Epithelial organoid cultures from patients with ulcerative colitis and Crohn's disease: a truly long-term model to study the molecular basis for inflammatory bowel disease?

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Abbreviations:

(d-)EpOC	(differentiated-) Epithelial Organoid Culture
UC	Ulcerative colitis
CD	Crohn's disease
CFU	Colony forming units
IBD	Inflammatory bowel disease

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Dear editor,

With great interest we read the paper by Dotti *et al*., who identified a set of differentially expressed genes in epithelial organoid cultures (EpOCs) derived from patients with ulcerative colitis (UC) compared to EpOCs from healthy controls.[1]

Similarly, we created an EpOC library from patients with UC (n=17), Crohn's disease (CD, n=12), and healthy controls (n=10). First, we assessed the potential of isolated intestinal crypts to grow into organoids (colony forming units, CFU). We observed that a similar percentage of organoids was formed from intestinal crypts isolated from controls and patients with UC or CD. Moreover, intestinal crypts isolated from macroscopically inflamed and non-inflamed tissue had similar potential to form organoids (figure 1A). Although we used a slightly divergent differentiation medium,[2] the expansion and differentiation capacity of our organoids was similar as demonstrated by Dotti *et al*.

Next, we characterized the organoids through mRNA expression (qPCR). Expression of genes involved in stemness and proliferation (*LGR5*, *PCNA*), differentiation into the downstream lineages (*ALPI*, *VIL1*, *CHGA*, *MUC2*), and tight junctions *(OCLN*, *TJP-1*) was similar in undifferentiated EpOCs from patients with inflammatory bowel disease (IBD) and controls. However, differentiated EpOCs (d-EpOCs) of patients with CD were characterized by decreased mRNA levels of *MUC2* compared to patients with UC and controls (p=0.04 and 0.046 respectively, figure 1B), while the other examined genes showed similar expression profiles between controls and patients with IBD. In addition, the expression of *ATOH1*, a transcription factor controlling differentiation into the secretory lineage, was decreased in EpOCs of patients with CD or UC compared to controls. Hence, we re-evaluated the authors'

publically available micro-array data and noticed, in line with our findings, a significantly lower *ATOH1* expression in UC d-EpOCs compared to non-IBD controls (p=0.007).

Dotti *et al*. confirmed their findings in a second cohort of intestinal biopsies from non-IBD controls, patients with UC in remission or with active disease. Expression of *LYZ, and CLDN18,* was upregulated in inflamed tissue, but not in tissue derived from patients with UC in remission. An important remark which we feel was not addressed by the authors, is the fact that inflamed tissue may quickly lose its inflammatory conditions once the trigger of this process has been resolved. Therefore, we compared intestinal biopsies and EpOCs derived from both inflamed and non-inflamed areas from the same patient. We found that the expression of *IFNγ* and *IL1β* were lower in EpOCs compared to the original biopsies used to form these EpOCs (figure 2). Strikingly, in the authors' micro-array dataset, a trend for lower expression of *IFNγ* (0.94 fold-change, p=0.012) in UC EpOCs compared to controls was observed, but these were patients with mostly no inflammation (six with Mayo 0 and two with Mayo 1). These data indicate that an inflammatory status at the mRNA level in mucosal biopsies may not be propagated to organoid cultures, and a stimulus is needed for continued expression of several markers. Recently, Hibiya *et al*. showed that 60 weeks continuous exposure of murine organoids to a mixture of cytokines and bacterial components led to induction of the NFκB-pathway, which remained elevated 11 weeks after withdrawal of the inducing components.[3]

In conclusion, the data obtained in our cohort of intestinal biopsies and organoids are in line with the observation of Dotti and colleagues. We therefore agree that the organoid model is a reliable tool for exploring the molecular basis of IBD. However, whether transcription

profiles of cultured organoids will remain identical on the long-term to those of the tissues of origin remains to be further elucidated.

1 Dotti I, Mora-Buch R, Ferrer-Picon E, Planell N, Jung P, Masamunt MC*, et al.* Alterations in the epithelial stem cell compartment could contribute to permanent changes in the mucosa of patients with ulcerative colitis. Gut 2016.
2 Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S*, et al.* Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 2011;**141**:1762-72.
3 Hibiya S, Tsuchiya K, Hayashi R, Fukushima K, Horita N, Watanabe S*, et al.* Long-term inflammation transforms intestinal epithelial cells of colonic organoids. J Crohns Colitis 2017, in press.

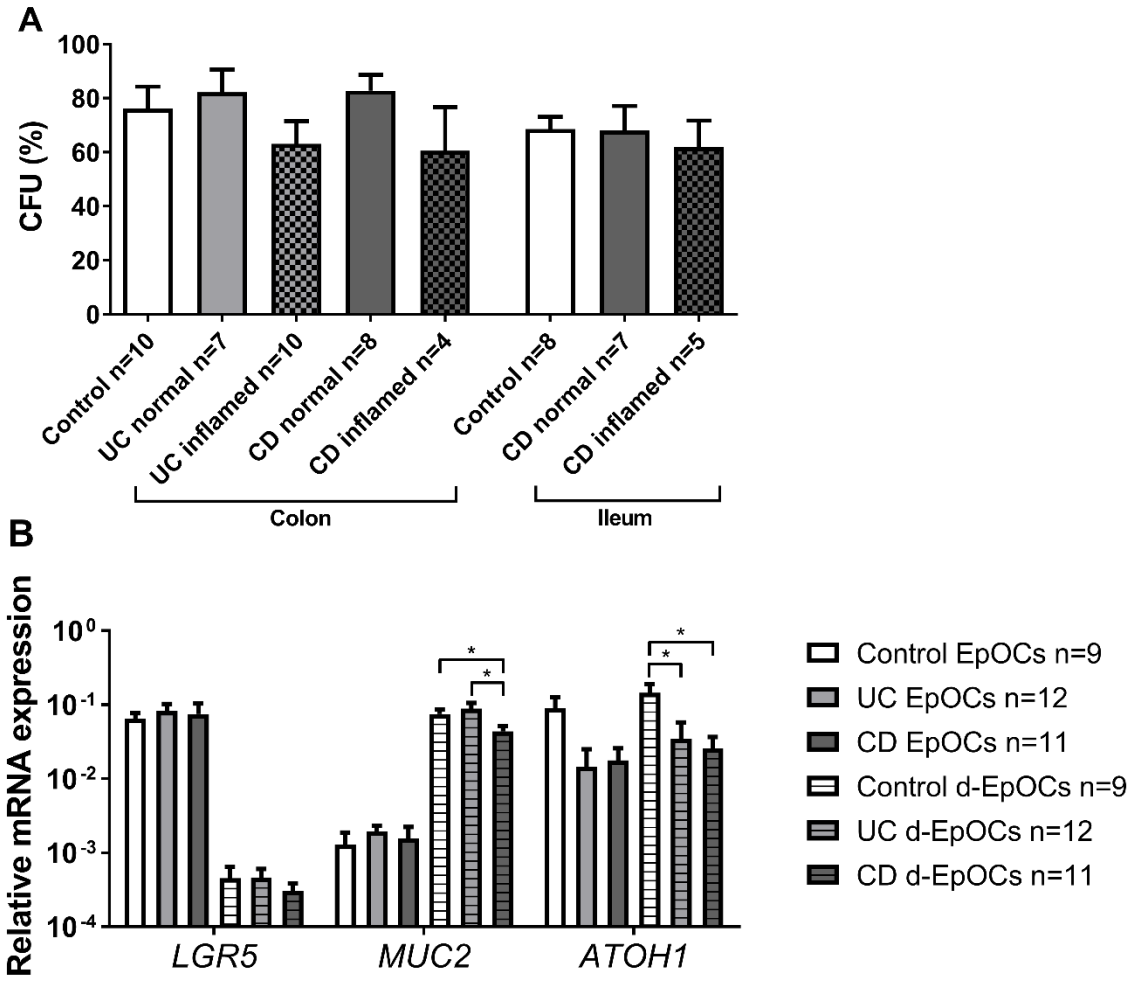

Figure 1 (A) Colony forming units (CFUs) determined by quantification of the number of seeded crypts at the day of isolation and organoid formation after 7 days. CFUs are expressed

as a percentage of organoids representative to the original number of seeded crypts. (B) Relative mRNA expression levels of leucine-rich repeat-containing G-protein coupled receptor 5 (*LGR5*), mucin2 (*MUC2*) and protein atonal homologue 1 (*ATOH1*). mRNA levels were normalized on the geometric mean of *RPS14*, *HPRT1* and *B2M* and expressed as log values. Statistical analyses were performed with Mann-Whitney U test, (*p<0.05). CD, Crohn's disease; dEpOC, differentiated epithelial organoid culture; EpOC, undifferentiated epithelial organoid culture; UC, ulcerative colitis.

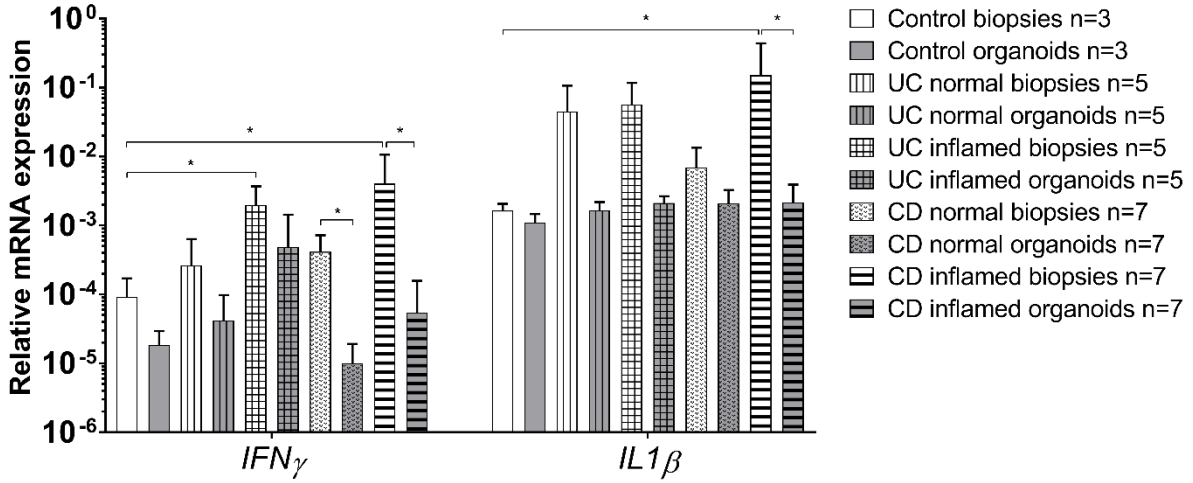

Figure 2 Relative mRNA expression levels of interferon-gamma (*IFNγ*) and interleukin-1β (*IL1β*). Organoids were grown from normal and inflamed mucosal biopsies, and RNA was extracted from EpOCs (undifferentiated epithelial organoid cultures) after 2 weeks in culture, as well as from original biopsies. Quantitative PCR was performed to quantify the expression of genes involved in inflammation. mRNA levels were normalized to three reference genes and shown as log values. Statistical analyses were performed with Wilcoxon matched-pairs signed rank test for matched pairs or Mann-Whitney U test (*p<0.05). CD, Crohn's disease; UC, ulcerative colitis.

Ethics Approval

The study was approved of by the ethics committee of the University Hospitals Leuven (ML7771).

Contributor Statement

Manuel Noben: conception or design of the work, acquisition, analysis or interpretation of data, manuscript writing

Bram Verstockt: data analysis, manuscript writing

Magali de Bruyn: conception or design of the work and manuscript writing

Gert Van Assche: acquisition, manuscript approval

Séverine Vermeire: acquisition, manuscript writing, manuscript approval

Catherine Verfaillie: analysis or interpretation of data, manuscript approval

Marc Ferrante: conception or design of the work, acquisition, analysis or interpretation of data, manuscript writing and final approval

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Competing interests

Manuel Noben
-PhD fellowship, Research Foundation – Flanders (FWO).

Bram Verstockt
-PhD fellowship, Research Foundation – Flanders (FWO).

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-Nothing to declare

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