*DISCLAIMER – Although this is actual IHC data on a specific protein target, the protein name is fictitious. Any resemblance to a protein name published in the literature is coincidental.

Immunohistochemistry Sample Report for Antibody LP-GLR on Rheumatoid Arthritis and Ulcerative Colitis Samples

Summary

Antibody LP-GLR, a Western positive rabbit polyclonal antibody targeting the amino terminus of the GLR protein, was evaluated in immunohistochemistry on normal synovium and synovium from a patient with rheumatoid arthritis and normal colon and a sample of inflamed colon from a patient with active ulcerative colitis.
Compared to normal synovium, the rheumatoid arthritis case showed increased staining of synoviocytes and subsynovial fibroblasts and histiocytes. Similarly, increased staining was seen in areas of reactive epithelium in ulcerative colitis compared to normal colon. These studies suggest that GLU is upregulated in response to inflammation, and may be a potential marker or target for inflammatory diseases such as rheumatoid arthritis and ulcerative colitis.

Methods

Antibody Titration and Study Protocol:

Antibody titration experiments were conducted with a Western positive affinity-purified anti-peptide antibody targeting the amino terminus of GLR, to establish concentrations that would result in minimal background and maximal detection of signal. Serial dilutions were performed at concentrations of 20 ug/ml, 10 ug/ml, 5 ug/ml, and 2.5 ug/ml. The serial dilution study demonstrated the highest signal-to-noise ratio at a concentration of 2.5 ug/ml on paraffin-embedded, formalin-fixed tissues. This concentration was used for the current study. The antibody to GLR was used as the primary antibody, and the principal detection system consisted of a Vector anti-goat secondary (BA-5000) and a Vector ABC-AP kit (AK-5000) with a Vector Red substrate kit (SK-5100), which produced a fuchsia-colored deposit.

Tissues were also stained with positive control antibodies (CD31 and vimentin) to ensure that the tissue antigens were preserved and accessible for immunohistochemical analysis. Only tissues that were positive for CD31 and vimentin staining were selected for the remainder of the study. The negative control consisted of performing the entire immunohistochemical procedure on adjacent sections in the absence of primary antibody.

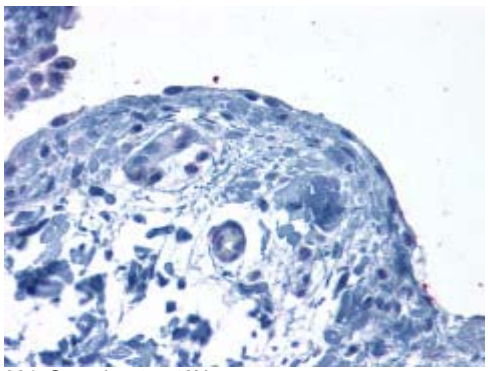
Slides were imaged with a DVC 1310C digital camera coupled to a Nikon microscope. Images were stored as TIFF files with Adobe Photoshop.

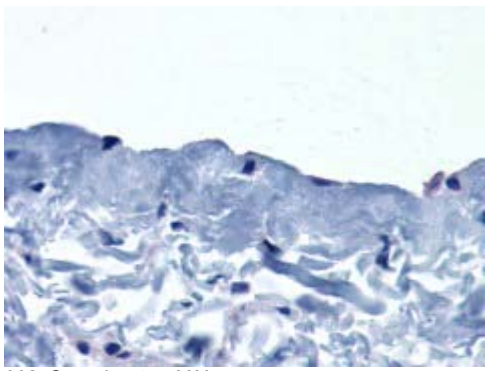
Results:

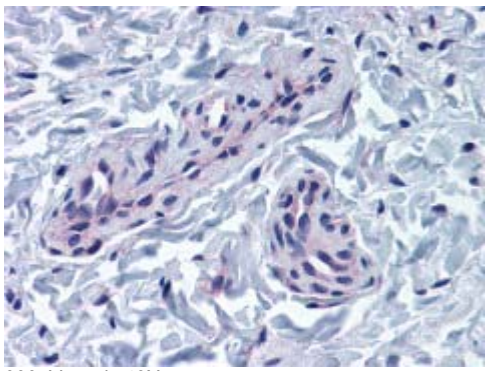
Normal Synovium

Synoviocytes, subsynovial fibroblasts, subsynovial histiocytes, and vascular endothelium were negative. Vascular smooth muscle was faintly positive.

Sample 1: This sample of normal synovium was obtained at surgery from a 31-year-old male with a medial meniscus tear.


001: Synoviocytes 40X


002: Synoviocytes 60X


003: Vessels 40X

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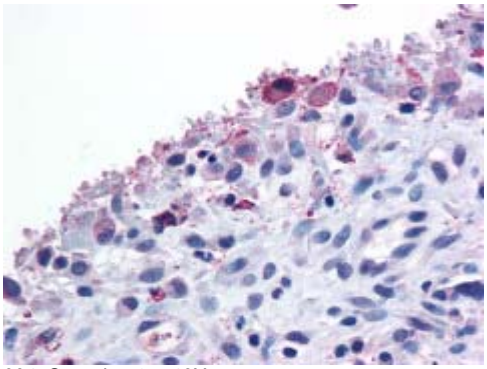
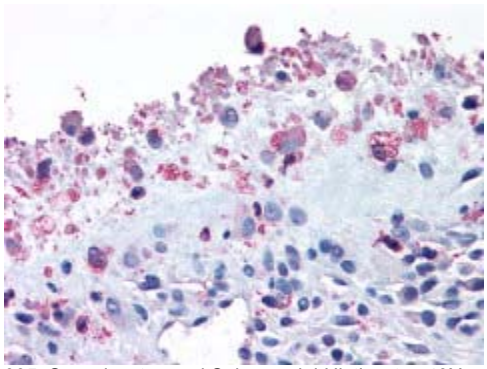
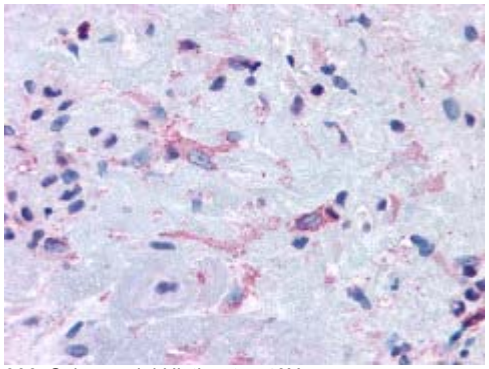
Synovium, Rheumatoid Arthritis

Sample 1: This sample of synovium was obtained at surgery from a 35-year-old female with a clinical history of rheumatoid arthritis.

Superficial synoviocytes exhibited faint to moderate positivity. Subsynovial fibroblasts and subsynovial histiocytes exhibited moderate to strong positivity. Capillary endothelial cells within the pannus were negative. Vascular smooth muscle displayed faint positivity. The lymphoplasmacytic infiltrate was negative. Neutrophils showed moderate to strong positivity. Macrophages showed moderate positivity. Compared to samples of normal synovium, this sample showed increased staining of synoviocytes and subsynovial fibroblasts and histiocytes.


004: Synoviocytes 40X

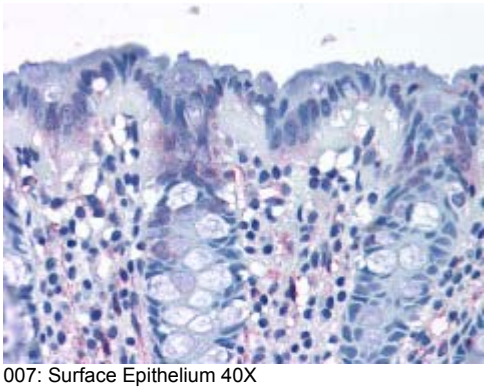

005: Synoviocytes and Subsynovial Histiocytes 40X

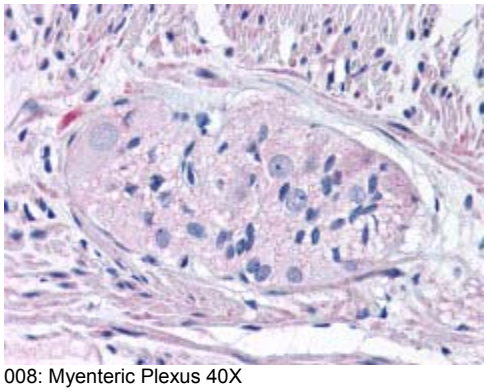

006: Subsynovial Histiocytes 40X

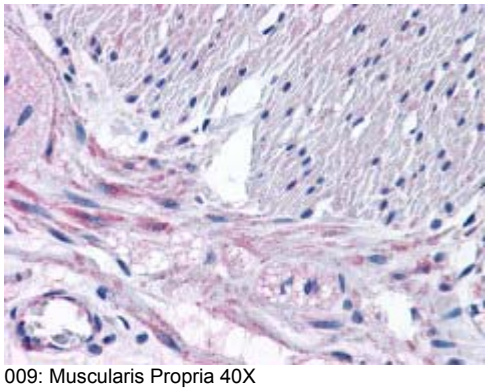
Normal Colon

Sample 1: This sample of normal colon was obtained at surgery from a 45-year-old female.

The surface epithelium was negative. Crypt epithelium was negative. Intraepithelial neuroendocrine cells were negative. The muscularis mucosa, muscularis propria, and endothelial cells and vascular smooth muscle within submucosal vessels were faintly to moderately positive. The myenteric plexus showed faint staining.


007: Surface Epithelium 40X


008: Myenteric Plexus 40X


009: Muscularis Propria 40X

Colon, Ulcerative Colitis

Surface epithelial cells exhibited occasional moderate staining, particularly in reactive areas. Crypt epithelium was negative or exhibited faint staining. Intraepithelial neuroendocrine cells and Paneth cells were negative. Lymphocytes in areas of chronic

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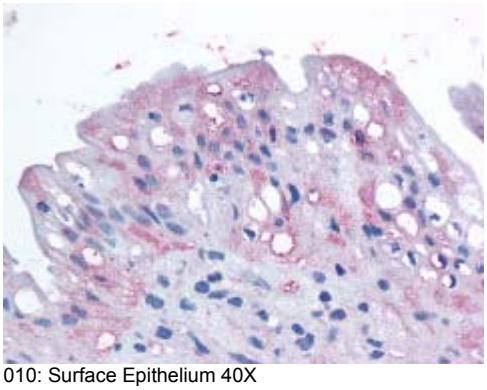
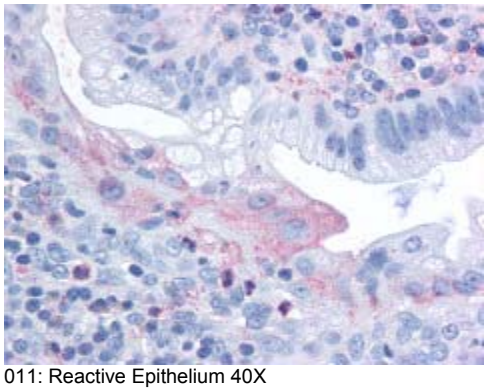
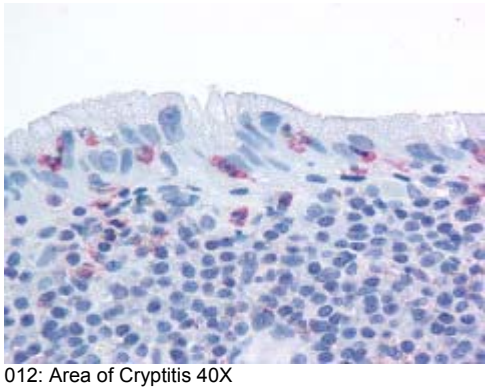
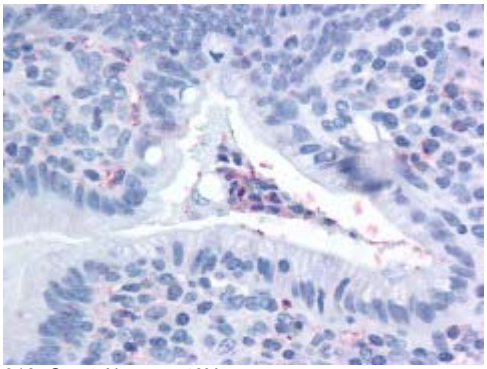
active inflammation were negative. Neutrophils in areas of chronic active inflammation were moderately positive. Eosinophils displayed moderate to strong positivity. Plasma cells were negative or displayed blush staining. Mast cells exhibited moderate positivity. Endothelial cells within the granulation tissue of ulcer beds were faintly to occasionally moderately positive. Lymphocytes within lymphoid follicles and within germinal centers were negative. The muscularis mucosa and muscularis propria exhibited faint to moderate staining. Compared to samples of normal colon, samples of ulcerative colitis showed increased staining of reactive epithelial cells.

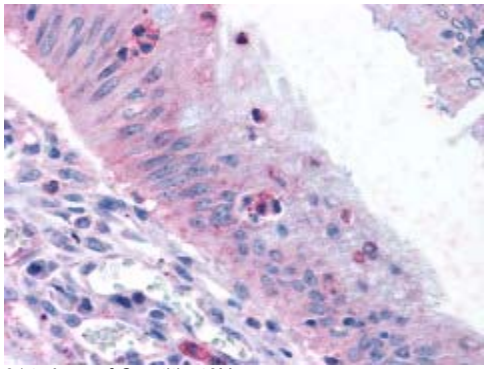
Sample 1: This sample of colon was obtained at surgery from a 36-year-old male.

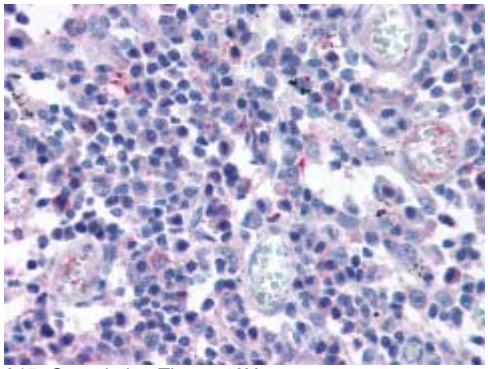

010: Surface Epithelium 40X


011: Reactive Epithelium 40X


012: Area of Cryptitis 40X


013: Crypt Abscess 40X


014: Area of Cryptitis 40X


015: Granulation Tissue 40X

Note: Although these results have been reviewed by a Pathologist, these studies are to be used for research purposes only and are not intended for clinical patient care. These results were obtained on a limited series of samples and tissues and therefore cannot be construed to represent a comprehensive picture of localization across the body. Further studies are recommended if one wishes to determine the true prevalence of staining within a particular tissue or disease with this antibody, or to obtain a more comprehensive distribution of staining across a broader variety of tissues.

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