Use of Ultivue InSituPlex® Multiplex Immunofluorescence to Localize and Quantify Regulatory T Lymphocytes in Crohn’s Disease and Ulcerative Colitis

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Abstract

The inflammatory bowel diseases ulcerative colitis (UC) and Crohn’s disease (CD) are chronic, relapsing inflammatory disorders of the gastrointestinal tract (GIT) that affect millions of individuals worldwide.1 The pathogenesis of these disorders is thought to involve dysregulation of mucosal immune homeostasis in the GIT in response to environmental factors in genetically susceptible individuals.2 Regulatory T cells (Treg) are CD4+ T lymphocytes that play a central role in peripheral immune tolerance, actively inhibiting inflammation upon antigen stimulation. There are two major populations of Treg: conventional Treg and TR1 cells.3 Conventional Treg arise from the thymus and are defined by the expression of CD4+CD25+FoxP3+. These cells are involved in the regulation of the immune response to environmental factors in genetically susceptible individuals.4 Thus, we sought to quantify conventional Treg and CTL populations in GIT tissue sections from IBD patients versus normal individuals by multiplex immunofluorescence.

Methods: Ultivue InSituPlex Assay

Using the InSituPlex technology, a custom antibody panel consisting of CD3, CD4, CD8, CD25, and FoxP3 was developed and the resultant multiplexed IHC assay was applied to de-identified FFPE specimens (Figure 1). Imaging was performed on the Zeiss Axiolab 21® 2E microscope using only the CY5 and Cy7 channels to avoid autofluorescence in the DAPI, FITC, and TRITC channels. To allow for multiple imaging rounds in the same two channels, a process of DNA-Exchange was employed. DNA-Exchange is a mild and specific removal of the labeled probe from a previous imaging round allowing for the application of a new set of probes and the detection of the next two markers. Image analysis was performed using HALO analysis software.

Results: Treg and CTL in Colon Specimens

Figure 2: Colon

Figure 3: Small Intestine

Conclusions

• CD8+ T cells were definitively identified, but were extremely infrequent among T cells in GIT sections from IBD patients and normal controls. CD4+ and CD8+ variants of CD25+FoxP3+ were observed.

References