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Inflammatory gene expression profiles in Crohn's disease and ulcerative colitis: A comparative analysis using a reverse transcriptase multiplex ligation-dependent probe amplification protocol

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Abstract

Background and aims: Cytokines and their receptors play a critical role in the pathogenesis of the inflammatory bowel disease (IBD). The aim of this study was to investigate the expression profiles of inflammatory genes in inflamed and non-inflamed colonic tissue samples in patients with Crohn's disease (CD) and ulcerative colitis (UC), and to identify molecular signatures for different IBD phenotypes.
Methods: Seventy-one patients diagnosed with IBD (38 CD, 33 UC) and 15 non-IBD controls have been included in the study. For each patient, biopsy samples were obtained during colonoscopy from inflamed (L) and healthy (N) mucosa. We investigated by commercially available reverse-transcriptase multiplex ligation-dependent probe amplification (RT-MLPA) kit the mRNA expression of a set of 40 genes involved in inflammation: cytokines, chemokines, receptors, signal transduction molecules and transcription factors.

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Results: In L biopsies from patients with CD, higher expression levels were found for IL-4 (p=0.009) and IL-12p35 (p=0.0005), whereas in L biopsy samples from patients with UC higher expression levels were found for IL-8 (p=0.03), chemokines SCYA3 (p=0.05), SCYA4 (p=0.01) and glutathione S-transferase P1 (p=0.01). In N biopsies of patients with CD higher expression levels were found for IL-1R (p=0.01) and IL-12p35 (p=0.007), whereas in N biopsies of patients with UC higher expression levels were found for IL-15 (p=0.009) and SCYA8 (p=0.001). The logistic regression analysis has indicated that low expression levels of IL-2 and IL-10, together with higher ASCA IgG titers were independently associated with penetrating/stricturing CD.
Conclusions: RT-MLPA is a sensitive and effective method for the evaluation of the profiles of inflammatory genes in IBD, with potential future applications for diagnosis, phenotypic stratification and targeted therapy.

1. Introduction

Inflammatory bowel diseases (IBD) are chronic relapsing conditions characterized by recurrent inflammation of intestinal mucosa, resulting from a dysregulated immune response to luminal antigens.[1] Recent studies have provided evidence that IBD are the result of a genetic predisposition that leads to a mucosal immune regulatory cell defect, barrier dysfunction and susceptibility to environmental triggers.[2] The activation of immune cell populations is accompanied by the production of a wide variety of nonspecific mediators of inflammation. These include cytokines, chemokines, growth factors, metabolites of arachidonic acid (e.g., prostaglandins and leukotrienes) and reactive oxygen metabolites such as nitric oxide.[2]

The expression of the proinflammatory cytokines is significantly increased in inflamed areas from both ulcerative colitis (UC) and Crohn's disease (CD). These mediators enhance the inflammatory process, being directly involved in the induction of tissue injury.[3] The maintaining of inflammation requires the recruitment of additional leukocytes from the vascular space to sites of disease activity and depends on the expression of various adhesion molecules (selectins, integrins) on vascular endothelial cells.[4] The abnormal influx of immune effector cells in the intestinal mucosa can be also explained by the changes in the pattern of expression of chemokines (or chemo-attractive cytokines). In IBD, a defect in chemokine production or a defect in the regulation of chemokine signal transduction pathways could contribute to the loss of epithelial integrity by inducing neo-angiogenesis and local production of free radicals.[5] Macrophage inflammatory proteins (MIPs) or small inducible cytokines – SCYA3, SCYA4, SCYA8, and IL-8 – are essential chemokines for immune response and inflammation. They activate granulocytes, which can lead to acute neutrophilic inflammation, a more prominent feature of UC than of CD.

Meantime, there is evidence suggesting that in the uninflamed/inactive areas of IBD there is also an abnormal immune activation as shown by the expression of increased levels of the proinflammatory cytokines IL-6, TNF-α, and IL-18, in intestinal biopsies from CD patients.[6]

The aim of our study was to evaluate, by means of a new polymerase chain reaction method – Reverse-Transcriptase Multiplex Ligation-dependent Probe Amplification (RT-MLPA) – the expression profile of inflammatory genes in inflamed and non-inflamed colonic tissue samples comparatively in CD vs UC, and to identify "molecular signatures" that could define different IBD phenotypes.

2. Materials and methods

2.1. Study population

Adult patients with a diagnosis of active colonic CD or UC evaluated in the Gastroenterology Departments of two academic hospitals in Bucharest, Romania – Fundeni Clinical Institute and Elias University Hospital – were enrolled in the study. The diagnosis of IBD was based on the endoscopic findings and histopathological analysis of biopsies taken from affected areas. Patients with an uncertain diagnosis of IBD and those with no active endoscopic lesions were not included in the study.

The clinical features of the patients were defined according to the Montreal classification.[7] For each patient, two biopsy samples were obtained for genetic analysis, one from the inflamed mucosa (L) and one from areas without endoscopic lesions (N), from at least 10 cm distance of any inflamed area. The unaffected areas from which biopsies were collected were defined by endoscopic criteria.

Informed consent was obtained from all patients prior to biopsy collection and the study protocol was approved by the local ethics committees.

2.2. Isolation of RNA and RT-MLPA protocol

Immediately after collection, all biopsy specimens were transferred into tubes containing RNAlater® Tissue Collection (Ambion Inc., USA). Total RNA was isolated using the RNAeasy® Mini Kit (Qiagen, Germany) following the manufacturer's protocol. RT-MLPA analysis was performed with the SALSA® RT-MLPA R009 Inflammation mRNA kit (MRC-Holland, The Netherlands) designed to quantify the expression levels of a panel of 40 mRNAs: cytokines, chemokines, receptors, signal transduction molecules and transcription factors. The inflammatory genes included in the MLPA kit are listed in Table 1. All samples were tested with the same batch of reagents. The PCR products were detected on a 3130 Genetic Analyzer (Applied Biosystems, USA) and analyzed using GeneMapper software v.3.7. The mRNA level for each gene was expressed as a normalized