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REVIEW

Th9 cells in inflammatory bowel diseases

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Abstract Inflammatory bowel diseases are chronic, relapsing, immunologically mediated disorders of the gastrointestinal tract. Emerging evidence suggests a critical functional role of transcription factors and T cell-related cytokines in ulcerative colitis and Crohn's disease. Gut-residing T cells from patients with inflammatory bowel disease produce high amounts of IL-9. Experimental models of colitis highlighted that IL-9-producing T cells critically interfered with an intact barrier function of the intestinal epithelium by impacting cellular proliferation and tight junction molecules. The blockade of IL-9 was suited to significantly ameliorate the disease activity and severity in experimental models of inflammatory bowel disease thereby suggesting that targeting IL-9 might function as a novel targeted approach for therapy.

Keywords IBD · Therapy · Cytokines · Immune response · Th9 cells · Il-9

Abbreviations

CD	Crohn's disease
UC	Ulcerative colitis
IBD	Inflammatory bowel disease

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Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) comprise two main disorders: Crohn's disease (CD) and ulcerative colitis (UC). Both diseases affect mainly young individuals and are characterized clinically by chronic diarrhea, rectal bleeding, and abdominal cramping in affected patients. However, there are key differences between these two disorders [1–3]: While inflammation in CD can affect the entire gastrointestinal tract (most frequently the terminal ileum and ascending colon), gut inflammation is restricted to the colon in UC patients. Moreover, at the histological level, gut inflammation in UC is usually restricted to the mucosa and submucosa, whereas transmural inflammation of the bowel wall is commonly seen in CD.

Gut inflammation in IBD is a key driver of disease-associated complications. Such complications include the presence of fistulas and strictures (usually seen in CD) as well as the development of IBD-associated neoplasias and cancer [4–8]. Risk factors for cancer development are the duration and the extent of colitis in IBD patients further highlighting the relevance of mucosal inflammation as a triggering factor for neoplasia development [9]. In addition, it should be highlighted that IBD are not restricted to the bowel wall, as many patients suffer from extraintestinal manifestations of the diseases such as erythema nodosum, pyoderma gangrenosum, primary sclerosing cholangitis, arthralgias, arthritis, uveitis, and conjunctivitis [1, 2, 10, 11].

In spite of marked progress in medical therapy of IBD in recent years, many patients still suffer from complications and have to undergo surgery [1, 12, 13]. This observation highlights the destructive and progressive nature of IBD [14] and underscores the need to better understand the pathophysiology of IBD in order to develop novel therapeutic approaches. We will thus first review the current understanding of the pathophysiology of IBD and subsequently highlight the role of Th9 cells in the disease process.

Pathophysiology of IBD

There is overwhelming evidence from genome wide association studies in recent years that genetic factors are import for disease manifestation in IBD. Specifically, single nucleotide polymorphisms in more than 200 genes have been associated with IBD development with specific findings in CD and UC, like the *NOD2* gene for a CD association [15–17]. However, a significant overlap between both disorders has been found. Most recent data support a continuum of disorders within IBD, much better explained by three groups (ileal CD, colonic CD, and UC). In any case, genetic factors play an important role in disease location and disease behavior over time [18].

In addition to these genetic factors, environmental factors have a fundamental role in disease manifestation of IBD. This is exemplified by the effect of smoking (protects from disease development in UC; risk factor for aggressive disease behavior in CD) and the protective effects of appendectomy in UC [15, 19–21]. In addition to these factors, other factors such as diet, oral contraceptives, nonsteroidal anti-inflammatory drugs, perinatal/childhood infections, or atypical infections have been suggested to play a role in IBD pathogenesis.

Given the prominent role of genetic and environmental factors in disease pathogenesis, development of IBD appears to require the interaction between specific environmental and genetic factors in individual patients. Thus, IBD can be regarded as complex disorder in a genetically susceptible host [15, 22]. As many genes (e.g., mucins, defensins) and environmental factors related to IBD appear to affect the intestinal barrier function, one may postulate that IBD are initially characterized by an altered barrier function thus allowing antigens from the commensal microflora or food antigens to reach the mucosa. Such impaired barrier function with translocation of bacteria has been demonstrated in vivo by confocal laser endomicroscopy in affected patients [23]. This process then appears to induce antigen presentation and immune cell activation resulting in specific T cell activation and proinflammatory cytokine production [24, 25]. The exaggerated proinflammatory cytokine response then alters the finely tuned balance between pro- and anti-inflammatory immune responses in the mucosa and leads to perpetuation and chronicity of the inflammatory process.

Based on the prominent role of T cells in the perpetuation of IBD, we will subsequently review the principles of T cell polarization and then focus on T cell polarization in IBD with special reference to Th9 cells.

T helper cell subsets

The identification of two different T helper cell subsets from CD4+ T cell clones was made in 1986. This discovery of Mossman and Coffman led to the Th1/Th2 paradigm, dividing the CD4+ T helper cells in two different compartments with distinct functions. Nowadays, it is well accepted that Th1 cells are important for cellular immunity, whereas Th2 cells are frequently associated with humoral immune responses [26]. T helper cell subsets are characterized by lineage-associated key transcription factors (e.g., T-bet in Th1 cells and GATA-3 in Th2 cells) and specific secreted signature cytokines (e.g., IFN-γ in Th1 cells and IL-4, IL-5 and IL-13 in Th2 cells) [27–31]. To stabilize the key features of each lineage and ensure lineage commitment [32], a positive feedback loop is known for many T helper cell subsets. These findings led to the idea that T helper cell commitment is lineage-restricted and static. On the contrary, lineage plasticity among different T helper cell subsets can be observed in many situations, particularly in chronic inflammatory disorders such as IBD.

Th9 cells: an IL-9 producing T helper cell subset

After the definition of the Th1 and Th2 cells and their signature cytokines, further cytokines with relevance for polarization and phenotype stabilization of CD4+ T helper cells were discovered. One of these cytokines was the T cell growth factor P40. This cytokine was produced by a T cell clone and was found to differ from the known growth factors IL-2 and IL-4 [33, 34]. Subsequently, it was shown that this growth factor P40 is identical with another factor called the T cell growth factor III (TCGFIII) and unable to stimulate the proliferation of naïve CD4+ T cells [35]. Moreover, the mast cell growth-enhancing activity factor (MEA) was identical with the P40 growth factor that was finally called IL-9 [36].

The expression and release of IL-9 by activated CD4+ T cells after T cell receptor (TCR) dependent stimulation was shown to be mainly driven by the local cytokine milieu. In vitro studies showed that under the influence of TGF-β, stimulated CD4+ T cells produce significant amounts of IL-9. Although IL-4 alone was not capable to induce IL-9 production, IL-4 had marked capacities to enhance IL-9 secretion in the presence of TGF-β. IL-4 signaling in T cells was found to induce the signal transducer and activator of transcription (Stat)-6 pathway, reduced the TGF-β induced expression of forkhead box P3 (Foxp3), and prevented the induction of Tregs [33, 37]. The differentiation of the IL-9 producing T cell phenotype was additionally dependent on IL-2 [38].

IL-9 released by T helper cells was thought to be associated with the Th2 subset for a long time, although a different regulation of IL-9 production compared to the other known Th2 cytokines like IL-4, IL-5, and IL-13 was observed. In subsequent years, however, it became clear that T cells exist that produce chiefly IL-9 leading to the classification of these cells as "Th9" cells [39, 40]. In these cells, IL-9 is not coproduced with the classical Th2, Th1, and Th17 cytokines [41]. It was suggested that the cytokine TGF-β converts the classical Th2

subset into an IL-9 producing phenotype. These cells displayed impaired expression patterns of classical Th2 cytokines, with the exception of IL-10. In addition, other signaling pathways for Th9 induction were discovered. These studies found that the TGF-β signal is indispensable for Th9 development, while the IL-4 signal can be substituted. Further studies showed that IL-1β, IL-18, and IL-33 are able to replace the IL-4 signal during Th9 differentiation [42, 43].

As the major T helper cell subsets were associated with the specific expression of transcription factors like T-bet for Th1, GATA3 for Th2, and RORγt for Th17 cells, various groups searched for transcription factors associated with Th9 cytokine production. Studies on IL-9 cytokine gene transcription revealed that IRF4 is able to bind to the *Il9* promoter. This transcription factor also upregulates IL-9 in a concentration dependent manner in Th9 differentiated CD4+ T cells [44]. Another transcription factor important for the Th9 phenotype is the ETS family transcription factor PU.1 [45] that was previously mainly associated with B cell and macrophage activation. PU.1 presumably controls the histone modification at the *Il9* promoter and may be involved in the downregulation of Th2 connected cytokines by direct interaction with the Th2 transcription factor GATA3 [44].

Funtionally, IL-9 shows growth stimulating capacities in mast cells and T cells. These signals are probably transmitted through the common γ-chain that is shared with other growth stimulating factor receptors like IL-2, -4, -7, -15, and -21. As IL-9 may show direct effects on CD4+ T cells and other immunomodulatory cells [46–49], the role of IL-9 in various chronic inflammatory diseases has been studied in recent years. Here, we will review its role in chronic intestinal inflammation and IBD.

IL-9 in inflammatory bowel diseases

IL-9 producing T cells have been discovered in a variety of different chronic inflammatory and autoimmune diseases, especially atopic diseases, asthma and IBD. Although T cells were not the only source for IL-9 production in these disorders, they appear to be a major source of IL-9 production in inflamed tissues.

CD4+ and CD8+ T cells accumulate in the inflamed intestine of patients with IBD [50–52]. Upon antigen presentation in the regional lymph nodes, T cells enter the circulation and may re-enter the mucosa via specific adhesion molecules such as α4/β7 that interact with their ligands (MAdCAM1) on gut endothelial cells. Moreover, T cells expressing αE /β7 may bind to E-cadherin on intestinal epithelial cells ensuring their stable positioning in the gut. Due to high expression of αE/β7 on Th9 cells, specific blocking of the αE/β7 integrins with antibodies leads to reduced numbers of Th9 cells in inflamed colonic tissue.

T cells in the gut of patients with IBD produce large amounts of proinflammatory cytokines that drive the inflammatory process leading to tissue damage [51, 53, 54]. There is reasonable consensus that the mucosa of patients with established CD is dominated by CD4+ lymphocytes with a type 1 cytokine phenotype (Th1), characterized by the production of interferon-γ and interleukin-2. In contrast, the mucosa in patients with UC has been found to have CD4+ lymphocytes with an atypical type 2 cytokine production (Th2). Interestingly, lamina propria CD4+ T cells in UC produce mainly IL-5 and IL-13 but only low amounts of IL-4 indicating the presence of an atypical cytokine phenotype. In addition to the above cytokines, CD4+ T cells in CD and UC have been found to produce other proinflammatory cytokines, most notably IL-6 and TNF [55]. TNF is not only produced as a soluble factor but can also be found as membrane bound TNF on the surface of immune cells such as CD14+ macrophages and T cells. The latter form of TNF can induce a co-stimulatory signal via interaction with its ligand TNFR2 in the mucosa [56]. The potential relevance of proinflammatory cytokines has been studied in numerous preclinical models and the results of these studies have suggested that controlling their expression, production and functional activity is a very promising approach for clinical IBD therapy [53, 57, 58]. This concept is underlined by the fact that antibodies to TNF are used for clinical therapy of both CD and UC [59]. Moreover, antibodies against IL-12/IL-23 p40 have been recently approved for therapy of CD in the USA [60].

Several studies looked at cytokine gene regulation and expression of transcription factors in lamina propria T cells from patients with UC. These reports showed an induction of GATA-3 and IRF-4 expression in lamina propria CD4+ T cells in UC, consistent with the idea of an induction of Th2 and Th9 T cell responses, respectively [61, 62]. Additional studies in IBD patients revealed that IL-9 and *Spi1* and *IRF4* mRNA levels were raised in lamina propria T cells from patients with active UC [63, 64]. In contrast, a much weaker induction of IL-9 mRNA expression could be detected in CD patients as compared to control patients. Double staining analyses demonstrated that IL-9 was mainly produced by lamina propria CD4+ T cells and only to a lesser extent by other cells. These observations suggested that Th9 cells may play a role in the pathogenesis of UC rather than CD whereas low levels of Th9 cells could be found under homeostatic conditions. The presence of IL-9 producing T cells in UC was remarkable, since augmented levels of TGF-β but not IL-4 as potential inducers of IL9 production have been described in this disease [58]. However, further studies showed that IL-33 plays an important role in inducing IL-9 production in the absence of IL-4 [65, 66]. As IL-33 is highly produced by intestinal epithelial cells in UC rather than in CD [67, 68], these observations were consistent with a model in which TGF-β plus IL-33 drive mucosal IL-9 production by T cells in UC.

Another recent study addressed IL-9 serum levels in IBD. In this study, IL-9 was detected in serum from many IBD patients (41%). Moreover, IL-9 serum levels correlated to severe prognosis and IL-6 production in IBD [69].

In initial studies on the potential functional role of IL-9 in the inflamed intestine, potential target cells were screened for IL-9R expression. These studies revealed high expression of IL-9R on gut epithelial cells from UC patients with active disease [63, 64]. To determine the functional relevance of IL-9 for epithelial cells, these cells were culture in the presence or absence of IL-9. Administration of IL-9 resulted in an impaired growth and proliferation of intestinal epithelial cells, similarly to previous reports on the effects on the function of IL-13 [64, 70]. Moreover, IL-9 stimulation increased pSTAT5 levels in gut epithelial cells and impaired mucosal wound healing in a scratch assay system using Caco2 cells. In summary, the above findings in human cells indicated that epithelial cells are a key target of IL-9 in the mucosa and that IL-9 exposure blocks proliferation of these cells and suppresses mucosal wound healing.

To study the functional role of IL-9 in colitis, murine models of chronic intestinal inflammation were used. Several different animal models of IBD are currently used, such as the T cell transfer colitis, hapten-induced (TNBS, oxazolone) colitis, and the dextran sodium sulfate-induced inflammation model [71–74]. Although none of these models truly mimics IBD in human, all models induce acute and/or chronic inflammation of the colon. Two of the most widely used chemically induced models of intestinal inflammation are the TNBS-mediated and the oxazolone-mediated colitis models, which are useful to study T helper cell dependent mucosal immune responses. The TNBS-mediated colitis model resembles a CD-related Th1-associated gut inflammation and T cells have been shown to play a central role in this model. In contrast, the oxazolone-mediated model is associated with a Th2-type cytokine response with augmented IL-13 production [71, 75].

First experiments to characterize the NKT cell-derived IL-9 in murine colitis models were done in the dextran sodium sulfate model. Here, IL-9 producing invariant NKT cells were able to protect mice from inflammation in an IL-4-independent manner [76]. The functional role of IL-9 was not studied with neutralizing antibodies, however. A recent observation in the DSS model revealed the inflammatory character of IL-9 in vivo. This study used anti-IL-9 antibodies and noted suppression of mucosal inflammation upon therapy [77]. Another study looked at the role of IL-9 producing T cells by using the T cell transfer model. Using this transfer colitis model Dardalhon et al. showed that adoptive transfer of IL-9- and IL-10-producing T cells into RAG1 KO mice induced colitis and promoted tissue inflammation [37]. These findings were consistent with a proinflammatory role of IL-9 in experimental colitis.

Concerning the hapten-induced oxazolone colitis model the absence of IL-9 resulted in protection from inflammation. In *Il9* knockout mice, colitis activity was suppressed as compared to wild-type mice. Moreover, *Spi1Cd4*^{Cre} mice with conditional inactivation of PU.1 in T cells had less inflammatory symptoms in oxazolone-induced colitis as compared to wild-type mice. Similar results could be observed with IL-9 knockout mice in the TNBS-mediated colitis model and these mice had decreased numbers of PU.1+ T cells in colonic tissue [78].

The importance of Th9 cells was further analyzed by the use of IL-9 citrine reporter mice in the oxazolone-induced colitis model [63]. These studies showed augmented reporter gene activity in mucosal T cells strongly suggesting the presence of Th9 cells in oxazolone colitis in vivo. Additionally, blocking anti-IL9 antibodies were successfully used to treat inflammation in this model in a preventive fashion. Moreover, anti-IL-9 antibodies suppressed chronic oxazolone colitis.

To functionally investigate, the role of IL-9 in intestinal barrier function proliferation of epithelial cells and expression of tight junction molecules were analyzed in oxazolone colitis. IL-9 was found to control claudin and occludine expression in experimental colitis suggesting that this cytokine directly controls barrier function [78]. Additionally, IL-9 reduced proliferation and increased apoptosis of epithelial cells in organoid culture systems. These findings together with the reduced epithelial barrier function demonstrated that IL-9 has the potential to regulate the activation and function of intestinal epithelial cells during colitis. Impaired barrier function in colitis was driven by IL-9 and allowed translocation of commensal bacteria into the intestinal wall which can serve as pacemaker of the inflammatory process. Consistently, more translocating bacteria could be observed in IL-9-treated colonic mucosa as well as a delayed wound healing capacity of the mucosa.

Table 1 Evidence of Th9 cells in described IBD models

Model	Th9 evidence
DSS-induced colitis	IL-9 producing invariant NKT-cells protect mice from inflammation [76]
T cell transfer colitis	Transferred IL-9 producing T cells in RAG1 KO mice induce inflammation [37]
Oxazolone-induced colitis	IL-9 KO mice are protected from inflammation [63]
TNBS-induced colitis	IL-9 KO mice are protected from inflammation [78]

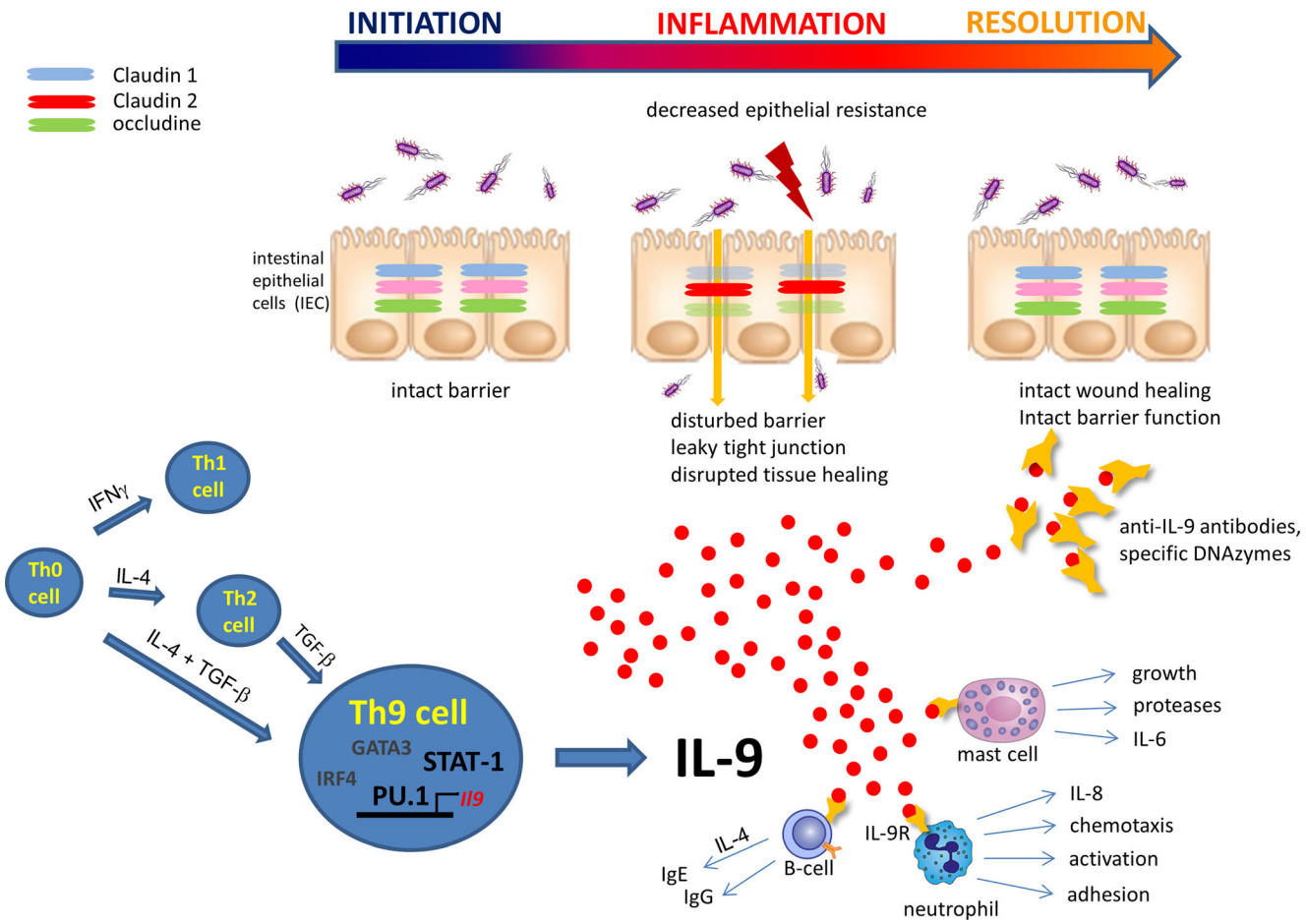

Fig. 1 Expression and function of IL-9 in the gastrointestinal tract in IBD patients. In patients with UC, IL-9 is mainly produced by mucosal CD3+ T cells that express the transcription factors IRF4 and GATA-3. IL-9 plays a major functional role on gut barrier function. It suppresses proliferation and regulates claudin expression in intestinal epithelial cells thereby impairing barrier function

In addition to suppression of IL-9 function via neutralizing antibodies, IL-9 expression might be targeted by inhibition of regulatory transcription factors controlling IL-9 gene expression. In this context, the production of IL-9 could be effectively suppressed by the use of GATA3 specific DNAzyme in oxazolone-mediated colitis. Such treatment led to reduced Th2 and Th9 cytokine production and DNAzyme-treated mice did not develop inflammation. Such DNAzymes are able to penetrate into cells and may allow to cleave a specific mRNA in order to suppress expression at the protein level. GATA3 DNAzyme may therefore block expression of various proinflammatory cytokines (including IL-9) simultaneously and thus emerges as potentially new approach for therapy of UC in humans [62].

In summary, these recent findings demonstrated a key regulatory role of IL-9 and Th9 cells in experimental colitis models (Table 1) and UC in humans. In the inflamed intestine, IL-9 is mainly produced by mucosal T cells and IL-9R expressing intestinal epithelial cells are major targets of IL-9 (Fig. 1). IL-9R signaling in gut epithelial cells regulates proliferation and claudin expression and thus has a major effect on intestinal barrier function. The above studies in experimental colitis models suggest that targeting of IL-9 is an interesting concept to suppress mucosal inflammation in patients with UC. Further prospective studies exploring this concept are highly warranted.

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