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Development of an *in vitro* co-culture model of inflammatory bowel disease to examine the effect of histamine H_4 receptor on gastrointestinal processes

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Introduction

Histamine, a mediator in immunological processes, reveals its pleiotropic functions upon binding to its receptors, such as the histamine H_4 receptor (H_4R), which is a GPCR mainly expressed in hematopoietic cells and in tissues such as the skin and intestine. Studies have described the pro-inflammatory role of H_4R on the pathophysiology of inflammatory bowel disease (IBD) in model systems such as mice. However, the effect of H_4R on intestinal inflammation on cellular and molecular level is yet to be elaborated. Our aim is the development of an *in vitro* co-culture model of intestinal inflammation suitable to examine further functions of H_4R.

Methods

As a model, we used Caco-2 cells, THP-1 cells, and cell culture inserts. On the apical side, Caco-2 cells differentiate upon confluency to form a polarized epithelial monolayer. On the basolateral side, THP-1-derived macrophage-like cells imitate the underlying immune environment. To examine differentiation of Caco-2 cells, the TEER was measured for 21 days. To derive macrophages, we stimulated THP-1 cells with 100 nM PMA for 24h to differentiate to resting macrophages (M0). M0 cells were differentiated to activated macrophages (M1) through co-stimulation with 20 ng/ml LPS and 20 ng/ml IFN-γ for 48h. IL-8 release, H_4R expression, and histamine release of THP-1, M0, and M1 cells were observed using ELISA, RT-qPCR, and HPLC-MS, respectively. We transfected Caco-2 cells with a plasmid containing human H_4R gene via electroporation. Expression and functionality of H_4R in transfected Caco-2 cells and wild-type Caco-2 cells were analyzed using RT-qPCR and calcium mobilization assay, respectively.

Results

Fig. 1: Conceptualization of the co-culture model of inflammatory bowel disease (IBD) and the effect of H_4R in the model. Schematic overview of the Caco-2/THP-1 co-culture model for stimulation with histamine and/or or H_4R-specific antagonist JNJ7777120 (JNJQ7).

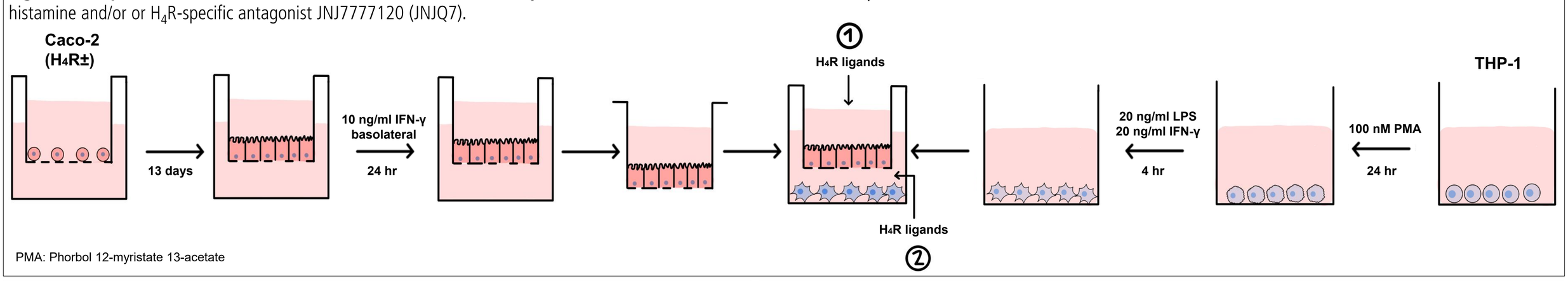


Fig. 2: Characterization of THP-1 cells and their derived macrophages. Interleukin-8 (A) and endogenous histamine (B) concentrations in cell culture supernatants of undifferentiated THP-1 cells, resting macrophages (M0), and activated macrophages (M1). After 24h treatment with PMA 100 nM, the medium was replaced with standard medium for M0 cells or with medium supplemented with 20 ng/ml LPS and 20 ng/ml IFN-γ for M1 polarization, followed by an additional 48h incubation. (A) **p<0.01 with one-way ANOVA with Tukey's test. (B) Lower limit of quantification (LLOQ) = 23,5 nM. Shown are the means ± SD (N=3).

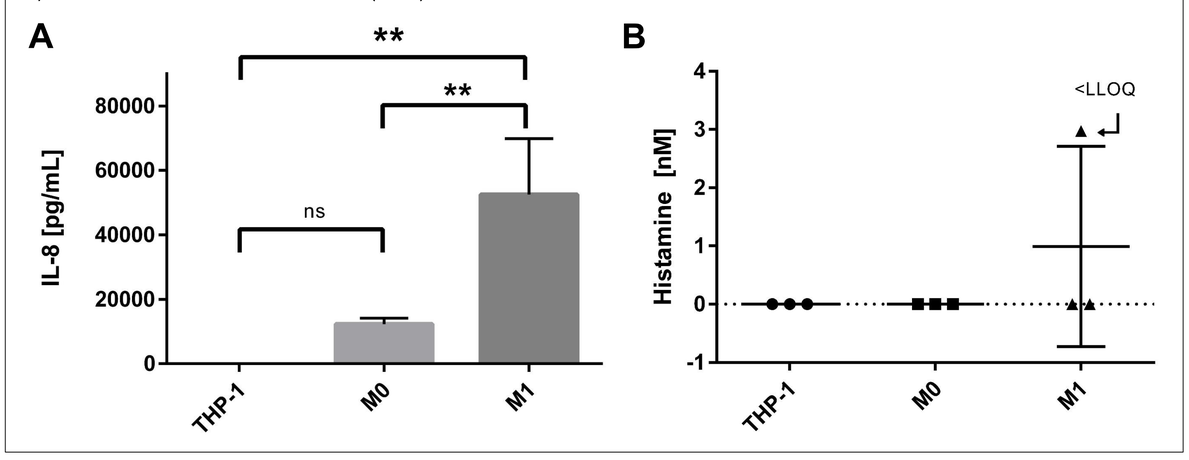


Fig. 3: Expression of H_4R in THP-1 and Caco-2 cell lines. (A) H_4R expression in undifferentiated THP-1 cells and their derived macrophage subsets. (B) H_4R expression in wild-type Caco-2 cells (WT), transfected Caco-2 cells (EP), and transfected Caco-2 cells (EP diff.) after 13 days of differentiation in cell culture inserts. H_4R expression is presented as fold change relative to undifferentiated THP-1 cells or wild type Caco-2 cells (WT), respectively. *p<0.05 with one-way ANOVA with Dunnet's test. Shown are the means ± SD (N=3).

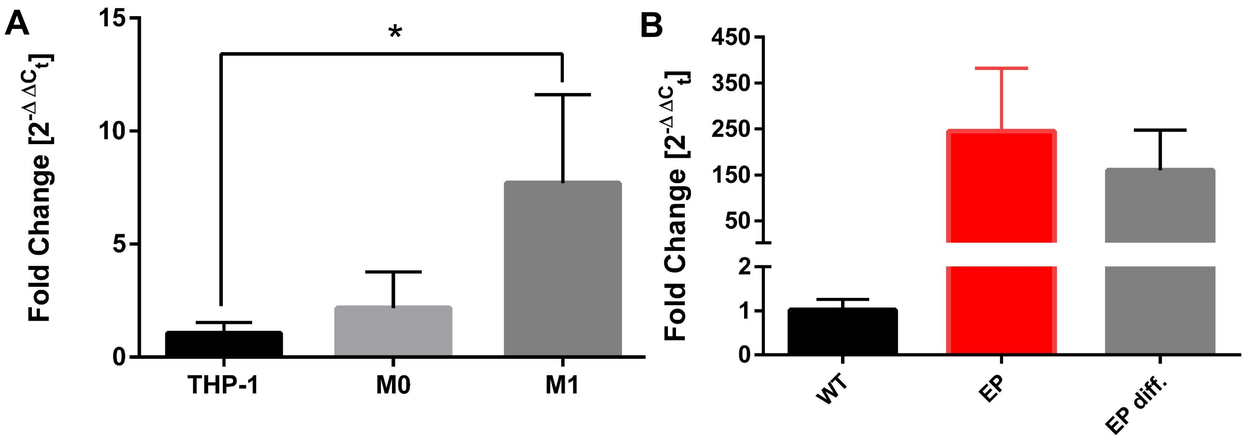
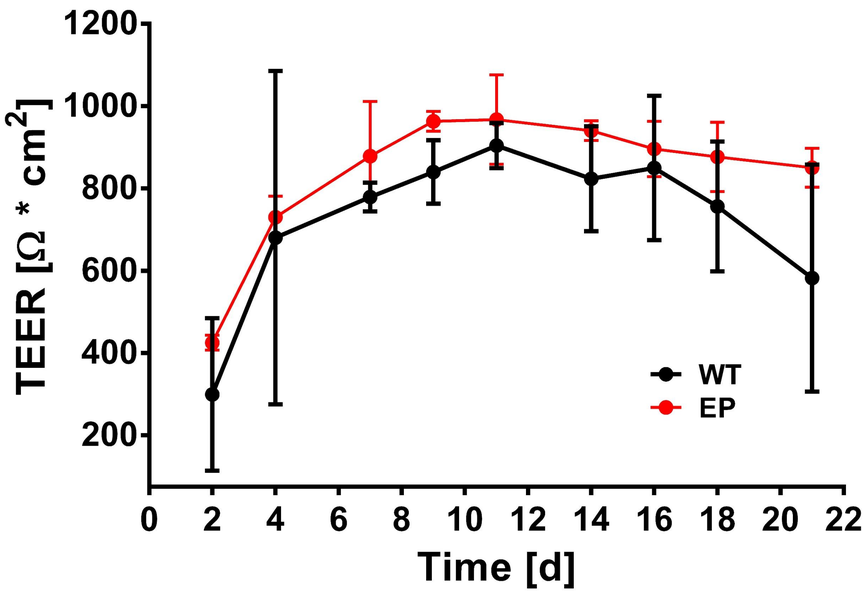
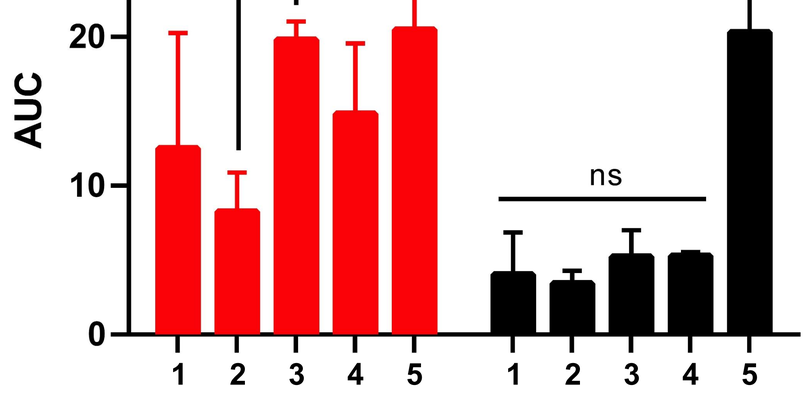


Fig. 4: Barrier integrity of Caco-2 monolayers in cell culture inserts. Wild-type Caco-2 cells (WT) and transfected Caco-2 cells (EP) were seeded at a density of 1.8 x 10^5 cells/cm^2 onto type I collagen-coated cell culture inserts. Transepithelial electrical resistance (TEER) was measured every 2-3 days, corrected for blank values, and normalized to the membrane surface area.





Stimulants	1	2	3	4	5
Assay buffer (HBSS + 20 mM HEPES)	+	+	+	+	+
100 µM Histamine	-	-	+	+	-
10 µM JNJQ7 (pre-incubated)	-	+	-	+	-
100 µM ATP	-	-	-	-	+

Fig. 5: Calcium mobilization in Caco-2 cells. Wild-type Caco-2 cells (WT) and transfected Caco-2 cells (EP) were loaded with fluorescent calcium indicator Calbryte™ 520 AM. Intracellular calcium responses were monitored following stimulation with assay buffer, histamine, and/or JNJQ7. Stimulation with ATP served as positive control. Calcium mobilization was quantified as the area under the curve (AUC), normalized to the baseline and maximum signal obtained with 5% Triton X-100. **p<0.01 with two-way ANOVA. With Tukey's test. Shown are the means ± SD (N=3-4).

Conclusion/Outlook

- Caco-2 cells were stably transfected with functionally active H_4R. H_4R expression remained elevated after 13 days of differentiation.
- During THP-1 differentiation into M1 macrophages, IL-8 production was significantly increased and H_4R expression gradually upregulated, whereas no histamine release was detectable.
- Following 14 days of epithelial differentiation, H_4R expressing Caco-2 cells will be co-cultured with M1 macrophages to establish an inflammatory epithelial-immune interaction model.
- Co-cultures will be stimulated with histamine and/or JNJQ7, applied either apically or basolaterally to assess compartment-specific effects.
- TEER of co-cultures will be measured at 0h, 4h, 18h, 24h, 28h, and 48h. After 48h, epithelial protein expression of occludin, ZO-1, and COX-2 will be analyzed by Western blot and cytokine secretion will be quantified using bead-based multiplex assay.

Acknowledgement
Mass spectrometric measurements have been done by the Research Core Unit Metabolomics of Hanover Medical School.

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