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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTER

Nod-Like Receptor Pyrin-Containing Protein 6 (NLRP6) Is Up-regulated in Ileal Crohn's Disease and Differentially Expressed in Goblet Cells

A contributing factor in the development of ulcerative colitis (UC) and Crohn's disease (CD) is aberrant signaling of the innate immune complex known as the inflammasome. The inflammasome regulates the production of interleukin (IL)1β and drives downstream inflammatory pathways. In this study of human tissue (ethics approval: H11930), we provide evidence for the disease-specific up-regulation of NLRP6 transcript and protein in ileal CD and describe an NLRP6-expressing goblet cell (GC) located predominantly in the upper portion of the intestinal crypt. Patient demographic details are provided in Supplementary Table 1.

By using paired quiescent and active biopsy specimens from UC and CD patients we found that the expression of *NLRP6* increased with disease activity. In ileal CD we report a 131-fold ($P < .001$) increase in *NLRP6* expression compared with a 3.9-fold ($P = .03$) increase in colonic CD (Figure 1*A*). The increase in *IL1β* expression was concomitant with increased messenger RNA levels of other inflammasome-related genes such as *NLRP1*, *NLRP3*, *NLRC4*, *NLRP12*, and *AIM2* (Figure 1*B* and Supplementary Figure 1*A* and *B*), suggesting IL1β

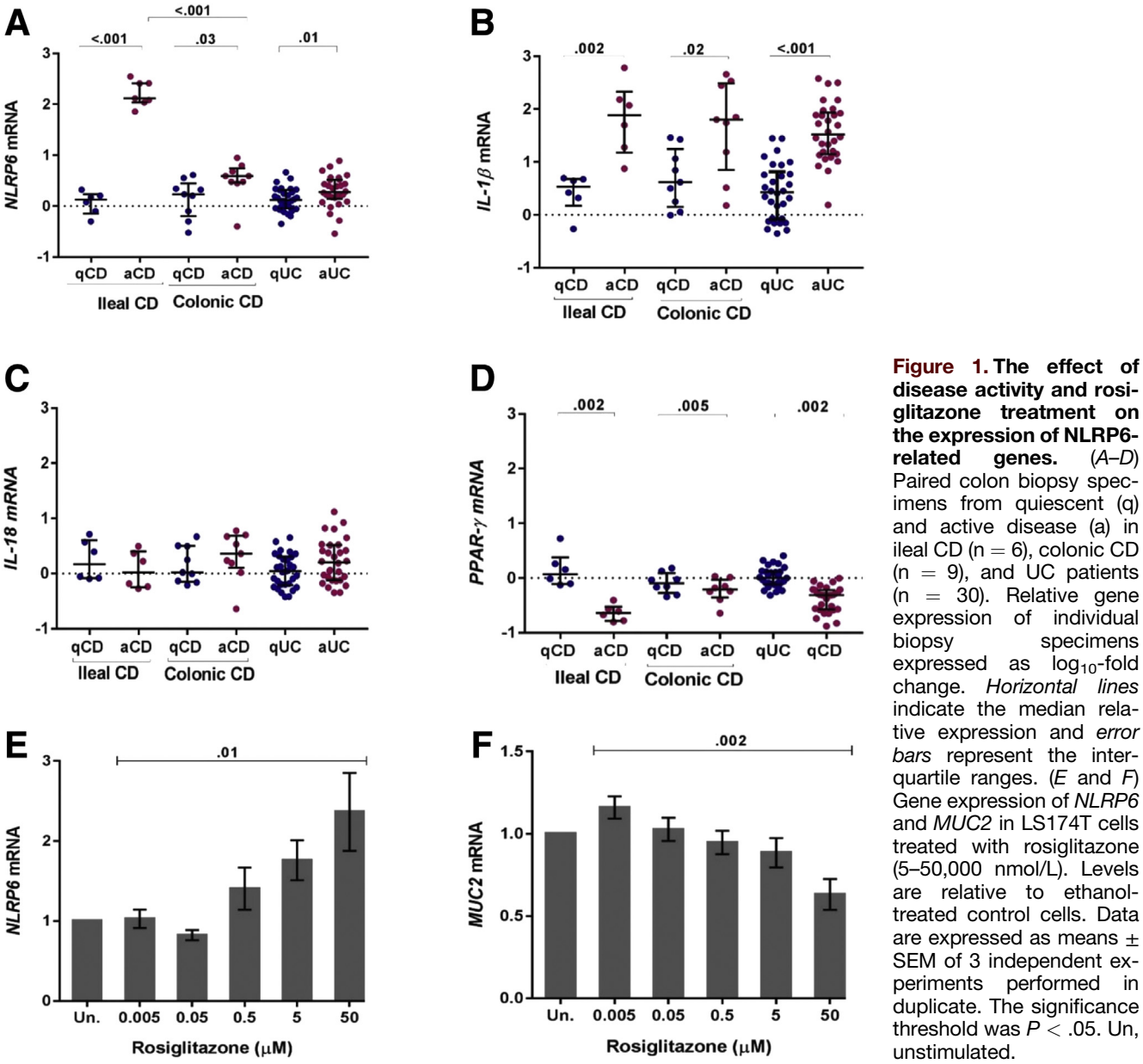

Figure 1. The effect of disease activity and rosiglitazone treatment on the expression of NLRP6-related genes. (*A–D*) Paired colon biopsy specimens from quiescent (q) and active disease (a) in ileal CD (n = 6), colonic CD (n = 9), and UC patients (n = 30). Relative gene expression of individual biopsy specimens expressed as $\log_{10}$-fold change. *Horizontal lines* indicate the median relative expression and *error bars* represent the interquartile ranges. (*E* and *F*) Gene expression of *NLRP6* and *MUC2* in LS174T cells treated with rosiglitazone (5–50,000 nmol/L). Levels are relative to ethanol-treated control cells. Data are expressed as means ± SEM of 3 independent experiments performed in duplicate. The significance threshold was $P < .05$. Un, unstimulated.

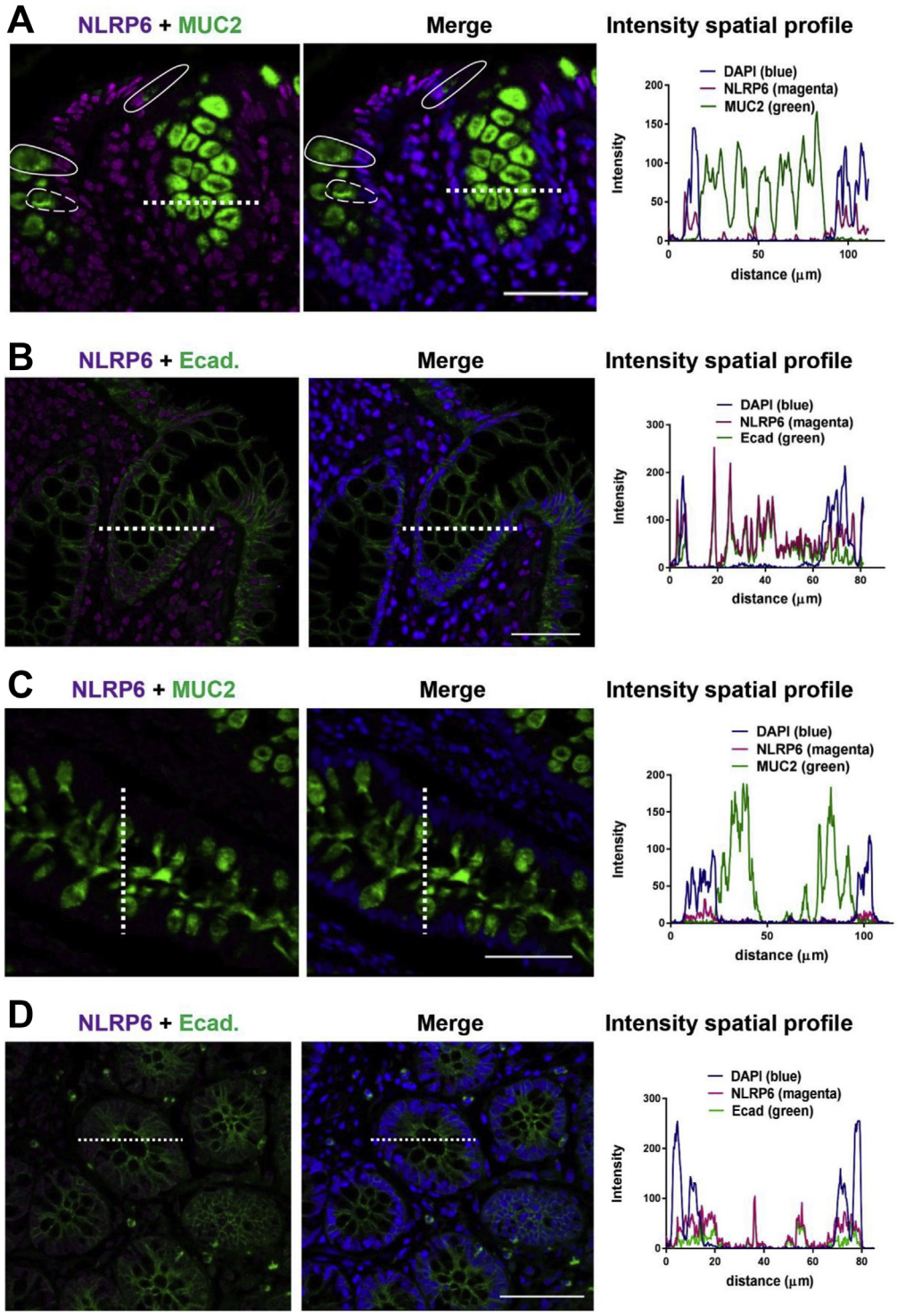

Figure 2. The differential NLRP6 expression in ileal CD goblet cells and overlap of NLRP6/E-cadherin fluorescence signals. Representative immunofluorescence confocal images of NLRP6 (magenta), MUC2 (green), in (*A*) active ileal CD colon biopsy specimens and (*B*) normal colon biopsy specimens. *Solid line* indicates goblet cells with high NLRP6 expression. *Dashed line* indicates goblet cell with minimal NLRP6 expression. Representative immunofluorescence confocal images of NLRP6 (magenta) and E-cadherin (green) in (*B*) active ileal CD biopsy specimens and (*D*) normal colon biopsy specimens. Spatial profiling is indicated by the *straight dotted line*. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, *blue*), original magnification for all images: 400×. *Scale bars*: 50 μm. Ecad, E-cadherin.

maturation by inflammasome activation is not solely NLRP6-dependent.

Interestingly, the expression of *IL18* remained constant despite variations in *NLRP6* expression (Figure 1*C*). Mouse models have indicated discrepancies regarding the association of NLRP6 with the production of IL18. For example, Nlrp6 deficiency has been associated with low levels of IL18[1] and the induction of intestinal IL18 has been shown to be NLRP6-dependent.[2] However, Normand et al[3] reported no NLRP6-dependent changes in the transcript abundance of IL18 in tumoral and nontumoral biopsy specimens procured from Nlrp6+/+ and Nlrp6-/-