EDITORIAL

Novel therapeutic concepts for inflammatory bowel disease—from bench to bedside

Viola Neudecker[1] · Sean P. Colgan[2] · Holger K. Eltzschig[3]

In this special issue of the *Journal of Molecular Medicine*, we are privileged to publish review articles on the latest advancements in mucosal inflammatory disease research. Four laboratories focused on translational research in the field of mucosal inflammation, in particular inflammatory bowel disease (IBD), outline the most recent achievements in the progress of translating research findings into therapeutic innovation (Fig. 1).

Epithelial cells lining tissue surfaces represent multifunctional barriers separating the body from the outside world. As part of innate immunity, epithelial cells function as a physical barrier, provide a portal for communication with the microbiome, and serve as a conduit for initiation of other aspects of innate and adaptive immune system. The mucosal integrity and functionality play a critical role in determining the organism's ability to absorb essential nutrients, eliminating metabolic waste products and to defending against invading pathogens. The perpetuation of the mucosal homeostasis requires tight regulatory networks in the mucosal layer, guaranteeing a functional barrier and adequate mucosal immunity. Disruption of this mucosal integrity and defective

immune responses can result in loss of barrier function and invasion or translocation of luminal microorganisms. In turn, this constellation can trigger systemic inflammation and sepsis, and might even result in multiple organ dysfunctions. Loss of barrier function also represents the grounds on which mucosal inflammatory disease might arise. In the two major mucosal organs, the intestinal and pulmonary mucosa, inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC) as well as acute lung injury, constitute inflammatory diseases in which defective mucosal barrier function might significantly determine disease endpoints. Novel concepts to rehabilitate the mucosal balance between the necessary permeability and a proper defense are encouraging and give reason for a justified optimism for translation from bench to the bedside in the near future.

Lanis et al. summarize our current understanding of tissue metabolism and mucosal inflammation [1]. A recently appreciated finding in mucosal inflammatory research is the relationship between active mucosal inflammation and tissue hypoxia. Studies on hypoxia-inducible factor (HIF), the major transcription factor orchestrating shifts in tissue metabolism, have revealed that HIF serves a protective role in maintaining mucosal homeostasis during changes in oxygen supply. HIF represents an essential oxygen sensing transcription factor, initially identified by the laboratory of Gregg Semenza in the early nineties [2], and its critical discovery was recently recognized with one of the most respected prizes in the field of biomedical research, the 2016 *Lasker Award*. In the mucosa, it is now appreciated that HIF stabilization functions to significantly support mucosal integrity and barrier protection, thereby preventing the onset of mucosal inflammation and disease such as IBD [1]. Latest research has focused on the microenvironmental metabolic conditions induced by mucosal inflammation and has demonstrated a fundamental connection

✉ Viola Neudecker
viola.neudecker@med.uni-muenchen.de

1 Department of Anesthesiology, University Hospital, LMU Munich, Munich, Germany

2 Department of Medicine and the Mucosal Inflammation Program, University of Colorado Denver, Aurora, CO, USA

3 Department of Anesthesiology, The University of Texas Health Science Center at Houston, McGovern Medical School, Houston, TX, USA

Fig. 1 Critical factors in Mucosal inflammation
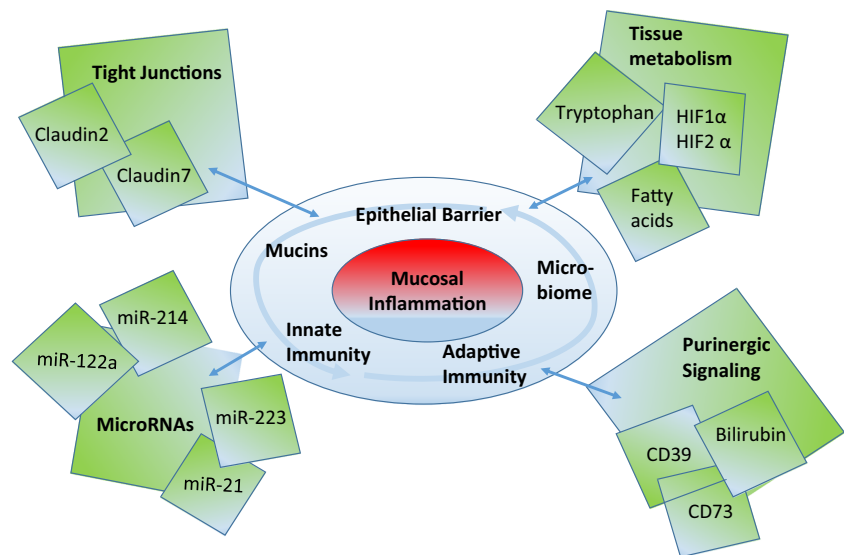
between infiltrating myeloid cells, in particular polymorphonuclear cells (PMNs), and the induction and stabilization of protective HIF pathways. When activated during acute inflammation, the oxygen consumption of the PMNs excessively increases to undergo respiratory burst, required for pathogen fighting. Strikingly, studies revealed that this increased oxygen consumption of PMNs imprints the surrounding inflammatory mucosal surfaces, leading to HIF stabilization and anti-inflammatory protection of the mucosal surface [3]. Moreover, the induction of HIF pathways during mucosal inflammation was also proven true in humans as HIF molecules and HIF indicative target genes could be detected in patient biopsies of inflamed mucosal intestine. Among the various pathways, HIF mediates metabolic actions on extracellular nucleotide signaling. Here, the production of adenosine is considered to provide a potent anti-inflammatory and tissue-protective function during mucosal inflammation [4]. Adenosine synthesis involved molecules such as the ectonuceotidases CD39 and CD73, as well as adenosine receptor molecules are critical for the inflammation-dampening effects of adenosine [5, 6]. In the case of adenosine, its simultaneous action in immune response suppression as a "metabokine," re-directing the adaptive T cell-mediated immune response, indicates the strong interrelation between inflammation and tissue metabolism. A third metabolic pathway with relevant contribution to preserve intestinal homeostasis and prevent unchecked inflammation is the metabolism of tryptophan. Indoleamine 2,3-dioxygenase (IDO1), the enzyme that catalyzes tryptophan to kynurenine, generally functions to promote immune suppression. Kynurenine functions as an endogenous ligand for aryl hydrocarbon receptor (AHR) that regulates the adaptive immune response contributing to the resolution of inflammation via stimulation of bacterial clearing through Th17 cell development. In addition, the induction of mucosal IL10-receptor expression fosters an IL-10-mediated immune suppression. Metabolic shifts resulting in oxygen consumption and HIF stabilization can also be induced by changes in microbial metabolism, emphasizing the relevance of the host microbiome in the pathogenesis of mucosal inflammation. The production of short fatty acids, produced during bacterial fermentation, can impact oxygen availability. Thus, reduced oxygen levels lead to increased HIF stabilization and maintenance of mucosal homeostasis. In contrast, anti-microbial therapies can promote increased detectable oxygen levels consequently preventing HIF stabilization potentially resulting in mucosal barrier disturbance and consecutive inflammation [7].

Longhi et al. focus on the potential role of IBD therapies based on influencing the purinergic signaling pathways [8]. Extracellular signaling mediated by purine nucleotides and nucleosides such as adenosine and adenosine triphosphate (ATP) predominantly associated with signaling in the circulatory system significantly gained attention in its immune response modulatory function. The review discusses the critical role of ectonucleotidase, ectonucleoside triphosphate diphosphohydrolase-1 (NTPDase1), CD39, in the immunomodulatory effect of adenosine-mediated signaling. In the signaling cascade, CD39 catalyzes the generation of adenosine monophosphate (AMP) that can be processed into adenosine by another ectonucleotidase, CD73, allowing the activation of specific adenosine receptors (A2AR) by adenosine binding. Therefore, CD39 represents the critical initial enzyme for the "CD39-CD73-adenosine-A2A receptor pathway," which mediates immune suppressive effects via adenosine on the innate, but also the adaptive immune system. Interestingly, CD39 expression is involved in the transition of Th17 cells into so-called suppressor-like Th17 cells. These Th17 cells appear dualistic with functional features of Tregs suppressing pro-inflammatory cell responses. At the same time, they also show effector Th17 features such as IL-17 production and decreased

levels of adenosine receptor A2A with corresponding reduction in adenosine protective effects mediated by this receptor. The latest findings indicate that microenvironmental conditions might affect the dual function of this particular lymphocyte subset. The upregulation of CD39 on immune cells can be induced by ligation of the AHR, which in turn is regulated by HIF. The strong impact on immune function and interrelation between purinergic signaling, hypoxia, and HIF stabilization is further reflected in its common involvement in circadian rhythm. Adenosinergic pathways involving HIF activity have been demonstrated in regulating immune cells intrinsic "clock." Interestingly, studies on the molecular basis of the known immunomodulating role of bilirubin demonstrated that its suppressive effects could also be traced back to interactions with AHR with consecutive induction of immune suppressive CD39. Recent in vivo studies in that direction further promote the idea of bilirubin inducing the immunosuppressive functions of Th17 cells in IBD by activating CD39. Consequently, the authors emphasize the worthiness of targeting this pathway for therapeutic treatment. Adenosine-dependent treatment, such as using agonists of A2A adenosine receptors, has the potential to ameliorate experimental colitis, while alterations in adenosine signaling—e.g., experimentally by genetic deletion of CD39 or reduced levels of CD39 due to single nucleotide polymorphisms in IBD patients—increase severity, respectively susceptibility of disease [9]. CD39 also gained additional attention as studies revealed that circulating microparticles (MP) associate with CD39. MPs can be considered as communication tools between host cells or, in the case of intestinal MPs, also mediate communication between the mucosa and microbiome. In the case of MP-associated CD39, it was suggested to modulate the intercellular crosstalk between leucocytes and vascular cells [10]. Besides host cells, the commensal bacteria are another source of extracellular ATP, again, presenting the primary components for purinergic responses. Therefore, the microbiome and its changed composition in disease or due to antibiotic treatment also affect the protective elements in purinergic signaling. Supporting this hypothesis, a recent study demonstrated that transfer of healthy-individual-derived intestinal microbiota to IBD patients revealed a shift to a donor-like microbiome composition and higher levels of suppressive immune cells [11]. CD39 is predominantly expressed in immune and endothelial cells. However, recent evidence calls for a similar important role for NTPDase-2 and NTPDase-3 in modulating purinergic signaling in muscle layers and the nervous system. Genetic deletion of either of these NTPDases results in more severe murine experimental colitis. A third candidate, NTPDase7, recently gained attention as it was found to be expressed on intestinal epithelial cells and its deletion to result in the increased presence of pro-inflammatory Th17 cells [12]. According to the authors, the finding of the dual expression localization of these NTPDases in the intestine and also in neuronal structures further implicates to also pursue novel innovative concepts of neuro-immune interactions within the scope of identifying new therapeutic targets in mucosal inflammation, in particular IBD.

Capaldo et al. highlight the current state of knowledge around intestinal barrier function in IBD [13]. Barrier disruption following aberrant immune responses and inflammation initiates and, due to increased uncontrolled leakage and microbial transition, further sustains and supports the progress of disease. Consequently, therapeutic approaches should provide an escape strategy from this vicious circle. The intact mucosal barrier function contains separating and communication-facilitating components. The mucins that form a three-layer mucus prevent pathogen invasion into the epithelium on the one hand, and remove them by being continuously produced and brushed out during intestinal peristaltic on the other hand, represent a critical component of an intact barrier function. Deletion of specific gene coding for mucins revealed consequences such as spontaneous colitis or increased susceptibility in the case of Muc2 deficiency in murine experimental colitis [14]. Studies in IBD patients revealed that not only the level of mucin production matters, but also secondary modifications are elementary. Another essential component of a proper barrier is comprised by the so-called tight junctions (TJ). They serve as paracellular seals blocking translocation and fencing the defensing components on the apical mucosa. TJ consist of claudins, proteins that form pores that are essential for intestinal ionic and water homeostasis, and whose compositions determine the permeability of the mucosal layer. It is not surprising that IBD has therefore been associated with TJ consisting of higher levels of pore-forming claudins. Importantly, it has been demonstrated that pathogens are capable of destructing and breaking down the paracellular junctions by different mechanisms and thereby pass through. Genetic presdisposition with impaired intestinal barrier function as well as environmental factors account for the onset of IBD. Experimental deletion of transcription factor hepatocyte nuclear factor alpha (HNF4α) that regulates the expression of intestinal expressed claudin 7 mimics the human IBD phenotype indicating a critical role of TJ functionality in the pathogenesis of IBD. Mucus and paracellular TJ seal comprise a dynamic yet separating barrier. Based on these findings, the identification of successful therapeutic approaches in IBD requires an integrated model of mucosal barrier function to identify the alterations in the structure and dynamics of the barrier elements that underlie the pathogenesis of IBD.

The last review of this special review series focuses on the role of microRNAs (miRNAs) in mucosal inflammation [15]. MiRNAs are small non-coding single-stranded RNAs discovered almost 25 years ago that are gaining rising interest as regulators of inflammatory processes. MiRNAs are considered to be mediators of inflammation by driving pro- or anti-inflammatory pathways through repression of their target

genes. Depending on the regulated target gene, miRNAs thereby modulate disease courses and illness progression. The mucosal surface can be seen as "under constant fire" being confronted with nutrients, antigens, and pathogens that might need to traverse or should be prevented from entering the body. In the essential interplay of mucosal barrier and immune functions, miRNAs are involved in the modulation of all of these components. The present review gives an overview on how miRNAs are produced and mechanisms of miRNA-dependent repression of gene expression. Subsequently, the authors outline some examples on how specific miRNAs can affect the functions of the pulmonary or intestinal mucosa.

As a very first example, a recent publication on the role of miR-223 in pulmonary mucosal inflammation is discussed [16]. The findings emphasize the importance of mucosal-leucocyte crosstalk in mucosal homeostasis. The data provide an essential role for miR-223 in the intercellular communication of infiltrating polymorphonuclear neutrophils (PMNs) with the resident pulmonary mucosa. During mucosal inflammation, PMNs come into very close spatial contact with the resident alveolar epithelium. Here, miR-223 was found to be secreted by PMNs and enveloped by alveolar epithelial cells. Once taken up by inflamed alveolar epithelial cells, miR-223 represses the pro-inflammatory target gene PARP-1, thereby dampening alveolar epithelial inflammation. In a murine model of acute lung injury, overexpression of miR-223 revealed to be protective. Interestingly, myeloid miR-223 was also recently demonstrated to play a crucial role in preventing excessive inflammation in the intestine. Here, miR-223 was identified to dampen IBD via the repression of the pro-inflammatory NLRP3 inflammasome [17]. Overexpression of miR-223 attenuated NLRP3 expression and resulted in ameliorated experimental colitis. Therefore, miR-223 may hold promise as a future therapeutic for attenuating mucosal inflammation in the lungs or the intestine. Other studies revealed correlations between altered miRNA expression patterns and various pathologic states and disease characteristic for the inflamed mucosa. Similarly, during intestinal mucosal inflammation, miRNA expression patterns differ from those of healthy individuals. One of those miRNAs differentially expressed during intestinal mucosal inflammation and of seminal importance in IBD appears to be miR-214. It was found to be involved in an inflammation driving feedback loop [18]. Epithelial miR-214 is induced by the pro-inflammatory cytokine IL-6 and further boosts the intestinal mucosal inflammation by promoting NF-κB activation. MiR-214 inhibition blocked this feedback loop resulting in ameliorated experimental colitis and was beneficial when treating colonic biopsies ex vivo. This is only a small excerpt of the miRNAs discussed in the review highlighting the strong involvement and relevance of miRNAs in mucosal inflammation. Not least because of their potential usefulness as biomarkers, but also because of recent discovery of extracellular miRNAs, they likely represent highly seminal

targets for future therapies of mucosal inflammation. Indeed, clinical approaches targeting miRNAs are currently being tested in clinical trials.

Taken together, the four reviews compiled in this special review series reflect the high potential and remarkable amount of translational ideas to approach new therapeutic strategies in mucosal inflammation. Adenosine signaling, hypoxia-induced pathways, HIF activation, and miRNA-mediated regulation all represent impactful levers to be tackled to improve control of mucosal inflammation. The challenge here is to bring these approaches into clinics. Because even though proof of principle exists and there are a few clinical trials, overall clinical studies for these novel concepts are still in their beginnings. Therefore, we hope that while additional mechanistic studies will continue to shed new light at the controls governing mucosal inflammation, some of the findings discussed in this series will be implemented in routine clinical care.

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