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Rogler, Gerhard

Abstract: Treatment of inflammatory bowel disease at present mainly targets mediators of inflammation to stop or suppress pro-inflammatory processes. Typical examples are steroids, suppression of T cells by thioguanine nucleotides, or antibodies against cytokines such as tumour necrosis factor, interleukin 12, or interleukin 23. In addition to suppression of inflammation, development of therapeutic strategies that support resolution of inflammation or that actively resolve inflammation might be desirable. Resolution of inflammation is now seen as an active process involving specific mediators (eg, lipid mediators or specific cytokines) that is mandatory to restore organ function and completely shut down inflammation. The molecular pathways involved in resolution of inflammation have been investigated in recent years and could be adopted in treatment strategies for inflammatory bowel disease. Among these approaches are anti-integrin strategies and means to produce or locally increase restitution or resolution factors, such as restoration of the activity of transforming growth factor- by anti-SMAD7 antisense oligonucleotides. The potential role of inflammation-resolving lipid mediators (eg, resolvins), however, still warrants further study and clinical development. This Review focuses on the specific role of active resolution of inflammation in inflammatory bowel disease pathophysiology. Potential therapeutic targets based on these pathways are also discussed.

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Resolution of inflammation in inflammatory bowel disease

Gerhard Rogler, MD PhD[1,2]

[1]Division of Gastroenterology and Hepatology, University Hospital, University of Zurich, Zurich, Switzerland

[2]Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

ADDRESS:

Gerhard Rogler, MD, PhD;

Division of Gastroenterology and Hepatology, University Hospital Zurich,

University of Zurich

Rämistrasse 100

8091 Zurich, Switzerland

Phone: +41-44-255-9519

Fax: +41-44-255-9497

E-mail: gerhard.rogler@usz.ch

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Search strategy and selection criteria: For this review a MEDLINE database search (https://www.ncbi.nlm.nih.gov/pubmed/) was performed from 1970 to August 2016 with the following search terms: Taking American and British spelling into consideration is was attempted to achieve a complete coverage of the topic: "inflammatory bowel disease" OR "Crohn's disease" OR "ulcerative colitis " AND "resolution of inflammation" OR "wound healing" OR "pro-resolving factors" OR "resolvins" OR "protectins". The reference list of the derived publications was checked for relevant reviews and other references of studies potentially eligible for inclusion in this review. Manuscripts to be included were selected by reading abstracts and results sections as well as conclusions. Furthermore, the National Institute of Health's study registry platform (*https://clinicaltrials.*gov*/)* was searched for clinical trials with pro-resolving factors (resolvins and protectins resp.) in August 2016.

Abstract:

Treatment of inflammatory bowel disease (IBD) at present mainly targets mediators of inflammation to stop or suppress pro-inflammatory processes. Typical examples are steroids, the suppression of T-cells by thioguanine-nucleotides or antibodies against cytokines such as anti-TNF- or anti IL-12/IL-23 antibodies. In addition to the suppression of inflammation it may be desirable to establish further therapeutic strategies that support the resolution of inflammation or actively resolve inflammation. In fact, resolution of inflammation is now seen as an active process involving specific mediators (such as lipid mediators or specific cytokines) that is mandatory to restore organ function and completely shut down inflammation. The molecular pathways that are involved in the resolution of inflammation are investigated in recent years and may be adopted in IBD treatment strategies. Among those approaches are anti-integrin strategies but also the local increase or production of "restitution or resolution factors" such as restoration of the activity of transforming growth factor beta (TGFβ) by anti-SMAD7 antisense oligonucleotides. The potential role of inflammation resolving lipid mediators (such as resolvins) on the other hand however still warrants further studies and clinical development. This review is focused on the specific role, the active resolution of inflammation plays in IBD pathophysiology. It will further discuss potential therapeutic targets for future IBD therapy based on these pathways.

Introduction

Therapeutic strategies for patients with inflammatory bowel disease (IBD) usually target to limit or suppress inflammation [1,2]. In the long run the therapeutic aim also is to restore mucosal functions (barrier function; innate immune functions; absorptive function; secretory function) after inflammation has resolved.

Interestingly in almost all clinical trials exploring a variety of different therapeutic approaches significant placebo success-rates have been observed [3]. Even when "mucosal healing" is used as a clinical endpoint in such trials there is a significant signal in the placebo group [4]. This indicates that there must be effective physiological mechanisms for the resolution of inflammation in place in the intestinal mucosa. These effective physiological mechanisms to resolve inflammation can explain placebo responses. They are – besides the potential disappearance of pro-inflammatory environmental factors - responsible for the "spontaneous" termination of IBD flares. The better understanding of those "mechanisms of resolution of inflammation" may not only help to increase (placebo-) responses upon established treatments but also reveal new treatment targets and strategies that might be more "physiological" than the ones we use currently.

There is good evidence that resolution of inflammation is a tightly coordinated and active process [5-9]. The concept of resolution of inflammation as an active process was brought forward by Serhan and colleagues [10-13]. Resolution of inflammation is not only initiated when either the initial triggers for inflammation (such as infective agents or danger signals) are no longer present or the problem that caused the inflammation (i.e. chemically induced damage to the mucosa) has been resolved [11-13]. Lipid mediators that are able to contribute to the resolution of inflammation are already produced and synthesized at onset of an inflammatory process [14]. This review focusses on key mechanisms relevant for the resolution of inflammation in the intestinal mucosa. Several strategies already well-established in IBD therapy use key pathways. Therapeutic trials in IBD patients in past years have told us which

mechanisms may be more and which less important.

The pathophysiology of intestinal inflammation in IBD - not only a defect in the resolution of acute intestinal inflammation

Inflammation is a prototypical reaction of the immune system in response to a variety of stimuli such as tissue or cell damage, microbial attack or invasion, radiation, hypoxia, pH changes and other threats to tissue homeostasis. The triggers that initiate inflammation in IBD patients are to some extend different from triggers in other diseases (Figure 1a). In IBD there is - according to widely accepted views - no clear microbial/infectious trigger of the inflammation (this is not the place to discuss the *Mycobacterium avium ssp. paratuberculosis* hypothesis) [15-17]. In contrast to most inflammatory reactions which are acute and self-limiting chronic inflammation develops in the intestinal mucosa of IBD patients.

In acute inflammatory responses a rapid recruitment of granulocytes (i.e., neutrophils, eosinophils, and basophils) to the inflammatory site occurs (Figure 1b). Granulocytes are required for the neutralization and removal of danger signals (DAMPs) and pathogen associated molecular patters (PAMPs) as well as for the defense against bacterial, fungal, and viral invasion. As acute inflammation depends largely on attraction of granulocytes, chemokine signaling involved in this recruitment. Neutrophils then secrete antibacterial proteins (e.g. of the S-100 family), myeloperoxidase (MPO), show an oxidative burst reaction with the secretion of reactive oxygen species to destroy microbes and secrete tissue degrading enzymes. Those processes, however, not just selectively destroy the pathogens, they also lead to local tissue damage and further inflammation characterized in the mucosa by hyperemia, edema and further epithelial damage (Figure 1b).

Therefore, granulocyte apoptosis after their activation plays an important role in the resolution of this type on inflammation. As mentioned above, the resolution of acute

inflammation, appears to be rather an active than a passive process (Figure 1c) [10-13]. It has been assumed that only when this active process of resolution of inflammation is ineffective and/or defective chronic inflammation may develop (Figure 1d). Some authors see the development of chronic inflammation mainly as a failure to resolve acute inflammation [18,19]. However, the induction of chronic inflammation in IBD is more complex. Important factors that induce chronification of inflammation in IBD have been identified making it unlikely that the pathophysiology of IBD reflects solely a failure to resolve inflammation.

In recent years more than 250 genetic susceptibility factors have been identified that increase the risk to suffer from IBD and contribute to the onset of chronic inflammation in 20–30% of IBD patients [20,21]. A large group of those genetic susceptibility factors plays an important role in the recognition of bacterial antigens (pathogen associated molecular patterns, PAMPs) via pattern recognitions receptors (PRRs). Many of those factors have a crucial role in the innate immune defense. Some of those genetic variants are shared between IBD and rheumatic diseases, such as PTPN22, IRF5, CCR6, or TNFRSF14 [20]. Other genetic risk factors for the occurrence of IBD have important roles in the adaptive immune system (such as interleukin (IL)-2, IL-23 or IL-10) [22-24]. Many of the identified genetic risk factors are not so much involved in resolution processes but reflect a genetically triggered impairment of intestinal barrier functions or an imbalanced immune reaction to commensal bacteria. The relevance of the mucosal barrier for the resolution of inflammation will be discussed below.

Besides genetic risk factors environmental factors are known to play an important role in the pathophysiology of IBD [20]. If only unresolved acute inflammation would cause IBD in a susceptible host it would be very hard to understand why a higher social and hygiene status (with most likely less acute intestinal infections and subsequent acute inflammatory responses) is associated with a higher risk to suffer from IBD. [25]Environmental factors such as titanium dioxide, aluminium or dietary

emulsifiers may directly or indirectly activate pro-inflammatory pathways (such as the inflammasome) and lead to an imbalance of pro-inflammatory and pro-resolving factors [26-28].

The mechanisms and pathways that support resolution of inflammation have been studied in detail in animal models and in different acute inflammatory diseases in patients. Cellular and molecular mechanisms have been identified that might become promising therapeutic targets for different inflammatory conditions. Despite the fact that IBD cannot simply and exclusively be seen as a defect in the active resolution-pathways of inflammation, it is of major importance to discuss these mechanisms in the context as they may provide future therapeutic strategies in IBD.

Mechanisms of resolution of acute inflammation – what can be learned for IBD?

As mentioned the resolution of inflammation involves several active processes rather than exclusively a stepwise disappearance of pro-inflammatory factors and mediators, dilution of chemokine gradients or subsequent reduction of leukocyte trafficking to the site of the inflammation [7-9,11-13,29]. Serhan and others have contributed to the important insight that the resolution of inflammation requires a production of "pro-resolving" factors and mediators which is tightly regulated and needs to be activated [7-9,11-13,29]. In recent years a multitude of actively formed factors and mediators have been identified that contribute to the active resolution of inflammation [11-13]. Among them are proteins and peptides (such as annexin A1, several anti-inflammatory cytokines or specific growth factors), small molecules (such as adenosine or carbon monoxide), lipid mediators (such as resolvins, protectins, maresins and lipoxins), as well as neurotransmitters (as a part of the neuroimmunological system). Those factors have been identified by both targeted and untargeted (unbiased) screening approaches [30].

In addition to the above mentioned lipid mediators, cytokines as well as growth factors contribute to the active resolution of inflammation either by direct action or via the induction of lipid mediators. Among the cytokines that limit inflammation are

IL-10 and IL-22. Genetic variants in the IL-10 receptor cause an early onset Crohn's disease (CD) with severe perianal involvement [24]. On the other hand, therapeutic administration of IL-10 was not successful in human disease and showed no benefit over placebo in active CD and as prophylaxis after surgery [31-33]. IL-22 is a more recently discovered member of the IL-10 family of cytokines that is mainly produced by both adaptive and innate immune cells [34]. It contributes to epithelial and subsequently barrier function restoration [34,35]. This is mediated by IL-22-induced proliferative and anti-apoptotic pathways [36]. The clinical utility of IL-22 is still under investigation.

Epithelial restoration also is induced by growth factors such as epidermal growth factor (EGF), GM-CSF and others. EGF not only stimulates proliferation and migration of epithelial cells, it also induced mucin production and mucosal ion transport associated with an improvement of barrier function [37-39]. EGF has been successfully tested for the treatment of ulcerative colitis [40,41], however, further clinical development has been hampered by the fear of overstimulation of epithelial proliferation and subsequent colorectal cancer development. Granulocyte macrophage colony-stimulating factor (GM-CSF) is a growth factor known to be essential for myeloid cell maturation, and dendritic cell differentiation [42]. GM-CSF also plays an important role in the pathogenesis of autoimmune diseases. Clinical trials indicated that administration of GM-CSF is followed by some clinical improvement in CD patients [43,44], however, the largest study was negative for the primary endpoint [43] leading to a suspension of further clinical development.

- **The role neutrophils in the resolution of inflammation in IBD**

Interestingly, neutrophils have been found to produce a number of these "resolution-factors", e.g. lipids mediators. The depletion of neutrophils in models of acute or chronic inflammation is followed by reduced secretion of these factors and by a delay or impairment in the resolution of inflammation [9,34,45].

Studies in patients with IBD have demonstrated altered neutrophil functions [46]. On one hand it has been described that neutrophils are – at least partially – responsible

for tissue damage by secreting nonspecific inflammatory mediators including reactive oxygen species ("oxidative burst"), pro-inflammatory lipid mediators, as well as proteases (such as cathepsins [47]). In IBD the tissue damage induced by neutrophils seems to be higher than "normal" due to an impaired apoptosis and subsequent phagocytosis of neutrophils and a subsequently extended lifespan [46] (Figure 2). This is in striking contrast to the finding that innate immune responses are impaired in IBD and especially in CD patients. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), an intracellular sensor protein detecting bacterial wall products such as muramyl dipeptide (MDP) was the first susceptibility gene described for CD and is still the risk factors with the clearest evidence [48]. Loss (reduction) of function variants are associated with the risk to develop chronic mucosal inflammation indicating that not an over-activation of the intestinal innate immune system triggers CD but a defect in appropriate responses.

In an interesting study this conclusion has been supported by clinical data: Marks and colleagues reported that patients with CD have reduced or impaired acute inflammatory responses in the gut mucosa as well as in the skin [49]. As compared to healthy subjects CD patients had a reduced neutrophil migration to sites of repeated colonic biopsies where obviously a defect of the intestinal barrier function is induced mechanically [49]. This was associated with lower levels of interleukin-8 (IL-8) secretion, a chemokine that mediates further neutrophil recruitment. Further, the investigators injected heat-killed *Escherichia coli* (E. coli) subcutaneously into the skin of CD patients and controls revealing again a reduced neutrophil migration and activation in patients [49].

A reduction of neutrophil recruitment and activation could not only be associated with impaired ability to clear invading bacteria but also with a reduced activation of subsequent inflammation-resolving processes leaving the field to other pro-inflammatory cell types that finally induce chronic inflammation. Indeed, bacterial wall LPS has been found in higher concentration in the portal blood and the intestinal mucosa of patients with CD [50]. However, several effects may contribute to this finding

– not only impaired neutrophil function but also an impaired mechanical barrier function caused by mucus depletion and epithelial cell dysfunction (see below).

An argument against the view of a severe defect of neutrophils in CD patients is the fact that in clinical patient care we use the neutrophil derived S-100 protein calprotectin as a surrogate marker for the degree/extent of mucosal inflammation. Calprotectin correlates very well with the endoscopic degree of inflammation and with tissue damage making a severe impairment of neutrophil recruitment in CD or UC very unlikely [51-53].

Could there be functional alterations of neutrophils in IBD [46]? During the course of acute mucosal inflammation there must be a switch from production of pro-inflammatory factors such as lipid mediators (e.g. pro-inflammatory eicosanoids, such as prostaglandin-E_2, thromboxane A_2 and leukotriene-B_4) to pro-resolving mediators (resolvins, protectins, anti-inflammatory lipoxins). There are not many studies in the field of IBD investigating this switch but Bento and coworkers demonstrated that a systemic treatment with resolvins in a nanogram range in mouse models of colitis improved clinical signs of disease as well as histology[54]. In addition, it reduced the expression of pro-inflammatory cytokines [54]. Further studies demonstrated that n-6 PUFA-derived lipoxins have anti-inflammatory effects in mouse models of colitis [55,56]. However, no clinical trials with these mediators have been initiated in IBD patients so far to translate these findings from bench to beside. Presently application of resolving is only studied in periodontal disease as well as in corneal transplant surgery.

Neutrophil apoptosis is central to the resolution of acute inflammation and limitation of inflammation. Recent data provide evidence that pro-resolving mediators, including lipid mediators [57] and proteins [58] promote neutrophil apoptosis as an active process. This process is likely to be impaired in IBD [46]. Impaired neutrophil apoptosis (partially associated with anti-apoptotic NF-κB activation) occurs in IBD patients.

Among the factors that delay apoptosis in the intestine are bacterial LPS, IL-1, IL-8, interferon-gamma (IFNγ) and granulocyte-macrophage colony stimulating factor (GM-CSF) [46]. What factors in contrast trigger neutrophil apoptosis? Reactive oxygen species (ROS) not only fight bacterial invasion they also induce apoptosis of neutrophils [57,59-61]. However, in IBD macrophages ROS production appears to be increased and not downregulated making it unlikely that this function significantly contributes to the impaired resolution of inflammation in IBD [62].

- **The role macrophages in the resolution of inflammation in IBD**

Monocytes/macrophages are an important component of the innate immune system and in the pathophysiology of IBD [63,64]. Tissue macrophages have been shown to play an important role for the resolution of inflammation [65,66]. One of their obvious functions is the phagocytosis of bacteria, bacterial wall components and apoptotic cells (such as neutrophils) [67,68]. In the intestinal mucosa their goal is to "clean the mucosa from debris" [67,68] (Figure 2).

In a recent review we highlighted the role of different macrophage populations in the intestinal mucosa [69]. Usually the differentiation of tissue macrophages in the mucosa is associated with a typical functional phenotype [63]: The majority of tissue macrophages found in IBD mucosa differs from the macrophages phenotype found in healthy mucosa [62,70-73]. In healthy mucosa more M2 type like macrophages are found which have tolerogenic and inflammations-resolving properties. In contrast, in IBD mucosa a M1 type with pro-inflammatory properties is predominant [74,75] (Figure 2). Normal intestinal macrophages isolated from healthy mucosa have low expression of co-stimulatory molecules such as CD80 or CD86 or PRRs such as TLR4 or TLR2 [72,76] making it unlikely that they normally induce T-cell responses and chronic inflammation. As the differentiation of mucosa-invading monocytes into typical intestinal macrophages is impaired by factors such as MCP-1 during IBD associated acute mucosal inflammation this may be a factor impairing the resolution of

inflammation [77] (Figure 2). Interestingly, resolvin and other mediators known to be involved in the resolution of inflammation can direct macrophage differentiation towards a M2 phenotype further supporting a potential therapeutic role also with respect to this mucosal innate immune cell population [78-80]. In addition, several cytokines are involved in the induction and differentiation of M2 macrophages [81]. Among them are TGFβ and IL-10. In addition, therapeutic anti-TNF antibodies contribute to the development of M2 macrophages thus actively triggering the resolution of inflammation.

- **The role of the adaptive immune system in the resolution of inflammation in IBD**

Besides neutrophils and macrophages components of the adaptive immune system have been shown to play a role during resolution of inflammation. This is best studied for regulatory T cells, but also effector T cells and B cells contribute to the resolution of inflammation. The administrations of regulatory T-cells to patients with refractory CD was found to be beneficial with a dose-dependent efficacy [82]. In addition, autologous stem cell transplantation has been discussed to mediate its effect mainly via changes in the adaptive immune system [83]. The mechanisms how these cell populations contribute to resolution of inflammation are complex and only partially understood.

Restoring barrier function to resolve inflammation?

A chronic "influx" of bacteria and bacterial antigens via a leaky or impaired intestinal barrier may contribute to the lack of resolution of inflammation and to chronification in IBD [74,84-86] (Figure 2). Indeed, there is experimental but also clinical evidence to support this concept. A number of clinical studies in recent years demonstrated that the restoration of the mucosa barrier associated with the so-called endoscopically visible "mucosa healing" predicts long-term remission and absence of acute or

chronic inflammation in IBD patients [2,87].

The intestinal epithelium is one of the most rapidly regenerating tissues in the human body. There are several molecular pathways that support the regeneration process such as the Notch and the Wnt pathways [88-90]. The not only regulate epithelial cell proliferation and differentiation but also secretion of defensins by Paneth cells in the small intestine [91]. Defensins are molecules that are anti-bacterial. They are an important component of the mucus layer on top of epithelial cells and prevent bacterial invasion thus contributing to the integrity of the intestinal barrier. Subsequently, intestinal barrier function not only refers to the physical barrier maintained by cell-to-cell junctions between epithelial cells but also to the antimicrobial proteins and factors secreted by those cells as well as components of the mucus layer such as mucins forming a barrier layer on top of the epithelial cells [92]. A depletion of mucus secreting goblet cells is a common histologic finding in patients with IBD. A reduction of mucin concentration in the gut may be associated with a reduced concentration of phosphatidylcholine incorporated into the mucus as demonstrated by Stremmel and coworkers [93]. Recent data indicate that a therapeutic administration of phosphatidylcholine increases the thickness of the mucus layer [94,95].

A rapid epithelial restoration also contributes to the resolution of inflammations by closing surface leaks that allow bacterial entry. Intestinal epithelial cell proliferation is induced by a number of growth factors such as TGFβ, EGF, FGF, KGF, or HGF that are either locally produced or secreted by surrounding tissues [39]. In the case of EGF clinical evidence has been obtained that there is therapeutic efficacy in mild-to-moderate ulcerative colitis (UC), however, further clinical development has been hampered by the fear that it might promote colitis associated colorectal cancer (CRC) [40,41]. Recombinant HGF was shown to improve mucosal repair and ameliorate (resolve??) inflammation in a rat colitis model.

These data indicate that improvement of the mucosal barrier function at different levels (e.g. cell-cell contact, epithelial repair, mucus, defensins, phosphatidylcholine) contributes to the resolution of inflammation in inflammatory bowel disease (Figure 2).

Epigenetic regulation of the resolution of inflammation – insights from IBD

As mentioned above a "shift" needs to take place to change from a pro-inflammatory state to a "resolution of inflammation "-state of involved cells. This may be associated with a "re-programming" of gene expression [96]. This reprogramming is frequently modified on an epigenetic level such as for the Toll-like receptor-NF-κB pathway in macrophages and neutrophils [97].

As another example, the re-differentiation of M1 macrophages into the M2 phenotype is associated with epigenetic modifications/alterations and chromatin remodeling [98,99]. However, the detailed mechanisms that facilitate this and subsequently potential ways to influence this are unknown making a therapeutic approach at this point currently unavailable.

Candidate targets such as sirtuins (a family of NAD+-sensing deacetylases that target histone and non-histone proteins and are able to generate heterochromatin) have been discussed and some experimental evidence suggest a role of sirtuins in the epigenetic regulation of resolution of inflammation. In addition, there are some data reported on the role of sirtuins in animal models of IBD [100-103]. It has been demonstrated that SIRT variants occur in patients with a rare form of diabetes that at the same time may be at risk for UC [104].

Further important epigenetic regulators are microRNAs. Several differences in microRNA expression have been described between IBD patients and healthy controls [105]. Some of them may be – besides other functions – involved in the

resolution of inflammation [106-108].

Environmental factors

It is very clear that environmental factors contribute to the onset and perpetuation of IBD. However, a causal relationship between environmental factors and the degree of mucosal inflammation in most cases is hard to prove. For some environmental factors however, potential pathophysiological mechanisms have been identified. An environmental risk factor clearly associated with more flares in both CD and UC are low vitamin D levels [109-111]. Vitamin D has been shown to act on a specific receptor and to be involved in the resolution of inflammation e.g. by regulating macrophage functions. Substitution therapy with vitamin D appears to have some effect in preventing flares of CD [112].

Cigarette smoking also is a well-studied environmental factors contributing to modulation of inflammation in IBD – increasing the risk of flares in CD and ameliorating inflammation in the case of UC [113-115]. Interestingly, smoking cessation changes the composition of the intestinal microbiota [116,117]. This points to the important role the intestinal microbiota may play in the resolution of inflammation [118-121].

Bacterial wall products and whole bacteria may well aggravate inflammation in a subject with a leaky barrier when they enter the mucosal wall. On the other hand, clinical trials provided evidence for successful therapy with probiotics such as *Escherichia coli* Nissle at least in UC indicating that probiotic bacteria may contribute to the resolution of inflammation [122].

In addition, there is evidence that bacteria may either produce or induce the production of pro-resolving lipid mediators [123]. Resolvins on the other hand have been demonstrated to shape the bacterial composition for example of the oral cavity

[124]. The bacterial composition in the GI tract is changed by inflammation (which is also associated with changes of the local pH that influences growth condition for bacteria). Therefore, it is not completely clear whether lipid mediators and resolvins have a direct effect on bacterial growth and subsequent composition of the microbiome or whether the composition is changed by the resolution of inflammation (and e.g. a subsequent change of the pH in the local microenvironment).

The bacterial composition in the gut that may contribute to the resolution of inflammation can also be changed by genetic variants associated with the risk to develop IBD. This further contributes to the complexity of the field making simples answers impossible.

A recent example has been described for caspase recruitment domain family member 9 (CARD9), a pattern recognition receptor (PRR) and an IBD risk gene. As demonstrated by Lamas and coworkers recently, CARD9 actively induced resolution of colitis by induction of IL-22 *in vivo* [125]. In Card9 deficient mice (which are more susceptible to colitis) the microbiota composition was found to be altered [125]. Interestingly the microbiota from these animals could not metabolize tryptophan into aryl hydrocarbon receptor (AhR) ligands (see below).

Diet derived compounds and mediators may either promote or prevent resolution of inflammation. Recently we demonstrated that food derived titanium dioxide nanoparticles can induce inflammasome activation in the mucosa wall and contribute to intestinal inflammation [26]. The nano-particles can penetrate cell membranes without needing a receptor and activate pro-inflammatory pathways – at least in individuals with a reduced mucus barrier – such as IBD patients [26]. In a similar way, other diet components or additives may activate the mucosal innate immune system such as aluminum [27] or emulgators [28].

The impact of various diets and dietary products on the course of IBD is still a matter of discussion. No benefit of a specific diet has been demonstrated so far. Certain

dietary factors have been studied in more detail: Omega-3 polyunsaturated fatty acids can attenuate intestinal inflammation in animal models [126]. They also represent a substrate for the production of resolvins and protectins. However, with respect to clinical trials a recent review concluded that available data "do not allow to support the use of omega-3 PUFA supplementation for the treatment of both active and inactive IBD" [127].

Dietary compounds and other environmental factors may mediate their effects via the aryl hydrocarbon receptor (AhR) (see above). AhR is a transcription factor that can be activated by a large number of environmental compounds and dietary factors [128,129]. It is regarded to be an important link between environmental factors and the adaptive as well as innate immune system [129] and their pathophysiology in IBD [128]. The activation of AhR by the mentioned factors is followed by expansion of adaptive and innate immune cells in the mucosa [129]. Among them are IL-22-producing innate lymphoid cells [130]. The potential role of IL-22 in the active resolution of inflammation has been discussed above.

However, it must be acknowledged that evidence from *in vitro* studies and animal models cannot be easily transferred into treatment options with respect to those dietary factors.

Resolution of inflammation as a therapeutic target in IBD?

There is already evidence that the therapeutic target of resolution of inflammation can be successful clinically applied in patients with IBD. Among those strategies is the active improvement of barrier function by phosphatidylcholine, the therapy with probiotics and clinical studies with growth factors (see above). Furthermore, epidemiological studies have demonstrated the long term benefit of mucosal healing (despite the fact that it is unclear how to best achieve mucosal healing). In addition, another recently adopted successful therapeutic strategy indicates that targeting resolution of inflammation may become more important in the future.

The successful clinical testing of anti-integrin antibodies is a proof of concept of supporting the resolution of inflammation. Natalizumab, Vedolizumab and other compounds soon to come (such as Etrolizumab) target to stop inflammatory cell recruitment into the inflamed mucosa [4,131-133]. Thereby they support the resolution of inflammation by shifting the balance or pro-inflammatory and inflammation-resolving cells (Figure 2). Mongersen, the anti-SMAD7 oligonucleotide may become another example for a treatment mechanism that supports resolution of inflammation rather than being directly anti-inflammatory [134] (Figure 3).

On the other hand, most of the clinical trials at present still are performed with substances that are directly anti-inflammatory (Figure 3). They target typical pro-inflammatory cytokines such as TNF, IL12/IL-23 or IL-6 (Figure 3). They target the suppression of T-cells such as thioguanines or steroids. Or they interfere with signaling downstream of cytokines receptors such as JAK-inhibitors. All those strategies are successful but only to a limited extend. The high response rates that have been reported for Mongersen (if confirmed in phase III studies) may be an indicator that the activation of resolution of inflammation can be more effective than interference with pro-inflammatory factors (Figure 3).

What therapeutic targets would be options to improve the resolution of inflammation? There are several mechanisms that need to be further investigated:

- induction of neutrophil apoptosis and clearance
- re-differentiation of intestinal macrophages (M1 to M2 shift)
- induction of regulatory cells
- induction of tissue repair/restoration of barrier function.

Further drugs or interventions that target at the resolution of inflammation need to be clinically tested in IBD. The view that resolution of inflammation will spontaneously occur when pro-inflammatory factors are neutralized or eliminated needs to be corrected. First examples (see above) indicate that the “resolution-of inflammation-strategy” is promising. The future of IBD therapy may be located in

these pathways.

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