REPORT

Cancer Science WILEY

Identification of characteristic subepithelial surface granulomatosis in immune-related adverse event-associated enterocolitis

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Abstract

Immune checkpoint inhibitors (ICIs) have provided an additional treatment option for various types of human cancers. However, ICIs often induce various immune-related adverse events (irAEs). Enterocolitis is a major irAE with poorly understood histopathological characteristics. In this study, we retrospectively investigated the histopathology of colon tissue samples from 17 patients treated with ICIs. There were two major histological patterns of colitis: an ulcerative colitis-like pattern and a graft vs host disease-like pattern. Although these two patterns of colitis were mutually exclusive, both patterns often showed a characteristic that we call "subepithelial surface granulomatosis" (SSG), which has not been reported in other types of colitis. SSG was found even in colon tissue without symptoms or endoscopic findings of colitis. Given the increasing reports of sarcoid reaction or exacerbation of tuberculosis after treatment with ICIs, granuloma formation could be a histological hallmark of systemic immune activation by ICIs. Although statistical significance was not obtained, probably because of the small sample size, SSG may be a surrogate biomarker of systemic anticancer immune activation. We propose that a prospective study with larger sample size be performed.

KEYWORDS
biomarker, colitis, immune checkpoint inhibitors, immune-related adverse event, subepithelial surface granulomatosis

1 | BACKGROUND

The increasing adoption of cancer immunotherapy with immune checkpoint inhibitors (ICIs) has given us an additional therapeutic option for various types of malignant tumors. However, the considerable success of ICIs has unfortunately increased immune-related adverse events (irAEs).[1] Enterocolitis is a relatively frequent irAE, particularly in patients treated with ipilimumab, an anticytotoxic T lymphocyte-associated protein 4 (CTLA-4) antibody. Diarrhea occurs in 12%-13% of patients administered antiprogrammed death receptor-1 (PD-1), 30%-35% of those administered anti-CTLA-4, and 10% of those treated with combination therapy.[2] Because irAEs greatly reduce quality of life and often show a clinical course different from that of ordinary autoimmune diseases, their appropriate diagnosis and treatment are important subjects. Based on our current understanding, irAE-associated enterocolitis does not show specific histological findings—any type of inflammation can be found.[2,3]

As described in this brief report, we investigated the histopathology of colon tissue from patients treated with ICIs. Interestingly, we discovered a characteristic feature that was a specific finding for the colon after treatment with ICIs. In addition, the theoretical mechanism of this feature would be consistent with efficient cancer immunity. In other words, this novel feature could be a potential surrogate marker of systemic anticancer immune activation.

2 | METHODS

2.1 | Patients and specimens

With approval of the institutional review board (322-134: Clinicopathological investigation of irAE caused by immune checkpoint inhibitors), the formalin-fixed, paraffin-embedded material archives of Sapporo Medical University Hospital, Hakodate Goryoukaku Hospital, Sunagawa City Medical Center, Asahikawa Red Cross Hospital, Otaru City General Hospital, and Kushiro City General Hospital were searched for colorectal biopsy or surgical resection specimens from patients administered ICIs. Informed consent was obtained through an opt-out on the website according to the guidelines of the Declaration of Helsinki. A total of 17 specimens were available: 16 were biopsies and one was a surgical resection specimen that was independent of the primary tumor. Patient histories were examined for relevant clinicopathological factors, including age, sex, primary tumor, type of ICI, intestinal symptoms, endoscopic findings, and other irAEs. The clinical effects of ICIs were evaluated according to RECIST (Response Evaluation Criteria in Solid Tumors) criteria. In each evaluation, the best clinical response before intestinal symptoms was analyzed. To obtain concordant results regarding the histological analysis, each slide was examined on a multiheaded microscope by three pathologists.

2.2 | IHC staining

Tumor tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections (4 μm thick) of formalin-fixed paraffin-embedded tumor tissues were stained with the following monoclonal antibodies after epitope retrieval using Target Retrieval Solution pH 9 (DAKO): mouse anti-CD4 monoclonal antibody (clone 4B12; Thermo Fisher Scientific), mouse anti-CD8 monoclonal antibody (clone C8/144B; DAKO), and mouse anti-CD68 monoclonal antibody (clone PG-M1; Nichirei Biosciences). These antibodies were used according to the manufacturers' instructions.

3 | RESULTS

3.1 | Patient characteristics

We analyzed colon tissue samples from 17 patients treated with ICIs. Clinicopathological and histopathological features are summarized in Table 1. The patients comprised eight men and nine women with a median age of 69 (range, 58-86) years. Six were receiving ICIs for lung adenocarcinoma, three had lung squamous cell carcinoma, three had urothelial carcinoma of the urinary bladder, two had malignant melanoma, and one each had lung carcinosarcoma, renal tumor (no histological confirmation was performed), and mucoepidermoid carcinoma. Most patients underwent chemoradiotherapy before treatment with ICIs (data not shown). Nine patients were administered pembrolizumab and six received nivolumab. One patient received atezolizumab and one received a combination of ipilimumab and nivolumab. In response to ICIs, five patients had progressive disease, whereas the others had disease control (complete response, six patients; partial response, five; stable disease, two). No patient had a history of inflammatory bowel disease, including ulcerative colitis (UC) or Crohn's disease.

3.2 | Clinical presentation and endoscopic findings

The most common symptom was diarrhea, reported by 13 of the 17 patients. Five patients had melena and one had abdominal pain. One patient had uveitis and one had dermatitis in addition to intestinal symptoms. Colonoscopy findings were typical for nonspecific colitis and UC-like severe inflammation. There was no tendency for a frequent inflamed region between the rectum and cecum.

3.3 | Histopathological findings

There were two major histological patterns of colitis: an UC-like pattern or a graft vs host disease (GVHD)-like pattern (Figure 1A). Remarkable crypt atrophy or distortion, implying chronic inflammation, was not clear in either pattern. The UC-like pattern exhibited an increased number of neutrophil and frequent cryptitis and/or crypt

TABLE 1 Clinicopathological features of the 17 patients treated with ICIs

	Age (years)	Sex	Tumor	Type of ICI (cycles)	Effect	Symptom	Endoscopic finding	Histology	SSG	Other irAE
1	69	M	Lung adeno Ca	Pembrolizumab: 200 mg/3 wk (8)	PR	Diarrhea	Nonspecific	GVHD-like	Positive	No
2	66	M	Lung adeno Ca	Nivolumab: 170 mg/2 wk (3)	PD	Diarrhea	Normal	UC-like	Negative	No
3	55	M	Lung adeno Ca	Nivolumab: 150 mg/2 wk (11)	PD	Diarrhea	Nonspecific	Nonspecific	Negative	No
4	69	M	Lung adeno Ca	Pembrolizumab: 200 mg/3 wk (16)	PD	Diarrhea	Nonspecific	UC-like	Negative	No
5	70	M	Lung adeno Ca	Pembrolizumab: 200 mg/3 wk (16)	PR	Diarrhea	UC-like	UC-like	Positive	Uveitis
6	67	F	Lung adeno Ca	Pembrolizumab: 200 mg (1)	PR	Diarrhea, Melena	UC-like	Nonspecific	Negative	No
7	86	F	Lung SCC	Nivolumab :155 mg/2 wk (5), 240 mg/2 wk (17)	SD	Diarrhea	Nonspecific	GVHD-like	Negative	No
8	58	F	Lung SCC	Atezolizumab: 1200 mg/3 wk (10)	SD	Diarrhea, melena	UC-like	UC-like	Positive	No
9	71	M	Lung SCC	Pembrolizumab: 200 mg/3 wk (3)	PR	None	Normal	None	Positive	No
10	69	F	Lung carcinosarcoma	Pembrolizumab: 200 mg/3 wk (1)	PD	Melena	Nonspecific	UC-like	Negative	No
11	66	M	Urothelial Ca	Pembrolizumab: 200 mg/3 wk (11)	CR	Diarrhea	UC-like	UC-like	Positive	No
12	77	F	Urothelial Ca	Pembrolizumab: 200 mg/3 wk (10)	PR	Diarrhea	Normal	GVHD-like	Positive	No
13	63	M	Urothelial Ca	Pembrolizumab: 200 mg/3 wk (15)	CR	Melena	Ischemic	Nonspecific	Negative	No
14	74	F	Melanoma	Nivolumab: 3 mg/kg/2 wk (6)	CR	Diarrhea, melena	UC-like	UC-like	Positive	No
15	74	F	Melanoma	Nivolumab: 3 mg/kg/2 wk (3)	PD	Diarrhea	Nonspecific	GVHD-like	Positive	No
16	64	F	Renal tumor	Ipilimumab:1 mg/kg + Nivolumab: 240 mg/3 wk (4)	CR	Abdominal pain	Normal	GVHD-like	Positive	No
17	67	F	Mucoepidermoid Ca	Nivolumab: 240 mg/3 wk (4)	PR	Diarrhea	Normal	GVHD-like	Positive	Dermatitis

Abbreviations: Ca, carcinoma; CR, complete response; GVHD, graft vs host disease; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; PR, partial response; SCC, squamous cell carcinoma; SD, stable disease; SSG, subepithelial surface granulomatosis; UC, ulcerative colitis.
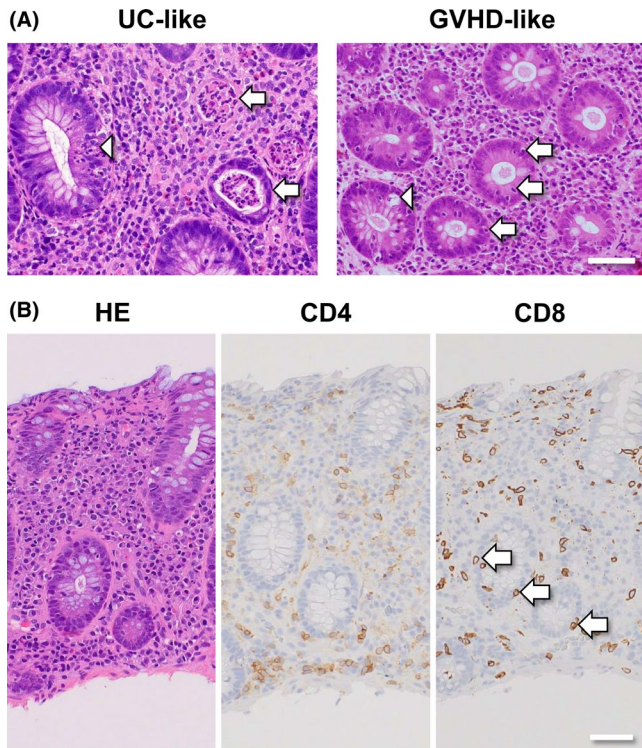
FIGURE 1 Histopathological images of the two patterns of immune-related adverse event (irAE)-associated enterocolitis. A, Two major histological patterns of irAE-associated enterocolitis. Left panel: ulcerative colitis (UC)-like pattern. Arrows and the arrowhead indicate crypt abscesses and cryptitis, respectively. Right panel: graft vs host disease (GVHD)-like pattern. Arrows and the arrowhead indicate lymphocyte infiltrates and an apoptotic body, respectively. Bar = 50 μm. B, Lymphocyte subtypes in GVHD-like pattern. Lymphocytes infiltrating into epithelial cells are CD8 positive (arrows), whereas CD4-positive cells are located in the interstitial region. Bar = 50 μm

FIGURE 2 Histopathological image of the subepithelial surface granulomatosis (SSG) found in the colon tissue after immune checkpoint inhibitor treatment. A, Representative histopathological image of SSG (arrows). Dotted lines indicate the epithelial-subepithelial margin. Bar = 50 μm. B, SSG may sometimes mimic the collagenous band. Bar = 50 μm. C, SSG (arrows) may sometimes mimic the aggregation of vascular endothelium. SSG is negative for CD31 and CD34. Bar = 50 μm. D, SSG lesions diffusely express CD68, implying a histiocytic lineage (arrows). Bar = 50 μm

abscess. On the other hand, lymphocyte infiltration into the epithelium and occasional apoptotic bodies were characteristic findings in the GVHD-like pattern. Lymphocytes infiltrating into epithelial cells were CD8 positive, whereas CD4-positive cells were located in interstitial lesions of the lamina propria (Figure 1B). We could not find statistically significant difference in clinical effect of ICIs between these two groups (P = .27, Fisher's exact test).

Interestingly, nine of the 17 patients showed a characteristic mucosal lesion involving subepithelial aggregation of cells with occasionally elongated ovoid pale nuclei and abundant eosinophilic cytoplasm (Figure 2A). Importantly, this feature was recognized by standard hematoxylin and eosin staining. It sometimes mimicked the morphological characteristics of collagenous colitis, although no apparent collagen band was identified (Figure 2B). In addition, this lesion is often confusing with the aggregation of vascular endothelium. We therefore performed immunohistochemical detection of CD31 and CD34, the well-recognized makers of vascular endothelium. They were positive only in the interstitial blood vessels (Figure 2C). On the other hand, this feature was positive for CD68, indicating a monocyte/macrophage lineage (Figure 2D). Together
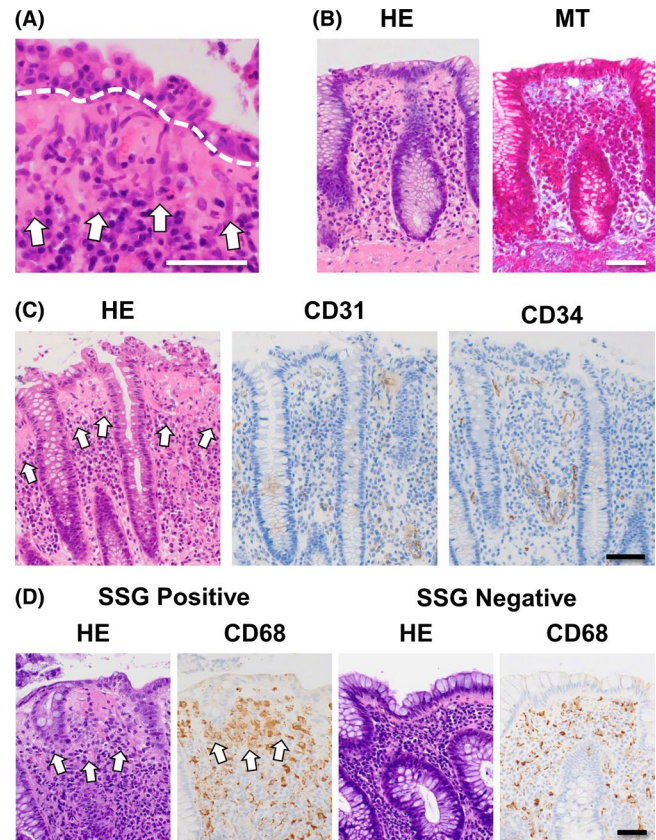
with these morphological and immunohistochemical findings, these formations were identified as granuloma-like lesions at the surface of the colon. We named this feature "subepithelial surface granulomatosis" (SSG), which is not found another type of colitis. Notably, these surface granulomas were located beneath the epithelium and often along the basal membrane, whereas small rounded granulomas are found deep in the colon in Crohn's disease. Cheese-like necrosis, a typical histological feature of tuberculosis, was also not observed. While the UC-like and GVHD-like patterns were mutually exclusive, surface granuloma was found in both types of colitis. In addition, surface granuloma was observed even in a patient without significant symptoms or histological evidence of colitis other than granuloma (Case 9). Interestingly, eight of the nine patients with SSG achieved disease control (complete response, partial response, or stable disease). Moreover, eight of the 12 patients with disease control exhibited SSG in the colon tissue. However, likely because of the small sample size, this finding did not reach statistical significance (P = .13, Fisher's exact test).
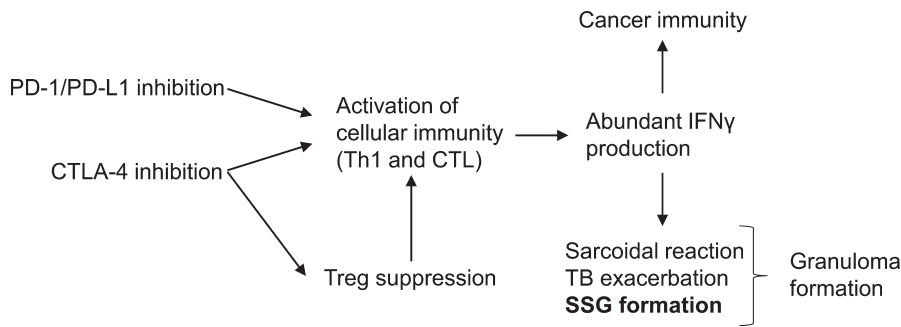
Cancer immunity

PD-1/PD-L1 inhibition ⟶ Activation of
cellular immunity ⟶ Abundant IFNγ
production

CTLA-4 inhibition ⟶ (Th1 and CTL)

⟶ Treg suppression ⟶

Sarcoidal reaction
TB exacerbation } Granuloma
SSG formation formation

FIGURE 3 Schematic diagram of the putative pathophysiology of granuloma formation as an immune-related adverse event

4 | DISCUSSION

In this study, we investigated histopathological findings in irAE-related colitis. The histological patterns of UC-like and GVHD-like pattern colitis have also been reported in some previous studies, although the differential mechanisms and clinicopathological importance of these patterns were unclear.[4-6] We identified SSG as a novel feature that was specific to colon tissue after ICI administration. PD-1/programmed death-ligand 1 (PD-L1) inhibition sometimes complicates granuloma formation disorders, including sarcoid reaction and exacerbation of tuberculosis.[7,8] SSG formation appears to be a more frequent event than these granuloma-forming adverse events. Granuloma formation after ICI administration can be attributed to a cellular immunity mechanism similar to that in mycobacterium tuberculosis infection. We assume that interferon-gamma (IFNγ) is the key factor underlying the granuloma formation induced by ICIs (Figure 3). Indeed, IFNγ is an essential cytokine for granuloma formation in mycobacterium infection.[9] In addition, mycobacterium tuberculosis-specific peripheral T lymphocytes release a large amount of IFNγ, which is used to diagnose tuberculosis infection. On the other hand, tumor antigen-specific activated cytotoxic T lymphocytes (CTLs) produce abundant IFNγ, which leads to cancer cell death. Type 1 helper T cells would also be involved. The surface location of the SSG could be due to a reaction to enteric contents, including commensal bacteria, a potential determinant of clinical efficacy of ICIs. Consequently, SSG formation can be a hallmark of systemic CTL activation. Therefore, the histological finding of SSG may be useful as a surrogate marker for estimating the clinical effects of ICIs. Further clinicopathological investigation is required to determine the clinical significance of SSG in irAE histology.

Our study has some limitations. First, this study is based on a retrospective review of a limited number of medical records. The evaluation time points of the clinical response to the ICIs varied among patients. Grades of the intestinal symptoms were not available. In addition, multiple types of malignant tumors were included in the study. To obtain more reliable evidence, an appropriately designed prospective study is required, as well as a larger sample size and optimal control group. In addition, the appropriate biopsy timing or site should be identified. Second, even though SSG may a surrogate marker of systemic immune activation induced by ICIs, this marker might not necessarily reflect clinical efficacy. Immune activation may be a necessary but not sufficient condition. Although the immune

system is activated, other immune escape mechanisms can inhibit cancer immunity. Third, only one patient who received a CTLA-4 inhibitor was included in this study. CTLA-4 inhibition may show a histology similar to that of autoimmune colitis in immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, a disease associated with regulatory T cell deficiency.[10] Further development of relevant biomarkers and their combination may provide a valid biomarker for estimating the clinical efficacy of ICIs.

In conclusion, we identified SSG as a characteristic histopathological feature of irAE-associated enterocolitis. Because this was a pilot study with a small sample size, further investigation in a larger population is required, particularly in terms of the type of ICI, clinical efficacy for cancer, or other parameters. However, we believe that the results of this pilot study are valuable and indicate a promising direction of future research.

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