Histological and immunological characteristics of colitis associated with anti-CTLA–4 antibody therapy

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There is no conflict of interest
Introduction

- Immunotherapy increasingly incorporated in the treatment algorithms for malignant neoplasms in recent years

- Ipilimumab fully human, IgG1, anti-CTLA-4 monoclonal antibody
  Approval for the treatment of metastatic melanoma in 2011, in both US and Europe

- Treatment of neoplasias with ipilimumab associated with immune-mediated adverse reactions, (hypophysitis, thyreoiditis, hepatitis, uveitis) and especially the development of immune-mediated colitis
Ipilimumab Blocks Negative Signaling From CTLA-4

Aim of the study

- The evaluation of the histological characteristics of ipilimumab-associated colitis (IAC) in patients with severe diarrhea after treatment for metastatic melanoma

- The determination of the mucosal immunophenotype
  1. mRNA expression patterns for the most important effector and regulatory cytokines and transcription factors in comparison to both healthy controls and patients with inflammatory bowel disease (IBD)
  2. The composition of lymphocytic cell populations that infiltrate the colonic mucosa in IAC
Material and Methods

- **Examined material**: colonic biopsies from 9 patients with severe diarrhea subsequent to initiation of ipilimumab therapy for metastatic melanoma

- **Control groups**: 8 healthy individuals and 9 naive IBD cases
Material and Methods

- Histological examination included
  - Inflammatory infiltrates
  - Number of neutrophils and eosinophils
  - Erosions/ulcerations
  - Crypts with flattened mucin depleted epithelium
  - Cryptitis
  - Crypt abscesses
  - Crypt distortion

- Colitis severity was estimated by disease activity that was graded as absent, mild, moderate and marked
Material and Methods

Assessment of mucosal immunophenotype

- by examination of the inflammatory infiltrate, including semi-quantitative immunohistochemical characterization of lymphocytic subsets (CD20, CD3, CD4, CD8)

- by measuring the relative mRNA expression for transcription factors and cytokines specific for Teff and Treg by real-time RT-PCR in biopsy-tissue specimens obtained during endoscopy
Results

Histological characteristics

- **Colitis:**
  - diffuse chronic active (6 cases/67%),
  - patchy chronic active (2 cases/22%) and focally active (1 case/11%)

- **Activity:**
  - mild (1 case/11%),
  - moderate (5 cases/56%) and severe (3 cases/33%)

- **Ulcerations and/or erosions** (8 cases/89%)
**Results**

**Histological characteristics**

- Cryptitis usually mild (8 cases/89%)

- Small and high numbers of crypt abscesses in 4 and 3 cases respectively

- Crypts with mucin depleted cell lining appearing thinned and elongated in sagittal sections almost constantly present (8 cases/89%)

- Neutrophils from small up to high numbers invariably present
Results

Immunohistochemical characterization of the lymphocytic population

- Small number of B (CD20 +) lymphocytes
- Moderate or high number of T (CD3 +) lymphocytes
- High CD4/CD8 ratio
**Table 4.** Histological characteristics of ipilimumab-associated colitis

<table>
<thead>
<tr>
<th>Case</th>
<th>Distribution / severity</th>
<th>Infiltrate</th>
<th>L</th>
<th>P</th>
<th>E</th>
<th>N</th>
<th>cryptitis</th>
<th>crypt abscess</th>
<th>Erosion / ulceration</th>
<th>crypt mucin depletion / flattening</th>
<th>crypt distortion</th>
<th>subtype</th>
<th>CD 20</th>
<th>CD 3</th>
<th>CD 8</th>
<th>CD 4</th>
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<td>+</td>
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<tr>
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<td>++</td>
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<tr>
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L, lymphocytes; P, plasma cells; E, eosinophils; N, neutrophils; CD, Crohn’s disease; UC, ulcerative colitis; IC, intermediate colitis
Results

Colonic mRNA expression of cytokines and transcription factors

- The expression of the major Th1 and Th17 cytokines, IFN-γ and IL-17, highly upregulated
  IFN-γ expression >10-fold and IL-17A >5-fold upregulated compared to baseline control

- No significant changes in the expression of the major Th2 cytokines, IL-4 and IL-13

Results

Colonic mRNA expression of cytokines and transcription factors

- Increased mucosa expression of both the Treg-specific transcription factor FoxP3 and the major Treg cytokine IL-10

- The TNF like cytokine, TL1A (TNFSF15) highly upregulated in both IBD and IAC

- The functional receptor DR3 (TNFRSF25) specifically upregulated in IAC

Results are expressed as mean ± SEM. * indicate statistically significant differences in comparison to healthy control baseline expression.
Conclusions

- The histological spectrum of IAC is consistent with IBD-like colitis

- Prominent neutrophilic infiltrations, no crypt distortion as well as the presence of crypts with flattened, mucin depleted epithelium speak in favor of ipilimumab-inflicted colitis in consideration with the clinical history
Conclusions

- The highly upregulated mucosal Th1/IFN-γ and Th17/IL-17A responses combined with heavy infiltration of the lamina propria with CD4+ cells support the hypothesis that CTLA-4 blockade may lead to unrestricted lymphocytic activation and exaggerated effector pro-inflammatory pathways.

- Increased mucosa IL-17A produced by effector Th17 cells may drive the increased numbers of neutrophils observed in IAC.
Conclusions

We support the similarity between IAC and IBD, by showing that the two conditions share similar histology, inflammatory mucosal signatures, dominated by exaggerated Th1 and Th17 responses as well as ineffective upregulation of FoxP3 and IL-10
Thank you for your attention!