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High mucosal plasma cell numbers and low serum TREM-1 levels may predict non-responsiveness to anti-TNF therapy in IBD

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Treatment with biologic agents targeting tumor necrosis factor (TNF) is highly effective in inflammatory bowel disease (IBD). However, approximately 30% of IBD patients do not respond to anti-TNF agents (Autoimmun Rev 2014;13:24-30), often requiring resectional surgery as a consequence. Unfortunately, there are currently no biomarkers in clinical practice able to predict responsiveness to biologic therapy in IBD, and at present, IBD patients are selected to receive anti-TNF therapy mainly on the basis of failure of previous treatments and lack of contraindications. The identification of baseline predictors of responsiveness or non-responsiveness to anti-TNF therapy in IBD is particularly relevant, especially as anti-TNF agents are burdened with significant costs and potential side effects.

Previous studies on colonic biopsies have identified baseline mucosal gene expression signatures associated with responsiveness to anti-TNF therapy in IBD (Gut 2009;58:1612-9, Inflamm Bowel Dis 2010;16:2090-8, Inflamm Bowel Dis 2014;20:2353-63), however no clinically useful biomarker has resulted from these studies so far. When analyzing heterogeneous tissue samples such as intestinal biopsies, which contain a multitude of different cell types, it is possible that the observed differences in gene expression may derive mainly from differences in cell type composition and from the specific gene expression profile of the overrepresented cell types. On the other hand, studying the gene expression of specific isolated cell types may lead to underestimate the effect of cell type composition differences on the overall gene expression profile in the biopsies. One way to overcome these limitations is to use computational deconvolution methodologies, which enable to extract cell-type specific information from heterogeneous samples (Curr Opin Immunol 2013;25:571-8).

In this study, Gaujoux and colleagues used computational deconvolution techniques to analyze previously published whole-genome expression data of colonic biopsies of IBD patients prior to anti-TNF treatment (discovery cohorts), in order to estimate the contribution of specific cell types to the gene expression differences detected in anti-TNF responders and non-responders. This approach revealed that the proportion of both macrophages and plasma cells was significantly higher in the colonic mucosa of non-responders than in responders, and that there was a significant decrease in the abundance of both cell subsets after treatment in responder Crohn's disease (CD) patients.

The authors subsequently used the same approach to test these findings on validation cohorts of IBD patients who received anti-TNF therapy. Responsiveness was defined as clinical and/or endoscopic improvement of IBD-related symptoms coupled with a decision to continue anti-TNF therapy. Analysis of colonic biopsies from a first validation cohort of 16 IBD patients, performed by a pathologist who was blinded to patient response status, confirmed that higher abundance of plasma cells and macrophages was associated with non-responsiveness to anti-TNF therapy. Plasma cells can be detected by immunohistochemistry using CD138 marker (Mod Pathol 1999;12:1101-6). The analysis of $CD138^{+}$ cells in colonic biopsies from a second validation cohort of 61 IBD patients confirmed that pre-treatment abundance of plasma cells is associated with non-responsiveness to anti-TNF therapy.

The authors reasoned that, on the other hand, mucosal gene expression differences between responders and non-responders may be masked by differences in cell

subset composition. On this basis, upon adjusting gene expression in IBD colonic biopsies from all (discovery and validation) cohorts for variations in plasma cells and activated monocytes, 15 pathways were found to be dysregulated between responders and non-responders. Of note, non-responsiveness to anti-TNF treatment was associated with MyD88 deficiency. At a single gene level, 166 genes were found to be differentially expressed between responders and non-responders. Among these, the ligand-receptor pair chemokine ligand 7 (CCL7)-chemokine receptor 2 (CCR2) was found to be up-regulated in non-responders. The authors analyzed the set of differentially regulated genes using the bioinformatics software Ingenuity Pathway Analysis (IPA), and identified triggering receptor expressed on myeloid cells 1 (TREM-1) as an upstream regulator of 6 of the 166 adjustment-derived differentially expressed genes, including CCL7.

Based on these findings, the authors assessed the expression of the TREM-1-CCL7-CCR2 axis in blood samples collected before infliximab treatment from a third validation cohort of 22 CD patients. CCL7 and CCR2 expression did not differ between responders and non-responders. Conversely, serum TREM-1 expression was significantly down-regulated in non-responders, and showed 94% accuracy in predicting non-responsiveness to anti-TNF therapy in CD.

Comment

Non-responsiveness to anti-TNF agents in IBD has been usually defined as “primary”, when response is lacking from the start of the treatment, or “secondary”, when the patient initially responds to treatment and subsequently loses response. The study by Gaujoux and colleagues focuses on primary non-responsiveness to anti-TNF in IBD, and investigates predictors of non-response in colonic biopsies and in the blood of IBD patients collected before the start of treatment.

The difference between primary and secondary non-responsiveness to treatment is purely dependent on time. Conversely, drug failure can also be classified into “mechanistic” or “pharmacokinetic” (Gastroenterology 2017;153:827-34). In particular, mechanistic failure occurs when the patient does not respond despite optimal drug trough concentrations. Pharmacokinetic failure occurs in the presence of undetectable or sub-therapeutic levels of the drug, and this can be further divided into “immune-mediated” or “non immune-mediated”, according to the presence or absence of anti-drug antibodies.

Multiple factors have been suggested to contribute to non-responsiveness to anti-TNF agents in IBD. In particular, a high degree of disease heterogeneity may be present in different IBD patients, in that intestinal inflammation may not be driven predominantly by TNF in some patients. Anti-drug antibodies exert a significant influence on the persistence of functional anti-TNF agents in the circulation and in the inflamed mucosa, and insufficient dosing has been shown to affect responsiveness to anti-TNF therapy in IBD. It has also been reported that loss of infliximab through the inflamed intestinal mucosa is associated with primary non-response in ulcerative colitis (UC) (Gastroenterology 2015;350-5). Furthermore, we have observed that up-regulated proteolytic enzymes can degrade and inactivate anti-TNF agents at the mucosal level in IBD, and this may influence responsiveness to treatment (Gastroenterology 2015;149:1564-74).

Gaujoux and colleagues observed that high numbers of mucosal macrophages and plasma cells, and up-regulation of CCL7 and CCR2 in the intestinal mucosa are associated with primary non-responsiveness to anti-TNF therapy in IBD. The authors provided an interpretation of these data. CCL7 has a chemotactic action on monocytes and promotes their migration into the colon, where they may differentiate into macrophages. These latter, in turn, are an important source of TNF, and high levels of TNF may support plasma cell survival (J Immunol 2003;171:1684-90). According to this interpretation, non-responsiveness would mainly be driven by high levels of TNF in the inflamed tissue and insufficient levels of the anti-TNF agent using standard dosing schedules.

Based on this interpretation, the authors suggested that a higher dose of anti-TNF would likely be effective in inducing remission in non-responsive patients. In support of this idea, Gaujoux and colleagues referred to the observation that the rate of colectomy during the infliximab induction phase in acute severe UC is lower when using an accelerated regimen with three infliximab 5 mg/kg infusions in 24 days compared with the standard dosing regimen at week 0, 2 and 6 (Clin Gastroenterol Hepatol 2015;13:330-5). However, it is important to note that, in the same study, there was no difference in the colectomy rate in the overall follow-up period of two years between patients who received accelerated infliximab induction compared to those treated with the standard dosing protocol (Clin Gastroenterol Hepatol 2015;13:330-5).

Putting cost considerations and toxicity to one side, there is an important and novel biological idea here. Is it simply that non-responders produce more TNF, and they would respond if given doses of anti-TNF therapy substantially higher than currently recommended? Looking at the data from the classical studies and clinical trials of anti-TNF therapy in IBD, there is only scant evidence to back up the concept that higher dose may have better efficacy. In particular, in the seminal study of a single dose infliximab induction in CD, 81% patients given 5 mg/kg showed response at 4 weeks compared to 50% of those given 10 mg/kg and 64% of those given 20 mg/kg (N Engl J Med 1997;337:1029-35). In the ACT-1 and ACT-2 studies on induction and maintenance therapy with infliximab in UC, there was no difference in response rates between 5 mg/kg and 10 mg/kg (N Engl J Med 2005;353:2462-76). Interestingly, with regards to adalimumab, the administration of 160 mg and 80 mg at week 0 and week 2, respectively, appeared to be more effective in inducing remission compared to smaller doses, both in the CLASSIC-I trial in CD (Gastroenterology 2006;130:323-33) and in the clinical trial in UC (Gut 2011;60:780-7). Indeed, 160 mg and 80 mg at week 0 and 2 is the standard dose regimen used for induction with adalimumab in clinical practice in IBD. Of note, however, in the CLASSIC-II maintenance trial in CD, weekly or fortnightly administration of adalimumab had comparable efficacy in maintaining remission at one year (Gut 2007;56:1232-9).

Finally, the idea that substantially increasing the dose of anti-TNF could bring a higher proportion of IBD patients into remission does not take into consideration the possible presence of anti-drug antibodies and their effect on anti-TNF trough levels and response to treatment, and also the possibility that the disease process may be driven by TNF-independent mechanisms.

On a more clinical perspective, Gaujoux and colleagues reported that pre-treatment down-regulation of serum TREM-1 is associated with non-responsiveness to biologic therapy in CD. Although this is perhaps the most relevant finding of the current study from a clinical point of view, it should be noted that only a small number of CD patients (n=22), and no patients with UC, were present in the blood validation cohort. Also, it has been previously observed that serum TREM-1 is elevated in IBD patients compared to control subjects, however no differences were reported in serum TREM-1 expression levels between active and quiescent IBD (J Crohns Colitis 2012;6:913-23).

Although the results of the study of Gaujoux and colleagues need further validation in larger studies, one of the strengths of these data is that they focus on measurements performed before the start of anti-TNF therapy. There have been studies on other non-invasive markers of response to biologic agents in IBD. For example, it has been observed that normalization of serum C reactive protein (CRP) levels is associated with long-term response to infliximab in Crohn's disease (Clin Gastroenterol Hepatol 2011;9:421-7), and that normal fecal calprotectin levels after induction is strongly associated with response to anti-TNF therapy in IBD (Inflamm Bowel Dis 2012;18:2011-7). Radiologic parameters have also been studied. It has been shown that improvement in small bowel motility on magnetic resonance enterography is associated with response to anti-TNF therapy in CD (Aliment Pharmacol Ther 2015;42:343-55). These studies all rely on measurements performed after the start of the anti-TNF therapy, and may provide useful information on who is going to achieve and maintain response early on in the treatment course. Nevertheless, it would be obviously even more useful to have information on likelihood of response when deciding about different treatment options in IBD, thereby optimizing results, safety, and costs. For this reason, the results by Gaujoux and colleagues are particularly promising, and warrant further investigation.

In conclusion, Gaujoux and colleagues report that pre-treatment mucosal abundance of plasma cells and macrophages, and down-regulation of serum TREM-1 may predict responsiveness to anti-TNF therapy in IBD. Studies on larger cohorts of both CD and UC patients before anti-TNF treatment start are needed to assess the predictive value of mucosal plasma cells and macrophages abundance and serum TREM-1 levels, alone or in combination with biomarkers of clinical activity in IBD, such as serum CRP, fecal calprotectin and radiologic markers.