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Immune Checkpoint Inhibitor–Induced Upper Gastrointestinal Tract Inflammation Shows Morphologic Similarities to, but Is Immunologically Distinct From, Helicobacter pylori Gastritis and Celiac Disease

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Context.—Immune checkpoint inhibitor (CPI) therapies are associated with multi-organ immune-related adverse events. Although colonic mucosal changes have been described, inflammatory changes incited by CPIs in the upper gastrointestinal tract have not been well characterized.

Objective.—To investigate morphologic and immunologic changes incited by CPI therapy in the upper gastrointestinal tract.

Design.—We compared the morphology and immune cell phenotype of gastric and duodenal biopsies from patients treated with anti–cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or anti–programmed death receptor-1/programmed death ligand-1 (PD-1/PD-L1) antibodies with biopsies from patients with Helicobacter pylori gastritis, patients with celiac disease, and normal controls.

Results.—Gastric biopsies from patients on CPIs showed chronic gastritis mimicking H pylori gastritis. However, CPI gastritis demonstrated greater numbers of CD8+ intraepithelial lymphocytes, less lamina propria inflammation, fewer plasma cells and CD20+ B cells, fewer lymphoid aggregates, and reduced CD4:CD8 ratio in both the lamina propria and the epithelial layer. There were no differences between anti–CTLA-4 and anti–PD-1/PD-L1 gastritis, except for more lymphoid aggregates in anti–PD-1/PD-L1 gastritis. Duodenal biopsies from patients on CPIs revealed chronic duodenitis with villous blunting, mimicking celiac disease. Compared with celiac disease, CPI duodenitis demonstrated higher prevalence of neutrophilic infiltrates and erosions, increased lamina propria CD3 and CD8 T cells, and reduced CD4:CD8 ratio. Upper gastrointestinal biopsies were more inflamed than concomitant colonic biopsies in the majority of patients.

Conclusions.—The morphologic and immunophenotypic distinctions between CPI-associated upper gastrointestinal injuries and common infectious and autoimmune diseases may provide useful discriminators when clinicians are confronted with gastric and duodenal inflammatory changes in patients receiving CPI therapy.

Immune checkpoint inhibitor (CPI) therapies are increasingly used in the treatment of malignant neoplasms. In the course of normal immune function, checkpoint proteins such as cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed death receptor-1 (PD-1) are expressed on the surface of T cells, initiating a series of signals that ultimately lead to the down-regulation of T-cell activity in settings in which tolerance is desirable (eg, to self-antigens). Malignant and immune cells in tumors often express abundant PD-1 and other checkpoint proteins that can co-opt this function and likewise inactivate the T-cell response, allowing tumor cells to evade the immune system. By blocking the binding sites of these molecules, CPI antibody therapies facilitate the activation of T cells against tumor cells and reinvigorate antitumor immune responses.1 In recent years, indications for CPI therapy have expanded from metastatic melanoma and non–small cell lung cancer to include renal cell carcinoma, gastroesophageal and other gastrointestinal malignancies, head and neck cancers, urothelial carcinoma, and other neoplasms.1-4

Notwithstanding promising therapeutic responses in a significant subset of cancer patients, CPIs come with the unfortunate side effect of multi-organ self-directed inflammation, so-called immune-related adverse events (irAEs). These autoimmune-like reactions are a by-product of the generalized suppression of T-regulatory cells. The most common sites of irAEs are the luminal gastrointestinal (GI) tract, endocrine organs, skin, lungs, and liver, with rarer reports of injury to a host of other organ systems.5-10 Studies have characterized the clinical, endoscopic, morphologic, and immunophenotypic effects of CPIs in the lower GI tract11-18; however, comparable studies in the upper GI tract...
are limited, especially with respect to anti–PD-1/anti–programmed death ligand-1 (PD-L1) therapies, consisting of case reports and small series.19–23 As CPI use is anticipated to increase, it is important that the morphologic characteristics of CPI-associated upper GI tract mucosal injury be better elucidated and accurately differentiated from other common inflammatory conditions. In addition, the concordance of upper and lower GI tract injury has not been established. In this study, we analyzed the morphologic and immunophenotypic effects of CPI therapy on gastric and duodenal mucosa with comparisons with Helicobacter pylori gastritis, celiac disease (CeD), and concomitant lower GI tract biopsies.

**MATERIALS AND METHODS**

After fulfilling institutional review board requirements, we searched the pathology files for patients on CPI therapy who underwent upper GI tract mucosal biopsies between 2005 and 2018. All patients identified were included in the study. Six of the 14 patients meeting criteria for inclusion in the study had concomitant esophageal biopsies, with 5 of 6 showing normal mucosa and 1 showing mild lymphocytic inflammation with negative fungal, cytomegalovirus (CMV), and herpes simplex virus stains. Because of the paucity of pathologic findings, esophageal biopsies were not further included in the analysis. In patients in whom colonoscopy was performed at the time of esophagogastroduodenoscopy, concomitant colonic mucosal biopsies were retrieved. Cytomegalovirus immunohistochemical stains were performed on all biopsies in the CPI cohort, and H pylori immunohistochemical stains were performed on all gastric biopsies in the CPI cohort. All CMV and H pylori immunohistochemical stains in CPI patients were negative. Patients with inflammatory bowel disease were excluded.

Gastric biopsies from patients with H pylori gastritis and duodenal biopsies from untreated CeD patients served as comparison inflammatory groups. Unremarkable gastric and duodenal biopsies served as normal controls.

Hematoxylin-eosin (H&E) stains of all mucosal biopsies were scored blindly by 2 pathologists simultaneously on a semiquantitative scale for degree of lamina propria (LP) inflammation (0, none; 1, mild; 2, moderate; 3, severe), lymphoid aggregates (0, absent; 1, present), activity, defined as neutrophilic infiltrates and/or erosions (0, absent; 1, present), apoptosis (0, absent; 1, rarely present; 2, prominently present) and number of intraepithelial lymphocytes (IELs) per 100 epithelial cells. In the stomach, IELs were counted in 100 consecutive surface epithelial cells, exclusive of the gastric neck and pit cells. In the duodenum, IELs were counted in 100 consecutive surface epithelial cells across the tops of villous tips (20 epithelial cells per villous tip), where these were present, or in 100 consecutive surface enterocytes in cases with villous blunting. In the colon, IELs were counted in 100 consecutive surface epithelial cells, exclusive of crypts. At all sites, epithelium in the vicinity of lymphoid aggregates was avoided when assessing IELs. Gastric biopsies were additionally scored for number of LP plasma cells by averaging the number of plasma cells counted in 2 high-power-field hot spots. Duodenal biopsies were additionally scored for degree of villous blunting in well-oriented sections (0, none; 1, mild; 2, moderate; 3, severe), crypt hyperplasia (0, absent; 1, present) and gastric foveolar metaplasia (0, absent; 1, present). Immunohistochemical stains with antibodies against CD4, CD8, and CD20 were used on all gastric and duodenal biopsies. CD3-stained slides were additionally analyzed in both the LP and epithelium in all duodenal biopsies. Positive cells for each stain in the LP were counted blindly in 2 high-power-field hot spots, and the stained intraepithelial lymphocytes were counted simultaneously by 2 pathologists as described above for IELs based on H&E stains. Morphology and immunohistochemistry scores were compared between all groups.

Statistical analysis was performed using Student t test for the comparison of all semiquantitative features and numbers of IELs and plasma cells as well as LP and intraepithelial immune cells. Fisher exact test was conducted for the comparison of binary features. Differences were claimed as statistically significant when the P value was less than .05.

**RESULTS**

**Patient Demographics**

Upper GI biopsies from 8 patients on anti–CTLA-4 therapy for metastatic melanoma (male to female ratio, 1:1; average age, 58 years; range, 37–74 years) were retrieved. For 5 patients both gastric and duodenal biopsies were obtained, for 2 only gastric biopsy, and for 1 only duodenal biopsy (total of 7 gastric biopsies and 6 duodenal biopsies). For 5 of 8 patients, concomitant colonic mucosal biopsies were obtained. One patient underwent a second esophagogastroduodenoscopy with biopsy sampling because of persistent symptoms despite withholding of anti–CTLA-4 therapy; only the first biopsy series was included in the statistical analysis. The indications for upper endoscopy were nausea and vomiting in 4 patients, diarrhea in 6 patients, low-grade fever in 3 patients, abdominal pain in 2 patients, melena in 2 patients, and the detection of positron emission tomography–avid upper GI tract regions in 2 patients. Biopsies did not reveal tumor in either of those patients, only 1 of whom had GI symptoms. On average, patients received 3 cycles of ipilimumab (range, 2–4 cycles) before undergoing upper endoscopy for GI symptoms.

Upper GI tract biopsies from 6 patients on anti–PD-1/PD-L1 therapy (male to female ratio, 1:5; average age, 58 years; range, 29–74 years) were retrieved. For 1 patient both gastric and duodenal biopsies were obtained, and for 5 patients only gastric biopsies were obtained (total of 6 gastric biopsies and 1 duodenal biopsy). For 3 of 6 patients, concomitant colonic mucosal biopsies were obtained. One patient underwent 2 follow-up esophagogastroduodenoscopies with biopsy sampling; only the first biopsy was included in the statistical analysis. The malignancies prompting anti–PD-1/PD-L1 therapy were diverse, consisting of breast carcinoma, renal cell carcinoma, urothelial carcinoma, non–small cell lung carcinoma, gastric carcinoma, and Hodgkin lymphoma. The indications for upper endoscopy were nausea and vomiting in 4 patients (1 of whom also had hematemesis), diarrhea in 2 patients, abdominal pain in 4 patients, melena in 2 patients, early satiety in 1 patient, constipation in 1 patient, and gastric outlet obstruction in 1 patient. On average, patients received 16 cycles of anti–PD-1/PD-L1 therapy (range, 2–70 cycles) before undergoing upper endoscopy to investigate GI symptoms.

Patients from both drug groups were on a variety of standard medications used in the care of oncologic patients, including vitamins and supplements, prednisone, analgesics, and antianxiety, antinausea, and anti-diarrheal compounds. From a clinical standpoint, these agents were not suspected as causes of the new onset of GI symptoms that prompted endoscopy, nor are they known to cause histologic abnormalities in the GI tract. Table 1 summarizes the demographics of patients treated with anti–CTLA-4 and anti–PD-1/PD-L1 therapies.

Biopsies from 8 H pylori gastritis and 8 untreated CeD patients were retrieved. Eight histologically unremarkable gastric and duodenal biopsies from 16 patients undergoing
upper endoscopy for symptoms including abdominal pain, gastroesophageal reflux, and diarrhea served as normal controls.

### Gastric Morphologic and Immunohistochemical Findings

**H pylori Gastritis Versus Normal Controls.**—As expected, gastric biopsies (Figure 1) from patients with *H pylori* gastritis demonstrated significantly increased scores for LP inflammation, LP plasma cells, lymphoid aggregates, activity, and intraepithelial lymphocytes compared with normal controls (Figure 2). There was no difference in apoptosis scores compared with normal controls (Table 2).

On immunohistochemical stains (Figure 3), *H pylori* gastritis demonstrated significantly increased LP CD4+ T cells (average, 82 versus 21; \( P < .001 \)), CD8+ T cells (average, 44 versus 10; \( P = .002 \)), and CD20+ B cells (average, 37 versus 4; \( P < .001 \)) compared with normal controls. There was no significant difference in intraepithelial CD4+ and CD8+ T cells or CD20+ B cells or in the intraepithelial or LP CD4:CD8 ratio (Figure 4) compared with normal controls (Table 2).

**CPI Gastritis Versus Normal Controls.**—All 13 gastric biopsies from patients on CPIs showed varying degree of chronic active gastritis, with increased intraepithelial lymphocytes and mixed neutrophilic and lymphoplasmacytic LP infiltrates (Figure 1). Compared with normal controls

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**Table 1. Summary of Demographics of Patients on Immune Checkpoint Inhibitor Therapy**

| Case | Age, y/Sex | Malignancy         | Therapy       | Cycles Received Prior to Biopsy | Sign(s) and/or Symptom(s) Prompting Endoscopy                        |
|------|------------|---------------------|---------------|-------------------------------|---------------------------------------------------------------|
| 1    | 66/F       | Melanoma            | Ipilimumab    | 3                             | Positron emission tomography–avid gastric mass                |
| 2    | 74/M       | Melanoma            | Ipilimumab    | 3                             | Positron emission tomography–avid gastroesophageal junction, melena |
| 3    | 71/M       | Melanoma            | Ipilimumab    | 4                             | Diarrhea, melena                                               |
| 4    | 37/F       | Melanoma            | Ipilimumab    | 2                             | Abdominal pain, nausea, vomiting, diarrhea                    |
| 5    | 55/M       | Melanoma            | Ipilimumab    | 2                             | Abdominal pain, nausea, vomiting, diarrhea, low-grade fever    |
| 6    | 43/M       | Melanoma            | Ipilimumab    | 4                             | Nausea, vomiting, diarrhea, low-grade fever                    |
| 7    | 62/F       | Melanoma            | Ipilimumab    | 2                             | Diarrhea                                                       |
| 8    | 53/F       | Melanoma            | Ipilimumab    | 3                             | Nausea, vomiting, diarrhea, low-grade fever                    |
| 9    | 44/F       | Breast carcinoma    | Durvalumab    | 10                            | Abdominal pain, nausea, vomiting, diarrhea                    |
| 10   | 62/F       | Renal cell carcinoma| Nivolumab     | 5                             | Abdominal pain, early satiety, nausea, vomiting, melena       |
| 11   | 70/F       | Urothelial carcinoma| Atezolizumab  | 7                             | Abdominal pain, constipation, melena                          |
| 12   | 74/M       | Non-small cell lung cancer | Pembrolizumab | 4                             | Diarrhea                                                       |
| 13   | 66/F       | Gastric carcinoma   | Pembrolizumab | 2                             | Gastric outlet obstruction, nausea, vomiting                  |
| 14   | 29/F       | Hodgkin lymphoma    | Nivolumab     | 70                            | Abdominal pain, nausea, vomiting, hematemesis                  |

Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated protein 4; PD-1/PD-L1, programmed death receptor-1/programmed death ligand-1.
biopsies from patients on CPIs showed significantly increased LP inflammation ($P < .001$), LP plasma cells ($P = .003$), activity ($P < .001$), intraepithelial lymphocytes ($P < .001$), and apoptosis ($P = .03$). There was no difference in scores for lymphoid aggregates compared with normal controls (Table 2).

On immunohistochemical stains (Figure 3), biopsies from patients on CPIs demonstrated increased LP CD4$^+$ T cells (average, 68 versus 21; $P < .001$), CD8$^+$ T cells (average, 65 versus 10; $P < .001$), and CD20$^+$ B cells (average, 15 versus 4; $P = .02$) compared with normal controls. Moreover, biopsies from the CPI cohort had increased intraepithelial

![Figure 2. Lamina propria (LP) inflammation (A) and intraepithelial lymphocytes (IELs) (B) in the stomach and duodenum of patients on immune checkpoint inhibitors (CPIs) (anti–cytotoxic T-lymphocyte associated protein 4 [anti–CTLA-4] and anti–programmed death receptor-1/programmed death ligand-1 [anti–PD(L)-1]) compared with those of Helicobacter pylori gastritis (HPG), celiac disease (CeD), and normal gastric/duodenal biopsies (control) as evaluated on hematoxylin-eosin. Data are expressed as means ± SD; *$P < .05$, **$P < .01$, ***$P < .001$.]

### Table 2. Morphologic and Immunophenotypic Scores of Gastric Biopsies

|                  | IEL/100 Epithelial Cells | LP Inflammation | Activity | Lymphoid Aggregates | Apoptosis    | CD4 | CD8 | CD20 |
|------------------|--------------------------|-----------------|----------|---------------------|--------------|-----|-----|-----|
| Controls         | 4                        | 8/8 unremarkable| 0/8      | 0/8                 | 4/8 absent   | 21  | 1   | 10  |
|                  |                          | 0/8 mild        | 0/8      | 0/8                 | 3/8 rare     | 1/8 |     |     |
|                  |                          | 0/8 moderate    | 0/8      | 0/8                 | 1/8 prominent|     |     |     |
|                  |                          | 0/8 severe      | 0/8      | 0/8                 |              |     |     |     |
| Helicobacter pylori gastritis | 7                        | 0/8 unremarkable| 8/8      | 7/8                 | 0/8 absent   | 82  | 2   | 44  |
|                  |                          | 0/8 mild        | 0/8      | 0/8                 | 4/8 rare     |     | 7   |     |
|                  |                          | 0/8 moderate    | 0/8      | 0/8                 | 4/8 prominent|     |     |     |
|                  |                          | 0/8 severe      | 0/8      | 0/8                 |              |     |     |     |
| Anti–CTLA-4 gastritis | 21                       | 0/7 unremarkable| 6/7      | 0/7                 | 0/7 absent   | 72  | 2   | 76  |
|                  |                          | 2/7 mild        | 6/7      | 0/7                 | 4/7 rare     |     | 28  |     |
|                  |                          | 5/7 moderate    | 0/7      | 0/7                 | 3/7 prominent|     |     |     |
| Anti–PD-1/PD-L1 gastritis | 22                       | 0/6 unremarkable| 5/6      | 3/6                 | 0/6 absent   | 64  | 2   | 52  |
|                  |                          | 3/6 mild        | 5/6      | 3/6                 | 4/6 rare     |     | 24  |     |
|                  |                          | 1/6 moderate    | 5/6      | 3/6                 | 2/6 prominent|     |     |     |
|                  |                          | 2/6 severe      | 5/6      | 3/6                 |              |     |     |     |

Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated protein 4; IEL, intraepithelial lymphocytes; LP, lamina propria; PD-1/PD-L1, programmed death receptor-1/programmed death ligand-1.
CD8+ T cells (average, 26 versus 3; \( P = .002 \)), with no difference in intraepithelial CD4+ T cells or CD20+ B cells compared with controls. CD4:CD8 ratios were lower in both the LP (average, 1.1 versus 2.1; \( P = .008 \)) and epithelium (average, 0.08 versus 0.33; \( P = .02 \)) compared with normal controls (Figure 4; Table 2).

**CPI gastritis versus H pylori gastritis.**—All gastric biopsies from both groups showed varying degrees of chronic active gastritis. However, biopsies from patients on CPIs showed less severe LP inflammation (\( P < .001 \)), fewer LP plasma cells (\( P < .001 \)), higher numbers of IELs (average, 21 versus 7; \( P < .001 \)) (Figure 2), and fewer lymphoid

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**Figure 3.** Lymphocyte phenotyping of Helicobacter pylori gastritis (A) and immune checkpoint inhibitor anti–programmed death receptor-1 nivolumab gastritis (D) shows no difference in the number of lamina propria CD4+ cells (B and E, respectively) or CD8+ cells (C and F, respectively) (hematoxylin-eosin and 3,3'-diaminobenzidine as chromogen, original magnification \( \times 200 \)).

**Figure 4.** Lamina propria (LP) CD20+ cells (A) and CD4:CD8 ratio (B) in the stomach and duodenum and LP CD3+ cells (C) in the duodenum in patients on immune checkpoint inhibitors (CPIs) (anti–cytotoxic T-lymphocyte associated protein 4 [anti–CTLA-4] and anti–programmed death receptor-1/programmed death ligand-1 [anti–PD(L)-1]) compared with patients with Helicobacter pylori gastritis (HPG), patients with celiac disease (CeD), and normal gastric/duodenal biopsies (control). Data are expressed as means \( \pm \) SD; * \( P < .05 \), ** \( P < .01 \), *** \( P < .001 \).
aggregates (P = .005) compared with *H pylori* gastritis. There was no significant difference in scores for activity (P = .30) or apoptosis (P = .86) (Table 2). *Helicobacter pylori* organisms were present in all *H pylori* gastritis–associated biopsies and in none of the CPI patients’ biopsies.

On immunohistochemical stains (Figure 4), fewer LP CD20⁺ B cells (average, 15 versus 37; P < .001) and higher numbers of intraepithelial CD8⁺ T cells (average, 26 versus 7; P = .004) were noted in CPI gastritis biopsies. There was no significant difference in LP CD4 and CD8⁺ T cells, intraepithelial CD4⁺ T cells, or intraepithelial CD20⁺ B cells between *H pylori* gastritis and CPI gastritis (Table 2).

Interestingly, although there was no significant difference in the absolute numbers of LP CD4⁺ and CD8⁺ T cells, the CD4:CD8 ratios in both the LP and epithelial layer were lower in CPI–associated gastritis (LP average, 1.1 versus 1.9; P = .01; IEL average, 0.08 versus 0.29; P = .006), reflecting a trend of increased CD8⁺ T cells in the LP in CPI gastritis.

**Anti–CTLA-4 Versus Anti–PD-1/PD-L1 Gastritis.**—Patients on anti–PD-1/PD-L1 therapy were significantly more likely to demonstrate lymphoid aggregates in gastric biopsies compared with patients on anti–CTLA-4 therapy (P = .03). There were no other significant morphologic or immunophenotypic differences between the 2 medication groups (Table 2).

**Duodenal Morphologic and Immunohistochemical Findings**

**CeD Versus Normal Controls.**—As expected, duodenal biopsies (Figure 5) from patients with untreated CeD demonstrated villous blunting, crypt hyperplasia, increased LP inflammation, increased intraepithelial lymphocytes, and increased apoptosis compared with normal controls. There was no difference in scores for lymphoid aggregates, foveolar metaplasia, or activity compared with normal controls (Table 3).

On immunohistochemical stains, CeD biopsies (Figure 6) demonstrated increased LP (60 versus 41; P = .03) and intraepithelial (54 versus 16; P = .006) CD8⁺ T cells, increased intraepithelial CD3⁺ T cells (average, 86 versus 22; P < .001) and increased LP CD20⁺ B cells (average, 16 versus 10; P = .02) compared with normal controls. There was no difference in LP or intraepithelial CD4:CD8 ratio, LP CD3⁺ (Figure 4, C) or CD4⁺ T cells, or in the intraepithelial CD4⁺ T cells or CD20⁺ B cells (Table 3).

**CPI Duodenitis Versus Normal Controls.**—All 7 duodenal biopsies from CPI patients showed villous blunting, active duodenitis, and increased LP inflammatory cells, including neutrophils (Figure 5). Compared with normal controls, biopsies from CPI patients demonstrated increased villous blunting (P < .001), LP inflammation (P < .001), activity (P < .001), IELs (P = .04), crypt hyperplasia (P < .001), and apoptotic bodies P < .001. There was no difference in lymphoid aggregates or foveolar metaplasia compared with normal controls (Table 3).

On immunohistochemical stains (Figure 6), duodenal biopsies from patients on CPIs demonstrated increased LP CD20⁺ B cells (average, 23 versus 10; P = .002), LP CD3⁺ T cells (average, 279 versus 175; P = .002), and LP CD8⁺ T cells (average, 156 versus 41; P < .001). There was no difference in LP CD4⁺ T cells, resulting in a significantly decreased LP CD4:CD8 ratio (average, 1.09 versus 3.00; P < .001) (Figure 4). Biopsies from patients on CPIs also demonstrated increased intraepithelial CD3⁺ T cells (average, 80 versus 22; P = .03). There was no difference in intraepithelial CD4⁺ and CD8⁺ T cells or CD20⁺ B cells or in the intraepithelial CD4:CD8 ratio (Table 3).

**CPI Duodenitis Versus CeD.**—As stated above, duodenal biopsies from patients on CPIs all showed villous blunting superficially mimicking CeD. The most striking morphologic difference was the presence of activity (neutrophilic infiltrates and/or erosions), which was present in all CPI–related duodenitis biopsies but none of the CeD biopsies (P < .001). There was no significant difference in degree of LP inflammation, IELs, presence of lymphoid aggregates, crypt hyperplasia, foveolar metaplasia, or apoptosis (Table 3).

On immunohistochemical stains (Figure 6, D), CPI therapy–associated duodenitis showed higher LP CD3⁺ T cells (average, 279 versus 159; P = .005) and CD8⁺ T cells (average, 156 versus 60; P = .01) compared with CeD. These findings correlated with a significantly decreased LP CD4:CD8 ratio compared with CeD (average, 1.1 versus 2.2; P = .01). There was no difference in LP CD4⁺ T cells and

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**Figure 5.** Duodenal biopsies in patients with celiac disease (CeD) (A) and in those receiving anti–cytotoxic T-lymphocyte associated protein 4 (CTLA-4) (B) and anti–programmed death receptor-1/programmed death ligand-1 (anti–PD-1/PD-L1) (C) therapy show marked villous blunting. Activity (neutrophilic infiltrates ± erosion) is significantly more likely in immune checkpoint inhibitor duodenitis compared with CeD (hematoxylin–eosin, original magnification ×400).
Although the inflammatory effects of CPIs in the colonic mucosa have been described, their effects on upper GI mucosa are less well characterized. To the best of our knowledge, this is the first clinicopathologic study comparing the morphologic and immunophenotypic manifestations of CPI-induced injury of the upper GI tract with commonly encountered inflammatory conditions, namely H pylori gastritis and CeD, as well as to concomitant lower GI morphologic and immunophenotypic scores of duodenal biopsies.

Table 3. Morphologic and Immunophenotypic Scores of Duodenal Biopsies

| Morphology          | CD3 | CD4 | CD8 | CD20 |
|---------------------|-----|-----|-----|------|
| Villous Blunting    | 23  | 0/8 | 0/8 | 8/8  |
| IEL/100 Epithelial Cells | 8/8 | 0/8 | 0/8 | 0/8  |
| LP Inflammation     | 0/8 | 0/8 | 0/8 | 0/8  |
| Activity            | 0/8 | 0/8 | 0/8 | 0/8  |
| Lymphoid Aggregates | 0/8 | 0/8 | 0/8 | 0/8  |
| Apoptosis           | 0/8 | 0/8 | 0/8 | 0/8  |
| Crypt Hyperplasia   | 0/8 | 0/8 | 0/8 | 0/8  |
| Foveolar Metaplasia | 0/8 | 0/8 | 0/8 | 0/8  |
| Controls            |     |     |     |      |
| Celiac disease      |     |     |     |      |
| Anti-CTLA-4 duodenitis |   |     |     |      |
| Anti-PD-1/PD-L1 duodenitis |   |     |     |      |
| Controls            |     |     |     |      |
| Celiac disease      |     |     |     |      |
| Anti-CTLA-4 duodenitis |   |     |     |      |
| Anti-PD-1/PD-L1 duodenitis |   |     |     |      |

Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated protein 4; IEL, intraepithelial lymphocytes; LP, lamina propria; PD-1/PD-L1, programmed death receptor-1/programmed death ligand-1.

DISCUSSION

Intraepithelial Lymphocyte Methodology: H&E Versus Immunohistochemistry

Comparing the 2 methods of IEL assessment used in gastric biopsies, good concordance between IEL counts performed by H&E and the sum of CD8 and CD4 CD3 stains were significant indicators of IEL counts in the upper GI tract. In 5 of 8 patients with both upper and lower GI biopsies from the same procedure, morphologic and immunophenotypic scores of the same patients were greater in the upper GI tract biopsies. In 2 patients, IEL counts were concordant in both GI tracts. This trend was especially true for the purposes of this analysis, the IEL counts found for the purposes of this analysis, the IEL counts found for the purposes of this analysis.
tract CPI-induced changes. From a clinical standpoint, and consistent with prior studies in the lower GI tract, symptoms prompting upper GI endoscopy occur sooner and after fewer doses in patients on anti–CTLA-4 therapy, compared with patients on anti–PD-1/PD-L1 therapy. Our results indicate that the histopathology and immunohistochemical features of CPI-associated gastroduodenitis superficially mimic 2 common disorders, *H pylori* gastritis and CeD. We also find that subtle histologic differences between CPI injury and these more common disorders can alert the astute pathologist to consider these differential diagnoses.

### Table 4. Comparison of Upper Versus Lower Gastrointestinal Tract Biopsy Findings in Patients on Immune Checkpoint Inhibitor Therapy

| Case | LP Inflammation | Activity | IEL/100 Epithelial Cells | Apoptosis | Comparison Summary |
|------|-----------------|----------|--------------------------|-----------|--------------------|
|      | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower |
| Anti–CTLA-4 | 1 | 2 | 3 | 1 | 1 | 27 | 8 | 2 | 2 |
| Anti–CTLA-4 | 2 | 2 | 0 | 1 | 0 | 58 | 11 | 2 | 0 |
| Anti–CTLA-4 | 3 | 3 | 0 | 1 | 0 | 30 | 13 | 2 | 1 |
| Anti–CTLA-4 | 4 | 3 | 1 | 0 | 40 | 9 | 1 | 1 |
| Anti–CTLA-4 | 5 | 3 | 3 | 1 | 0 | 150 | 27 | 2 | 1 |
| Anti–PD-1/PD-L1 | 1 | 3 | 1 | 0 | 53 | 10 | 2 | 1 |
| Anti–PD-1/PD-L1 | 2 | 1 | 3 | 1 | 1 | 23 | 10 | 1 | 2 |
| Anti–PD-1/PD-L1 | 3 | 1 | 3 | 0 | 1 | 21 | 7 | 1 | 2 |

Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated protein 4; IEL, intraepithelial lymphocytes; LP, lamina propria; PD-1/PD-L1, programmed death receptor-1/programmed death ligand-1.
diagnoses. Specifically, in the stomach CPI-associated gastritis is characterized by less severe LP inflammation, largely because of fewer plasma cells and LP CD20 B cells, and demonstrates more IELs, primarily because of significantly higher numbers of intraepithelial CD8 T cells. Checkpoint inhibitor gastritis also demonstrates fewer lymphoid aggregates compared with \textit{H pylori} gastritis. Aside from a higher incidence of gastric lymphoid aggregates in patients on anti–PD-1/PD-L1 therapy, no morphologic or immunohistochemical differences between the 2 drugs were found in gastric mucosal biopsies. In the duodenum, CPI-associated mucosal injury was characterized by marked villous blunting and intraepithelial lymphocytes, mimicking CeD. The main histologic discriminator was the presence of activity (defined as neutrophilic infiltrates and/or erosions) found in 100% of CPI-associated duodenitis biopsies, but not in CeD. However, because acute inflammation can occasionally be seen in CeD, the morphologic similarities between the 2 conditions has the potential to be diagnostically misleading. Further, it has been established that CPI therapy can induce the manifestation of CeD in genetically at-risk individuals, as described in a recent case report\textsuperscript{24} of diet-responsive CeD developing after anti–CTLA-4 therapy. In addition, CPI injury was postulated to exacerbate the immune response to \textit{H pylori} infection in a patient receiving anti–CTLA-4 antibody leading to severe hemorrhagic gastritis.\textsuperscript{21}

Increases in tissue CD8\textsuperscript{+} T cells and decreases in the CD4:CD8 T-cell ratio are hallmarks of CPI-associated irAEs, in keeping with the mechanism of action of these therapies to suppress Regulatory T-cell activity.\textsuperscript{1,2,24} Consistent with previous findings, the current study confirms this trend in the upper GI tract mucosa and suggests that these findings may be a discriminating feature compared with both \textit{H pylori} gastritis and CeD.\textsuperscript{1,2,24} However, we fully acknowledge that immunohistochemical lymphocyte phenotyping is not needed in the diagnostic setting to discriminate between these disorders.

It is noteworthy that all patients identified in our database who underwent esophasagogastroduodenoscopy and biopsy in order to investigate the possibility of CPI-induced pathology did, in fact, have morphologic changes attributable to drug injury, suggesting that symptoms relatable to the upper GI tract are a highly reliable indicator of the presence of tissue pathology in these patients. Patients with advanced malignancies on CPI therapy are often immunosuppressed. Hence, it is important to exclude concurrent viral infection, especially CMV gastritis or duodenitis. The inflammatory infiltrate associated with active CMV infection may resemble CPI gastritis or duodenitis morphologically. Immunohistochemical stains for CMV should be considered when a diagnosis of CPI-induced gastritis or duodenitis is invoked. Of note, in 5 of 6 patients with concomitant esophageal biopsies, only 1 esophageal biopsy showed mild lymphocytic chronic esophagitis (with negative viral and fungal stains), whereas 5 esophageal biopsies showed normal esophageal mucosa. These findings may suggest that squamous esophageal mucosa does not manifest CPI-induced injury as commonly as columnar lined portions of the GI tract.

Concomitant upper and lower GI tract mucosal inflammatory changes were the rule in our cohort, with 6 of 8 patients with simultaneous upper and lower endoscopic procedures showing inflammation in both upper and lower mucosal biopsies. In these patients, there was a tendency for the upper GI tract biopsies to exhibit more severe inflammation than the paired colonic mucosal biopsies. Although this finding should be confirmed in larger studies, it may suggest that CPIs induce a generalized, not a localized, inflammatory response in the GI tract. This observation is reminiscent of studies of graft-versus-host disease, in which the concordance of upper and lower GI tract mucosal injury has been used to argue that flexible sigmoidoscopy may suffice to detect injury in most patients.\textsuperscript{25,26} Whether such considerations should impact choices of endoscopic approach in patients receiving CPI therapies will require additional data.

Any study of GI mucosal inflammation must acknowledge the polypharmacy that exists in complex patients, including those receiving CPI therapy. Many medications cause gastric and duodenal pathologic changes and must be considered in the differential diagnosis of CPI-induced mucosal injury. In addition to excluding infections in our cohort, concurrent medications were reasonably excluded as the cause of the inflammatory changes seen, based on temporal relationship of drug to symptoms, prior literature on known drug reactions of agents used, and responses to immunosuppressive therapy for presumed CPI-induced injury. In general, close correlation with medication history, the finding of typical histologic changes, and the dynamic evaluation of pathologic changes after CPI therapy will help confirm if an injury is likely to be an irAE. Appropriate differential diagnoses should be provided in pathology reports to account for the possibility that other medications or infections are relevant.

In summary, although it is acknowledged that the sample size is relatively small, these data provide evidence that CPI-induced upper GI tract inflammation is immunologically distinct from, yet exhibits morphologic similarities to, \textit{H pylori} gastritis and CeD. The distinction between these entities can usually be readily achieved using standard morphologic and serologic data. From a diagnostic viewpoint, lymphocyte phenotyping by immunohistochemical stains for CD3, CD4, CD8, or CD20 is not necessary in the consideration of causes of GI tract mucosal injury. However, as the use of CPIs and prevalence of subsequent irAEs increase, the molecular characterization of the drivers and effectors of the immune response in these reactions may help foster understanding of the pathogenesis of irAEs, as well as the pathophysiology of the autoimmune and infectious diseases they mimic. The morphologic and immunophenotypic distinctions noted herein between these entities may provide useful discriminators when clinicians are confronted with gastric and duodenal inflammatory changes in patients undergoing CPI therapy.

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