Deciphering the Immune System in Small Intestine: Immune Related Antibodies and Multiplex Strategies for Inflammatory Bowel Disease

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Introduction
Inflammatory bowel disease (IBD) can affect the lower part of the small intestine (ileum) and the colon.1,2 When defining IBD, the typical clinical manifestations are Crohn’s disease or ulcerative colitis.2 Patients with IBD increasing the risks may be at increased risk for developing osteoarthritis.3 Although CRC results from genetic and cellular context, it is a disease that occurs in approximately 1 to 2% of all cases of IBD; however, CRC is considered a serious complication that accounts for approximately 12% of deaths in patients with IBD.4 Recent IBD studies have shown great promise for potential therapeutic interventions.5 Evidence suggests that several factors may play a role in the balance between homeostasis and intestinal inflammation, and thus, present future challenges for the development of tools to improve diagnosis, provide a more accurate prognosis, and potential immunotherapies for IBD.5-8

In this study, we use normal small intestine (SI) as a model to identify multiple immune targets using monoclonal mouse and rabbit antibodies. Peyer’s patches (PP) are lymphoid tissues in the wall of the SI. PP are involved in the development of immunity to antigens presented in the surrounding area.9 Morphological locations of immune interest include the germinal center (GC), the mantle zone (MTZ), which is located on the outer ring of GC; the marginal zone (MZ), located outside the MTZ and adjacent to non-lymphoid tissue; and intestinal villi.10,11 SI and cases of IBD were evaluated as potential models using immunohistochemistry (IHC) to identify categories of immune cells including cytotoxic T-cells, costimulatory cells (activators), macrophages, T-regulatory cells (TREGs), and to examine expression patterns of immune checkpoint proteins.

Figure 1

Formalin-fixed paraffin-embedded human cases of SI and IBD were cut at 4 microns and stained with hematoxylin and eosin (H&E). Mouse and rabbit monoclonal antibodies including CD8, CD163, CD103, CD137, OX40, FOXP3, LAG3, GATA3, T-bet, RORγT, PD-1 and PD-L1 (Biocare Medical) were titered for staining optimization and evaluated by IHC. Antibodies were detected with a one-step polymer detection system and visualized with DAB chromogen.

The staining pattern of each antibody was categorized according to cell type, cell function, cell location, and morphological location in SI. Mouse and rabbit monoclonal antibodies including CD8, CD163, CD103, CD137, OX40L, FOXP3, LAG3, GATA3, T-bet, RORγT, PD-1 and PD-L1 (Biocare Medical) were verified for staining optimization and evaluated by IHC. Antibodies were detected with a one-step polymer detection system and visualized with DAB chromogen.

FOXP3 (black) + CD8 (red) & RORγT (brown)

Additionally, several cases of IBD were selected for single and multiplex stains including PD-1 + CD8, FOXP3 + CD8, OX40 + CD8 and a triple stain with FOXP3 + CD8 & RORγT antibodies.

Design

Figure 10: Inflammatory Bowel Disease

Figure 7: Small Intestine

Figure 8 - 9: Inflammatory Bowel Disease

Figure 10 Above: PD-1 (black) + CD8 (red). Notice co-expression of PD-1 + CD8 (green arrow) FOXP3 (black) + CD8 (red)

OX40 (black) + CD8 (red)

FOXP3 (black) + CD8 (red) and RORγT (brown)

PD-1 (black) + CD8 (red) & FOXP3 (royal blue)
Results
An IHC section of a Peyer patch SI with defined morphological zones demonstrated GC, MTZ, MZ and intestinal vili (Figure 1). Antibodies tested were by cell type, cell function, cellular location and morphological location in SI (See Table 1).

Small Intestine
Cellular Location (Table 1)

The IHC cellular location of each antibody tested included intraepithelial lymphocytes, epithelial, nuclear and cell surface stains. Epithelial T-cells were cell membrane/epithelial; macrophages demonstrated cytoplasmic staining; we observed nuclear staining in F4/80 with the exception of IBA1 (anti-Iba1), and immunohistochemical visualization demonstrated cell invasion staining.

Morphological Locations (Table 1)

PD-L1 (PD-1 ligand) showed weak staining in GC and moderate to strong staining in MTZ, MZ and SI (Figure 2). CD8 (cytotoxic T-lymphocytes) showed moderate to strong staining in all zones (Figure 3). CD4 (helper T-lymphocytes) showed weak to moderate staining in SI and SI (Figure 4A), and CD137 (costimulatory molecule) strongly stained follicular dendritic cells (FDCs). CD68 (macrophages) demonstrated cytoplasmic staining in all zones (Figure 5B). CD103 (CXCR3 ligand) showed strong selective staining in GC, MTZ and MZ (Figure 5C). CD80 (B7-1) and CD86 (B7-2) showed strong staining in all zones (Figure 6A, B). ICOSL (costimulatory molecule) showed strong staining in all zones (Figure 6C). PD-L1 (PD-1 ligand) showed moderate to strong staining in GC, MTZ and MZ (Figure 7A, B). CD137 (4-1BB) and OX40 (CD134) showed strong selective staining in GC, MTZ and MZ (Figure 7C, D).

Inflammatory Bowel Disease

Table 1

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cell Function</th>
<th>Cellular Location</th>
<th>Gastrointestinal</th>
<th>Intestinal</th>
<th>Skin</th>
<th>Mucosal</th>
<th>Hematological</th>
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<tbody>
<tr>
<td>CD8</td>
<td>Cytotoxic T-cell</td>
<td>Killing target cells</td>
<td>Mononuclear/plasmacytes</td>
<td>Low expression</td>
<td>High expression</td>
<td>High expression</td>
<td>Moderate expression</td>
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<tr>
<td>CD46</td>
<td>Macrophage</td>
<td>Engulfing and degrading target cells</td>
<td>Mononuclear/plasmacytes</td>
<td>Moderate expression</td>
<td>Moderate expression</td>
<td>High expression</td>
<td>High expression</td>
</tr>
<tr>
<td>CD103</td>
<td>Integrin-mediated phagocytosis</td>
<td>Activation of epithelial T-lymphocytes</td>
<td>Mononuclear/plasmacytes</td>
<td>No expression</td>
<td>Moderate expression</td>
<td>High expression</td>
<td>Moderate expression</td>
</tr>
<tr>
<td>CD80</td>
<td>Activated T-cell activator</td>
<td>Checkpoint co-stimulatory for effecter T-lymphocytes</td>
<td>Mononuclear/plasmacytes</td>
<td>Moderate expression</td>
<td>Moderate expression</td>
<td>Trace expression</td>
<td>Moderate expression</td>
</tr>
<tr>
<td>CD86</td>
<td>Follicular dendritic cells and T-cell</td>
<td>Checkpoint co-stimulatory for effecter T-lymphocytes</td>
<td>Grid membrane</td>
<td>High expression</td>
<td>Low expression</td>
<td>Moderate expression</td>
<td>Low expression</td>
</tr>
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<td>PD-1</td>
<td>T-lymphocyte</td>
<td>T-cell receptor protein</td>
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<td>No expression</td>
<td>No expression</td>
<td>Moderate expression</td>
<td>Moderate expression</td>
</tr>
<tr>
<td>PD-L1</td>
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<td>Inhibitory function</td>
<td>No expression</td>
<td>No expression</td>
<td>No expression</td>
<td>Moderate expression</td>
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<td>T-cell and macrophage</td>
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<td>Moderate expression</td>
<td>Low expression</td>
<td>Trace expression</td>
<td>Moderate expression</td>
<td>Trace expression</td>
</tr>
</tbody>
</table>

CD103 (CXCR3 ligand) showed strong selective staining in GC, MTZ and MZ (Figure 6C). CD137 (costimulatory molecule) showed strong selective staining in GC, MTZ and MZ (Figure 7A, B). CD137 (4-1BB) and OX40 (CD134) showed strong selective staining in GC, MTZ and MZ (Figure 7C, D).

Conclusion

Small intestine is a good model to develop IHC for a host of immune cell targets. Enhanced visualization of these targets is possible with double and triple stains, and thus may help facilitate acquisition of important diagnostic and prognostic information. We posit that these approaches may also support new therapeutic strategies for inflammatory bowel disease by leveraging immunotherapeutic agents.

References