to distinguish irAE colitis from other forms of colitis.

Introduction

Surgery, chemotherapy, and radiation have been the mainstays of cancer treatment for several decades. Recent new types of cancer therapy, including such immune checkpoint inhibitors (ICPIs) as ipilimumab, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor, and nivolumab, a programmed cell death-1 (PD-1) inhibitor, are becoming more widely used for treating cancer and have shown good results [1–3]. However, these agents can induce a wide spectrum of immune-related adverse events (irAEs) [4–6]. IrAEs are differentiated from the adverse events (AEs) associated with conventional systemic

chemotherapy [7], being defined as AEs with a potential immunologic cause and necessitating frequent monitoring [8–11].

Colitis is one of the most common irAEs, with 30% of patients using ICPIs suffering from diarrhea [12]. Severe irAE colitis may require colectomy and lead to perforations and even death [13–16]. In fact, Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or 4 irAE colitis was present in 27%, 2%, and 24% of patients treated with ipilimumab, nivolumab, or their combination, respectively [17]. The recommended treatment for irAE colitis is steroid therapy. In patients unsatisfactory therapeutic effects, anti-tumor necrosis factor alpha agents are usually administered next [12, 18, 19].

The mechanisms of irAEs associated with ICPIs are gradually being elucidated. IrAEs are presumed to result from the activation of T cells that recognize self-proteins or commensal microorganisms [20]. Moreover, predominantly CD8+ lymphocyte and macrophage infiltration in inflamed organs are typical in irAEs [21–23]. However, the precise etiology of irAE colitis remains to be clarified.

The clinical characteristics of colitis associated with ICPIs are very similar to those of inflammatory bowel disease (IBD), especially ulcerative colitis (UC). Endoscopic findings are also comparable among the diseases (Fig. 1). It is clinically important to distinguish between irAE colitis and other forms of colitis for optimal treatment. This study therefore aimed to determine whether the histological infiltration of CD8+ lymphocytes could be a biomarker for diagnosing irAE colitis.

Methods

Study population

This was a single-center, retrospective analysis of data comprised of consecutive newly diagnosed, untreated colitis patients who underwent endoscopic biopsies from colon mucosa at Shinshu University Hospital, Matsumoto, Japan, from July 2010 to June 2020. Total of 102 patients [12 of irAE colitis, 37 of UC, 22 of Crohn's disease (CD), and 31 of ischemic colitis (IC)] were included in this study. Among the UC patients, 20 had pancolitis, 7 had left-sided colitis, and 10 had proctitis. The CD patients included 8 with colitis and 14 with ileocolitis. Patients who had received immunosuppressive treatment, such as glucocorticoids, biologic therapies, and immunomodulators, were excluded. This study was approved by the ethics committee of Shinshu University Hospital (approval number: 5079). Our institutional review board waived the requirement for informed consent, and patients were provided with the opportunity to opt-out of the study. The study was conducted according to the principles of the Declaration of Helsinki.

Clinical criteria

Patient data including demographic information, clinical notes, endoscopic findings, and laboratory results were collected from medical records and reviewed by a multidisciplinary team consisting of gastroenterologists, radiologists, oncologists, and pathologists to assess for associations and causality in irAE colitis, UC, CD, and IC. The diagnosis of irAE colitis was based on the following criteria: (i) diarrhea,

bloody stool, or abdominal pain while receiving ICPIs or after the cessation of ICPI therapy; (ii) clinical and/or histological exclusion of other causes of these symptoms; and (iii) improvement of symptoms with immunosuppressive therapy. The diagnosis of UC, CD, and IC was made according to the following criteria: (i) typical clinical and pathological findings [24-26]; and (ii) exclusion of other forms of colitis. For each colitis type, infectious etiologies were excluded through microbiological studies, including stool culture, stool ova and parasite examination, Clostridioides difficile toxin screening, and cytomegalovirus DNA testing.

Histological methods

Colonic biopsy samples were obtained from the areas of highest inflammation on colonoscopic examination. Certified gastrointestinal pathologists performed biopsy analysis for all 102 patients. Tissue specimens were fixed in formalin and embedded in paraffin. Serial paraffin sections of 3 μm thickness were cut and stained with hematoxylin-eosin (H&E). Immunohistochemical staining was conducted using an automated slide preparation system (BenchMark ULTRA automated slides stainer, Ventana Medical Systems, Tucson, AZ) for CD4 (clone 1F6; Leica, Germany) and CD8 (clone SP16; Abcam, Cambridge, UK).

Quantification of CD4+ and CD8+ lymphocytes

Immunostained sections were visualized using a BX-51 light microscope (Olympus, Tokyo, Japan). The high-power field (HPF) (x400 magnification) containing the most inflammation as determined by the observer was examined for each case. We counted the number of CD4+ and CD8+ lymphocytes outside of lymphoid follicles and recorded the location of infiltrated lymphocytes in the same field. The number of CD4+ and CD8+ lymphocytes as well as CD8+/CD4+ ratio were compared among the colitis groups. Lymphocyte quantification was carried out by a single observer after training by an experienced pathologist.

Statistical analysis

Statistical analyses were performed using StatFlex version 6 software (Artech Co., Ltd., Osaka, Japan). Mann-Whitney U test was used to compare CD4+ and CD8+ lymphocyte infiltration. For multiple comparisons, a one-way analysis of variance followed by the Dunn test for multiple comparisons was used. Predictive values were calculated employing receiver operating characteristic (ROC) analysis. The Youden index [27] was adopted to estimate optimal cut-off points. Categorical variables were analyzed with multiple comparison tests. A p-value of < 0.05 was considered statistically significant.

Results

Clinical features

Table 1 summarizes the characteristics of patients with irAE colitis. The 12 patients included 9 men and 3 women of a median age of 67 years (range: 57-82 years). Six patients received nivolumab monotherapy, 1 received ipilimumab and 5 received a combination of nivolumab and ipilimumab. ICPIs were used to treat malignant neoplasms, which included melanoma (n = 8), gingival carcinoma (n = 2), pharyngeal carcinoma (n = 1), and renal cell carcinoma (n = 1). CTCAE grade 1 (n = 3), grade 2 (n = 3), and grade 3 (n = 6) were found. The median time to symptom onset was 11 weeks from the first dose, and the median number of doses to the onset of symptoms was 3. Regarding irAE treatment, glucocorticoids were administered in all patients, and biologic therapy (infliximab) was given to 4 patients. Seven patients experienced multiple irAEs, including liver dysfunction (n = 5), hypothyroidism (n = 4), and sclerosing cholangitis (n = 2). As expected, IBD was significantly more prevalent in younger patients as compared with irAE colitis and IC, and C-reactive protein was significantly higher in CD patients (Supplementary Table 1 and 2).

Table 1
Clinical features of patients with irAE colitis

Total (cases)	**12**
Age (years)	
Median (range)	67 (57-82)
Sex (cases)	
Male	9
Female	3
ICPI regimen (cases)	
Nivolumab	6
Ipilimumab	1
Combination	5
Tumor classification (cases)	
Melanoma	8
Gingival carcinoma	2
Pharyngeal carcinoma	1
Renal cell carcinoma	1
CTCAE grade (cases)	
Grade 1	3
Grade 2	3
Grade 3	6
Median time to first onset of symptoms (weeks) (range)	11 (1-42)
Median number of doses to first onset of symptoms (times) (range)	3 (1-20)
Treatment (cases)	
Corticosteroid use at admission	12
Biologic therapy (infliximab) use at admission	4
Types of other irAEs (cases) [a]	

[a] Includes multiple irAEs.

Abbreviations: *CTCAE* Common Terminology Criteria for Adverse Events, *ICPI* immune checkpoint inhibitor, *irAE* immune-related adverse event

Total (cases)	12
Liver dysfunction	5
Hypothyroidism	4
Sclerosing cholangitis	2
None	5

[a] Includes multiple irAEs.

Abbreviations: *CTCAE* Common Terminology Criteria for Adverse Events, *ICPI* immune checkpoint inhibitor, *irAE* immune-related adverse event

Infiltration Of Cd4+ And Cd8+ Lymphocytes

Histological analysis revealed more abundant CD8+ lymphocytes in irAE colitis than in the other types of colitis (Fig. 2). Both CD4+ lymphocytes and CD8+ lymphocytes were predominant in the interstitial tissue. In irAE colitis, the number of CD8+ lymphocytes was significantly higher than that of CD4+ lymphocytes ($p < 0.01$) (Fig. 3A). In contrast, CD4+ lymphocytes were significantly more numerous in IC ($p < 0.05$). CD4+ and CD8+ lymphocyte amounts were comparable in UC and CD. The number of CD4+ lymphocytes was significantly greater in UC and CD than in IC (both $p < 0.01$). No significant differences in infiltrated CD4+ lymphocytes were found between irAE colitis and other colitis forms (Fig. 3B). On the other hand, the number of CD8+ lymphocytes was significantly higher in irAE colitis as compared with UC ($p < 0.05$), CD ($p < 0.05$), and IC ($p < 0.01$) (Fig. 3C).

Cd8+/cd4+ Ratio

The CD8+/CD4+ ratio in irAE colitis was significantly higher than in the other forms of colitis ($p < 0.01$ vs. UC, CD, and IC, respectively), with comparable ratios for UC, CD, and IC (Fig. 4A).

Higher CD8+/CD4+ ratio and higher number of CD8+ lymphocytes predict irAE colitis

We compared the colitis groups using ROC analysis to determine the optimal CD8+/CD4+ cut-off point for diagnosing irAE colitis. The area under the ROC curve was 0.909. Using the Youden index, the optimal CD8+/CD4+ ratio cut-off was 1.17, with a sensitivity of 83% and specificity of 84% (Fig. 4B). Using the same method for CD8+ lymphocyte number, the area under the ROC curve was 0.881 and the optimal cut-off point for the number of CD8+ lymphocytes was 102 cells/HPF, with a sensitivity of 75% and specificity of 81% (Fig. 4B). In comparisons of irAE colitis with UC, CD, and IC to determine the ideal CD8+/CD4+ cut-off point (Supplementary Fig. 1), both CD8+/CD4+ ratio and CD8+ lymphocyte number appeared suitable to diagnose irAE colitis.

T lymphocytes were seen to infiltrate the interstitial tissue preferentially to the epithelium. However, as shown in Fig. 2, lymphocytes were more abundant in the epithelium in irAE colitis as compared with the other forms of colitis. In the epithelium, the number of CD8+ lymphocytes was significantly higher in irAE colitis versus UC ($p < 0.01$), CD ($p < 0.05$), and IC ($p < 0.01$) (Fig. 5A), with no remarkable differences in the number of CD4+ lymphocytes. A similar trend was seen for CD8+ lymphocytes in the interstitial tissue ($p < 0.01$ vs. UC, CD, and IC, respectively) (Fig. 5A). The number of CD4+ lymphocytes was significantly higher in UC and CD than in IC in the interstitial tissue (both $p < 0.01$) (Fig. 5A).

In determining the optimal number of CD8+ lymphocytes in the epithelium to diagnose irAE colitis, the area under the ROC curve was 0.908. According to the Youden index, the optimal cut-off point was 15 cells/HPF, with a sensitivity of 83% and specificity of 87% (Fig. 5B).

Discussion

This study revealed that the number of histologically infiltrating CD8+ lymphocytes and the CD8+/CD4+ ratio were significantly higher in irAE colitis than in UC, CD, and IC. These results suggested that irAE colitis may be easily and accurately diagnosed through the immunohistochemical investigation of biopsy specimens.

IrAE colitis, UC, CD, and IC all share the similar digestive symptoms of diarrhea, bloody stools, and abdominal pain. IrAE colitis and UC in particular have comparable endoscopic images and pathological histology, which often lead to confusion. Moreover, both conditions exhibit a loss of vascular transparency, edematous membrane, and granular mucosa with multiple erosions in endoscopic images (Fig. 1). Several features have been reported distinguish between irAE colitis and UC. UC patients generally have more severe histopathologic abnormalities with increased cryptitis, crypt abscesses, crypt distortion, and basal plasmacytosis, whereas irAE colitis displays significantly more apoptotic bodies [28]. However, the differentiation of UC exacerbation and irAE colitis is extremely difficult in clinical practice [29].

CD8+ lymphocytes have been reported to infiltrate the heart, lung, and liver tissues of irAE-induced myocarditis, pneumonia, and liver injury, respectively [22, 23, 30], in which PD-1 and CTLA4 were considered to be mainly expressed by CD8+ cytotoxic lymphocytes. Immunohistochemically, biopsy specimens of irAE colitis due to CTLA-4 agents were found to have lower CD20+ lymphocyte density than those of UC, with similar CD3+ lymphocyte involvement [28]. From those findings, we hypothesized that cytotoxic CD8+ lymphocyte infiltration was more abundant in irAE colitis than in other forms of colitis. Indeed, infiltrated CD8+ lymphocytes were significantly more numerous in irAE colitis as compared with UC, CD, and IC. In the diagnosis of irAE colitis, only 1 colonic biopsy specimen from an inflamed area may be necessary for assessing CD8+/CD4+ ratio and/or CD 8+ lymphocyte density.

We observed significant differences among the groups in the location of infiltrated CD4+ and CD8+ lymphocytes. In the epithelium, the number of infiltrated CD8+ lymphocytes was significantly higher in irAE colitis. Oble et al. reported that increased intraepithelial lymphocytes and elevated epithelial cell

apoptosis were helpful for diagnosing irAE colitis [31]. They also described a statistically significant increase in CD8+ lymphocytes in both the lamina propria and intraepithelial compartment [31]. Thus, it may be possible to diagnose irAE colitis by the number of infiltrated CD8+ lymphocytes alone.

Lastly, IrAEs are presumed to result from the activation of CD8+ lymphocytes that recognize self-proteins or commensal microorganisms due to the immune collapse of ICPIs [20]. The MHC class I required for CD8+ lymphocyte activation exists systemically, and so CD8+ lymphocytes can infiltrate any organ throughout the body and irAEs may occur body-wide. In this study, 7 of 12 patients with irAE colitis had irAEs in other organs in the form of liver dysfunction, hypothyroidism, and sclerosing cholangitis. Careful attention is warranted for other organ involvement when using ICPIs.

This retrospective study had several limitations. First, there were no comparisons with normal cases. Second, since the endoscopic, pathological, and radiological data were all based on patient charts, there could have been inter-observer variance that affected our results. The selection of the biopsy site and HPF for evaluation were also subjective in nature. Third, although we were able to collect a relatively large sample size, our subgroup analyses were still underpowered. To the best of our knowledge, however, this is the first study to reveal an association of irAE colitis with CD8+ lymphocyte infiltration and/or CD8+/CD4+ ratio.

In conclusion, a high number of infiltrating CD8+ lymphocytes and/or CD8+/CD4+ ratio may be simple and accurate biomarkers to distinguish irAE colitis from other forms of colitis. More comprehensive analyses are required to validate our findings.

Conclusions

In summary, our results suggest that greater CD8+ lymphocyte infiltration and a higher CD8+/CD4+ ratio may be simple and useful biomarkers. Accordingly, assessment of CD8+ lymphocyte infiltration and a CD8+/CD4+ ratio may distinguish irAE colitis from other forms of colitis.

Abbreviations

AE: adverse event; CD: Crohn's disease; CTCAE: Common Terminology Criteria for Adverse Events; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; H&E: hematoxylin-eosin; HPF: high-power field; IBD: inflammatory bowel disease; IC: ischemic colitis; ICPI: immune checkpoint inhibitor; irAE: immune-related adverse event; PD-1: programmed cell death-1; ROC: receiver operating characteristic; UC: ulcerative colitis

Declarations

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Author's contributions

YT, TN, YI, TO, AH, MI, and TU participated in the concept and design of the study, participated in acquisition/collection of data, analysis and interpretation of data, and drafted/revised the manuscript for important intellectual content. TU participated in the analysis and interpretation of the data and drafted/revised the manuscript critically for important intellectual content. All authors approved the final version of the manuscript for submission, including the authorship list.

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Availability of data and materials

The data underlying this article cannot be shared publicly due to (describe why the data cannot be shared, e.g. for the privacy of individuals that participated in the study). The data will be shared on reasonable request to the corresponding author.

Ethics approval and consent to participate

Informed consent was obtained for all participants and the study was approved by the Shinshu University Hospital Ethics Board.

Consent for publication

Not applicable

Competing interests

The authors have declared that no conflict of interest.

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Supplemental Figure

Supplementary Figure 1 is not available with this version.

Figures

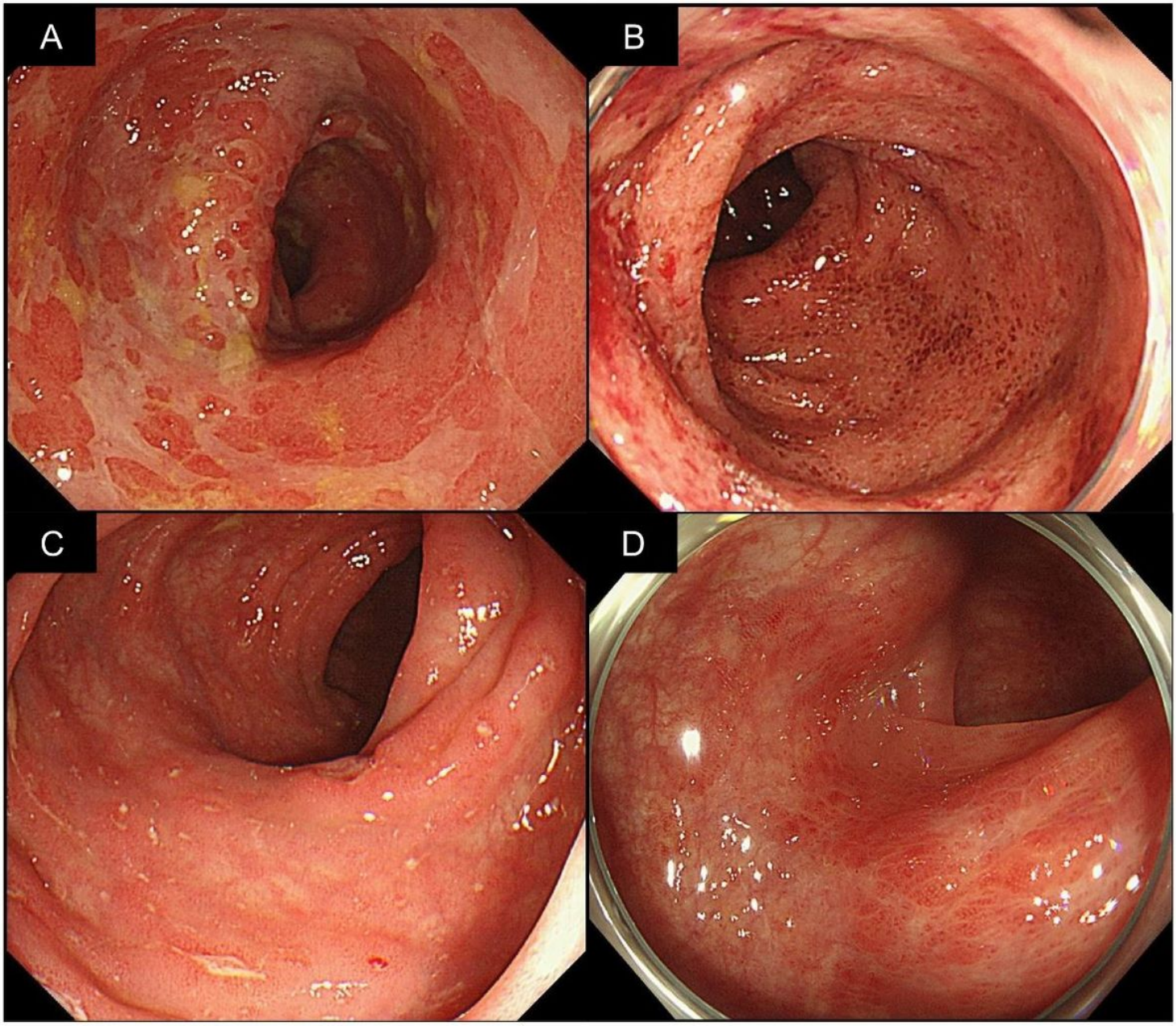

Figure 1

Endoscopic images of irAE colitis, UC, CD, and IC. (A) Endoscopic image of irAE colitis. Shallow ulcers and a reddish mucous membrane are observed. (B) Endoscopic image of UC. Red, rough mucous membrane is evident. (C) Endoscopic image of CD. Many aphthae and small erosions are present. (D) Endoscopic image of IC. Red mucosa and ulcers are noted. All images were taken in the sigmoid colon. CD Crohn's disease, IC ischemic colitis, irAE immune-related adverse event, UC ulcerative colitis

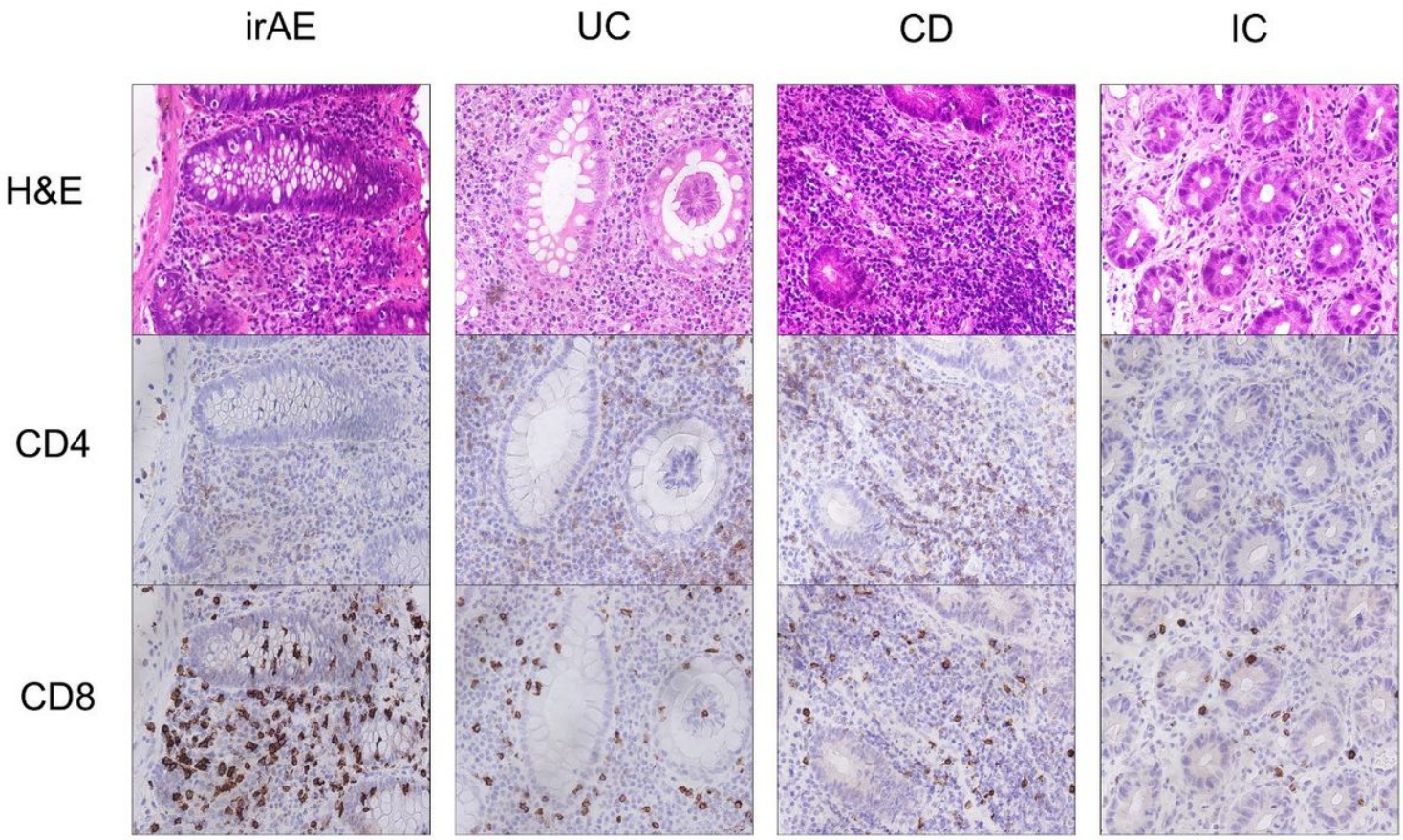

Figure 2

Serial sections from paraffin-embedded biopsies stained with H&E and automated immunohistochemistry. In H&E staining, some degree of inflammatory cells, including lymphocytes, plasma cells, and neutrophils, infiltrated the interstitial sites of the lamina propria in all colitis forms (upper panels). Infiltrated CD4+ lymphocytes appear comparable (middle panels). On the other hand, infiltrated CD8+ lymphocytes are visibly higher in irAE colitis, with greater infiltration in the epithelium as well (lower panels) (x400 magnification). Upper panels are stained with H&E, middle panels are immunostained for CD4, and lower panels are immunostained for CD8. H&E hematoxylin-eosin

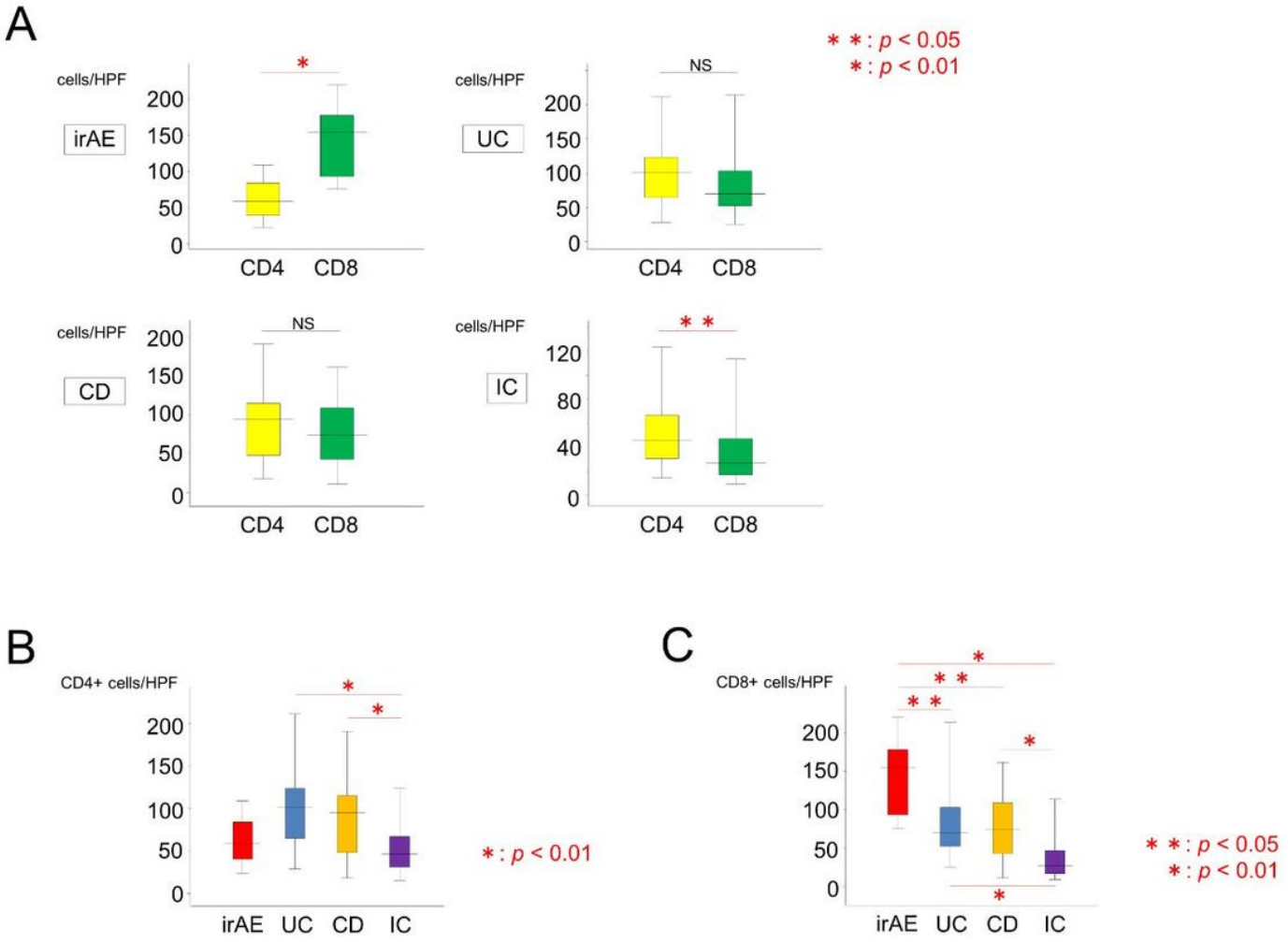

Figure 3

Quantitative comparisons of the number of infiltrated CD4+ and CD8+ lymphocytes. (A) CD8+ lymphocytes were significantly more numerous than CD4+ lymphocytes in irAE colitis. The number of CD4+ lymphocytes was significantly higher than that of CD8+ lymphocytes in IC. (B) The number of CD4+ lymphocytes was significantly greater in UC and CD as compared with in IC. (C) The number of CD8+ lymphocytes was significantly higher in irAE colitis than in UC, CD, and IC. CD8+ lymphocyte number was significantly higher in UC and CD than in IC. HPF high-power field, NS not significant

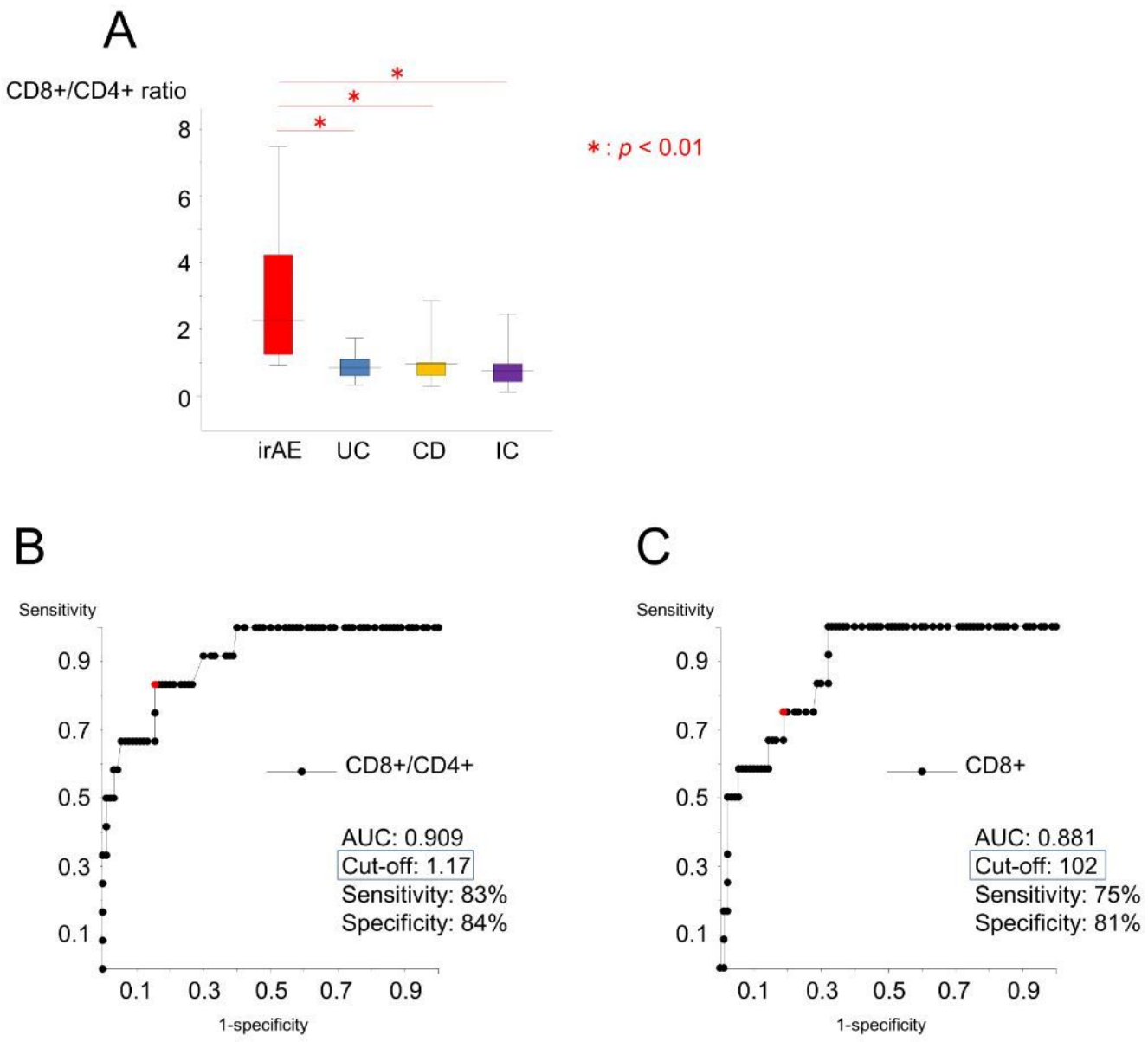

Figure 4

CD8+/CD4+ ratio and ROC analysis. (A) Comparison of CD8+/CD4+ ratio in each form of colitis. The CD8+/CD4+ ratio in irAE colitis was significantly higher than those in UC, CD, and IC. (B) In ROC analysis of irAE colitis vs. other forms of colitis, the area under the ROC curve was 0.909. The optimal cut-off point for CD8+/CD4+ ratio was 1.17, with a sensitivity of 83% and specificity of 84%. (C) In ROC analysis of irAE colitis vs. other forms of colitis, the area under the ROC curve was 0.881. The optimal cut-off point for the number of CD8+ lymphocytes was 102 cells/HPF, with a sensitivity of 75% and specificity of 81%.

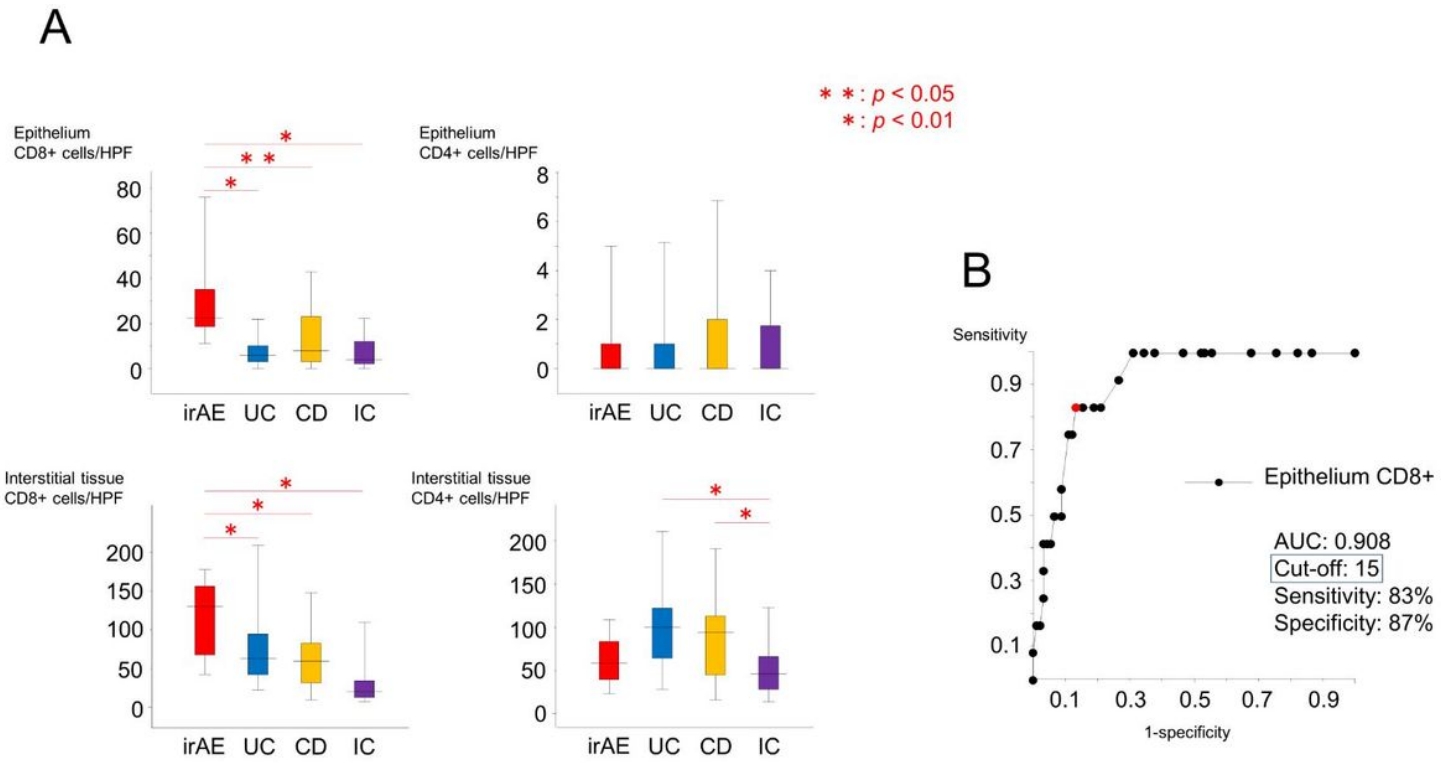

Figure 5

Quantitative comparisons of the number of infiltrated CD4+ and CD8+ lymphocytes and infiltration location as well as ROC analysis of infiltrated CD8+ lymphocytes in the epithelium. (A) Comparisons of CD4+ and CD8+ lymphocytes according to location in each form of colitis. (B) In ROC analysis of irAE colitis vs. other forms of colitis, the area under the ROC curve was 0.908. The optimal cut-off point for the number of CD8+ lymphocytes in the epithelium was 15 cells/HPF, with a sensitivity of 83% and specificity of 87%.

Supplementary Files

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- SupplementaryTable1.docx
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