Predicting Therapeutic Response by in vivo Molecular Imaging in Inflammatory Bowel Diseases

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Key Words

Apoptosis · Inflammatory bowel diseases · Membrane-bound TNF · Molecular imaging · Anti-TNF antibodies

Abstract

Background: Different invasive and non-invasive imaging modalities are indispensable tools in the management of inflammatory bowel disease (IBD) patients. Standard imaging procedures like white light endoscopy or MRI are used to define gut inflammation based on structural changes and altered morphology of the mucosa. Nevertheless, it has thus far not been possible to analyse biological processes at the cellular level, which drive intestinal inflammation in IBD patients. The recent advent of molecular imaging in the field of IBD has opened new promising avenues to allow personalized medicine approaches based on in vivo-detected molecular findings. **Key Messages:** Recent clinical studies have attempted to address the issue of predicting therapeutic response to anti-tumor necrosis factor (TNF) treatment in IBD patients based on the molecular mechanism of action of these agents and corresponding in vivo assessment of mucosal immune responses. Several experimental studies have indicated that one of the main functions of efficacious anti-TNF therapy in IBD is the induction of intestinal cell apoptosis. Fittingly, a corresponding molecular-imaging study using single-photon emission CT for the localization and quantification of cell apoptosis, demonstrated that induction of mu-

cosal T-cell apoptosis correlated with the therapeutic response to anti-TNF therapy in Crohn's disease patients. There was moreover a predictive capacity regarding therapeutic efficacy. As the main biological properties of anti-TNF antibodies in IBD are mediated through binding to membrane-bound TNF (mTNF) expressing intestinal cells, another study used molecular imaging for in vivo visualization of these cells via fluorescent anti-TNF antibodies to predict therapeutic efficacy of these agents. It could be shown that patients with high amounts of mTNF positive cells showed significantly better response rates compared to patients with low amounts of mTNF positive cells. **Conclusion:** In vivo molecular imaging in IBD has the potential to have an impact on our current treatment approaches and may allow us to individualize specific therapies based on molecular level analysis.

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Introduction

Inflammatory bowel diseases (IBD) comprise Crohn's disease and ulcerative colitis and are defined as chronic remittent inflammatory disorders of the gastrointestinal tract not due to specific pathogens [1–3]. As the pathogenesis of IBD is still incompletely understood, therapeutic approaches have mostly been limited to rather unspecific suppression of the adaptive immune system [4, 5]. Recent advances in understanding the underlying immunopathogenetic mechanisms of IBD have led to the devel-

opment of biological therapies, which aim to selectively inhibit crucial mediators of the inflammatory process [6–8]. First, tumor necrosis factor (TNF) was identified as one of the central pro-inflammatory cytokines [9], which resulted in the development of agents that neutralize its biological activity and subsequently demonstrated therapeutic efficacy in inducing and maintaining remission in IBD patients [10–12]. Another crucial pathway in the inflammatory cascade is the aberrant migration of cells from the systemic circulation into the gastrointestinal tract [13]. Therefore, different anti-adhesion molecule therapies have been developed to expand the therapeutic possibilities in IBD, among which an antibody against the $\alpha 4\beta 7$ integrin was the first to be approved for IBD treatment [14]. Currently, the anti-TNF antibodies Infliximab (chimeric IgG1 monoclonal antibody), adalimumab (fully human IgG1 monoclonal antibody), certolizumab pegol (pegylated humanized Fab' fragment monoclonal antibody), and golimumab (fully human IgG1 monoclonal antibody) as well as the anti-adhesion molecule vedolizumab (humanized IgG1 monoclonal antibody) have been licensed for treatment of one or both IBD entities in different countries.

However, across all trials with approved biological agents in IBD, about 40% of the patients do not respond to treatment with the applied biological substance, and subsequent repeated treatments produce a therapeutic effect in only a small proportion of these patients [15]. A central aspect in the rational clinical management of IBD patients is therefore to identify biological therapy responders and to establish clinically applicable predictive markers. This would enable the treating physician to directly introduce the patient to the best suited biological therapeutic option, which would enable a better and time-efficient control of disease for the patient. Reliable prediction of therapeutic response would also be essential in order to avoid exposure of non-responders to an inefficient biological therapy and associated potential side effects [16, 17]. In view of current economic constraints in health-care systems, the development of reliable predictors of response to these relatively expensive treatments is of central importance and might be essential to their future use. Only limited amount of data are available in this regard and have mainly been collected for anti-TNF antibodies. Thus far, it could be shown that Crohn's disease patients with elevated CRP-levels have a higher probability of efficacious anti-TNF therapy [18]. Further studies relying on clinical, genetic or serological predictors of response have yet not been validated in further studies or implemented in clinical practice [19]. In the search for novel

promising biomarkers, focus has lately been directed on potential predictors of therapeutic response that are directly derived from insights into the molecular mechanism of action of the biological therapy. This might possibly enhance its significance for specific prediction of treatment success. Such an approach is based on basic science advancements regarding insights into the molecular mechanism of biological agents, which may then be transferred to the identification of potential biomarkers for prediction of treatment response [20]. Molecular-imaging techniques represent a promising approach to implement these insights into clinical application and help to further optimize treatment of IBD patients.

In the following sections, we would like to present in vivo molecular-imaging studies in IBD patients that have been based on advancements in basic science knowledge regarding the mechanism of action of these substances.

Insights into the Mechanism of Action of Anti-TNF Antibodies in IBD

TNF is a key regulatory cytokine that plays a multifaceted role in the immunopathogenesis of mucosal inflammation in IBD [21]. Accordingly, several studies have demonstrated that TNF production is elevated in the intestinal mucosa and serum of IBD patients [22–24]. TNF is initially synthesized as the transmembrane precursor protein membrane-bound TNF (mTNF), that is being cleaved by the metalloproteinase TNF-converting enzyme into its soluble form (sTNF). TNF is mainly produced by monocytes, macrophages and T cells [25, 26]. Both sTNF and mTNF aggregate as homotrimers and mediate their biological functions through binding to 2 distinct TNF receptors (TNFRs). TNFR1 is ubiquitously expressed on various cell types and its activation can lead to induction of pro-inflammatory cytokines, inhibition of apoptosis or induction of caspase-dependent apoptosis [27]. The expression of TNFR2 on immune cells is highly regulated. TNFR2 binds members of the TNFR-associated factor family that induce activation of the transcription factor nuclear factor B causing dysregulated production of proinflammatory cytokines such as interleukin (IL)-6, which exerts anti-apoptotic effects. It can moreover also confer T-cell activation and proliferation as well as pro-apoptotic signals [25]. While both sTNF and mTNF can generally activate the TNFR1, sTNF preferentially binds to TNFR1 owing to a heightened dissociation rate from TNFR2 than from TNFR1. This has led to the ligand-passing assumption that sTNF bound to TNFR2 is

quickly passed on to TNFR1, through which most of the biological activities of sTNF are mediated through [28]. The TNFR2 on the other side can only be fully activated by mTNF and therefore plays an important role during cell–cell interaction [26]. It could be shown that mTNF not only acts as a ligand but can also act as a receptor that is activated by TNFRs and transmits intracellular signals in a process termed reverse signaling [29].

Recent studies have shown that mTNF plays a pivotal role in the inflammatory process in IBD. This has been demonstrated most prominently in an experimental colitis model, where mucosal inflammation was induced upon transfer of naïve T cells into mTNF RAG2^{-/-} mice, which had a mutated TNF that could not be cleaved into sTNF [30]. These data imply that a systemic effect of TNF is not needed for the onset and perpetuation of mucosal inflammation. Fittingly, specific neutralization of mTNF instead of sTNF was able to exert anti-inflammatory effects in a T-cell mediated colitis model [31]. Accordingly, it was demonstrated that anti-TNF antibodies exert their main therapeutic functions by binding to mTNF expressed on mucosal immune cells rather than neutralization of sTNF [32]. Several effects have been described that are mediated by binding of anti-TNF antibodies to mTNF-bearing intestinal cells, including suppression of pro-inflammatory cytokine production, antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity [33, 34]. The most important cellular effect mediated by anti-TNF antibodies on mTNF expressing target cells in IBD is the induction of apoptosis [35, 36]. It is general consensus that heightened activation and resistance of lamina propria T cells is one of the key factors in the perpetuation of the inflammatory cascade in IBD [37–39]. It could be shown that there is direct induction of apoptosis through reverse signaling in mucosal T lymphocytes through activation of different caspases, pro-apoptotic proteins (bak, bax) and cytochrome c release but independent of CD95/Fas signaling [32, 40]. Furthermore, indirect induction of T-cell apoptosis by anti-TNF antibodies was demonstrated in a recently published study. It could be shown that binding of anti-TNF agents to mTNF expressing macrophages led to the induction of apoptosis in TNFR2 bearing T cells [41]. The targeting of the mTNF/TNFR2 pathway and indirect induction of T-cell apoptosis underlines the importance of mTNF for the therapeutic efficacy of anti-TNF agents in IBD.

Taken together, these basic science findings indicate that the therapeutic effects of anti-TNF antibodies are mediated by binding to mTNF expressing mucosal immune cells, subsequent induction of apoptosis and thereby removal of

inflammatory immune cells in the mucosa. Advanced understanding of the mechanistic of efficacious biological therapy is essential for the development of molecular-imaging procedures that aim to guide therapeutic decision processes. These insights in basic science have been rapidly transferred from the laboratory to clinical implementation in 2 recently conducted clinical molecular-imaging studies in IBD. These studies aim to further analyse the effects of anti-TNF antibodies in Crohn's disease patients and examine the predictive capacity of single phenotypic biomarkers to predict therapeutic response to therapy.

Clinical Molecular-Imaging Studies in IBD

As the described research results have clearly implicated that the induction of apoptosis is one of the key features in the mechanism of action of anti-TNF antibodies, an imaging study was conducted to depict intestinal cell apoptosis in patients with Crohn's disease. Visualization of apoptosis was achieved by the application of ^{99m}Tc-annexin V. Apoptotic cells display phosphatidyl serine on their surface, which is bound by annexin V; this is therefore an ideal molecular probe to detect the molecular signature of the apoptotic process in vivo. Radionuclide imaging of annexin V was achieved by administering ^{99m}Tc-annexin V in 14 patients with Crohn's disease in its active form, followed by single-photon emission CT (SPECT) for the detection of apoptotic cells. SPECT scanning was performed at baseline and 24 h after application of the anti-TNF antibody Infliximab. It could be shown that that intestinal uptake of ^{99m}Tc-annexin V significantly increased to 98.7% in Crohn's disease patients who clinically responded to anti-TNF therapy compared to 15.2% in non-responders. Clinical response was defined as reduction of the Crohn's Disease Activity Index >100 points at week 2. The region of apoptosis did correspond to the inflamed intestinal segment. Subsequent analysis of mucosal biopsies identified mucosal T cells as the potential target cell undergoing apoptosis [42]. These results were for the first time able to demonstrate in vivo that anti-TNF treatment leads to the induction of T-cell apoptosis and even more important, that it correlated with clinical response in the examined Crohn's disease patients. The performed study obtained data only from a small number of patients and was not followed up by any larger prospective analyses till now; nevertheless, this study serves as an indicator that molecular-imaging approaches might be used for the prediction of therapeutic efficacy of biological treatments. While this study in-

Fig. 1. Molecular imaging with a fluorescent anti-TNF antibody in combination with CLE. Topical application of fluorescent anti-TNF antibodies leads to their binding to mTNF, which is expressed on the surface of some mucosal cells. The CLE is able to

excite the fluorochrome with a laser light of 488 nm wavelength. The emitted light from the fluorochrome is then detected by the CLE scope, thereby enabling visualization of mTNF bearing mucosal cells.

vestigated prediction of therapeutic response following the initiation of anti-TNF therapy, another study concentrated on the predictive value of a molecular-imaging approach prior to initiation of anti-TNF therapy.

As current data indicate that anti-TNF agents mediate their immunosuppressive effects by binding to mTNF on mucosal target cells, a diagnostic *in vivo* molecular-imaging procedure to detect mTNF expression on intestinal cells was used for predicting individual patient response to subsequent anti-TNF therapy [43]. Detection of mTNF bearing mucosal cells was achieved by topical application of a GMP-conform fluorescent anti-TNF antibody. Confocal laser endomicroscopy (CLE) was employed to assess the number of mTNF positive cells. The study was performed with a CLE instrument where the miniaturised laser scanning microscope was integrated into the distal tip of an otherwise conventional white light endoscope. The solid-state laser provided an excitation wavelength of 488 nm that is focused onto a distinct imaging plane within the tissue. The intensity of the light reflected from a given point was then measured in order to compute an electronic image from these data. This procedure provides *in vivo*, real-time, fluorescent imaging at 1000-fold magnification of the captured mucosal structures. Infiltration depth of

the blue laser light is 0–250 μm and the optical slice thickness of the scanned mucosa is 7 μm . The axial resolution is 0.7 μm [44]. CLE in conjunction with the topically applied fluorescent anti-TNF antibody, which was sprayed onto the most inflamed area of the mucosa, was for the first time used to predict therapeutic response to treatment. It was possible to localize the mTNF expressing cells in the lamina propria with this diagnostic approach (fig. 1). There was moreover a correlation between mucosal mTNF expression and subsequent clinical response to anti-TNF therapy. It could be demonstrated that Crohn's disease patients with high amounts of mTNF+ cells showed significantly higher response rates at week 12 (92%) upon subsequent anti-TNF therapy as compared to patients with low amounts of mTNF+ cells (15%). Clinical response was defined as the reduction of the Crohn's Disease Activity Index >100 points at week 12 after the initiation of the anti-TNF therapy. A high number of mTNF positive cells also predicted sustained clinical response at week 52 and decreased steroid use [43]. These findings suggest that this procedure could be used as a screening tool to stratify patients into possible responders and non-responders to anti-TNF therapy, thereby decreasing mortality and optimizing health resource utilization. This pro-

cedure might serve as a model for similar applications in other chronic inflammatory disorders that are treated with biologics and allow implementation of personalized medicine approaches.

Conclusion and Outlook

Molecular-imaging approaches that aim at the characterization of mucosal lesions *in vivo* due to their molecular composition rather than their morphological structure alone have made their way into clinical studies with IBD patients [45, 46]. These studies clearly underline that molecular imaging is a rapidly evolving field in diagnostic medicine and has the potential to have a significant impact on our daily clinical practice. Based on the identification of crucial molecules that are involved in disease pathogenesis, a growing number of possible molecular targets arise. In the performed studies, a direct correlation between the expression levels of target molecules and the response to the associated biological therapy directed against them was observed. It would be interesting to examine, if this procedure might also be used to predict response to already or soon-to-be-approved biological therapies, like anti adhesion molecule antibodies or agents targeting IL-12/23. Another clinical area where molecular imaging might have a major impact in IBD is detection of neoplastic mucosal lesions through improved imaging contrasts, differentiating normal and dysplastic tissue. Recently, a first approach in that direction was performed in a study where a topically applied enzymatically activatable probe (gGlu-HMRG), which fluoresces in the presence of γ -glutamyl transpeptidase,

an enzyme that is specifically associated with cancer, was applied in a mouse model for colitis-associated cancer. Fluorescence imaging was feasible within minutes after topical application. Fluorescence colonoscopy allowed the detection of gGlu-HMRG fluorescent lesions mostly in tissue with high-grade dysplasia or cancer even in small (<1 mm) lesions on a background of microscopic, but not endoscopically, active inflammation. Importantly, mucosal inflammatory infiltration also had variable fluorescence but much lower in signal than neoplastic lesions [47]. A similar approach in IBD patients with long-standing colonic disease would be highly attractive, as it might improve current endoscopic surveillance examinations, which are often hampered by the difficulty to differentiate between surrounding mucosal inflammation and neoplastic lesions.

In summary, molecular imaging in IBD has the potential to have an impact on our current treatment approach and may in future allow us to individualize specific therapies based on molecular level analysis.

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