

Characterization of JAK/STAT Pathway Activation in Ulcerative Colitis: A Methodology for Identifying Activation Patterns Towards Personalized Treatment Approaches

Mariña Durán-Rubí¹, David Moreira-Alvarez¹, Iria Brocos-Mosquera¹, Cristina Calviño², Manuel Barreiro², María Isabel Loza¹, Antón Martínez¹

1. Singular Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), Santiago de Compostela, Spain
2. University Hospital Complex of Santiago de Compostela (CHUS), Santiago de Compostela, Spain



INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory condition of the colon, characterized by ulcer formation and a relapsing-remitting course. It disrupts intestinal homeostasis, overstimulating the immune system and causing persistent inflammation. Despite treatment progress, many patients remain unresponsive, likely due to variability in inflammatory pathways such as JAK/STAT.

The study's hypothesis is that UC patients may have increased JAK/STAT signaling. This would show as increased protein phosphorylation in the inflamed colon. This study will allow us to observe possible variations in the activation of this pathway among individuals.

Objectives:

- To establish a method for characterizing the activation of the JAK/STAT pathway in colon biopsies from inflamed (I) and non-inflamed (NI) areas of patients with UC, and to compare the activation levels between both regions.
- To analyze the activation pattern of phosphorylated proteins in the JAK/STAT pathway in colon biopsies from the inflamed (I) region as a potential stratification approach for patients.

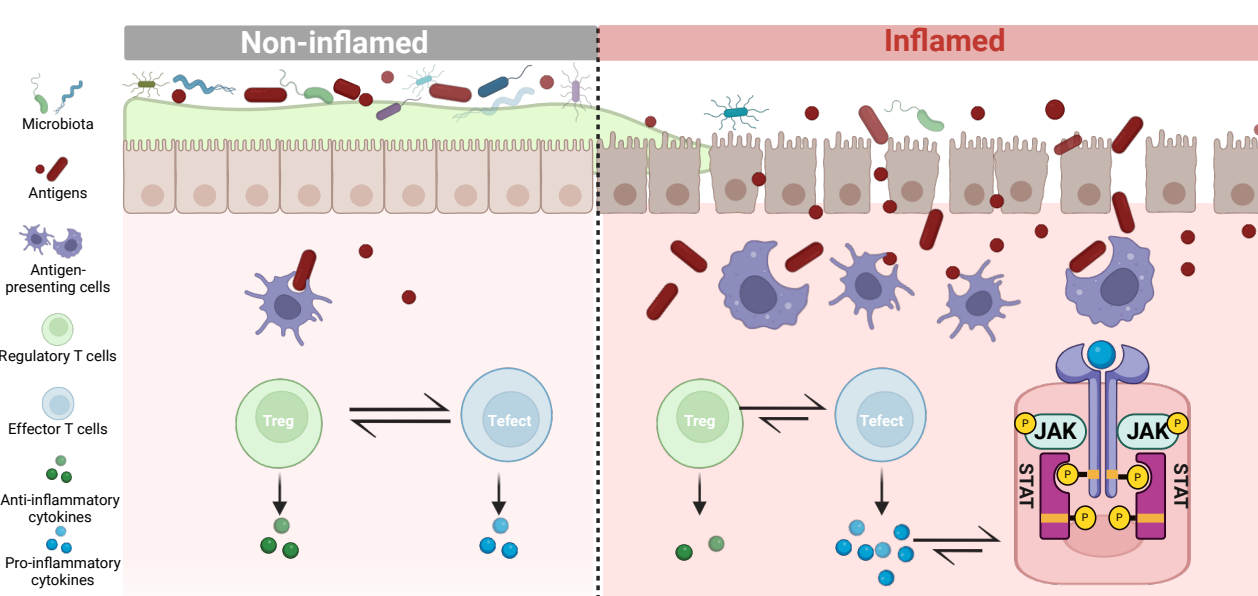
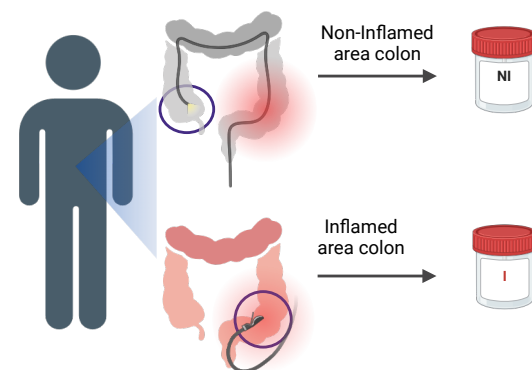


Figure 1. Disruption of homeostasis and chronic inflammation in UC. Created by the author using BioRender^{1,2,3}.

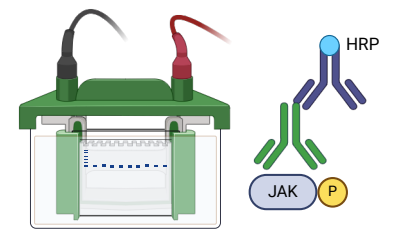
METHODS

1) 58 Patient recruitment



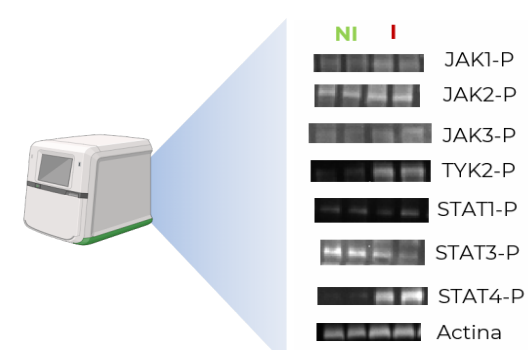
- Colon biopsy
- Digestive Service, Santiago de Compostela Hospital

2) Sample processing and Western blot



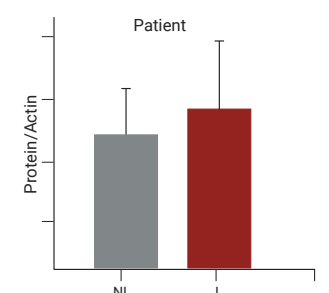
- Lowry protein quantification
- Gel electrophoresis; Semi-dry transfer
- Incubation with 1° and 2° antibodies

3) Densitometry



- Detection (iBright)
- Quantification of band intensity (ImageJ Software)
- Corrected by internal control (actin)

4) Analysis



- GraphPad Prism
- Data: mean ± S.D.; N=2 and n=2
- Unpaired two-tailed Student's t-test

Figure 2. Methodology.

This study has the approval of the Ethical Committee for Drug Research of Galicia (CEIm-G).

RESULTS

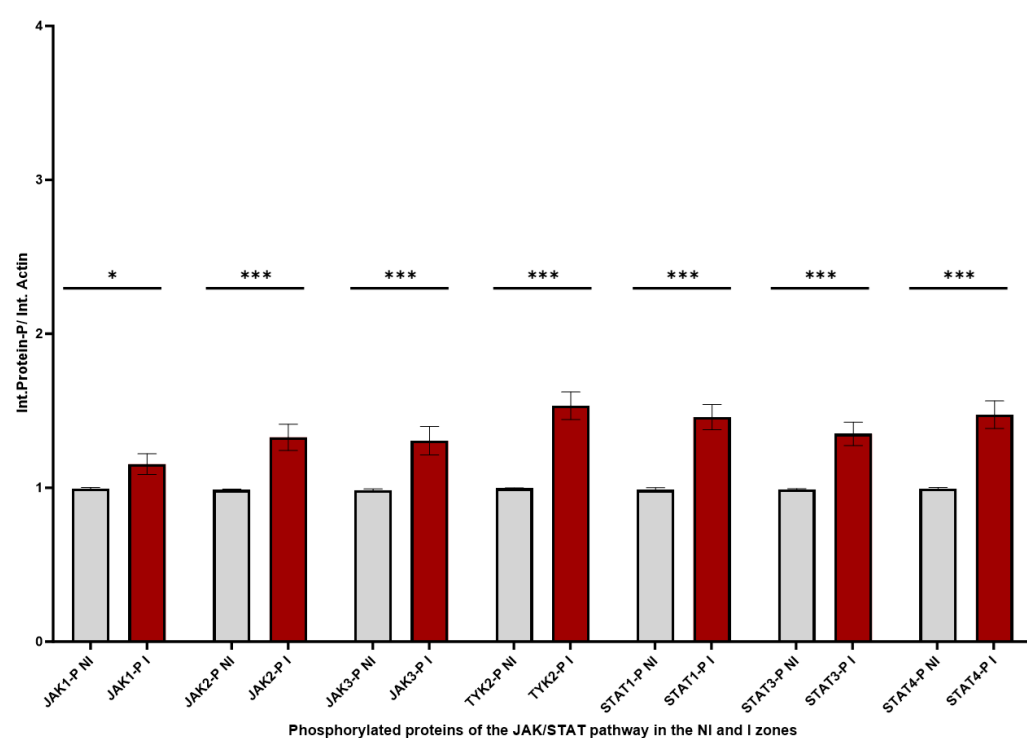


Figure 3. Graph showing the mean ± S.D. of each phosphorylated protein in the NI and I regions. *p< 0.05, ***p< 0.001 (Student's t-test).

Characterization of the JAK/STAT pathway from colon biopsies of 58 UC patients.

To characterize the phosphorylation of proteins in the JAK/STAT pathway, the concentrations of the phosphorylated isoforms JAK1, JAK2, JAK3, TYK2, STAT1, STAT3, and STAT4 were quantified.

The activation of these 7 phosphorylated proteins in the JAK/STAT pathway was evaluated based on the average results obtained from colon biopsies of the inflamed (I) and non-inflamed (NI) areas of each of the 58 UC patients (Figure 3).

The average levels of the seven phosphorylated JAK/STAT proteins analyzed were significantly higher in the inflamed (I) region than in the non-inflamed (NI) region.

Interindividual variability in the activity of the JAK/STAT proteins in the inflamed (I) areas of the colon of 58 UC patients.

The activation of the 7 phosphorylated isoforms of the JAK/STAT proteins was analyzed in the inflamed (I) region of each patient (Figure 4).

The analysis found a different pattern in the activation of the phosphorylated JAK/STAT proteins among the 58 patients.

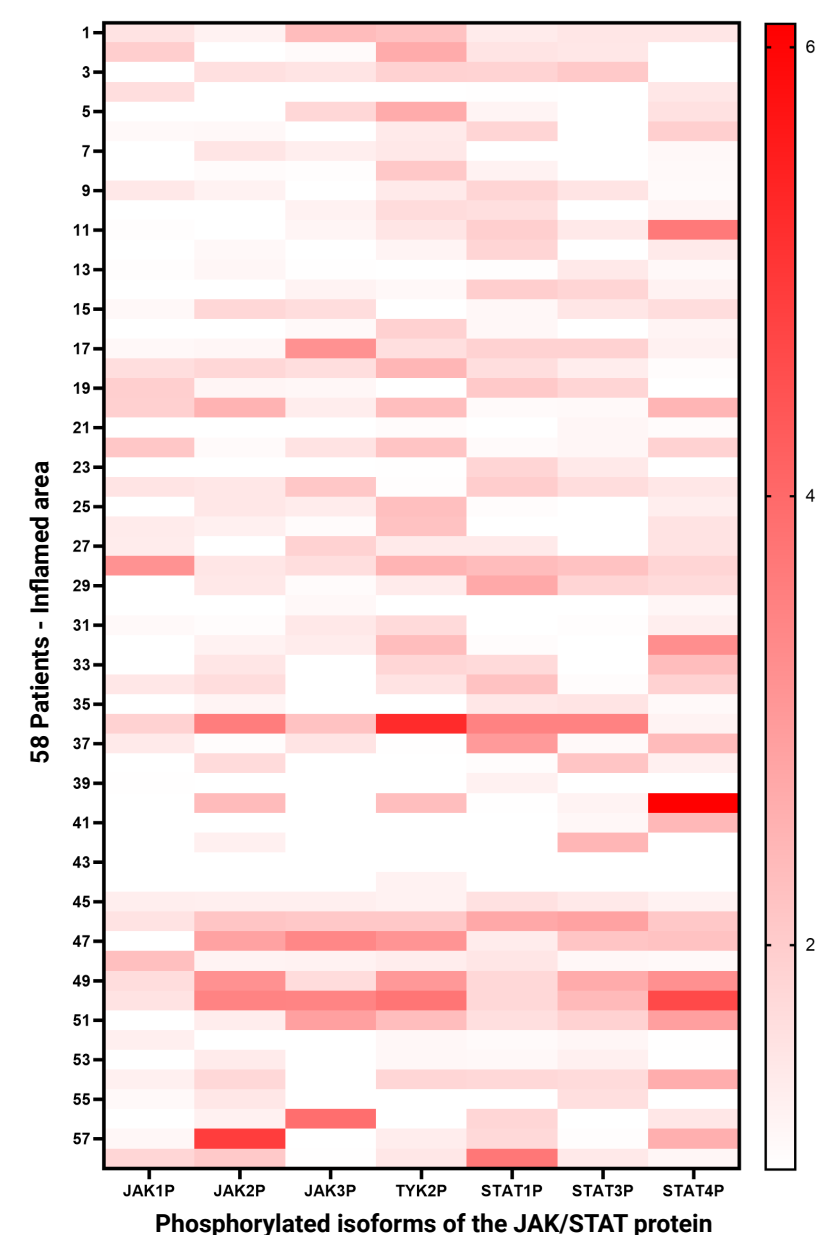


Figure 4. Heatmap of JAK/STAT activation in the inflamed area.

In the inflamed region of the colon, the JAK/STAT pathway is significantly activated, but among the 58 UC patients, there are qualitative variations (which phosphorylated isoform is activated) and quantitative variations (the levels of activation).

CONCLUSION

- A method was developed to characterize the activation of the JAK/STAT pathway in colon biopsies from UC patients. In a study of 58 patients, phosphorylation levels of JAK/STAT proteins were significantly higher in inflamed compared to non-inflamed areas, supporting the pathway's role in UC inflammation.
- The analysis revealed significant interindividual variability in the activation patterns of phosphorylated JAK/STAT proteins in the inflamed regions of the colon among 58 UC patients, which paves the way for using JAK/STAT activation patterns for stratification in patients with UC.

