

Supplemental material

Supplementary methods

Ussing chamber experiments Rectal biopsies were mounted in modified Ussing chambers (exposed area 1.76 mm²; Harvard apparatus, Holliston, MA, USA) as previously described.¹ To ensure tissue viability, potential difference (PD) was recorded throughout the experiments²⁰ and at the end of experiments an adequate response to forskolin was monitored.² Previous studies in our laboratory validated the Ussing method for human endoscopic biopsies. A potential difference (PD) that is less negative than -0.5mV at the start of experiments is a marker of non-viability.²⁰ In addition, Forskolin was added at t120 min, to assess viability at the end of the experiments. Forskolin is a cell permeate and activates cAMP-driven, CFTR-mediated Cl⁻ secretion. In viable biopsies forskolin stimulates a visible peak in in short circuit current (I_{sc}) and PD, indicating responsiveness of the tissue secretory machinery. Biopsies were excluded from further analysis when not demonstrating tissue viability as according to these criteria.

The transcellular marker horseradish peroxidase (HRP) (type VI; Sigma Chemical Co, St Louis, Missouri, USA) and the paracellular probe ⁵¹Chromium (Cr)-EDTA (Perkin Elmer, MA, USA) were added to the mucosal sides and serosal samples were collected at 0, 10, 30, 60, 90 and 120 min. ⁵¹Cr-EDTA permeability was measured by gamma-counting (1282 Compugamma, Sweden) and is presented as apparent permeability coefficient (P_{app}) during steady state permeability (t30-t90). P_{app} is defined as the flux of compound through a membrane normalized by membrane surface area and donor concentration and was calculated from the equation $P_{app} = \frac{\Delta Q}{\Delta t} \times \frac{1}{AC_0}$ where $\Delta Q/\Delta t$ is the steady-state flux (mol/s), A is the surface area of the exposed tissue (cm²) and C_0 is the initial concentration in the donor chamber at each time interval (mol/L) and given as (10⁻⁶ cm/s).²⁰ HRP passage was analysed with QuantaBlu™ Flourigenic peroxidase substrate kit (Pierce, Rockford, USA).³ Permeability to HRP is given as flux during t30-t90 (pmol/cm²/h).

Supplementary table 1a. Gene expression assay probes used for Q-RT-PCR

Gene symbol	Gene name	TaqMan assay
<i>PCK1</i>	Phosphoenolpyruvate carboxykinase 1	Hs01572978_g1
<i>DUOX2</i>	Dual oxidase 2	Hs00204187_m1
<i>CR2</i>	Complement component 3d receptor 2	Hs00153398_m1
<i>MS4A1</i>	Membrane spanning 4-domains A1	Hs00544819_m1
<i>TCL1A</i>	T-cell leukemia/lymphoma 1A	Hs00951350_m1
<i>BANK1</i>	B-cell scaffold protein with ankyrin repeats 1	Hs01009378_m1
<i>CD22</i>	CD22 molecule	Hs00233533_m1
<i>FDCSP</i>	Follicular dendritic cell secreted protein	Hs00395131_m1
<i>CCL19</i>	C-C motif chemokine ligand 19	Hs00171149_m1
<i>CCL21</i>	C-C motif chemokine ligand 21	Hs00989654_g1
<i>CXCL13</i>	C-X-C motif chemokine ligand 13	Hs 00757930_m1
<i>CCR7</i>	C-C motif chemokine receptor 7	Hs01013469_m1
<i>CXCR5</i>	C-X-C motif chemokine receptor 5	Hs00173527_m1
<i>TPSB2</i>	Tryptase beta 2	Hs02576518_gH
<i>PPIA</i>	Cyclophilin A	Hs 99999904_m1

Supplementary table 1b. Gene expression assay probes for Q-RT-PCR of barrier-related proteins

Gene symbol	Gene name	TaqMan assay
<i>JAM-A</i>	Junctional Adhesion Molecule A	Hs 00375889_m1
<i>CLDN2</i>	Claudin 2	Hs 00252666_s1
<i>ZO1</i>	Zonula occludens protein 1	Hs 00268480_m1
<i>ZO3</i>	Zonula occludens protein 3	Hs 00274276_m1

Supplementary table 2 Background data of 16 healthy volunteers (10 female, 6 male) included in the analysis

	Median	Range	Range instrument
Age	23.5	20-29	
STAI-T	30	23-45	20-80
Current stress	48	15-65	20-80
Life event stress	65	15-145	0-1155
Rome III	0	0-8	0-37

Trait anxiety measured by the State and trait anxiety index (STAI-T™); reference values for ages 19-39 according to the manual: 35 ± 10 . Life event stress= Stressful life events the six months preceding the experiment as measured by the Social Readjustment Rating Scale. Reference values: Low level of stress 0-150, Medium level of stress 150-299, High levels of stress >299. Current stress = Perceived level of stress the week preceding the experiment measured by a version of the Everyday life stress scale modified by Arbetshälsoinstitutet. Reference values: Low level of stress 15-30, Medium level of stress 30-50 High level of stress 50-65. M=male. F = female.

Supplementary table 3 . Networks identified by IPA, based on focus genes interactions

ID	Top Functions	Score	Focus Molecules	Molecules in Network
1	Inflammatory Response, Cell Signaling, Cellular Function and Maintenance	32	15	Akt, APOH, ATP2B4, BCR, BLNK, C3, CCL19, CCL20, CCL21, CCR6, CCR7, CCRL1, CD3, CD22, CD52, CR2, CXCL13, CXCR5, DEFB103A/DEFB103B, DUOX2, ERK, ERK1/2, Fc gamma receptor, IGHM, MS4A1, NFkB(complex), NLRP12, P38 MAPK, Pmca, SELL, SYK/ZAP, TCL1A, TCR, TLR10, VAV
2	Cell Death, Cell-toCell Signaling and Interaction, Hematological System Development and Function	4	2	C1q, CR1, Cyclin A, IFNG (includes EG:15978), IL2, IL10, IL27, SH2D1A, SLAMF6
3	Cell-To-Cell Signaling and Interaction, Cell-mediated Immune Response, Cellular Development	2	1	FDCSP, CD40
4	Antigen Presentation, Antimicrobial Response, Cell-To-Cell Signaling and Interaction	2	1	FAIM3, TLR7, TLR9
5	Cell Death, Cell Morphology, Cellular Development	2	1	CSF2, FAM65B, IL5, SATB1
6	GastrointestinalDisease, Hepatic System Disease, Cancer	2	1	CASP3, CASP6, CASP8, CD180, STAB1
7	Connective Tissue Development and Function, Tissue Development, Lipid Metabolism	2	1	FSH, HNF1A, Ifn gamma, NEDD9, PCK1 (includes EG:18534), UBR5

Supplementary table 4. Biofunctional analysis of Inflammatory Response identified in differentially expressed genes in the colonic mucosa

Functions Annotation	p-value	Molecules
Chemotaxis of B lymphocytes	2,03E-09	CCL19, CCL20, CCL21, CXCL13
Immune response	5,72E-09	C3, CCL19, CCL20, CCL21, CCR7, CD180, CR1, CXCL13, DUOX2, MS4A1, TCL1A
Chemotaxis of dendritic cells	1,45E-07	CCL19, CCL20, CCL21, CCR7
Migration of dendritic cells	2,10E-07	CCL19, CCL20, CCL21, CCR7
Chemotaxis of leukocytes	4,32E-07	C3, CCL19, CCL20, CCL21, CCR7, CXCL13
Cell movement of phagocytes	5,44E-07	C3, CCL19, CCL20, CCL21, CCR7, SELL
Chemotaxis of T lymphocytes	7,07E-07	CCL19, CCL20, CCL21, CXCL13
Migration of monocyte-derived dendritic cells	1,72E-06	CCL19, CCL21, CCR7
Chemoattraction of Langerhans cells	2,22E-06	CCL19, CCL20
Chemotaxis of phagocytes	2,52E-06	C3, CCL19, CCL20, CCL21, CCR7
Chemotaxis of naive T lymphocytes	6,67E-06	CCL19, CCL21
Chemotaxis of memory T lymphocytes	4,65E-05	CCL19, CCL21
Antimicrobial response	3,69E-04	CCL19, CXCL13, DUOX2

Supplementary table 5. Expression of barrier-related genes in QT-PCR of colorectal mucosa of healthy volunteers following acute experimental stress

Gene	Relative mRNA expression Stress/Control (Mean \pm SEM)	<i>p</i>-value
<i>JAM-A</i>	1.26 \pm 0.33	0.72
<i>CLDN2</i>	0.94 \pm 0.13	>0.99
<i>ZO-1</i>	0.93 \pm 0.04	0.5
<i>ZO-3</i>	1.10 \pm 0.02	0.25

Probes used for each of the genes are presented in supplementary table 1b.

References

1. Wallon C, Braaf Y, Wolving M, et al. Endoscopic biopsies in Ussing chambers evaluated for studies of macromolecular permeability in the human colon. *Scand J Gastroenterol* 2005;40:586-95.
2. Gustafsson JK, Hansson GC, Sjoval H. Ulcerative colitis patients in remission have an altered secretory capacity in the proximal colon despite macroscopically normal mucosa. *Neurogastroenterol Motil* 2012;24:e381-91.
3. Wallon C, Persborn M, Jonsson M, et al. Eosinophils express muscarinic receptors and corticotropin-releasing factor to disrupt the mucosal barrier in ulcerative colitis. *Gastroenterology* 2011;140:1597-607.