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TOPIC HIGHLIGHT

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Mucosal biomarkers in inflammatory bowel disease: Key pathogenic players or disease predictors?

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Abstract

Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders of the bowel, including ulcerative colitis and Crohn's disease. A single etiology has not been identified, but rather the pathogenesis of IBD is very complex and involves several major and minor contributors, employing different inflammatory pathways which have different roles in different patients. Although new and powerful medical treatments are available, many are biological drugs or immunosuppressants, which are associated with significant side effects and elevated costs. As a result, the need for predicting disease course and response to therapy is essential. Major attempts have been made at identifying clinical characteristics, concurrent medical therapy, and serological and genetic markers as predictors of response to biological agents. Only few reports exist on how mucosal/tissue markers are able to predict clinical behavior of the disease or its response to therapy. The aim of this paper therefore is to review the little information available regarding tissue markers as predictors of response to therapy, and reevaluate the role of tissue factors associated with disease severity, which can eventually be ranked as "tissue factor predictors". Five main categories are assessed, including mucosal cytokines and chemokines, adhesion molecules and markers of activation, immune and non-immune cells, and other mucosal components. Improvement in the design and specificity of clinical studies are mandatory to be able to classify tissue markers as predictors of disease course and response to specific therapy, obtain the goal of achieving "personalized pathogenesis-oriented therapy" in IBD.

INTRODUCTION

During the past decade, major insights into the pathogenesis of inflammatory bowel disease (IBD) have been achieved, and investigators generally agree that factors including the external environment, genetic makeup, intestinal microbial flora, and immune system are all involved and functionally integrated in the generation of

the chronic intestinal inflammatory reaction that characterizes IBD[1].

Among all factors, those considered to contribute mainly to IBD pathogenesis are genetics, along with mucosal immunity. New insights in mucosal immunity have allowed for the introduction of biological agents, which have led to great changes in clinical management of IBD, as well as the course of the disease.

Biological drugs block key molecules that are involved in the induction and maintenance of intestinal chronic inflammation through several pathways, differently represented among patients. However, although considered pathogenesis-oriented drugs, a lot still needs to be done in order to achieve "personalized pathogenesis-oriented therapy" in IBD. The complex pathogenesis that involves several major and minor contributors, involves different inflammatory pathways that can have diverse roles in different patients.

These components should be taken into consideration, when choosing from the variety of emerging therapies available for IBD patients, especially biological agents and immunosuppressants. These potent treatments may be associated with significant side effects, in particular serious infections and increased risk of malignancy. In addition, they might not be needed for the subgroup of patients destined to have a more benign course. Their high cost also requires optimal treatment-to-patient adjustment.

As a consequence, there is a pressing need to differentiate between patients who may have more aggressive phenotypes from those with a potentially more benign course[2], with the use of markers that are capable of predicting therapeutic responses.

Major attempts regarding the prediction of therapeutic efficacy have been made on clinical characteristics (e.g. smoking is associated with lower response rates[3]), concurrent medical therapy, serological markers (anti-Saccharomyces cerevisiae antibody and anti-neutrophil cytoplasmic antibody)[4-6], outer membrane porin C, CBir1-flagellin, antibodies against I2 protein and the anti-glycan antibodies) and genetic markers[2]. All the above markers have been assessed for their response to anti-tumor necrosis factor (TNF)-α therapy. Emerging evidence, mostly related to the capacity of biological agents to induce mucosal healing, has suggested that mechanisms related to mucosal disease/healing are helpful in completing the scenario of predictors of specific therapy in IBD.

However, how mucosal and tissue inflammatory pathways can influence or predict the disease remains largely unknown, therefore making both prediction of disease course and clinical management of IBD a challenging task.

In fact, only few reports exist on how mucosal/tissue markers can predict clinical response, mostly related to infliximab treatment (summarized in Table 1 and briefly reported below).

Arijs *et al*[7] have shown that mucosal extracts that overexpress five genes, which encode osteoprotegerin (TNFRSF11B), stanniocalcin-1, prostaglandin-endoper-oxide synthase 2, interleukin (IL)-13 receptor α 2 and IL-11, predict response to infliximab therapy with 89% accuracy in patients with refractory ulcerative colitis (UC). Notably, those genes encode for proteins involved in signaling in the adaptive immune response, inflammation and TNF-α pathways.

Another study from Van den Brande *et al*[8] has suggested that higher levels of apoptosis in the intestinal mucosa, as shown by 99mTc-annexin V uptake SPECT-CT images comparing before and after infliximab infusion, correlated with clinical response to infliximab in active Crohn's disease (CD) patients.

Schmidt *et al*[9] have demonstrated that, in 19 steroid-refractory CD patients treated with infliximab or cyclophosphamide, lower mucosal TNF-α concentration predicted long-term remission with a sensitivity of 100% and a specificity of 87.5%.

Similar to the study mentioned above, Olsen *et al*[10] have reported that an inverse association between pre-treatment TNF-α expression levels and clinical/endoscopic remission following infliximab treatment exists in UC patients with moderate to severe disease activity. In the same study, neither age, sex, steroid therapy, immunosuppression, pancolitis, endoscopic sub-score, disease duration, C-reactive protein, IL-4, IL-10 nor interferon (IFN) predicted mucosal or clinical remission.

Arsenescu *et al*[11] have revealed that, in a cohort of 69 CD patients, those with low levels of RelA subunit of nuclear factor (NF)-κB, A20 (a negative regulator of NF-κB), polymeric immunoglobulin receptor (pIgR), TNF-α and IL-8, had moderate to severe disease and poor responses to immunosuppressive and anti-TNF-α therapy, while patients characterized by low expression of RelA, A20, and pIgR, normal TNF-α and elevated IL-8, had acute inflammation that responded well to therapy.

Few reports are available on tissue factors as predictors of response to corticosteroids. Ishiguro[12] have found a higher protein level of IL-6 and TNF-α in biopsies from patients with intractable disease who were receiving corticosteroids compared to those with non-intractable disease receiving corticosteroids; these findings have not been confirmed in other studies[13].

Alternately, Raddatz *et al*[14] have found that, in patients with IBD, glucocorticosteroid receptor (GR) expression was not different from controls, although it was decreased in biopsies from UC patients who were non-responsive to glucocorticosteroids. In those patients, the inhibitory subtype GRb was expressed 100-1000 times less than GRa. On the contrary intercellular adhesion molecule-1 (ICAM-1) mRNA expression, although elevated in biopsies from UC patients with active disease, was no different in responders or patients with impaired response[14].

Another study has shown by univariate analysis that, in a total population of 561 UC patients evaluated with a median follow-up of 21.4 years since disease onset, mean histological inflammation and steroid use were predictors of colectomy[15].

Moving to tissue markers as predictors of disease

relapse, Yamamoto *et al*[16] have shown by multivariate analysis that higher rectal mucosal IL-8 level, younger age, and greater number of prior relapses were independent significant risk factors for future relapse in patients with quiescent UC. In the same study, IL-1, IL-6 and TNF-α levels in the rectal mucosa were not associated with relapse.

In light of these experiments, the aim of this paper is to review the role of tissue factors associated with disease severity, which may eventually be ranked as predictors of disease course and response to specific therapy. A schematic representation of the five proposed main categories of mucosal biomarkers, including mucosal cytokines and chemokines, adhesion molecules and markers of activation, immune and non-immune cells, and other mucosal components, is shown in Figure 1.

MUCOSAL CYTOKINES AND CHEMOKINES

Alterations in the production of many cytokines have been described in patients with active IBD[17]. Although the significance of these findings in the pathogenesis of IBD remains a controversial issue, as it is unclear whether they are primary or secondary defects in the regulation of the intestinal immune system, they can be considered as markers in the differentiation of groups of patients.

In active IBD, a disturbed balance between regulatory and effector cells has been described, which mainly involves effector T cells (Th1 and Th2) and regulatory T cells (Tregs, Th3). CD is associated with a Th1 T-cell cytokine profile, including IFN-γ, TNF-α and IL-12[18], whereas UC is associated with a modified Th2 type response cytokine profile including IL-15 and IL-10[18]. In addition, these findings have been recently complemented by the discovery of the IL-23/IL-17 axis that is part of the effector T cell immunological response, and seems to be involved in IBD. Levels of expression of IL-23 and IL-17 are increased in patients with active IBD[19].

TNF-α, IL-1, IL-6
IL-1 and TNF-α share a multitude of pro-inflammatory properties and are crucial in the amplification of mucosal inflammation in IBD. Both cytokines, primarily secreted by monocytes and macrophages, activate intestinal macrophages, neutrophils, fibroblasts, and smooth-muscle cells, inducing them to secrete prostaglandins, proteases, and other soluble mediators of inflammation and injury, as well as other chemotactic cytokines[20].

TNF-α is increased in the colorectal mucosa and stools of both forms of IBD[21,22], and correlates with a higher endoscopic inflammation score[23]. TNF-α is significantly increased in the non-inflamed mucosa of CD patients compared to controls[13,21], as well as in organ culture taken from macroscopically normal mucosa[24].

Other studies[13,22] have shown that several cytokines other than TNF-α are also upregulated in the intestinal mucosa of active IBD patients, including IL-1 and IL-6.
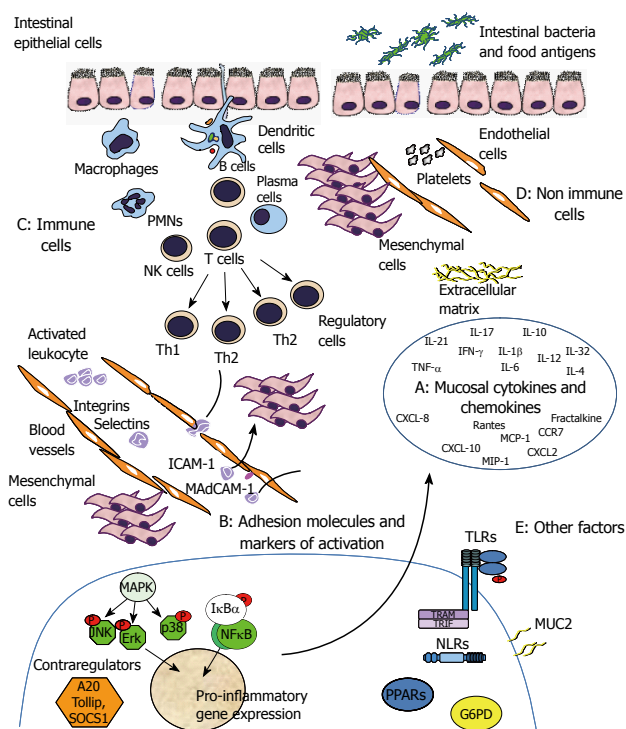

Figure 1 Mucosal biomarkers in inflammatory bowel disease (IBD). Based on available data from IBD pathogenesis, there are five major categories of mucosal biomarkers which can be assessed as predictors of disease severity and also response to therapy. A: Mucosal cytokines and chemochines [tumor necrosis factor (TNF)-α, interleukin (IL)-6, CXCL-2, *etc.*]; B: Adhesion molecules (MadCAM, ICAM-1, *etc.*) and intracellular markers of activation [mitogen-activated protein kinase (MAPK), nuclear factor (NF)-κB, A20, *etc.*]; C: Immune cells (dendritic cells, monocytes, macrophages, lymphocytes, NK, plasma cells, *etc.*); D: Non immune cells (endothelial cells, mesenchymal cells, platelets, *etc.*); E: Other factors [toll-like receptors (TLRs), NLRs, mucin (MUC), G6PD, *etc.*].

In addition TNF-α, IL-1β and IL-6 mRNA expression is increased compared to controls, with IL-6 being particularly high in the inflamed mucosa.

IL-4
IL-4 seems to play a crucial role in the Th2 response. One study has found that IL-4 is expressed in most biopsies from UC patients but not in CD[25], whereas others have not found any association between IL-4 levels and any form of IBD[13,26], probably due to technical issues[27].

IL-10
IL-10 is an anti-inflammatory cytokine produced by T cells, which inhibits the production of IL-1, IL-6 and TNF-α. Although a polymerase chain reaction (PCR)-based qualitative study on IL-10 in IBD has revealed that IL-10 is less frequently detected in UC compared to controls and CD[25], other studies have reported elevated mucosal IL-10 expression in UC and CD[28,29] or in UC alone[13,30,31].

IFN-γ
IFN-γ is elevated in all genetic animal models of IBD and is crucial in the development of Th1 responses.

Table 1 Studies assessing tissue markers as predictors of disease severity or drug response

Target	Techniques	Use	Ref.
Osteoprotegerin, STC1, PTGS2, IL13Rα2 and IL11	Microarray and PCR on mucosal extract	Predict response to IFX in UC	Arijs *et al*[7]
TNF-α levels and IL8, IL18	PCR and Immunohistochemistry on mucosal biopsies, ELISA on organ culture	Clinical/endoscopic remission in UC, response to corticosteroids, response to therapy in CD	Olsen *et al*[10] Ishiguro *et al*[12] Arsenescu *et al*[11] Schmidt *et al*[9]
RelA, A20, pIgR	PCR and Immunohistochemistry on mucosal biopsies	Severe disease and poor responses to immunosuppressive and anti-TNF-α therapy in 69 CD patients	Arsenescu *et al*[11]
GR expression	RT-PCR on mucosal biopsies	Decreased in biopsies from UC patients non responsive to glucocorticosteroids	Raddatz *et al*[14]
Mean histological inflammation and steroid use	Histology on colon biopsies	For univariate analysis, were predictors of colectomy in 561 patients UC	Hefti *et al*[15]
Mucosal IL-8 level	ELISA on mucosal biopsies culture	In multivariate analysis, independent risk factors for future relapse in patients with quiescent UC	Yamamoto *et al*[16]

GR: Glucocorticosteroid receptor; UC: Ulcerative colitis; CD: Crohn's disease; TNF: Tumor necrosis factor; IL: Interleukin; pIgR: Polymeric immunoglobulin receptor; ELISA: Enzyme-linked immunosorbent assay; RT-PCR: Reverse transcriptase polymerase chain reaction; IFX: Infliximab.

Mucosal IFN-γ expression is elevated in clinically active CD[32] but not in UC[33-35], which supports the theory that an imbalance in cytokine expression occurs in UC and CD; however, other studies have not reported such differences between UC and CD patients[36,37].

Other mucosal cytokines and chemokines

Other cytokines in the intestinal mucosa that are associated with active IBD include IL-21, IL-18, IL-8, monocyte chemotactic protein (MCP)-1, RANTES, epithelial neutrophil activating protein 78 (ENA-78), and IL-32[38].

Western blotting analysis of biopsies from patients with IBD and controls has demonstrated that IL-21 (a cytokine involved in the Th17 response) is overproduced in the inflamed intestine of patients with CD, in comparison to patients with UC and normal controls[39].

IL-18 is upregulated in intestinal mucosal cells of patients with IBD[40], and animal models have shown to benefit from anti-IL-18 antibody therapy with reduced severity in colitis[41].

In the proximal and distal regions of the colonic mucosa of UC patients, there was a greater than 10-fold increase in IL-8 levels compared to control subjects. A 3-5-fold increase in leukotriene B4 levels was observed along with a significant increase in myeloperoxidase levels throughout the colonic mucosa in patients with UC[42].

Other studies have reported elevated levels of MCP-1 and MCP-3, ENA-78, macrophage inflammatory proteins 1a and 1b, IFN inducible protein 10, stromal cell derived factor 1, and fractalkine[43-49].

Of particular interest is the demonstration that RANTES expression is not only increased in human IBD, but also plays a crucial role in the transition of acute to chronic disease in experimental models of colitis. In addition, RANTES triggers leukocyte adhesion to the inflamed intestinal microvasculature[50-52].

Furthermore, Kawashima *et al*[53] have observed a significant increase in CCR7 receptor only in CD and not in UC patients or healthy subjects. These observations are consistent with the reported increase in CC ligand expression in CD.

Studies assessing mucosal cytokine/chemokine panels

Several studies have attempted to explore the possibility of detecting tissue factors as predictors of disease severity by combining the analysis of different components simultaneously. However, none of these approaches has been validated and none are used for clinical purposes.

Real-time PCR quantification of CXCL8, CXCL10, calgranulin B and CXCL2 in colonic biopsies correlated with clinical activity index and endoscopic activity index in 27 UC patients, which suggests the use of these molecules as biomarkers and objective tools in clinical trials for the evaluation of anti-inflammatory and immunomodulatory regimens[54].

Another study with real-time PCR analysis (some confirmed by western blotting and enzyme-linked immunosorbent assay) of biopsy specimens from patients with UC and CD has shown increased expression levels of IFN-γ, TNF-α, IL-6, IL-15, IL-18, and IL-23 in affected and unaffected areas of IBD mucosa, compared to those found in healthy controls. Conversely, IL-1β, IL-6, IL-12, and IL-27 levels were higher in affected areas compared to unaffected ones in UC mucosa, but not in CD. A correlation between cytokine mRNA levels and inducible nitric oxide synthase and granzyme B has been observed[43].

ADHESION MOLECULES AND MARKERS OF ACTIVATION

Lymphocyte infiltration into the intestinal tract in IBD is mostly mediated by the interaction between α4β7 integrin expressed on lymphocytes and its specific ligand, mucosal vascular addressin CAM-1 (MAdCAM-1), which is expressed on the endothelial cells of the microvasculature in the inflamed intestinal tissue. Integrins form a large family of transmembrane proteins that are required for leukocyte-endothelium interaction, as well as cell-cell

interactions in the intestinal mucosa. These molecules together with the NF responsible for the signaling transduction pathway, such as NF-κB and mitogen-activated protein kinase (MAPK), are particularly abundant in the inflamed mucosa in UC and CD.

In one study[55], MAdCAM-1-expressing venules were more abundant in CD than in UC, whereas E-selectin was equally expressed in both diseases. Furthermore, CD was characterized by the presence of MAdCAM-1-expressing venules deep into the intestinal tissue, mainly localized in lymphoid aggregates.

Another group has reported[56] increased endothelial ICAM-1 expression in areas with dense lymphocyte infiltration, and areas close to crypt abscesses and ulcerations in the intestinal mucosa of active IBD patients. Ulcerations were covered by a continuous layer of macrophages and epithelial cells expressing ICAM-1.

We have reported previously[57] CD40 overexpression on mucosal endothelium in CD patients, which was reduced markedly after infliximab treatment, and reached a level similar to that seen in the normal mucosa of control subjects. In the same study, we reported vascular cell adhesion molecule-1 overexpression in the intestinal microvasculature of CD patients, which also disappeared after infliximab treatment.

Nikolaus *et al*[58] have shown that CD patients that relapse after induction of remission following infliximab administration, are characterized by increased TNF-α secretory capacity and mucosal nuclear NF-κB p65 before reactivation of clinical symptoms. Conversely, extended downregulation of nuclear concentrations of colonic mucosal NF-κB p65 is associated with sustained induction of remission.

Our group has reported increased phosphorylation of all three MAPKs (p38, extracellular signal-regulated kinase and Jun N-terminal kinase) in the microvasculature and mesenchymal cells of bowel preparations from patients with CD and UC, compared to controls and non-inflamed IBD mucosa[59]. In the same study, activation of these major signal transduction pathways was associated, *in vitro*, with increased production of inflammatory cytokines, as well as increased leukocyte adhesiveness to intestinal endothelial cells and fibroblasts.

IMMUNE CELLS

The uncontrolled inflammatory reaction in IBD is most likely the result of the interplay between genetic deficiencies in the innate immune system and an exaggerated T-cell-driven adaptive immune response[20].

IL-17-positive cells have been detected by immunohistochemical staining in the inflamed mucosa of CD and UC patients[60]. A recent study has confirmed the presence of Th17 cells in CD mucosa but the authors also found a previously unreported subset of mucosal T cells that share features with Th1 and Th17 cells (i.e. cells concomitantly producing IFN-γ and IL-17)[61].

Abnormalities in Tregs in human IBD have been described but are unclear because of the few inconclusive studies reported so far. It has been reported that, in the course of active UC and CD, the number of circulating Tregs is decreased compared to controls[62-64], but these differences do not persist during remission, which suggests that, in active IBD, Tregs migrate to the inflamed gut. In fact, paralleling the clinical activity of the disease, the number of Tregs during UC and CD has been reported to increase in the intestinal lamina propria and mesenteric lymph nodes[62,63,65-67]. One study from Japan has found that the relative proportion of CD4+CD25+ Tregs is significantly increased in patients with active IBD[68], whereas a German study has demonstrated that the frequency of CD4+CD25+ Tregs varies according to IBD activity[62]. In the latter study, Tregs were functional, but their number was reduced in the peripheral circulation and only moderately expanded in the inflamed mucosa.

Neutrophils and monocytic cells also play an important role in the pathogenesis of IBD and in the maintenance of active inflammation. Recently[69], infliximab has been reported to decrease histological disease activity in Crohn's ileocolitis, by lowering the number of neutrophils and mononuclear cells in intestinal mucosal biopsies. Furthermore, the number of lamina propria mononuclear cells is also reduced because of a global reduction in CD4(+) and CD8(+) T lymphocytes and CD68(+) monocytes.

Mucosal dendritic cells (DCs), the main antigen-presenting cells in the gut, display an activated phenotype in IBD tissues, which is indicative of their involvement in the local chronic inflammatory reaction. In particular, they have been shown to express elevated levels of CD40 in mucosal extracts from UC and CD patients. These levels decrease after patients are treated with anti-TNF-α[70]. Another group[71] has reported an imbalance in intestinal DC subpopulations in a group of pediatric CD patients that underwent surgical bowel resection, following different therapies. In particular, immature DCs (CD11c+CD83-CD68-DC-SIGN+) were only found in non-inflamed control colonic tissue, whereas mature (CD11c+CD83+CD68+DC-SIGN+) DCs were markedly reduced (60% and 30%) in CD tissue samples compared to controls. In addition, tissue samples from CD patients undergoing corticosteroid therapy display marked depletion of DCs compared to tissue from untreated patients or those treated with other drugs. Colonic tissue with severe inflammation shows reduced numbers of CD11c+ and CD83+ DCs in the lamina propria and submucosal compartments, with a concomitant increase in DCs in the muscularis, compared to moderately inflamed and non-inflamed CD tissue[71].

Active CD is also associated with an increased number of macrophages in the ileal and colonic mucosa. A recent study[72] has shown increased numbers of macrophages expressing the scavenger receptor CD163 in colonic mucosa of CD patients concomitantly affected by spondyloarthritis. Macrophages or DCs that express RANK (receptor activator of NF-κB ligand), CD68 and S100

protein are increased in the colon of CD patients, particularly in inflamed areas compared to normal colon[73].

NON-IMMUNE CELLS

Other important cell types that participate in the chronic inflammatory response of IBD, include epithelial, mesenchymal and endothelial cells, and platelets, which exert many of the functions traditionally attributed to classical immune cells, such as cytokine production or expression of major histocompatibility complex class Ⅱ antigens[18].

Immunohistochemical studies have shown that intestinal epithelial cells (IECs) inappropriately express the class Ⅱ antigen HLA-DR in actively inflamed mucosa of UC and CD patients[74]. More recently, IBD IECs have been reported to inappropriately express members of the B7 family of costimulatory molecules[56], a finding that suggests alterations in B7-ICOS costimulatory pathways in IBD.

Other studies have shown that, in IECs, expression of toll-like receptor (TLR)3 is significantly downregulated in active CD but not in UC, whereas TLR4 is strongly upregulated in both CD and UC[75]. In the mucosa of patients with postcolectomy active pouchitis, expression of TLR2 and TLR4 is also strongly upregulated[76]. In addition, patients with CD show a reduced content of defensins in the affected ileum, and this reduction is independent from the degree of mucosal inflammation[77].

The involvement of fibroblasts in IBD traditionally has been viewed as being restricted to the production of extracellular matrix components and the pathogenesis of a common and serious complication, such as intestinal fibrosis[78]. However, fibroblasts are also involved in gut injury because they represent a major source of matrix metalloproteinases (MMPs), a family of proteolytic enzymes that are directly responsible for the tissue destruction that occurs during inflammation.

In the course of IBD, upregulation of MMPs occurs in the intestinal tissue, and this increase is lowered significantly by the anti-TNF-α antibody infliximab. These observations have been confirmed by the work of Meijer *et al*[79]. They have reported increased expression of MMP and tissue inhibitor of metalloproteinase (TIMP) in the intestinal mucosa from patients with IBD, compared with control samples. Furthermore, infliximab downregulated expression of MMP-1, -3 and -9 but not TIMP-1 and -2, depending on genotype.

Wang *et al*[80] have confirmed these findings. They have shown that mRNA and protein expression of MMP-1 and TNF-α in the ulcerated and inflamed colon mucosa of patients with UC is significantly higher than that in non-inflamed mucosa of normal controls. Furthermore, the expression of MMP-1 and TNF-α in the ulcerated area is significantly higher than that in the inflamed area of patients with UC.

Endothelial cells play an essential role in inflammation as a result of their central gatekeeper function, which controls the quality and quantity of leukocytes that transmigrate from the vasculature into the interstitial space. This process is complex and is mediated by a number of molecules, including cytokines, chemokines and adhesion molecules.

Bardin *et al*[81] have shown that, in active disease, high expression of CD146 is observed on endothelial cells in intestinal biopsies from patients with CD and UC. In addition, there is a decrease in the soluble form of CD146 in relation to active disease and extent of CD and UC. Lower levels of soluble CD31 have also been detected in UC, but not in CD. This suggests CD146 as a novel marker of the endothelial intercellular junction, which reflects endothelial remodeling more effectively than soluble CD31 does.

Angiogenesis has been suggested to play an integral part in IBD pathology[82]. Vascular endothelial growth factor (VEGF) has long been considered to play a central specific role in angiogenesis. Our group has reported[82] increased microvessel density in IBD mucosa, with an increase in αVβ3 expression on new mucosal vessels. These observations are associated with increased mucosal levels of VEGF, which in turn are able to induce increased angiogenesis in corneal and chorioallantoic membrane assays. Other groups[83] have confirmed our findings, and have shown increased VEGF expression in CD and UC compared to controls. Furthermore, immunohistochemical staining for CD146 is also higher in both forms of IBD, as well as CD34 expression, which indicates a possible role of angiogenesis in the pathogenesis of IBD.

Endothelial junction adhesion molecules, such as CD146, recently have been suggested to play a role in angiogenesis. CD34 is expressed on the vascular endothelium, and is upregulated in IBD.

In active CD and UC, a loss in the expression of endothelial EPCR (the receptor for protein C) and thrombomodulin has been reported, therefore impairing the activated protein C pathway in the intestinal microvasculature[84].

OTHER MUCOSAL COMPONENTS

A positive correlation has been found between mucosal levels of total lactate dehydrogenase (LDH) isoenzyme M monomers and LDH 5 monomers for each of the histological subgroups of UC, while glucose-6-phosphate dehydrogenase activity seems to be independent of inflammatory changes[85].

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of transcription factors whose activities are regulated by high affinity binding of small lipophilic ligands such as steroid hormones. PAR2 methylation status in rectal mucosa has been shown to correlate with more severe disease phenotypes of UC[86].

Mucin 2 (MUC2) precursor biosynthesis and total MUC2 levels are significantly decreased in UC patients with active inflammation, compared to controls. In contrast, both these parameters returned to control values

during remission from inflammation, which demonstrates that colonic biosynthesis and total amounts of MUC2 vary according to disease activity. However, *MUC2* mRNA levels are similar in all patients and are independent of disease activity, which indicates that these variations in MUC2 synthesis are regulated post-transcriptionally[87].

Immunohistochemical analysis[88] of cryostat sections, using polyclonal and monoclonal antibodies specific for TLR2, TLR4 and CD14, have shown a significant increase in TLR2 expression in the terminal ileum of patients with inactive and active UC compared with controls. Significant upregulation of TLR4 expression relative to controls has been found in the terminal ileum and rectum of UC patients in remission and in the terminal ileum of CD patients with active disease. CD14 expression is upregulated in the terminal ileum of patients with active and inactive CD. It is also upregulated in the cecum of UC patients in remission and with active disease, and in the rectum of patients with active UC.

CONCLUSION

The identification of tissue markers, together with other predictors, is crucial for clinical assessment and management of IBD. Although several attempts have been made, it is clear that more efforts should be made in creating a wider analysis, which involves more IBD centers and patients. To achieve this, a prospective observational study to identify precursors for each subgroup of patients is necessary. More consistent data from immunohistochemistry or western blotting of cytokines on mucosal extracts are needed. Interesting data also can be derived from tissue indicators of activation, such as NF-κB, MAPK or markers of other pathways, which are also involved in IBD pathogenesis. Finally identifying whether an immune or non-immune cell type is involved in mucosal inflammation through specific markers of activation/function, also represents another important aspect to be addressed by further research.

In conclusion, we must admit that, to date, despite great potential, no tissue factor can be considered as a predictor of disease progression or response to therapy in everyday clinical practice devoted to the management of patients with IBD.

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