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The oxysterol receptor GPR183 in inflammatory bowel diseases

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Abstract

Immune cell trafficking is an important mechanism for the pathogenesis of inflammatory bowel disease (IBD). The G-protein-coupled receptor 183 (GPR183, also called EBI2) and its ligands, dihydroxylated oxysterols can mediate positioning of immune cells including innate lymphoid cells (ILCs). *GPR183* has been mapped to an IBD risk locus; however, another gene, *UBAC2*, is encoded on the reverse strand and associated with Behçet's disease and the role of GPR183 as a genetic risk factor requires validation.

GPR183 and production of its oxysterol ligands are upregulated in human IBD and murine colitis. *Gpr183* inactivation reduced severity of colitis in ILC3-dependent colitis and in IL-10 colitis but not in dextran sodium sulphate colitis. Irrespectively, *Gpr183* knockout strongly reduced accumulation of intestinal lymphoid tissue in health and all colitis models.

In conclusion, genetic, translational and experimental studies implicate GPR183 in IBD pathogenesis and GPR183-dependent cell migration might be a therapeutic drug target for IBD.

List of abbreviations

CCR7	C-C chemokine receptor type 7
CD	Crohn's disease
CH25H	cholesterol 25-hydroxylase
CLPs	colonic patches
CPs	cryptopatches
cSILT	colonic solitary intestinal lymphoid tissue
CXCL8	C-X-C motif chemokine ligand 8
CXCR5	C-X-C chemokine receptor type 5
CYP7B1	cytochrome P450 family 7 subfamily member B1
DCs	dendritic cells
DSS	dextran sodium sulphate
EBI2	Epstein-Barr virus-induced G-protein-coupled receptor 2 (in this review referred to as GPR183)
EIM	extraintestinal manifestations (of IBD)
GM-CSF	granulocyte macrophage colony-stimulating factor
GPR183	G-protein coupled receptor 183 (also called EBI2)
GWAS	genome wide association study
HSD3B7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7
IBD	inflammatory bowel diseases
ICAM-1	intercellular adhesion molecule-1
ICOS	inducible T cell co-stimulator
IL	interleukin
ILCs	innate lymphoid cells
ILFs	isolated lymphoid follicles
LPS	lipopolysaccharide
LTi	lymphoid tissue inducer cells
LTo	lymphoid tissue organizing cells
LT	lymphotoxin
LXR	liver X receptor
MAdCAM-1	mucosal addressin cell adhesion molecule-1
MLN	mesenteric lymph nodes
MS	multiple sclerosis
NASH	non-alcoholic steatohepatitis
NFκB	nuclear factor κB
OR	odds ratio
PML	progressive multifocal leukoencephalopathy
PPs	Peyers' patches
RAG1	recombination activating gene
RORγt.	retinoic acid related orphan receptor gamma-t
S1PR	sphingosine-1-phosphate receptor
S1P	sphingosine-1-phosphate
SILT	solitary intestinal lymphoid tissue
SNP	single nucleotide polymorphism
TLR	toll like receptor
TLT	tertiary lymphoid tissue
TNBS	trinitrobenzene sulfonic acid
TNF	tumour necrosis factor
TRUC	*Tbx21*$^{-/-}$*Rag2*$^{-/-}$ ulcerative colitis

UBAC2	ubiquitin-associated domain-containing gene 2
UC	ulcerative colitis
VCAM-1	vascular cell adhesion molecule-1
7α,25-diHC	7α,25-dihydroxycholesterol
25-HC	25-hydroxycholesterol

Introduction

In its early days, the field of immune cell migration was dominated by studies on classical adhesion molecules and the chemokine-chemokine receptor system. The study of these systems had opened up exciting therapeutic opportunities in the clinic. However, more recently, additional signals that finely control the movement of distinct immune subsets and their positioning in specific tissues have been identified. Excitingly, these pathways include small molecule metabolites which are well characterised in other systems and link effects of diet, metabolism and the microbiota. Here we focus on one of these systems, the G-protein-coupled receptor 183 (GPR183[*]), also known as Epstein-Barr virus-induced G-protein-coupled receptor 2 (EBI2) and its cholesterol-derived ligands. We will discuss genetic information, observations in IBD patients and experimental studies implicating GPR183 in IBD pathogenesis and morphogenesis of the intestinal immune system.

Role of GPR183 in the migration and activation of immune cells

7α,25-diHC is the main ligand of the oxysterol receptor GPR183

GPR183/EBI2 was discovered in 1993 as an Epstein Barr virus–induced orphan receptor in Burkitt lymphoma cell lines (8). GPR183 ligands remained unknown until 2011, when two landmark publications identified dehydroxylated oxysterols as GPR183 ligands (9, 10). Oxysterols arise from oxidation of cholesterol. 7α,25-dihydroxycholesterol (7α,25-diHC) has the strongest affinity to GPR183, 7α,27-diHC shows second highest affinity whereas other oxysterols (monohydroxylated oxysterols 25-HC and 7α-HC) show substantially lower activity (9, 10). Synthesis of the GPR183 ligand 7α,25-diHC requires two hydroxylation steps (Figure 1) at position 25 by the enzyme cholesterol 25-hydroxylase (CH25H) and at position 7α, by cytochrome P450 family 7 subfamily member B1 (CYP7B1). Degradation of 7α,25-diHC is catalysed by the enzyme hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7 (HSD3B7) (9, 10).

[*] In this article we follow the drug/ molecular target nomenclature of the Concise guide to Pharmacology 7. Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, et al. The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. British journal of pharmacology. 2013;170(8):1459-581.. Further, nomenclature of proteins and genes follow international conventions: Proteins will be written non-italic (GPR183/ Gpr183), genes in italic *(GPR183/ Gpr183*). Human genes and proteins will be capitalized (*GPR183*/ GPR183), for mouse proteins genes and proteins only the first letter will be capitalized (Gpr183/ *Gpr183*). If we refer to the gene/ protein independent from the species, the capitalized form will be used (GPR183/ *GPR183*).

GPR183 function and its dependence on an oxysterol gradient

GPR183 is expressed on B cells, T cells, dendritic cells (DCs), macrophages and innate lymphoid cells (ILCs) (3, 5, 9-13). In peripheral blood mononuclear cells, highest levels of GPR183 are observed on memory lymphocytes, in particular B and $CD4^+$ T cells but to a lesser extent on $CD8^+$ T cells (2, 14). Binding of oxysterols to GPR183 leads to the release of intracellular calcium (9), suppression of cAMP (10) and internalization of GPR183 (10). However, the most important consequence of GPR183 activation is migration of GPR183-expressing cells towards higher 7α,25-diHC concentrations (3, 5, 9-13, 15).

In secondary lymphatic organs, enzymes for production of 7α,25-diHC (i.e. *Ch25h* and *Cyp7b1*) are expressed by the same and/or neighbouring cells while the enzyme for 7α,25-diHC degradation (*Hsd3b7)* has a divergent expression pattern (9, 10, 13, 16). This suggests that spatial differences in the synthesis and degradation of 7α,25-diHC may result in gradients of oxysterols in lymphoid organs. In agreement with this hypothesis, knockouts of all 7α,25-diHC-producing and -degrading enzymes (i.e. *Ch25h*, *Cyp7b1* and *Hhsd3b7*) recapitulate important aspects of the phenotype of *Gpr183* deficiency with respect to B and T cell positioning (9, 10, 13, 16). While it is clear that the spatial organization of the enzymatic machinery regulating 7α,25-diHC concentrations in the tissue is indeed important for GPR183 function (9, 10, 13, 16), the postulated 7α,25-diHC gradient has not been visualized yet.

GPR183-dependent positioning of DCs, T and B cells

For any given antigen only few naïve B and T lymphocytes expressing a cognate antigen receptor exist. Due to this large search space, the probability that a given lymphocyte would encounter a specific antigen by chance is very low. Consequently, the generation and coordination of adaptive immune responses rely on cell migration and proper positioning of the interacting cell types. Sophisticated anatomical structures and mechanisms of cell migration have evolved to increase the likelihood of productive cell-cell encounters and enable the efficient generation of adaptive immune responses.

The principal cell types to initiate adaptive immunity are DCs. DCs take up antigens, process them and transport them to specific sites where they encounter and activate lymphocytes. Thereby, DC migration, proper positioning of DCs and lymphocytes within immune compartments and the anatomical organization of the immune system increase the probability of antigen-presenting DCs to encounter cognate lymphocytes and hence the efficacy of adaptive immunity. Two main subsets of conventional DCs (cDCs) have been described. Broadly, the cDC1 subset plays a key role in CD8 T cell responses whereas the cDC2 subset preferentially interacts with CD4 T cells (17, 18). While upon toll-like receptor (TLR) stimulation, GPR183 expression is upregulated on cDC2s, cDC1s don't seem to express GPR183 (19). GPR183 contributes at various levels to the coordination of cell–cell encounters by modulating the migration and positioning of DCs, T cells and B cells.

GPR183 supports localization of cDC2s at the bridging channel in the spleen and the periphery of lymph node T cell zones, both of which are locations where antigens accumulate and antigen uptake and presentation to T cells may occur (11, 12). Additionally, GPR183 promotes

migration of lymphocytes to the interface between lymph node follicles and the T cell zone, aiding contact with DCs (13). These contacts facilitate differentiation of activated T cells to follicular helper T cells by inducible T cell co-stimulator (ICOS) – ICOS-L interactions and DC-mediated quenching of CD25 signalling (13).

GPR183 specifically facilitates co-localization of cDC2s with CD4 T-helper cells in the peripheral T cell zone as well as antigen-dependent CD4 T cell activation and expansion. In mice, Gpr183 was required for CD4 T cell–dependent protection against helminths and successful plasmodium vaccination (20).

For the activation of B cells in secondary lymphoid organs, GPR183 synergizes with the C-C chemokine receptor type 7 (CCR7) to position B cells at the B–T zone interphase (Figure 3). After having received T cell help, B cells downregulate CCR7 and move to interfollicular and outer follicle regions (9, 10, 16, 21-23). Importantly, vaccination-induced plasma IgG1 responses were reduced in *Gpr183* knockout mice as compared to wild type animals (9). Thus, GPR183 is important for rapid and efficient B cell activation.

GPR183-dependent migration of ILCs in the intestine

Besides supporting the migration of lymphocytes and DCs, GPR183 coordinates the migration of innate lymphoid cells (ILCs). ILCs are innate counterparts of T lymphocytes. Like conventional T cells, ILCs are potent producers of cytokines and are derived from the same common lymphoid progenitor. However, unlike T cells, ILCs lack specific T cell receptors (24-28). ILC1 mirror the functionality of Th1 cells and respond to intracellular pathogens, ILC2s secrete cytokines, which play an important role in anti-parasite and allergic responses and ILC3, like their Th17 counterparts, can participate in defence against extracellular microbes. Since ILCs do not require clonal expansion for their function, they are able to respond without delay to appropriate signals.

In the intestinal mucosa, ILC3s are the dominant type of ILCs and contribute to immunity and barrier integrity (29). ILC3s are characterized by expression of transcription factor retinoic acid related orphan receptor gamma-t (RORγt). ILC3s contain commensal bacteria (30) and support defence against enteropathogens (27, 29). Interleukin (IL)-22 is a key cytokine produced by ILC3s that stimulates secretion of antibacterial peptides (31) and also contributes to antiviral defence (32).

Gpr183 is expressed at higher levels on ILC3s in the intestinal lamina propria (3, 5) compared to blood (2) and ILC3s have been shown to migrate towards a 7α,25-diHC gradient (5). GPR183 can promote accumulation of ILC3s in the intestine. In experiments with mixed bone marrow chimeras, Gpr183-expressing ILC3 cells clustered in solitary intestinal lymphoid tissue (SILT) of colonic and small intestinal tissue (3), while *Gpr183*$^{-/-}$ cells accumulated in the mesenteric lymph nodes (5).

Gpr183 expression on ILC3s mediates protection against enteric bacterial infections and *Gpr183* deficiency resulted in more severe inflammation in the model of *Citrobacter rodentium* induced colitis. In line with a role of ILCs in this phenotype, *Rag1*$^{-/-}$ *Gpr183*$^{-/-}$ mice had lower

numbers of ILC3 and lower numbers of IL-22 producing ILC3 in the small intestine and in the colon (5).

Fine-tuning of oxysterol gradients

As described above, the generation of the high-affinity GPR183 ligand 7α,25-diHC requires the sequential activity of *CH25H* and *CYP7B1*. However, *Ch25h* knockout mice only partially replicate the phenotype of *Gpr183* deficiency (2, 3, 19). Similarly, *Ch25h* and *Cyp7b1* knockouts differ with respect to their effects on DC positioning. These observations suggest a more complex machinery producing and degrading GPR183 ligands. Indeed, the enzyme *Cyp27a1* with 27-hydroxylating activity is able to generate the alternative GPR183 ligand 7α,27-diHC (19). 7α,27-diHC basically recapitulates 7α,25-diHC signalling via GPR183, even though 10-fold higher concentrations are required (9, 10, 19) and 7α,27-diHC might result in lower Gpr183 desensitization than 7α,25-diHC (19). Thus, local 7α,27-diHC and 7α,25-diHC availability in secondary lymphoid organs might fine-tune GPR183 signalling. Close spatial expression (including co-expression by the same cell) of the enzyme pairs *Ch25h* and *Cyp7b1* or *Cyp27a1* and *Cyp7b1* would favour generation of 7α,25-diHC or 7α,27-diHC, respectively.

Oxysterol levels can also be regulated by degradation pathways. In the spleen, activation of the cDC1 subset (not expressing *Gpr183,* see above) further influenced positioning of the *Gpr183*-expressing cDC2 subset. cDC1s express the oxysterol-degrading enzyme Hsd3b7 and the activity of Hsd3b7 may prevent movement of GPR183 expressing cells into the vicinity of cDC1s by the degradation of oxysterol gradients (19).

So far, oxysterol gradients in tissues cannot be directly visualized. GPR183 ligands are generated via 7α-hydroxylation (CYP7B1) and 25-hydroxylation (CH25H, CYP3 and CYP46A1) (33) or 27-hydroxylation (CYP27A1) activity (19). A more detailed understanding of the GPR183-oxysterol system will require a better definition of the spatio-temporal dynamics of GPR183 ligand generation and degradation.

Direct effects of GPR183 activation on immune cell function

GPR183 is a well-characterized G-protein coupled receptor and many biological effects of GPR183 described so far involve cellular migration and positioning. However, there is mounting evidence suggesting additional intracellular effects of GPR183. Interestingly, GPR183 has been discovered in an siRNA screen as a host factor for *Mycobacterium tuberculosis* infection in human macrophages (34). In murine macrophages, overexpression of *Gpr183* promoted intracellular *Mycobacterium tuberculosis* replication while knockdown, mutation or chemical inhibition of *Gpr183* reduced *Mycobacterium tuberculosis* infection (35). Gpr183 expression and activity was also critical in a tissue culture model of non-alcoholic steatohepatitis (36). Further investigation into the direct effects of GPR183 on effector cells of the immune system would open a new layer of complexity and opportunities for more discoveries.

GPR183 in lymphoid organogenesis

Development of the intestinal immune system

The gut associated lymphoid tissue (GALT), comprising distinct immune compartments such as large encapsulated Peyer's patches (PPs) and isolated intestinal follicular structures, initiates and regulates intestinal immune responses. Recent reports reveal how GPR183 contributes to the organization of intestinal lymphoid tissue (2, 3, 5). Before reviewing the role of GPR183 in lymphoid organogenesis, we will briefly summarize key aspects of intestinal lymph organogenesis using the example of Peyer's patches and introduce key features of different GALT structures (see textbox).

Like lymph nodes, PPs are formed during gestation (37). A centre stage in PP organogenesis is taken by hematopoietic lymphoid tissue inducer cells (LTi), which are a subtype of ILC3s. LTi interact with stromal cells known as lymphoid tissue organizing cells (LTo). LTi express lymphotoxin, which binds to lymphotoxin receptor on LTo. Lymphotoxin signalling triggers expression of the chemokine CXCL13 to recruit LTi expressing the chemokine receptor CXCR5 and induces survival and retention of LTi. Clusters of LTi subsequently recruit additional immune cells for formation of mature lymphoid structures (38, 39).

PPs resemble lymph nodes with respect to their typical organization of distinct B and T cell zones (even though at different proportion in PP as compared to lymph nodes). The colonic equivalent to small-intestinal PPs are colonic patches (CLPs). Like PPs, CLPs are formed during gestation. They are located in the muscularis mucosae and 'touch' the intestinal lumen (Figures 3, 4). However, intestinal immune responses are also supported by additional mucosal lymphoid tissues (see below). Some intestinal immune structures, such as PPs, develop independently from inflammation as secondary lymphoid tissues but tertiary lymphoid tissue is also induced in response to chronic inflammation.

Solitary intestinal lymphoid tissue (SILT) is a type of GALT with features between *bona fide* secondary and tertiary lymphoid tissues (Figures 3, 4). SILTs are strictly submucosal structures, close to the intestinal epithelium of the small and large intestine (1). By definition, SILTs never invade or penetrate the muscularis propria. In contrast to lymph nodes, PPs and CLPs, SILTs only appear after birth (37) and development of SILTs requires the presence of LTi (40), lymphotoxin (41) and TLR signalling (1, 42, 43), compare textbox.

Different maturation stages of SILTs can be distinguished. The smallest structures are referred to as cryptopatches (CPs) and comprise mostly LTi and LTo cells, surrounded by DCs. Larger SILTs are known as isolated lymphoid follicles (ILFs). ILFs can reach the size of a PP or CLP and also contain organized B cell follicles. At least in humans, T cell areas and MadCAM-1^{+} vessels for immune cell recruitment were demonstrated (44). Recruited B cells have been shown to undergo T cell–independent class-switch recombination within ILFs. Thus ILFs may be a critical source of T cell–independent IgA (45).

Impact of GPR183 on the development of colonic SILTs (cSILTs)

Several recent studies demonstrated that GPR183 is required for the development of lymphoid tissue in the colon. *Gpr183* knockout mice show a lower number of B-cell positive lymphoid

structures (2, 3, 5). Microscopic examination revealed that colonic SILTs (cSILTs) were mainly affected, while the number of CLPs was constant (2) or only slightly reduced (3). Detailed experiments with conditional *Gpr183* knockouts demonstrated that GPR183 expression by LTi-like ILC3s is required for generation of colonic SILTs while it is dispensable for the formation of colonic patches (3).

Gpr183 knockout impacted less on the development of SILTs in the small intestine: in one study, the overall number of ILC3s in the small intestinal lamina propria was lower in *Gpr183*$^{-/-}$ and *Ch25h*$^{-/-}$ mice and there were also fewer cryptopatches in the small intestine (5), while in another study, the number of small intestinal SILTs was only marginally reduced (3). Most likely, the GPR183-7α,25-diHC axis is also active in the small intestine, mediating recruitment of immune cells. However, B cells seem to be able to compensate for loss of LTi-like ILC3 function in the small intestine, but not in the colon (3).

Immune cell migration into CPs and ILFs is dependent on Ch25h activity, which is required for synthesis of the GPR183 ligand 7α,25-diHC. More detailed evaluations revealed expression of the 7α,25-diHC producing enzymes Cyp7b1 and Ch25h in CD34^{-}podoplanin^{+} fibroblasts within ILFs, while CD34^{+}podoplanin^{+} fibroblasts outside ILFs produced the 7α,25-diHC degrading enzyme Hsd3b7 (3). This differential enzyme expression pattern suggests existence of a 7α,25-diHC gradient around SILTs which would mediate the recruitment of Gpr183 expressing ILC3s, B cells and DCs into SILTs.

Despite these profound alterations of the GALT of *Gpr183* knockout mice, their intestinal immune system remained intact and no further impairment of functionality could be detected in the absence of environmental challenges: The number of mesenteric lymph nodes and the total number of B cells in the colon was normal with normal faecal IgA levels (2, 3). Furthermore, no differences in microbiota composition were found in *Gpr183* deficient mice and the fraction of IgA coated bacteria remained normal (2). Interestingly, in colonic ILC3s, Il-22 production was reduced upon *Gpr183* knockout (3).

GPR183 in intestinal inflammation

The *GPR183* locus is a risk locus for inflammatory bowel diseases

Inflammatory bowel diseases are chronic inflammatory conditions and comprise the major subgroups Crohn's disease (CD) and ulcerative colitis (UC). While in UC disease activity is restricted to the colon with strongest inflammatory activity in the rectum, CD can affect the whole intestinal tract. The pathogenesis of IBD is unknown but genetic and environmental risk factors have been identified (46, 47).

Genome wide association studies (GWAS) have linked the GPR183-oxysterol system to IBD. GWAS identified >240 genetic regions and most identified genes increased the risk for both, UC and CD (48-51). One IBD risk gene maps to the genetic region encoding GPR183. The single nucleotide polymorphism (SNP) rs9557195 on chromosome 13 within an intron of *GPR183* (Figure 5) is associated with CD (odds ratio (OR) of 1.12; 95% CI: 1.08-1.16) and UC (OR 1.1; 95% CI 1.06-1.15) (48). Association of SNP rs9557195 with both UC and CD, suggests a potentially more general role of the *GPR183* locus for inflammatory mechanisms

and/or organization of the gastrointestinal immune system. Very recent data from our group indicate increased GPR183 expression on the surface of immune cells in individuals with the CC-allele of rs9557195 [F. Ruiz, C. Pot, B. Misselwitz manuscript submitted], providing the first experimental confirmation of these genetic data in human samples.

The *UBAC2* gene is a second gene at the *GPR183* locus and associated with Behçet's disease

The architecture of the human *GPR183* gene locus is complex. The ubiquitin-associated domain-containing gene 2 (*UBAC2*) is located on the complementary DNA strand and contains several intron and exon regions overlapping with *GPR183* (Figure 5). *GPR18*, another G-protein coupled receptor, also overlaps with *UBAC2*. Several studies associated SNPs within the *UBAC2* gene with the risk for Behçet's disease (52-54). Behçet's disease is a rare condition but more frequently occurring along the ancient silk road from Eastern Asia to Mediterranean with most cases in Turkey (55).

Behçet's disease is a small vessel vasculitis, diagnosed upon occurrence of recurrent bipolar (oral and genital) aphthae and additional systemic manifestations with involvement of eyes, skin (erythema nodosum and pyoderma gangraenosum), the nervous system and joints. Gastrointestinal involvement occurs in 10-15% of patients with abdominal pain, diarrhoea and intestinal ulcerations frequently in the ileocecal region (56, 57).

Manifestations of Behçet's disease may thus overlap with intestinal and extraintestinal manifestations (EIM) of CD, making a correct disease diagnosis challenging (56). Further, IBD and Behçet's disease respond to similar medical treatments (57) and have genetic overlap (58). It is therefore possible that Behçet's disease mutations contribute to the risk of EIM in IBD but the clinical relevance of the overlap of IBD with Behçet's disease remains unknown.

UBAC2 expression was increased in peripheral blood mononuclear cells in patients carrying risk alleles of two Behçet's disease–associated SNPs (rs7999348 and rs3825427, Figure 5) (52, 53). UBAC2 is part of an endoplasmic reticulum (ER) membrane protein complex, which can inhibit wnt/β-catenin signalling, and defects of other proteins in this complex result in severe lymphocyte dysfunction (59). In another study, UBAC2 restricted trafficking of ubiquitin-like-domain-containing protein 8 (also called FAF2) from the ER to lipid droplets (60). Besides UBAC2, other ubiquitination-related genes (UBASH3B, SUMO4) have also been associated with Behçet's disease, suggesting a crucial role of ubiquitination in the pathogenesis of this condition (52, 53, 61). However, it remains unclear how UBAC2 can contribute to organ inflammation.

The IBD-associated SNP rs9557195 is located within introns of both genes, *GPR183* and *UBAC2*, and it remains unclear how much each of those genes contributes to IBD risk. rs9557195 might act via GPR183 since it is associated with the risk for both CD and UC, while Behçet's disease mainly overlaps with CD. Further, our unpublished observations show increased GPR183 expression in individuals with the rs9557195-CC allele (see above).

However, in CD patients resistant to anti-tumour necrosis factor (TNF) treatment, an expression quantitative trail loci (eQTL) study identified elevated UBAC2 levels in individuals

with the rs9557195-TT genotype in peripheral blood and the intestine (62). Further, rs3742130, another SNP within UBAC2/GPR18 was associated with anti-TNF non-responsiveness (63), raising the possibility of involvement of UBAC2 in IBD as well. However, none of the studies of either GPR183 or UBAC2, rigorously excluded the effects of the other gene, hence a role of both genes in IBD is possible.

Murine models of IBD suggest proinflammatory effects of GPR183

Experimental observations indicate an important role of GPR183 in inflammation and pathogenesis of colitis. In the anti-CD40 colitis model, colon inflammation is induced by injection of the anti-CD40 antibody into *Rag1*$^{-/-}$ mice. Reduced overall inflammation and fewer inflammatory foci containing Gpr183 expressing ILC3s and myeloid cells were observed in *Rag*$^{-/-}$ *Gpr183* $^{-/-}$ animals, compared to animals with functional Gpr183 (3). *Gpr183* knockout also reduced granulocyte macrophage colony-stimulating factor (GM-CSF) production (3), which had been suggested as a key pro-inflammatory activity of ILC3s in innate colitis.

Similarly, GPR183 confers pro-inflammatory effects in the IL-10 model of chronic colitis: *Gpr183*$^{-/-}$*Il-10*$^{-/-}$ animals had less severe inflammation compared to *Il-10*$^{-/-}$ controls (2). Notably, the IL-10 colitis model shows at least some relevance for human IBD, since *IL-10* mutations have been linked to the human disease (64). Interestingly, in IL-10 colitis, the *Gpr183* phenotype was restricted to male animals (2). The reason for this sex-difference is unclear but related observations have also been reported in other colitis models (65). Further, in humans, polymorphisms in the IL-10 gene can also act in a gender specific manner (66) and *CYP7B1*, one of the enzymes necessary for 7α,25-diHC production, is a male dominant gene (67). In any case, mechanistic effects how Gpr183 increased colon inflammation in Il-10 colitis remain unclear.

In contrast, in acute and chronic chemical (dextrane sodium sulphate: DSS) colitis, knockout of *Gpr183* did not affect intestinal inflammation (2). This suggests that GPR183 effects on intestinal inflammation depend on the particular system and mode of pathogenesis.

Intestinal inflammation affects balancing of the GPR183–oxysterol system

GPR183 mRNA levels as well as mRNA of enzymes producing 7α,25-diHC (*CH25H*, *CYP7B1*) are upregulated in biopsies of UC patients with active disease compared to patients in remission or healthy controls (2). Expression levels correlated with disease activity and established inflammatory markers including TNF or C-X-C motif chemokine ligand 8 (CXCL8) expression (2, 3). *GPR183* expression was also induced by lipopolysaccharide (LPS) on human macrophages (10, 68, 69), with possible repression in the murine system (69, 70), possibly related to ligand-induced GPR183 downregulation.

In murine colitis, activation of the GPR183-7α,25-diHC axis was also observed and confirmed by oxysterol measurements. Most experiments were done in the acute and chronic DSS colitis model, which relies on chemical destruction of the intestinal barrier and innate immune activation. Measurements in acute DSS colitis confirmed increased expression of *Gpr183*, *Cyp7b1* and *Ch25h* in the gut (2, 71) and higher levels of the oxysterols 4β-HC, 25-HC and 7α,25-diHC in the gut mucosa (2, 72, 73). Similar changes, correlating with colitis severity,

were observed in chronic DSS colitis (72). In one study, 7α,25-diHC was elevated in colon and stool and usage of 7α,25-diHC as a biomarker was suggested for IBD (73). Trinitrobenzene sulfonic acid (TNBS) induced colitis resulted in increased levels of 4β-HC, 25-HC but not 7α,25-diHC (72). Increased *Ch25h* expression and production of an activity, stimulating Gpr183 dependent cell migration was also observed in innate anti-CD40 colitis (3).

While an increase in 25-HC and 7α,25-diHC was repeatedly observed, expression levels of *Cyp27a1* (responsible for production of 27-HC and the alternative GPR183 ligand 7α,27-diHC) were not affected in IL-10, DSS or TNBS colitis (2, 72), while Hsd3b7 levels (responsible for degradation of most oxysterols) were normal or even decreased (2, 72).

Upregulation of oxysterol synthesis and increased oxysterol concentrations were also observed in human and murine models of non-alcoholic steatohepatitis (NASH) but knockouts of *Gpr183*, *Cyp7b1* or *Ch25h* did not affect severity of NASH (74).

In summary, there is strong and consistent evidence for increased expression of the enzymes synthesizing the GPR183 ligand 7α,25-diHC in human samples and four different mouse models of colitis and enzyme levels correlate with severity of inflammation. In biochemical analyses, higher intestinal 7α,25-diHC levels were observed in murine tissue upon inflammation in most models (2, 3, 72, 73). However, levels of other oxysterols varied to a higher degree. Most likely, the degree of systemic and local inflammation differed according to the mouse models used. Further, mass spectrometry determinations of di-hydroxylated oxysterols can be challenging, likely explaining most discrepancies.

Non-GPR183 mediated effects of oxysterols in intestinal inflammation

While pro-inflammatory effects of GPR183 in inflammation are increasingly recognized, it has long been established that oxysterols are immune-modulatory molecules with broad activities (75) (Table 1).

The induction of *CH25H* is a general feature in inflammation. Several TLR agonists increased *CH25H* mRNA levels and increased 25-HC production in human and murine macrophages (10, 68, 76-80). In line with these results, in human healthy volunteers, serum 25-HC concentration increased upon LPS injection (77).

25-HC also has broad immune-modulatory effects. It is a powerful inhibitor of human viruses (78, 79) and was very recently identified as an efficient antibacterial molecule in *Listeria monocytogenes* and *Shigella flexneri* infection (81). Antibacterial effects are mediated by redistribution of membrane cholesterol and reduction of accessible membrane cholesterol (81). 25-HC also inhibits inflammasome assembly, caspase activation and IL-1 and IL-18 production in macrophages (82) while IL-1 can in turn induce Th-17 differentiation (83).

Therefore, not all effects of oxysterols in intestinal inflammation are mediated by GPR183 (Table 1) and the oxysterol receptors liver X receptor (LXR)-α and LXR-β have a role in colitis. LXR-α and LXR-β are activated by oxysterols including 25-HC and 27-HC (84). More severe DSS colitis was observed upon knockdown of *Lxr-β* and/ or *Lxr-α* while the LXR agonist GW3965 improved clinical course of DSS colitis (85). Thereby, Lxr-β and Lxr-

α reduced activation of CD11b$^+$ immune cells, while Lxr-β in addition downregulated secretion of inflammatory mediators in colonic epithelial cells (85). Recent experiments have also associated Ch25h with intestinal fibrosis in two animal models (71).

Moreover, dihydroxylated oxysterols such as 7α,27-diHC and 7β,27diHC are agonists of RORγt and regulate Th17 cell differentiation (86). Since Th17 cells have been shown to be of relevance in IBD pathogenesis (87) oxysterol mediated RORγt stimulation is likely relevant in IBD. Distinguishing GPR183-mediated effects of oxysterols from effects on LXR, RORγt and pleiotropic oxysterol effects (see below) remains a challenge.

GPR183-dependent accumulation of lymphoid tissue in chronic colitis

Whereas colonic patches are clearly distinct in their anatomical localization and structure, cSILT and TLT in the colon cannot easily be distinguished by histology and foremost differ in their developmental requirements. Thus, lymphoid tissue observed in the inflamed colon might represent true TLT and/or more mature and/or hypertrophic cSILT.

Build-up of colonic lymphoid tissue seems to require a prolonged inflammatory stimulus: no changes of cSILT/TLT numbers were seen in acute DSS colitis, while in chronic DSS colitis a pronounced increase of cSILTs/TLTs could be observed (88). Accumulated lymphoid tissue comprises B cells with germinal centres, DCs and CD4 T cells but no T cell areas. High endothelial venules are frequently observed around inflammation-induced TLTs, which would facilitate further recruitment of immune cells (89).

Accumulation of TLTs in chronic inflammation is dependent on GPR183: in chronic chemical (DSS) colitis, in *Gpr183*$^{-/-}$ animals, low cSILT/TLT numbers were seen at baseline without any inflammation dependent increase, despite similar levels of inflammation in *Gpr183*$^{-/-}$ and wildtype animals (2). Interestingly, the increase in cSILT/TLT was only partially dependent on *Ch25h*, suggesting activity of either Cyp27a1 (for production of 7α,27-diHC) or activity of other enzymes with 25-hydroxylation activity (see above).

Inflammation in chronic Il-10 colitis also increased the number of cSILTs/TLTs with a strong correlation of the number of lymphoid structures and the level of inflammation (2). Upon *Gpr183* knockout, an overall lower number of SILTs was observed even in animals with similar levels of inflammation (2). In innate anti-CD40 colitis, *Gpr183* was also required for an inflammatory migratory response and induction of lymphoid tissue during inflammation (3). Therefore, there seems to be a universal requirement for Gpr183 for the induction of inflammatory lymphoid tissue, independent from the way colon inflammation was induced.

For induction of SILTs in inflammation, B cells might be able to take on the function of LTis. In chronic DSS colitis, accumulation of lymphoid tissue required lymphotoxin but not RORγt and the authors demonstrated a critical function of B cells for induction of lymphoid tissue upon RORγt knockout (88). Since GPR183 is expressed on ILCs and B cells, GPR183 might support both, the classical LTi (ILC3)-dependent induction of cSILTs as well as the alternative B cell dependent pathway, described above. However, this still needs to be confirmed directly.

A recent study indicated that ILC3s were predominantly localized in CPs but ILC3 motility increased after induction of anti-CD40 colitis resulting in preferential ILC3s egress from CPs

(90). It is unclear whether this change of motility depends on GPR183 and whether it is related to inflammation and/ or accumulation of lymphoid tissue.

The detailed function of the accumulated colonic lymphoid tissue in chronic colitis is unclear. It is unknown whether they have more pro-inflammatory or anti-inflammatory properties in the context of infection or IBD and discrepant results have been described in different models (91). While in some murine studies, colitis was dependent on accumulated cSILT/TLT (3, 88, 92, 93), in other studies no correlation between cSILTs/TLTs and chronic inflammation was observed (2, 94). Accumulation of lymphoid follicles and lymphangitis are also a hallmark of human small intestinal Crohn's disease but pathogenesis and implications for management of these findings are insufficiently understood (91, 95, 96).

In summary, recruitment of immune cells into the colon upon chronic inflammation remains a complex process (Table 1) and CXCL13, IL-22, IL-23, lymphotoxin, the microbiota (6, 88, 92) and possibly also the CXCR5-CXCL13 axis (97) have been implicated. Recent data also indicate a role of GPR183 in this process (2). Further studies of GPR183 in intestinal inflammation might also reveal relevance and functional impact of lymphoid tissue accumulation in colitis.

The GPR183-oxysterol axis as a potential drug target for IBD

Targeting immune cell migration

Inhibition of immune cell migration is a well-established therapy principle in IBD and other inflammatory conditions. Current approaches include integrin inhibition, sphingosin-1-phosphate receptor (S1PR) modulation and chemokine receptor blockage. GPR183 modulation might constitute a new therapeutic approach, non-redundant with established therapies and with potentially unique effects.

Integrin inhibitors have been the first approved drugs to block cellular migration. Homing of immune cells into the gut is mediated by integrin-α4β7. Natalizumab (blocking the integrin-α4 subunit) and vedolizumab (blocking integrin-α4β7) are of therapeutic value in IBD (98-101). Natalizumab (102) (but not the gut specific vedolizumab (103)) is associated with the risk of the opportunistic infection progressive multifocal leukoencephalopathy (PML). Therefore, natalizumab is rarely if ever used in IBD. However, in patients with multiple sclerosis (MS) under natalizumab treatment, GPR183 expression and activity is increased on the surface of CD4 memory T cells compared to before treatment (14), suggesting high GPR183 expression in immune cells destined to target inflamed tissues such as the brain in MS and/ or the intestine (104).

S1PR mediate exit of lymphocytes from secondary lymphoid organs. Lymphocytes follow a sphingosine-1-phosphate (S1P) gradient with higher S1P concentrations in blood. S1PR blockade results in functional inactivation of lymphocytes by trapping them within lymphoid organs (105). Ozanimod (blocking S1PR1 and S1PR5) showed some activity in UC patients. In line with entrapment of lymphocytes as the mode of action for ozanimod, peripheral lymphocyte counts decreased by almost 50% (106, 107).

Chemokine receptors CCR6, CCR9 and CXCR3 and its ligands mediate recruitment of immune cells into the gut in health and/ or inflammation; however, clinical tests of blocking these chemokine receptors in IBD remained disappointing (108-112), most likely due to redundant migratory signals for immune cell recruitment into the inflamed gut.

Effects of GPR183 inhibition would likely differ from inhibition of integrins, S1PR and chemokine receptors. In animal experiments, GPR183 knockout did not reduce the overall number of intestinal lymphocytes, but affected immune cell distribution in the intestine with reduction of SILTs (2, 3) and entrapment of lymphocytes in mesenteric lymph nodes (5). In inflammation, GPR183 mediated accumulation of tertiary lymphoid follicles in the mucosa with pro-inflammatory effects in some but not all animal models (2, 3). Since our understanding of the function of SILTs in health and inflammation is limited, effects of GPR183 inhibition in human IBD is not foreseeable, but the opportunity of a new drug target in intestinal inflammation is highly welcome.

Blocking of the GPR183–oxysterol axis

A chemical inhibitor which prevents binding of 7α,25-diHC to GPR183 and hence 7α,25-diHC dependent migration of immune cells can be used as an GPR183 blocker (113, 114). In mice, this inhibitor replicated DC positioning phenotypes of the *Gpr183* knockout (19), but has not been tested in murine colitis.

Targeting oxysterol synthesis, such as blocking Cyp7b1 by clotrimazole (10), could be used as additional therapeutic strategy. However, to the best of our knowledge, knockouts of *Cyp7b1*, *Ch25h* or *Cyp27a1* have not been tested in Gpr183 dependent colitis models so the therapeutic potential for IBD remains unexplored. Conversely, in mice, application of 25-HC drastically changed oxysterol levels (including 7α,25-diHC) but did not alter severity of DSS colitis (72).

Conclusion and outlook

The immune system is organized in a multi-layered, fractal-like manner. GPR183 is a key factor involved in the spatial organization of the immune system and in particular the development of lymphoid tissues in the intestine. Human genetics and animal experiments have suggested a role of GPR183 in colitis. However, for now, we are still far from understanding the function of GPR183 in intestinal inflammation. Of note, it remains unclear whether the effects of the IBD polymorphism rs9557195 are mediated via GPR183 or UBAC2. Furthermore, the mechanisms by which GPR183 promotes intestinal inflammation in some, but not all animal models has not been clarified. Moreover, the impact of GPR183 mediated accumulation of lymphoid tissue in colitis, and its impact on inflammation and healing are unclear. Finally, broad immune-modulatory effects of oxysterols and possible direct effects of GPR183 on immune cells have been described and need further study to be distinguished from the effects of GPR183 on cell motility and migration. Notably, downstream effects of GPR183 inhibition, disrupting intestinal immune cell distribution may be unique and different from all other cellular migration inhibitors and warrant further efforts to assess the therapeutic potential for GPR183 inhibition in IBD.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to the corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUHAR/BPS Guide to pharmacology (115), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (7).

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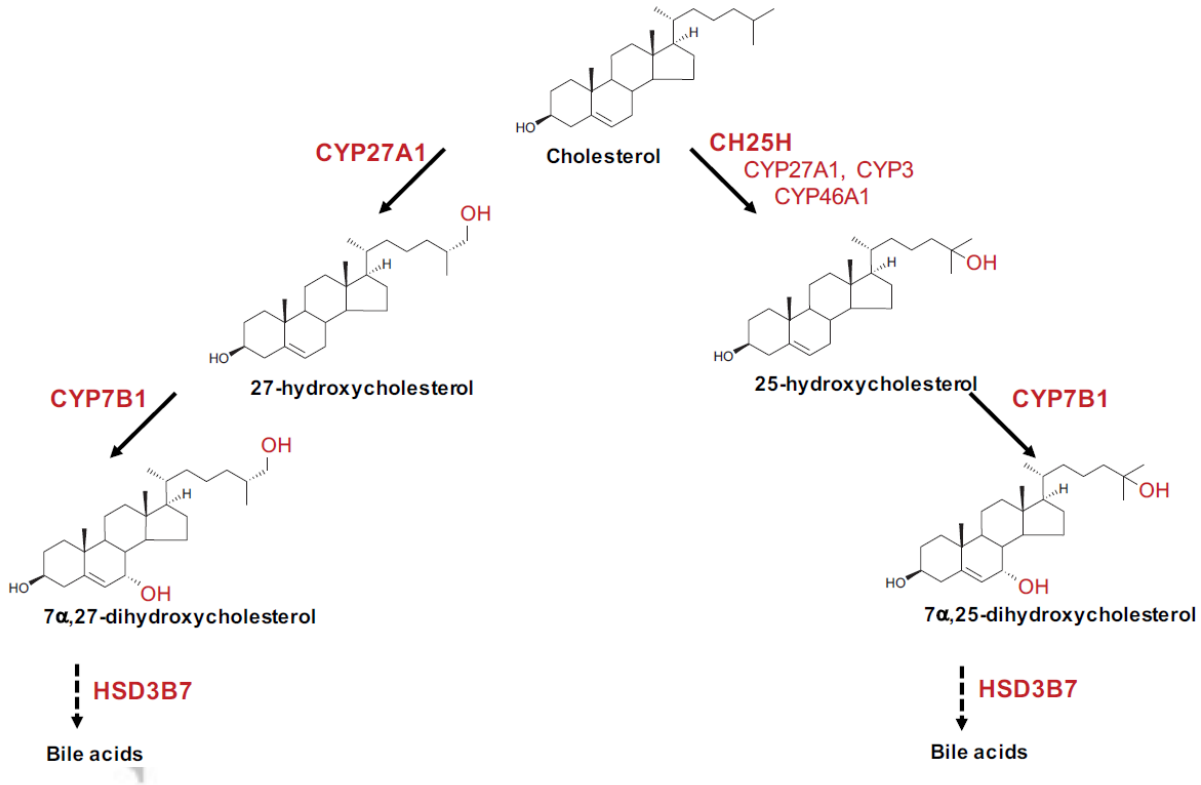

Figure 1: Biosynthesis and degradation of oxysterols.

Oxysterol biosynthesis starts with oxidation at the 25-position by the enzyme cholesterol 25-hydroxylase (CH25H) or at the 27-position by cytochrome p450 family 27 member A1 (CYP27A1), yielding 25-hydroxycholesterol (25-HC) and 27-hydroxycholesterol (27-HC), respectively. Further 7α-hydroxylation by CYP7B1 results in production of 7α,25-diHC and 7α,27-diHC, respectively. Oxysterols are ultimately degraded to bile acids by the activity of hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7 (HSD3B7).

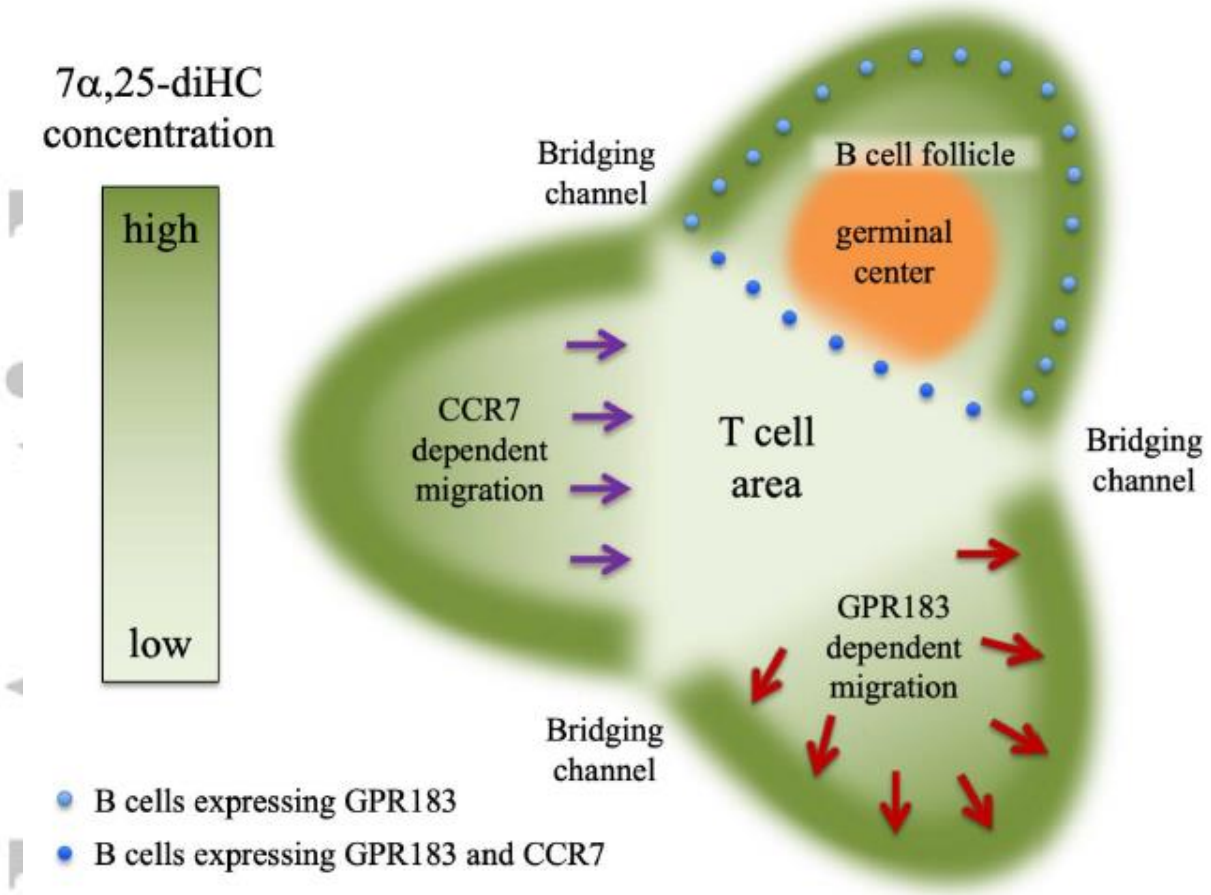

Figure 2: GPR183 dependent movements of immune cells in secondary lymphoid organs

B cell follicles with germinal centre (orange) and the T cell area are indicated. A gradient of the GPR183 ligand 7α,25-diHC is expected in secondary lymphoid organs according to expression levels of the 7α,25-diHC synthesizing enzymes Cyp7b1 and Ch25h. Highest 7α,25-diHC concentrations are expected in the outer follicle (dark green). GPR183 expressing B cells, T cells and dendritic cells follow the 7α,25-diHC gradient (red arrows) and some B cells move to the outer follicle (light blue circle). For other immune cells with concurrent expression of other chemokine receptors (e.g. CCR7, purple arrows) positioning reflects the effects of more than one cytokine and B cells are also found in the interfollicular area (dark blue circles). GPR183 also supports positioning of T cells and DCs (not indicated).

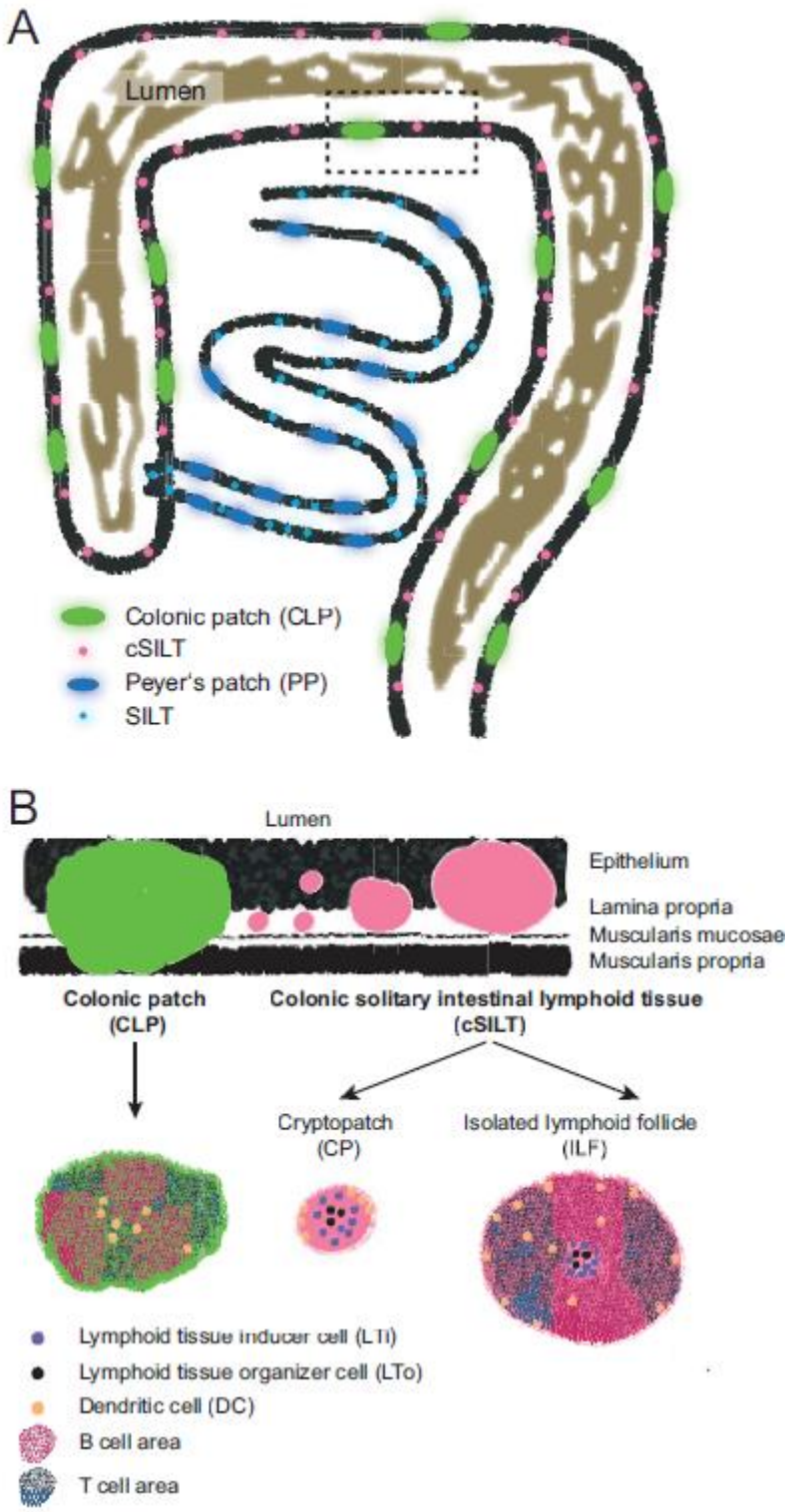

Figure 3: Schematic view of lymphoid structures in the colon and the small intestine.

(A) Overview of the small intestine and the colon with intramucosal solitary intestinal lymphoid tissue (SILT) and colonic SILT (cSILT) as well as larger mucosal and submucosal Peyer's patches and colonic patches (CLP)

(B) Schematic view of location of lymphoid structures and its cellular content. Please note the strict intramucosal of cSILT comprising small cryptopatches and larger isolated lymphoid tissue

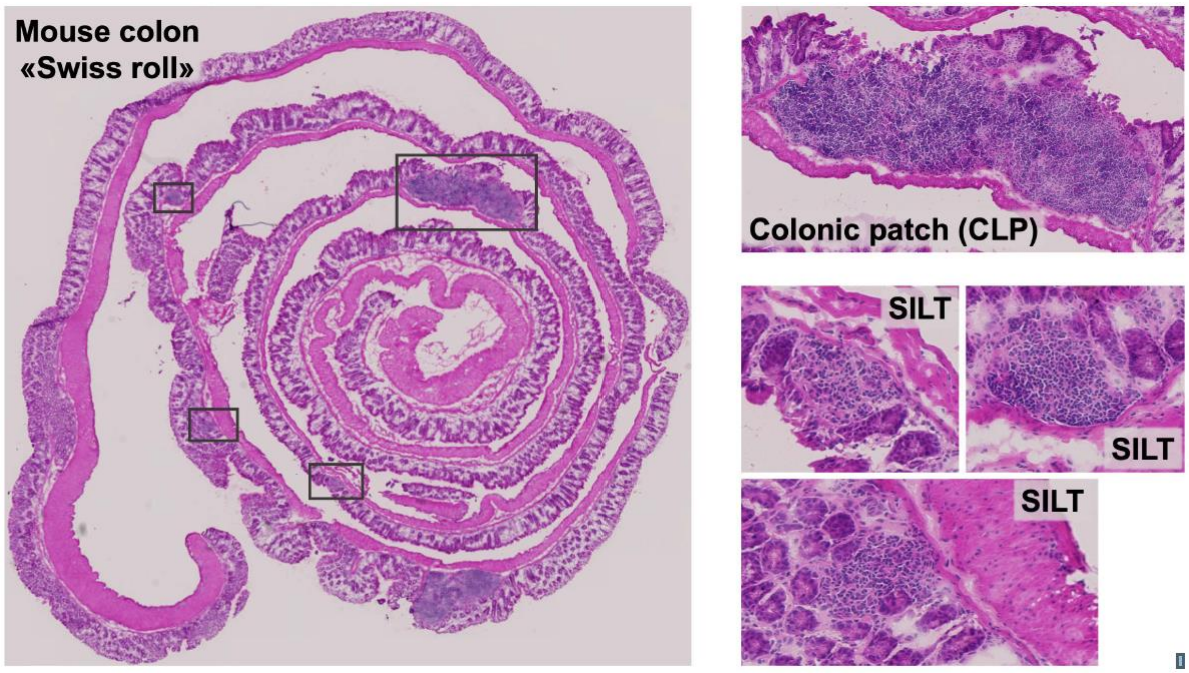

Figure 4: Key structures of the colonic immune system.

Left panel: complete mouse colon as a "Swiss roll". Inserts indicate colonic patches (CLP) and solitary intestinal lymphoid tissue (SILT), shown on the right. While CLPs replace mucosa and submucosa, SILTs are strict mucosal structures, respecting the tunica muscularis mucosae (arrows). Cryptopatches are small SILTs comprising mainly lymphoid tissue inducer (LTi) cells and dendritic cells (DCs) but lack B cells (not shown). Acquisition of B cells yields larger SILTs, called intestinal lymphoid follicles (ILF).

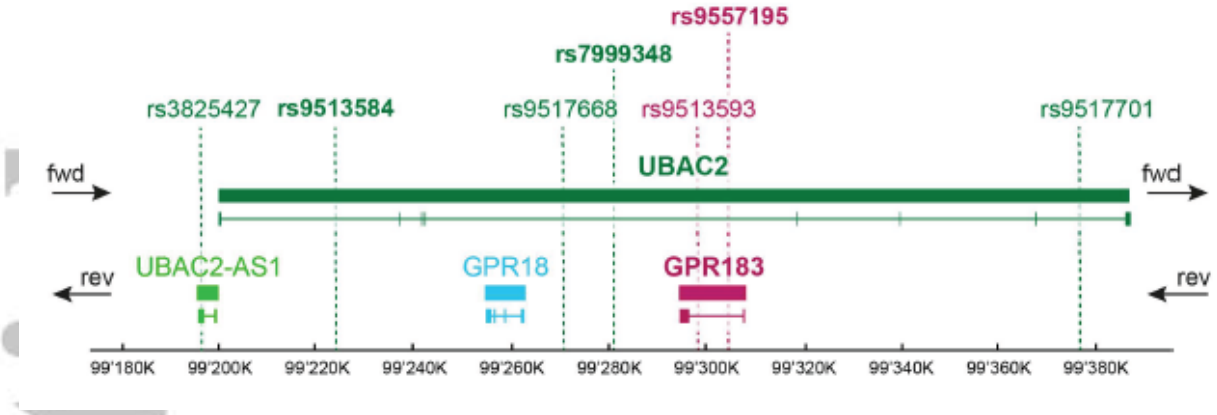

Figure 5: Architecture of the GPR183/ UBAC2 gene locus on chromosome 13

The UBAC2 gene (green) is located on the forward (fwd) strand of chromosome 13, while the GPR183 gene (pink) is located on the reverse (rev) strand. Neighbouring genes encoding G-protein coupled receptor 18 (GPR18) and UBAC2 antisense RNA 1 (UBAC2-AS1) are also indicated. The major transcript of the respective genes with its introns (horizontal line) and exons (rectangles or vertical lines) are indicated in grey. Relevant SNPs (for instance the IBD-SNP rs9557195, the psoriasis-SNP rs9513593 or the Behçet's disease-SNPs rs3825427, rs799348) are highlighted with the colour of the associated gene.

Table 1: Cellular targets of oxysterols and downstream effects.

Oxysterol ligand	Cellular target	Exemplary downstream effects and effects in the intestine (if applicable).	Reference
7β,27-diHC, 7α,27-diHC	RORγt ↑	Th17, ILC3 differentiation, induction of SILTs in the colon	(3, 38-42, 73)
25-HC, 27-HC	LXR-α, LXR-β ↑	Anti-inflammatory effects in colitis, cellular metabolism and immunity	(71, 72, 109)
	INSIG ↑, SREBP ↓	Inhibition of cholesterol synthesis, effects in the intestine unclear	(110)
25-HC	Inflammasome ↓	Inflammasome inhibition, reduced production of IL-1 and IL-18	(69)
	Antibacterial defence ↑	Reduction of accessible membrane cholesterol. Inhibits replication of *L. monocytogenes*, *S. flexneri*	(68)
	Antiviral defence ↑	Inhibits replication of a number of viruses	(65, 66)
7α,25-diHC, 7α,27-diHC	GPR183 ↑	Positioning of B cells, T cells, DCs, ILC3s. Formation of SILTs in the colon, pro-inflammatory effects in colitis (see text for details).	(2, 3, 5, 8, 9, 26-28, 34)