Orion™: 15-Plex Single-Step Stain & Imaging of Tonsil Tissue with Reactive Lymphoid Hyperplasia

RareCyte, Inc.

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Precision Biology for Life Sciences

Overview

This is the same whole-slide tissue section of a tonsil with reactive lymphoid hyperplasia stained with a 15-plex immuno-oncology biomarker panel and imaged with the Orion system in a single staining and scanning process (figure 1).

In tonsil, B cell follicles with germinal centers and T cell interfollicular zones define the lymphoid compartment that is lined by epithelial crypts, which are invaginations of the squamous mucosa of the pharynx. Tonsillar architecture is illustrated here, with B-cell follicles (CD20), surrounding inter-follicular T-cell regions (CD4, white), and epithelial cells of the tonsillar crypts (Pan-Cytokeratin and E-Cadherin). Cell proliferation is indicated by the nuclear marker Ki67 and tissue morphology is provided by measuring autofluorescence.

Reactive Tonsil H&E-Stained Section

This is a hematoxylin and eosin (H&E) stained whole-slide tissue section of a tonsil with reactive lymphoid hyperplasia. Lymphoid hyperplasia is an increase in the number of lymphocytes that are contained in lymph nodes which often happens as part of the body's reaction to a chronic infection (figure 2).

Cell Proliferation in the Follicle

The follicle is the micro-anatomic site of clonal expansion of B cells in response to foreign antigens. In reactive lymphoid tissues, these follicles are enlarged and have distinct compartments, including a highly proliferative germinal center and a surrounding mantle zone (figure 3).

Nuclear staining (Hoechst) demonstrates the mantle zone (white circle) is comprised of small, densely-packed cells surrounding the germinal center. Germinal center cells are clearly visualized using cellular proliferation markers Ki67 and PCNA. Indeed, in this view, many cells appear orange-yellow indicating co-expression of both markers within the cell nucleus. Macrophages (CD68) act to eliminate apoptotic B cells and, in this image, are found interspersed within the germinal center.

A benefit of Orion collecting all markers in a single scan is that localization of individual markers may be robustly interrogated at subcellular resolution as singletons or in combination.

T Cells in the Follicle

Helper T cells (CD4) participate in the activation of B cell (CD20) expansion. Numerous helper T cells are seen in the germinal center with some in the mantle zone. Cytotoxic T cells (CD8) are also seen in the mantle zone (figure 4).

Spatial multiplexing demonstrates that the immune checkpoint receptor PD-1 is distinctly expressed by CD4 helper T cells within the germinal center in this field, but not in helper T cells in the T cell zone, and not in CD8 cytotoxic T cells.

PD-L1 Expression

PD-L1 and PD-1 are cognate immune checkpoint receptors involved in immune system tumor evasion, and their expression on tumor cells, and within the tumor microenvironment, impacts checkpoint inhibitor therapy response (figure 5):

- PD-L1 is expressed at high levels (red arrows) in the tonsillar crypt epithelium (Pan-CK).
- PD-L1 is expressed at moderate levels (magenta circle) in follicle germinal centers (CD20) to the left of the crypt.
- PD-L1 is expressed at low levels (yellow circle) in stromal cells of the T cell zone, shown above the germinal center.

Memory Cells in the Follicle

Memory lymphocytes can be identified by the marker CD45RO. In the follicle most of the memory cells co-express CD45RO and CD4, indicating that they are helper T cells. Numerous memory helper T cells can be seen in the T cell zone, as well as CD4 cells that are CD45RO negative. Most B cells (CD20) are negative for CD45RO. This is most clearly seen in the mantle zone (figure 6).

Lymphocyte Trafficking in the High Endothelial Venule (HEV)

Recirculation of lymphocytes into lymphoid tissues occurs via HEVs: vessels specialized for the attachment and transmigration of circulating cells. HEVs are present in the T cell zone and contain characteristic endothelial cells which have ample cytoplasm as indicated by the marker CD31 (figure 7).

Arrows show the following cells within this longitudinal section of the HEV lumen:

- CD4 (green arrow) T cells including FoxP3 (magenta arrow) T regulatory cells.
- CD8 (red arrow) T cells.
- CD20 (white arrow) B cells.

Macrophage Subsets

Macrophages express different markers depending on their function, which may be mapped spatially to location within a tissue. In the follicle, germinal center macrophages express CD68 (green arrows) alone. Just outside the follicle in the T cell zone, HEV (CD31) macrophages co-express both CD163 and CD68 (orange arrows) (figure 8).

Note: Macrophages have processes that extend beyond the cell body. When these are cut in cross-section and stained the resulting image may appear as a point or wispy fragment.

H&E and IF on Same Section

Morphology of cells and spatial location with biomarker phenotype may be correlated by staining the same tissue section with multiplexed IF and H&E (HEV, white arrows) (figures 9 & 10).

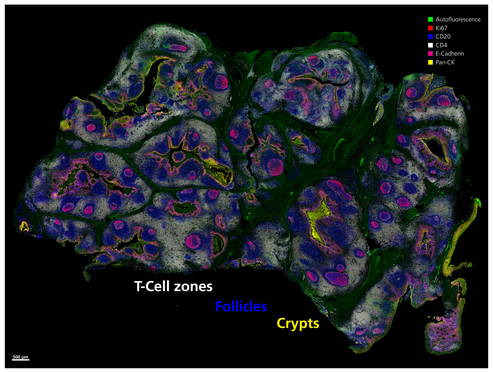

Fig 1. Whole-slide tissue section of a tonsil with reactive lymphoid hyperplasia stained with a 15-plex immuno-oncology biomarker panel and imaged with Orion.

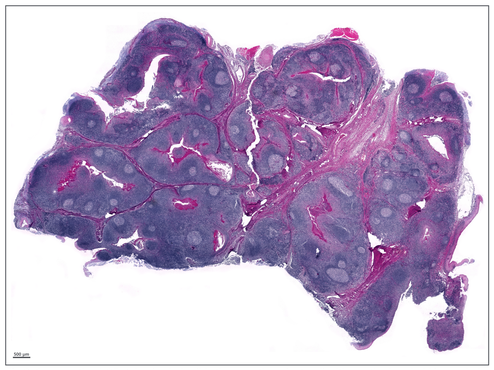

Fig 2. Same section H&E stained, whole-slide tissue section of a tonsil with reactive lymphoid hyperplasia.

Summary of Orion Workflow

Tissue Staining and Scanning Protocol

- Mount sections on glass slides
- De-paraffinize and perform antigen retrieval
- Quench autofluorescence
- Stain slides with panel of ArgoFluor™ conjugated antibodies
- Coverslip with ArgoFluor Mounting Medium and cure overnight
- Image whole slides at 20X magnification using Orion instrument
- Process to ome.TIFF and analyze
- De-coverslip in aqueous solution
- Perform H&E staining and scanning on same section

Tonsil Sample Information

The FFPE tonsil section was stained with a 15-plex immunofluorescence (IF) panel in one staining round followed by whole slide imaging with the Orion instrument in one imaging round.

- Tissue autofluorescence was imaged and isolated as an additional fluorescence channel
- H&E staining was performed after IF imaging on the same section and imaged by brightfield microscopy

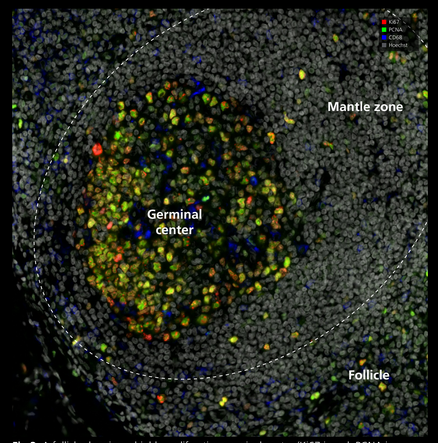

Fig 3. A follicle showing a highly proliferative germinal center (Ki67 in red, PCNA in green, co-expression of both in yellow) and a surrounding mantle zone (Hoechst, grey).

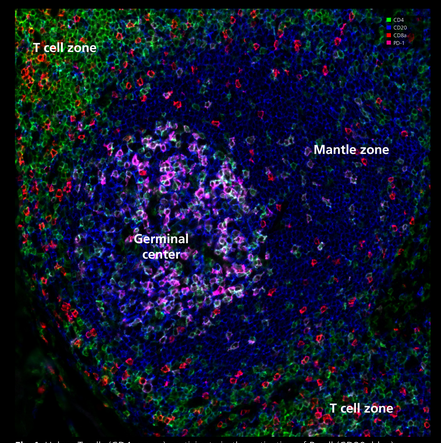

Fig 4. Helper T cells (CD4, green) participate in the activation of B cell (CD20, blue) expansion.

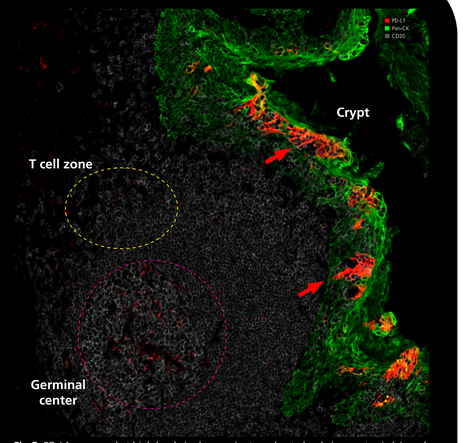

Fig 5. PD-L1 expressed at high levels (red arrows), at moderate levels (magenta circle), and at low levels (yellow circle).

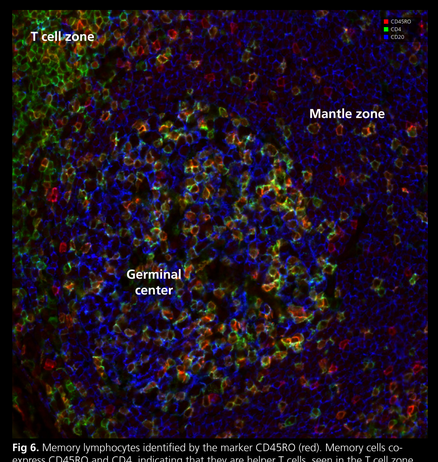

Fig 6. Memory lymphocytes identified by the marker CD45RO (red). Memory cells co-express CD45RO and CD4, indicating that they are helper T cells, seen in the T cell zone.

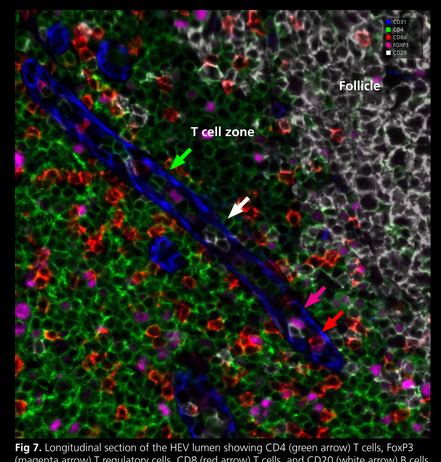

Fig 7. Longitudinal section of the HEV lumen showing CD4 (green arrow) T cells, FoxP3 (magenta arrow) T regulatory cells, CD8 (red arrow) T cells, and CD20 (white arrow) B cells.

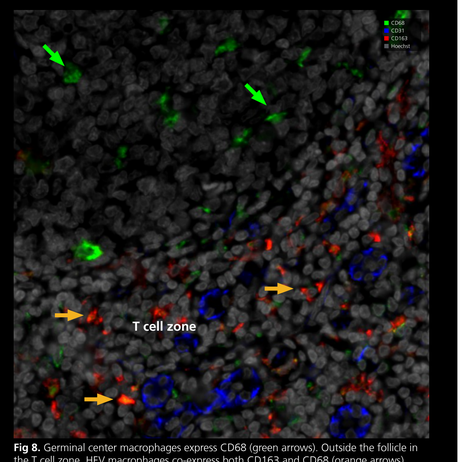

Fig 8. Germinal center macrophages express CD68 (green arrows). Outside the follicle in the T cell zone, HEV macrophages co-express both CD163 and CD68 (orange arrows).

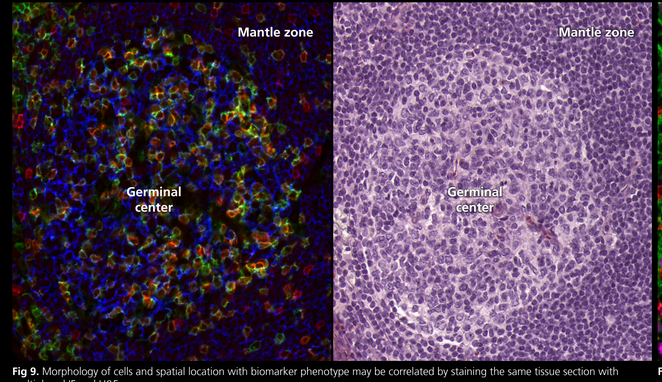

Fig 9. Morphology of cells and spatial location with biomarker phenotype may be correlated by staining the same tissue section with multiplexed IF and H&E.

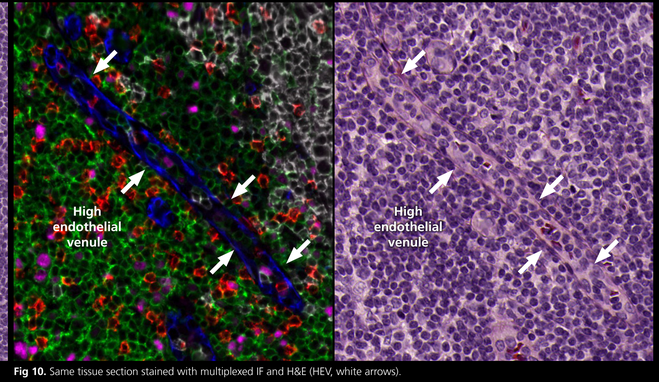

Fig 10. Same tissue section stained with multiplexed IF and H&E (HEV, white arrows).

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