

Intestinal Inflammation

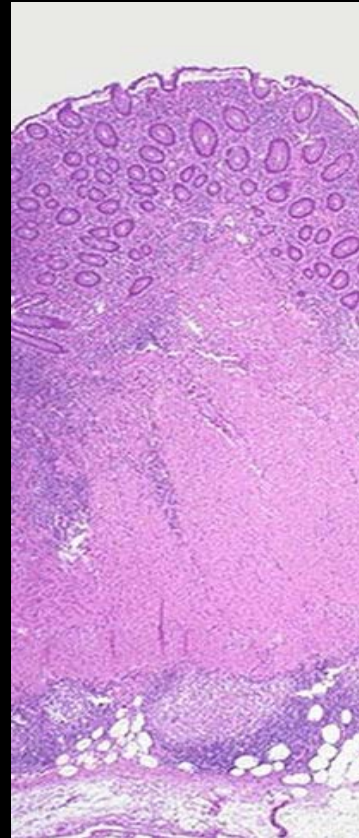
- I Epithelial-dependent antigen interactions at the luminal surface of the large intestine
- II Inflammation, myofibroblasts and toll-like receptors in the large intestine
- III Regulation of neutrophil migration across the intestinal epithelium

Titus A. Reaves, Ph.D.
Assistant Professor
Cell Biology and Anatomy
Medical University of South Carolina

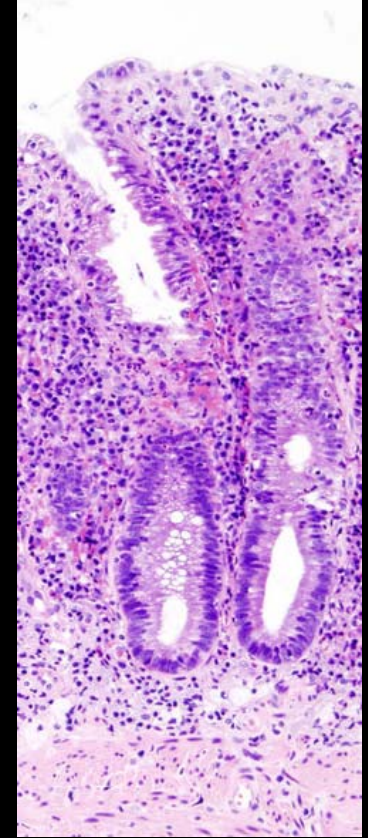
Introduction

- Inflammatory Bowel Disease (IBD) is a major cause of gastrointestinal disorders in children and adults
- IBD consists (primarily) of Crohn's Disease (entire GI tract, mostly terminal ileum and large intestine) and Ulcerative Colitis (localized to large intestine and rectum)
- Features: leukocyte migration (intestinal lumen); epithelial destruction; crypt abscess formation (causes considerable epithelial damage); auto-immune characteristics

Crohn's Disease

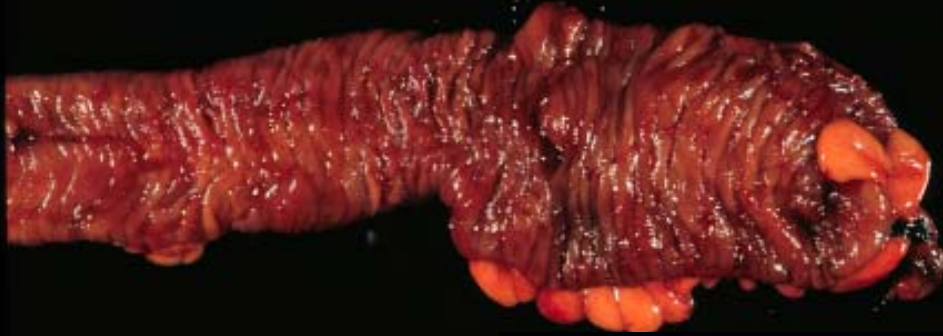


Ulcerative Colitis

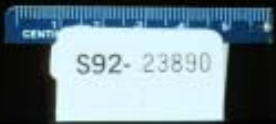


Etiology is unknown

Consequences of IBD on intestine



← Large Intestine
Normal



Large Intestine
IBD



Introduction: epithelial surface

- The luminal surface (epithelium) of the large intestine serves as the first line of defense against pathogenic microbes and toxins
- Microbial flora ($\sim 10^{14}$) are contained in the lumen of the large intestine, and are major activators of the immune system
- Studies have shown that this microbial flora is different in patients diagnosed with IBD
- Potential cause of IBD is the interaction of the normal flora with antigens on the intestinal epithelial surface

Leads to a dysregulation of the immune system

Epithelial-dependent antigen interactions at the luminal surface of the large intestine

- Collaboration with the Digestive Disease Center and the Department of Gastroenterology and Hepatology at MUSC
- 40 blood samples were taken from patients diagnosed with IBD; such patients were from various ethnic and socioeconomic groups
- The serum was isolated and analyzed for reactivity against epithelial cells

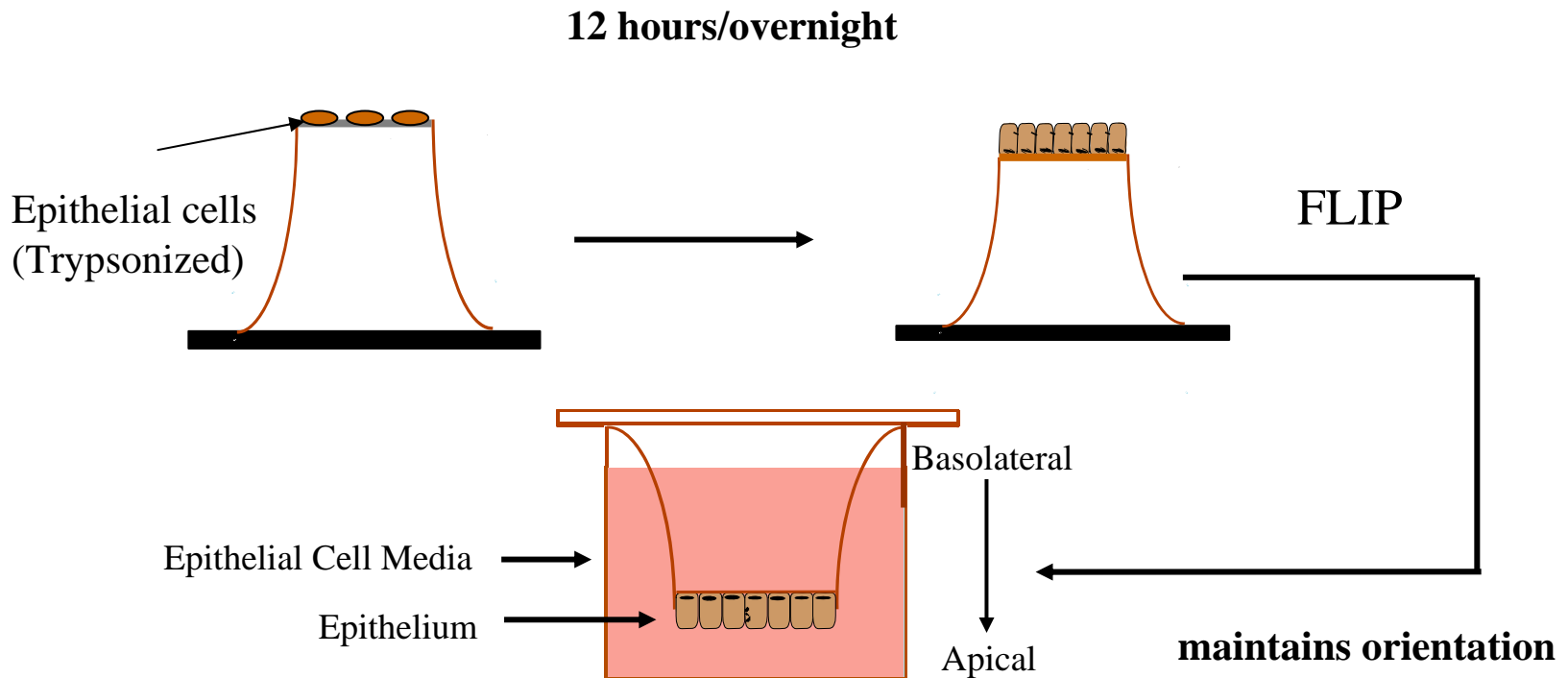
Culturing of Intestinal Cells

Human Intestinal Epithelial Cells (polarized)

T84: Large intestine (High resistance)

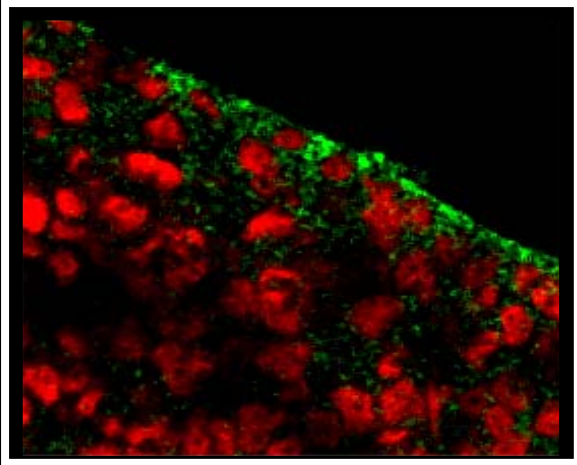
HT-29: Large intestine (Low resistance)

CaCo-2: Small intestine

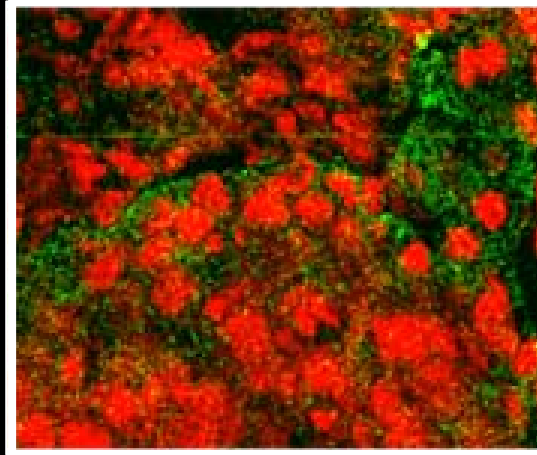


Immunofluorescence: human serum reacts to HT-29 cells

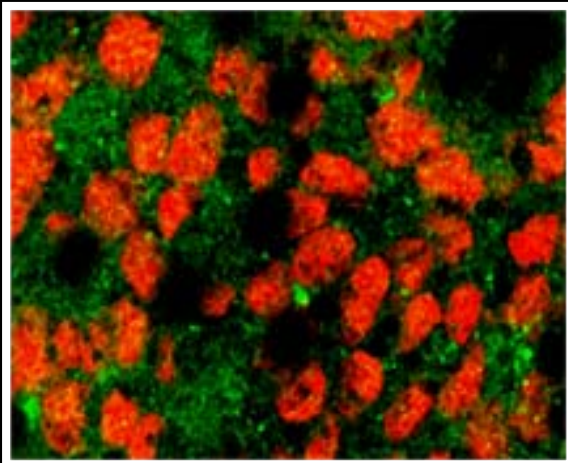
CM-42



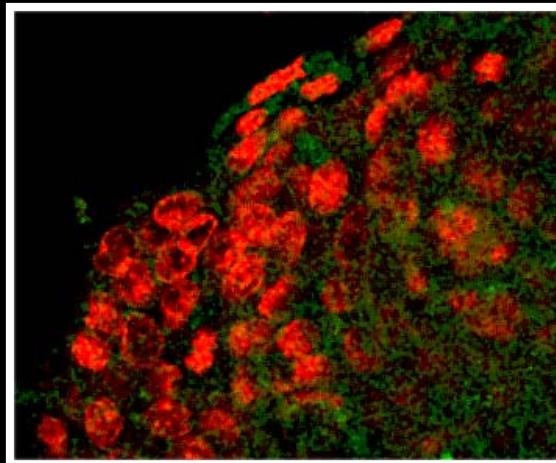
ST-35



KS-33



MG-32



Antigen

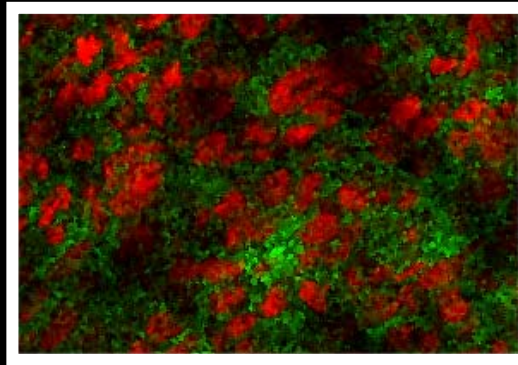


Nuclei

Immunofluorescence: serum TH-10 strongly reacts to the surface of HT-29 cells

Subject TH-10

X-Y

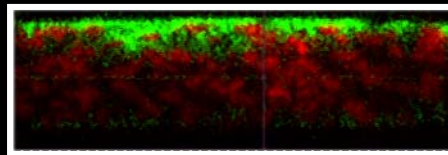


Antigen



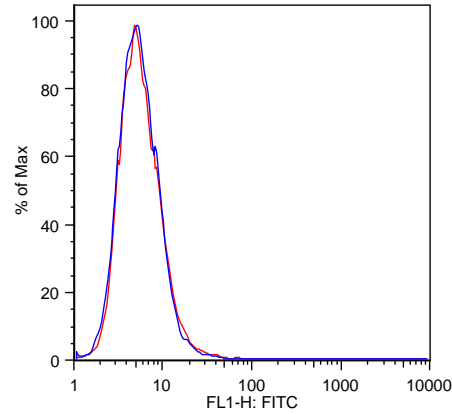
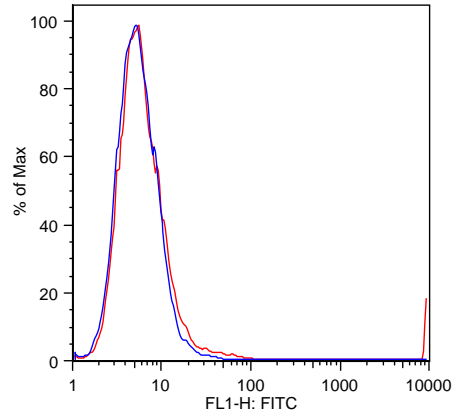
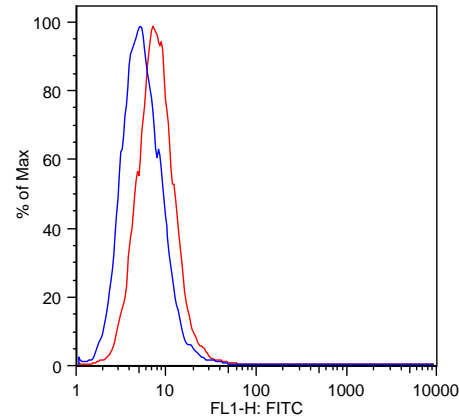
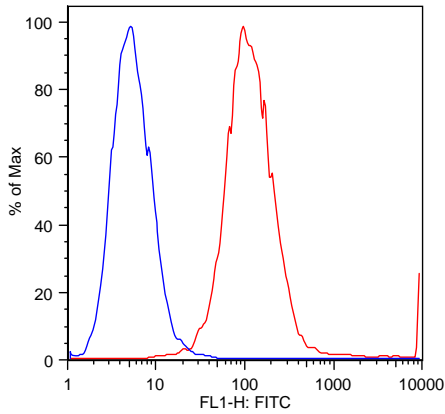
Nuclei

X-Z



No Expression in CaCo-2 cells

TH-10 expression in HT-29 intestinal epithelial cells



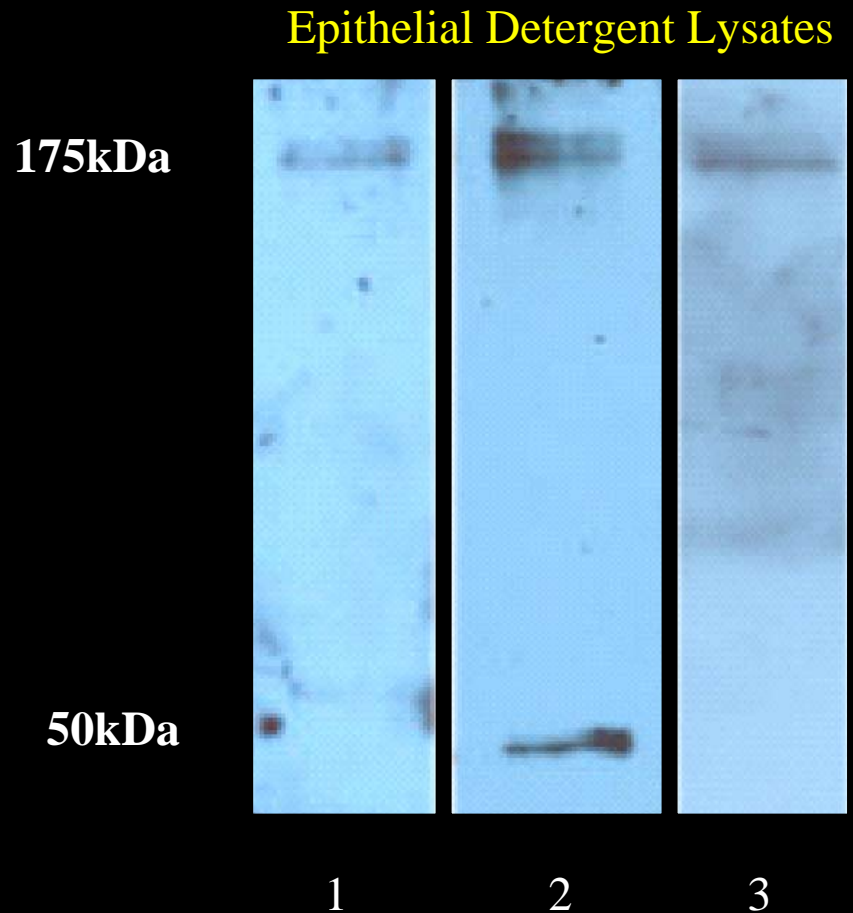
Negative results in CaCo-2 cells

- Antigen specific for HT-29 and T84 cells, but not CaCo-2 cells
- Epithelial cells from other areas have not been examined (e.g., skin and lung)

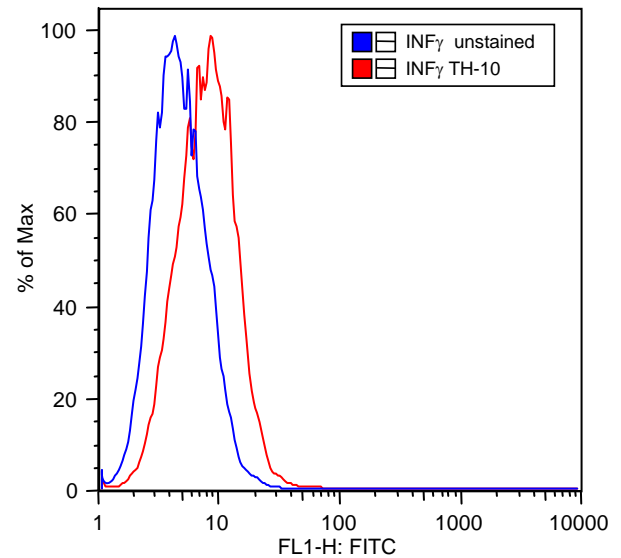
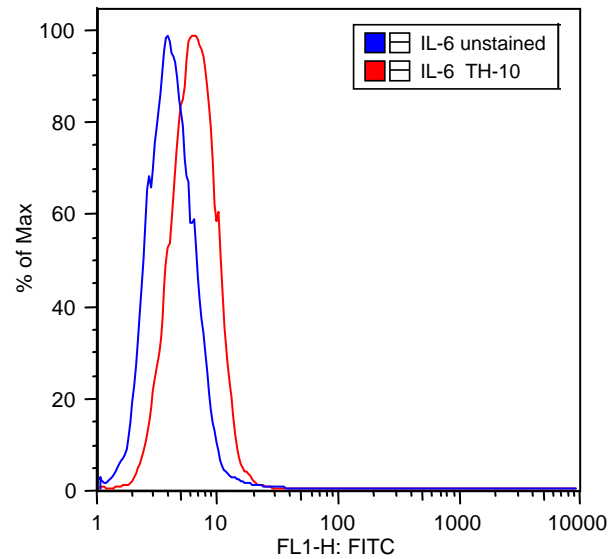
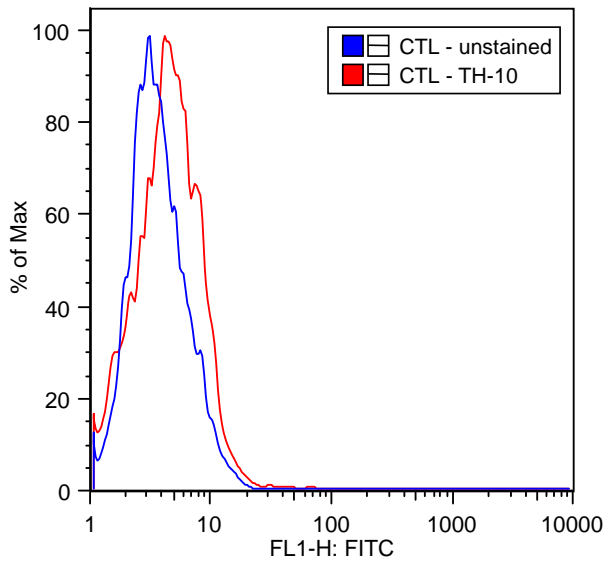
Interferon- γ induced expression of antigen defined by antibody TH-10 (human serum)

Immuno-blots of antibody TH-10 to HT-29 lysates

1. Normal
2. Interferon Gamma (γ)
(inflammatory cytokine)
3. Interleukin-10
(anti-inflammatory cytokine)



Expression of antigen defined by antibody TH-10 can be regulated by inflammatory cytokines



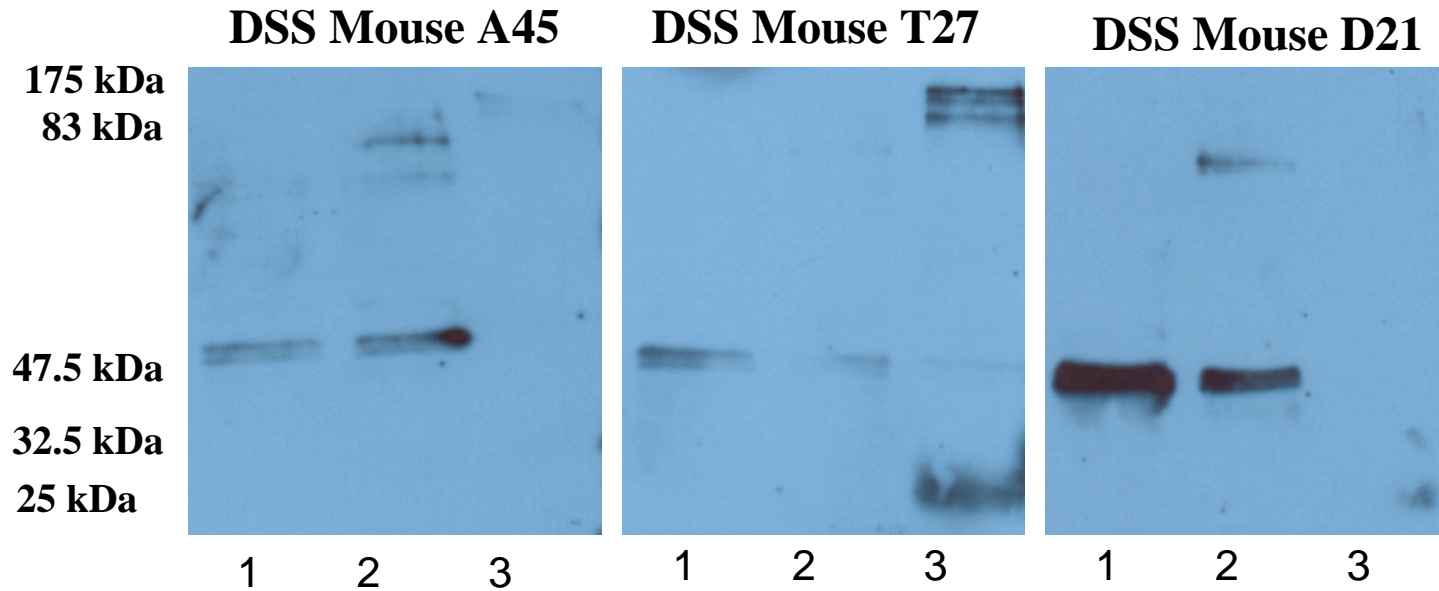
Mouse Model of Colitis

- Dextran Sulfate Colitis Model—intestinal characteristics: epithelial destruction, leukocyte transmigration, ulcerated crypt epithelium
- Mice receive 2% dextran sulfate in drinking water
- Evidence of Colitis: blood in the stool, intestine characteristics

Day 10 and 21 mice were euthanized and bled out

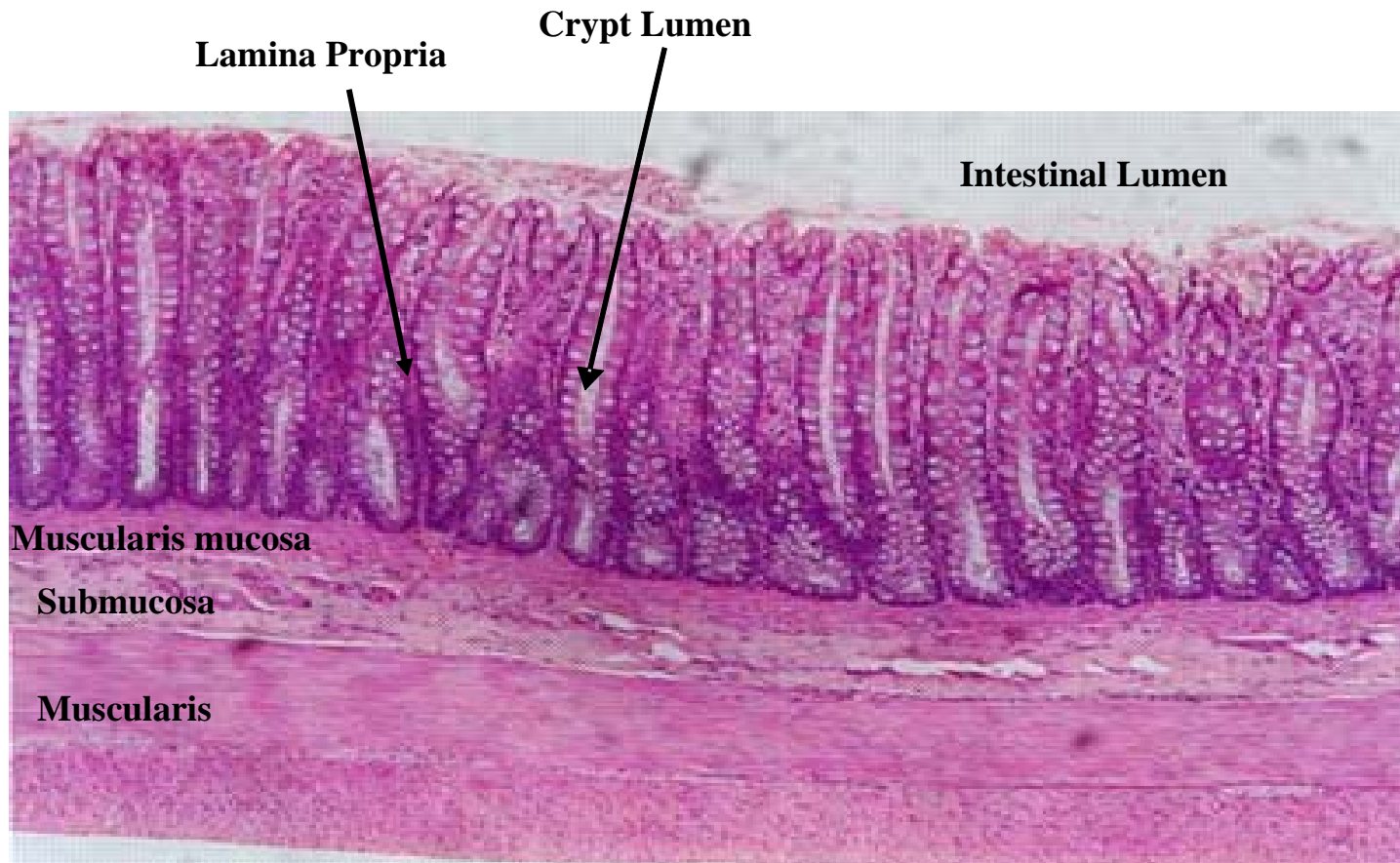
Immuno-blots: DSS mice produced antibodies similar to antibody TH-10

epithelial lysates probed with mouse serum

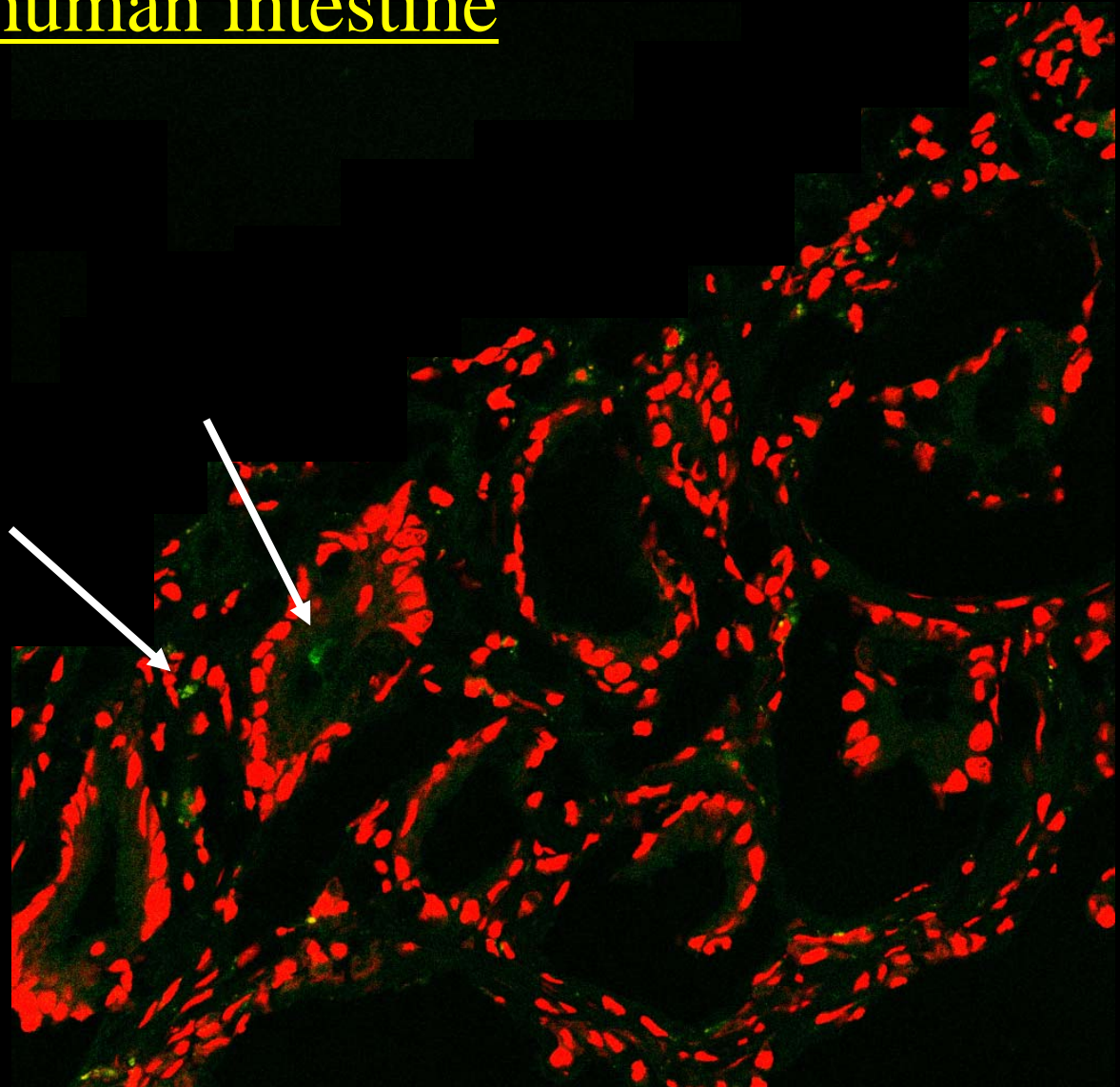


1. Normal (untreated) HT-29 cells probed with mouse serum
2. IFN γ treated HT-29 cells probed with mouse serum
3. TH-10 column isolate (affinity chromatography of epithelial lysates) probed with mouse serum

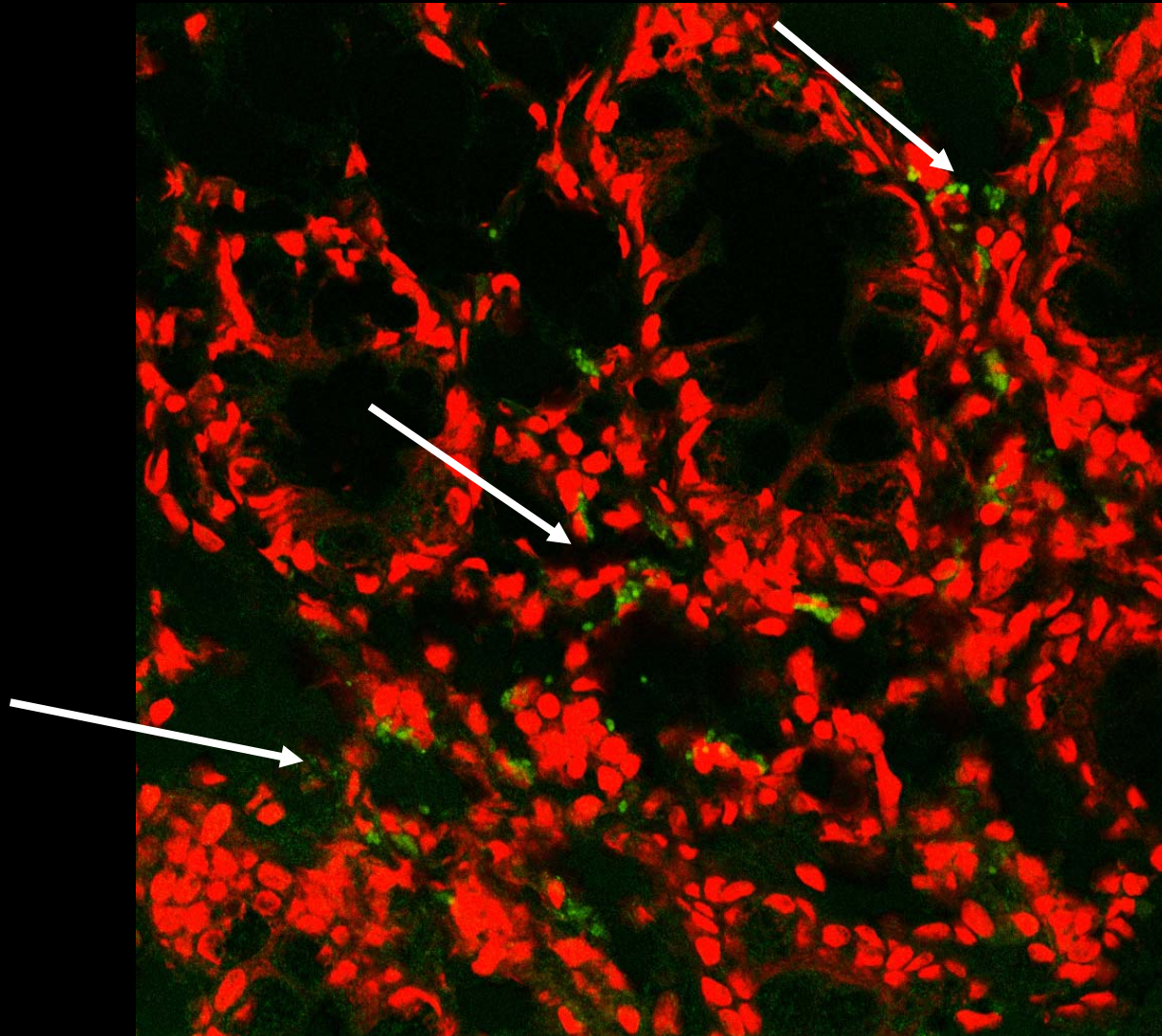
Anatomy: large intestine



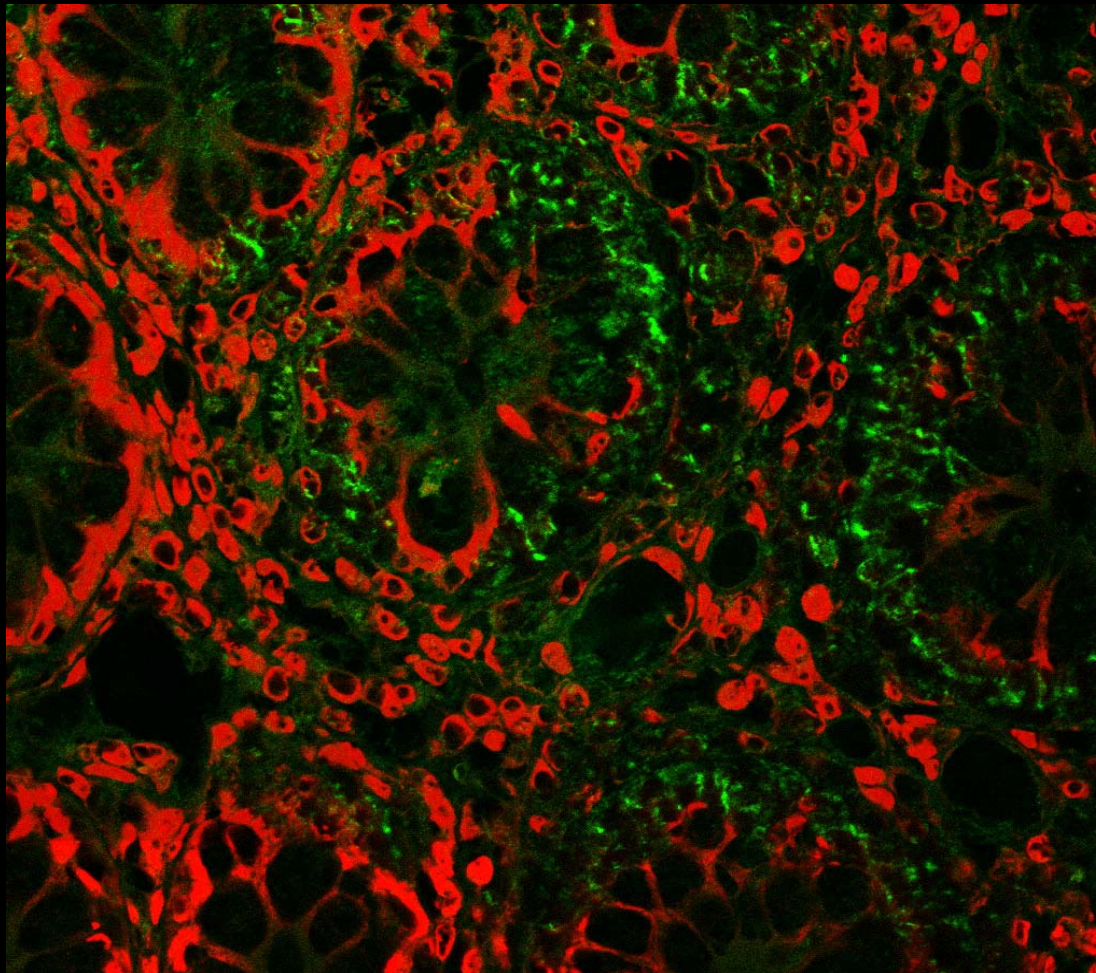
Serum from mouse T27 reacts to
normal human intestine



Serum from mouse T27 strongly reacts to Ulcerative Colitis intestine (human)

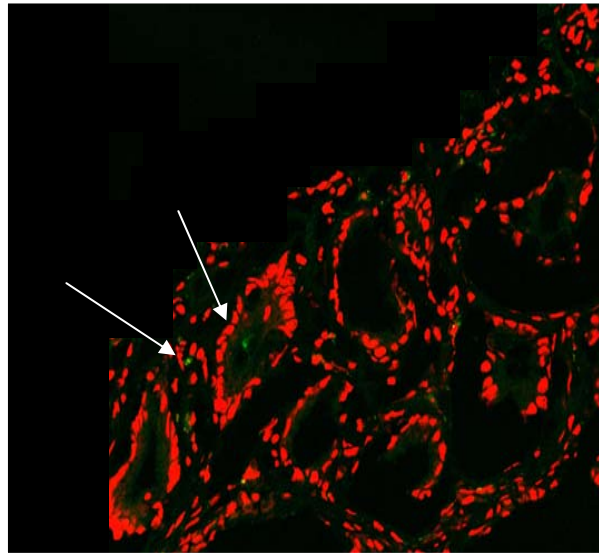


Serum from mouse T27 reacts to Crohn's Disease
intestine (human)

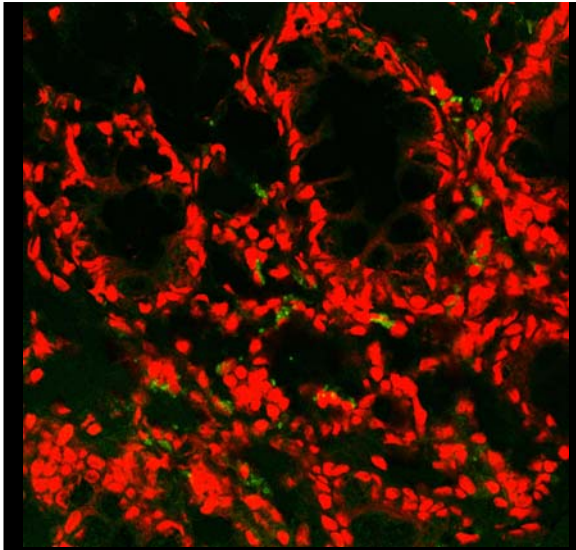


Increase in Mouse T27 Reactivity

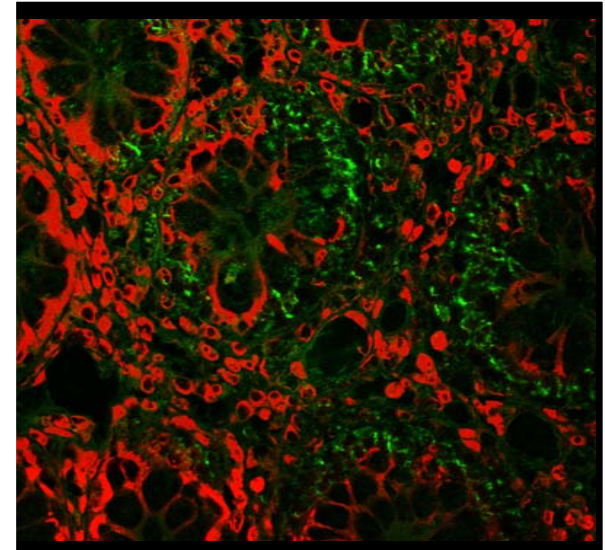
Normal



Ulcerative Colitis



Crohn' Disease

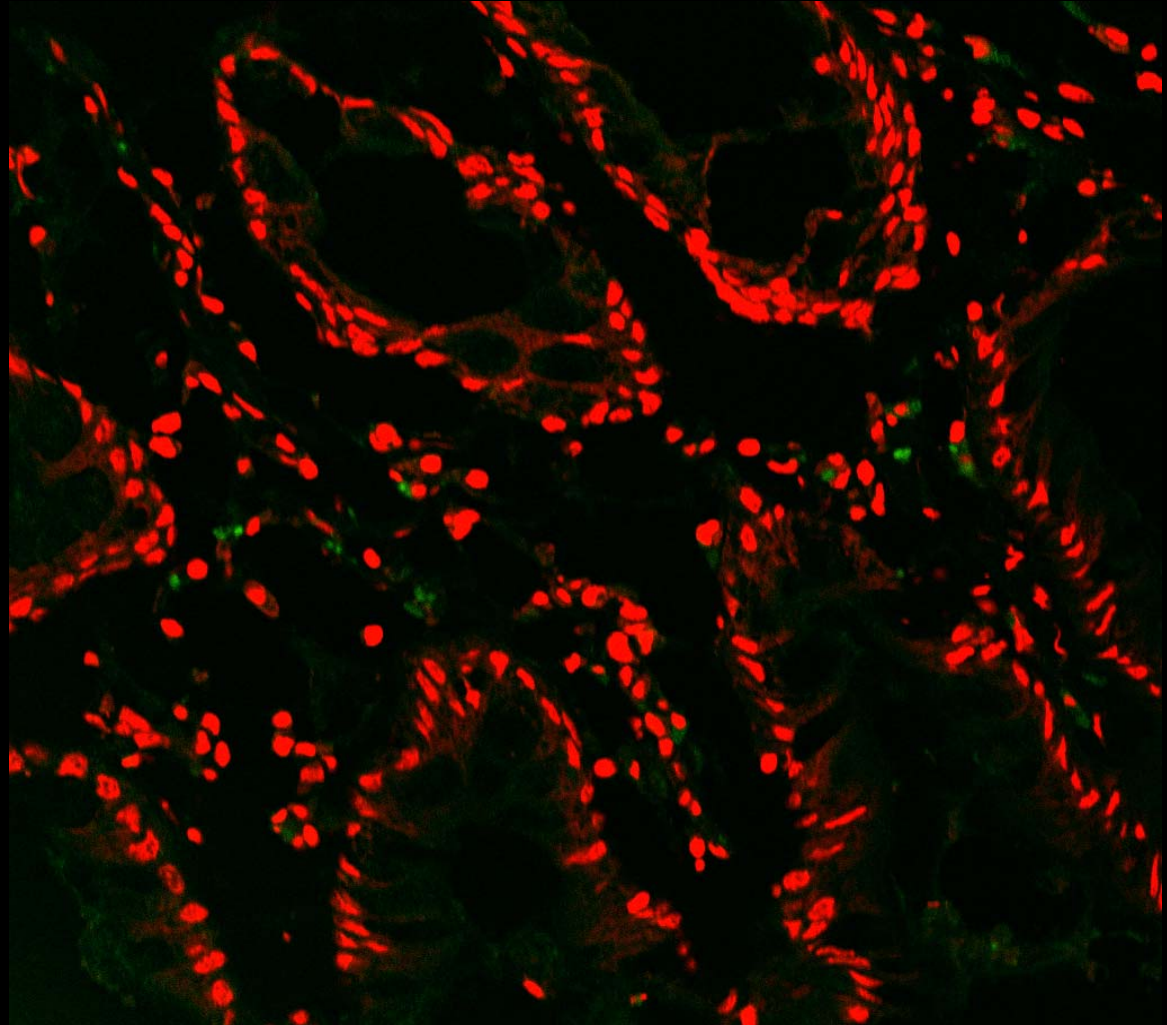


Correlates to the antigen defined by Antibody TH-10

Active Ulcerative Colitis Intestine (human)

Non specific

Serum from
control
mouse



Summary

- Epithelial antibodies are produced by patients diagnosed with IBD
- Epithelial antibodies are also produced by mice (with colitis), which are similar to human epithelial antibodies
- Expression of such antigens can be regulated by inflammatory cytokines
- The state of the epithelium determines expression of the antigen and probably affects antibody

Future Directions

- Identify the antigen
- Examine interaction of antibody with leukocytes (neutrophil)
- Examine modifications of antigen with cytokines

Acknowledgments

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