GLOBAL RESEARCH IMMERSION PROGRAM FOR YOUNG SCIENTISTS

Flow Cytometry Analysis of Immune Profiles in Lamina Propria Mononuclear Cells from TNBS and DSS-Induced IBD Mouse Models

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OBJECTIVES

- To characterize the progression and severity of colitis in mouse models.
- To investigate the role of immune cells in inflammation.
- Analyze the immune cell populations in TNBS- and DSS-induced colitis models using flow cytometry.

BACKGROUND

Inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, are chronic conditions characterized by intestinal inflammation.[1] Mouse models are often used to study these diseases and develop treatments.[1] Two common methods to induce colitis in these models are TNBS injection and DSS administration in drinking water.[1,2]

Figure 2. Illustration highlighting the differences between a normal intestine and an intestine suffering from Crohn's disease. Identifiable by a thickened wall and additional fat wrapping.

In contrast, DSS added to drinking water causes cellular damage in the colon, leading to immune cell infiltration, inflammation, and ultimately colitis.[2] This condition more closely resembles ulcerative colitis in humans, as it is characterized by superficial inflammation and mucosal damage.[2]

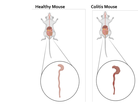

Figure 1. Illustration showing a healthy mouse colon compared to a diseased colon, characterized by shorter length and inflammation.

TNBS is a hapten, a small molecule that stimulates an immune response when attached to a larger carrier molecule like a protein.[3] When injected into the colon, TNBS triggers a T-cell mediated immune response, damaging the intestinal epithelial barrier and inducing inflammation.[2] This process shares similarities with Crohn's disease in humans, notably transmural inflammation and granuloma formation.[2]

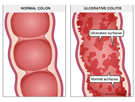

Figure 3. Illustration highlighting the differences between a normal colon and a colon suffering from ulcerative colitis. Identifiable by the presence of ulcerated surfaces.

METHODS

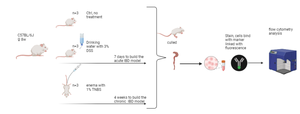

Figure 4. Workflow of the experiment. 4 steps including (1) IBD mouse modeling with 2% DSS or 1% TNBS, (2) tissue collection and single cell acquisition, (3) staining of markers and (4) analysis using flow cytometry.

TNBS-Induced Colitis

1. Lightly anesthetized the mouse and administered 1% TNBS solution via enema using a lubricated needle inserted ~4 cm into the colon.
2. Daily monitoring for weight loss, as significant weight loss (~20%) can indicate intestinal damage.
3. Euthanized at the end of the fourth week, at which point the colon is dissected and assessed for inflammation via flow cytometry.

DSS-Induced Colitis

1. Provided mice with DSS-containing water to induce acute colitis and monitored them daily.
2. The DSS-containing water replaced with regular water for recovery period.
3. Mice weighed weekly and euthanized at the end of the experiment, at which point the colon is dissected and assessed for inflammation via flow cytometry.

Lymphocyte Isolation

1. Prepared dissociation (epithelial cell removal), digestion (collagen breakdown) buffers and Percoll solutions (40% + 80%).
2. Colon collected and cleaned in ice-cold PBS, fat and visible fecal material removed.
3. Tissue is then cut into 1 – 1.5 cm pieces and incubated in dissociation solution.
4. After supernatant filtration, cell resuspended in 10ml 40% Percoll (for further digestion).
5. Cell suspension then centrifuged in Percoll gradient, allowing for separation of cells based on density.
6. The target cells, lamina propria lymphocytes, are collected from the middle layer.
7. Cells stained for flow cytometry analysis.
8. Cells fixed, and resuspended in staining buffer for storage.
9. Samples were then analyzed by a flow cytometer.

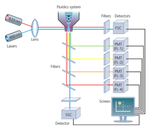

Figure 5. Principle of flow cytometry. Fluorescently tagged cells are excited as they pass through the lasers, resulting in lights of different wavelengths being detected by sensors.

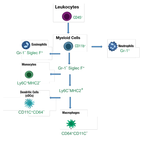

Figure 6. Myeloid cell surface markers. These surface markers are connected to the fluorescent dyes. These markers are expressed differently and used to identify different cell types.

RESULTS

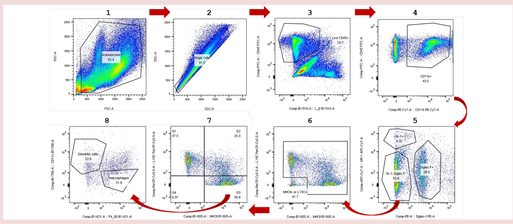

Figure 7. A gating strategy for flow cytometry data analysis. A polygon shape is used to acquire target cells. (1) The area of the cells is detected by the forward scatter (FSC) and side scatter (SSC) to identify cell size. (2) The height and area of the cell is used by SSC to differentiate single cells from doublets. (3-8) The X and Y axes represent different surface markers.

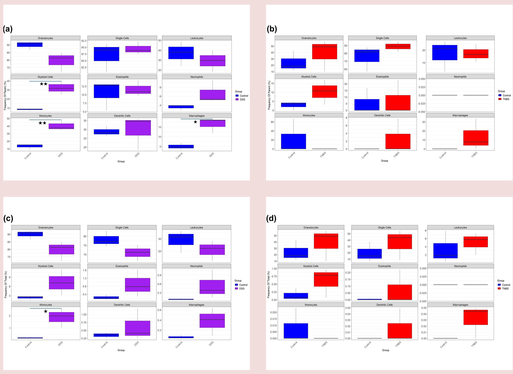

Figure 8. Comparison of different cell types belonging to diseased and healthy colon mice models. Frequency of parent (%) regarding 9 cell types (a) from DSS and control groups where significant differences were found among the myeloid (P=0.0056), monocyte (P=0.0044) and macrophage (P=0.0104) cell types; (b) from TNBS and control groups. Frequency of total cells (%) regarding 9 cell types from (c) DSS and control groups where a significant difference is found in the monocyte (P=0.0223) cell type; (d) from TNBS and control groups. (*p<0.05, **p<0.01).

DISCUSSION

- In this research project, we constructed two IBD mice models induced by TNBS or DSS to investigate myeloid immune cell dynamics. We analyzed nine different myeloid immune cell types from the colon using flow cytometry, focusing on their frequencies relative to parent populations and total cell percentage.
- In the DSS-induced model, we observed significant increases in monocytes and macrophages (frequency of parent). We also noted increasing trends in eosinophils and neutrophils (frequency of total), meaning these cell types were generally more abundant compared to the control, though not to a statistically significant degree. Interestingly, leukocytes exhibited a decreasing trend, suggesting their numbers were generally lower in the DSS group compared to the control, but not significantly so. In the TNBS-induced model, we observed a trend increase only in myeloid cells for both frequency measures (parent and total), indicating a general but not statistically significant increase in these cell types.
- Monocytes and macrophages play crucial roles in IBD inflammation progression as key innate immunity mediators. These cells produce pro-inflammatory cytokines, phagocytose pathogens and cellular debris, and present antigens to T cells, effectively linking innate and adaptive immune responses. Moreover, monocytes can differentiate into macrophages within tissues, potentially amplifying the inflammatory cascade in IBD.
- The TNBS results were compromised due to sample mishandling (left without ice for 1.5 hours during preparation), resulting in insufficient cells for comparable data analysis. This underscores the critical importance of maintaining consistent experimental procedures, especially in multi-model studies where timing and sample handling can significantly impact results.

CONCLUSION

- This flow cytometry-based approach enhances our understanding of immune cell population dynamics during IBD progression. While not all observed trends were statistically significant, they provide valuable insights into the underlying mechanisms of IBD. Despite limitations, combining chemical methods for IBD model construction with flow cytometry analysis offers a precise examination of cellular-level changes, aiding in the standardization and evaluation of IBD models.
- In summary, TNBS and DSS-induced colitis mouse models remain valuable tools for IBD research. Their distinct pathological characteristics, when integrated with advanced analytical techniques like flow cytometry, offer a comprehensive approach to studying IBD. Future studies using these models in combination with emerging technologies hold great promise for unraveling the complexities of IBD and developing more effective treatments.

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