The chemokine CCL28 is elevated in the serum of patients with celiac disease and decreased after treatment

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Abstract: Accumulating evidence show that many inflammatory cytokines are involved in pathophysiology of celiac disease (CD). CCL28 known as mucosa associate epithelial chemokine (MEC) is produced by mucosa and chemotacts IgA-producing B cells into the mucosa. However, its levels in patients with CD have not yet been elucidated. CCL28 levels and anti-tTG (IgA) were detected in the serum of 28 new cases of CD, 32 cases of treated patients and 32 normal individuals by Elisa. Moreover, the effect of gluten on intestinal cells, Caco-2, was examined by RT-PCR. Our data show that (i) the levels of CCL28 is significantly higher in patients with CD than normal individuals, (ii) CCL28 levels is reduced in patients with CD who had gluten-free diets. Accordingly, we observed that CCL28 expression is upregulated in a dose-dependent manner when the Caco-2 cells were cultured in the presence of gluten. In conclusion, gluten enhances CCL28 expression and that CCL28 could be a novel biomarker for diagnosis and following up the patients with CD. However, further investigation in a larger number of patients is required.

Keywords: CCL28, celiac disease, gluten, anti-tTG IgA

Introduction

Celiac disease (CD) is a T cell mediated enteropathy disorder induced by gluten in genetically susceptible individuals. Modification of gluten by the enzyme tissue transglutaminase (tTG) increase the binding of these peptides to human leukocytes antigen (HLA)-DQ2 and DQ8 resulting in potentiating T cell stimulation [1]. CD patients develop autoantibodies (IgA and IgG) against tTG which can be used for screening and diagnosis of CD [2]. However, the histopathological analysis of small intestine remains to be the gold standard diagnostic procedure for diagnosis of CD [3, 4]. From histopathological point of view, active CD is characterized as many changes in small intestinal mucosa including villous atrophy, infiltration of T and plasma cells into lamina properia and enhanced infiltration of intraepithelial lymphocytes (IEL). It is well known that both innate and adaptive immune responses participate in pathogenesis of CD. Direct damage to small intestinal epithelial cells is considered to be the primary caused by migration of IEL [4, 5].

It has been recently shown that IL-15 contributes to tissue protection by promoting the elimination of infected cells. However, when the expression of IL-15 is chronically dysregulated, it involves in development of T cell-mediated disorders such as CD [6, 7]. The levels of CXCL10 have been shown to be much higher in the serum of patients with CD than control individuals. Consequently, the expression levels of IL-15, TNF-alpha, IL-10, and TGF-beta were detected in the surface epithelium of untreated CD with respect to control. Interestingly, the expression levels of IL-15 were much higher in the surface epithelium than the lamina propria. In contrast, the expression levels of IL-21 and IL-17 were higher in lamina pro-
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Figure 1. The levels of CCL28 is higher in patients with CD. Serum samples from 28 newly diagnosed patients and 32 normal donors were analyzed for Anti-tTG-IgA (A) and CCL28 (B).

peria than the surface epithelium [8], indicating that many chemokines and cytokines play crucial roles in pathobiology of CD.

CCL28 has been, firstly, known as mucosae-associated epithelial chemokine (MEC) and is expressed by columnar epithelial cells in gastrointestinal tract and lung cells and mediates trafficking of many inflammatory cells expressing CXCR10 receptors in the mucosa [9, 10]. CCL28 can recruit CCR10+ IgA or IgE antibody-secreting cells to intestinal and non-intestinal mucosal tissues or to respiratory epithelium in asthma [11]. Moreover, CCL28 is reported to constitutively expressed in colon epithelial cells and upregulated by proinflammatory stimuli such as IL-1 and bacteria-derived components [12]. A recent study has demonstrated that the expression levels of CCL28 are remarkably increased in synovial tissue lining macrophages and sublining endothelial cells compared to normal [10]. However, the expression levels of CCL28 in the serum of patients with CD as an inflammatory disorder of gastrointestinal tract have not been yet elucidated.

Material and methods

Patients

28 newly diagnosed CD patients, 32 treated patients and 32 normal donors who serological test for CD were negative were enrolled in this study. Diagnosis was carried out in gastroenterology ward at Tohid Hospital University based on the biopsy of duodenal and serological tests for CD. Clinical and histopathological data were collected from the information contained in medical records. The diagnosis of CD required the classical symptoms of malabsorption, positive for CD serological tests [13].

Detection of CCL28

Serum sample from all the participants were kept at -80°C until use. The levels of CCL28 were detected using human Elisa CCL28 kit (R&D systems, Minneapolis, MN) according to the manufacturer’s instruction.

Cell culture and reverse transcription polymerase chain reaction

The Caco-2 cell line was purchased from Iran Pasteur Institute (Tehran, Iran) and were cultured in RPMI 1640 supplemented with 10% FBS (Gibco, Manchester, UK) at 37°C in a humid incubator with 5% CO₂. The cells were cultured in the presence of different concentration of gluten for 24 h as previously reported [14]. Then, RNA was isolated using a RNA extraction kit (Bioflux, Basel, Switzerland) and RNA was transcribed into cDNA using of Bioneerkit (Bioneer, Daejeon, South Korea). Re-
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verse transcription-polymerase chain reaction (RT-PCR) procedures were carried out using primer for CCL28 (Sense: AGCCATACTTCCA-TTGCC, antisense: ATCTCTTGCCCTGACATG) and β-actin as housekeeping gene (sense: AG-ATCATGGCTCCTCCTGAG, antisense: CTAGTCA-TAGTCCGCCTAG). Amplification was performed with a thermocycler (Mastercycler, Eppendorf, Westbury, NY) and the PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide. The PCR products were visualized by a gel document system.

Results

CCL28 levels are elevated in the serum of patients with CD

First of all, we detected anti-tTG IgA in the serum of all the participants and found that the levels of it are remarkably higher in the patients with CD (Figure 1A). Moreover, the concentrations of CCL28 is significantly elevated (10.4 folds) in patients with CD than the normal individuals (Figure 1B).

The CCL28 concentrations are decreased after treatment

Next, we compared the levels of anti-tTG IgA in patients after a 6-month of gluten-free diet, we found that the levels of this autoantibodies significantly decreased (Figure 2A). More importantly, the amount of CCL28 were also reduced (2.4 folds) in patients after a 6-months of gluten-free diet (Figure 2B). Furthermore, we examined the concentration of anti-tTG IgA and CCL28 and in the serum of 5 patients before and after treatment and found that the levels of CCL28 are decreased in parallel with the titer of anti-tTG IgA (Figure 3).

Gluten upregulates CCL28 in Caco-2 cells

In order to determine the direct effect of gluten on CCL28 expression, the Caco-2 cells were cultured in the presence of different concentrations of gluten for 24 h. As shown in Figure 4, gluten enhanced CCL28 expression at mRNA levels in Caco-2 cells.

Discussion

Human CCL28 was originally identified as a chemokine which mainly expressed by columnar epithelial cells in gastrointestinal tract and shown to play a crucial role in trafficking of inflammatory cells in mucosa [10]. However, its levels in many gastrointestinal inflammatory disorders have not yet been elucidated. In the current study, we demonstrate that CCL28 is increased in the serum of patients with CD and that CCL28 is decreased along with anti-tTG IgA reduction, indicating that when anti-tTG IgA
could induce an inflammatory condition within intestine in which results in upregulation of CCL28 in the blood of patients with CD.

Accumulating evidence demonstrate that many cytokines and chemokines play important roles in pathogenesis of CD [15]. Track G and et al have recently reported that many novel parameters such as IL-8, IL-17 and sCD25 elevated in the serum of patients with active CD [16]. IL-15 is known to be the hallmark of the disease and mounting evidence from in situ and ex vivo studies exhibited that IL-15 is upregulated in lamina properia of intestine of patients with CD [7]. In addition, another study exhibited that the expression levels of IL-21 at both protein and mRNA levels is higher in patients with active CD than control [17], indicating that a number of immunological parameters are key players in the pathogenesis of CD. This notion was supported by our data showing that CCL28, as a chemokine which mostly produced by epithelial cells, is elevated in serum of patients with CD. Several studies have shown that there are some alternations in the levels of inflammatory mediators including cytokines following gluten-free diet compared to the time of diagnosis [18-20]. Therefore, it was a great importance to examine the effect of gluten-free diet on serum levels of CCL28. Our data show that the levels of CCL28 were reduced along with decreasing levels of anti-tTG IgA, implying that reduction of anti-tTG IgA may lead to create a less inflammatory condition in the intestines in which could results in downregulation of CCL28. We have to consider, however, that although the concentrations of CCL28 are increased in CD, the exact mechanism of CCL28 in pathogenesis of CD is still unknown and need to be addressed.

Gluten is known to induce both innate and adaptive immunity in CD in individual who are genetically susceptible. To evaluate the direct role of gluten and its derivatives on expression
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of inflammatory cytokines, many studies challenged the patients with gluten and analyzed their expression in intestinal mucosa. Brottevit M et al, have recently demonstrated that challenge of CD patients with gluten results in upregulation of IL-8 and TNF-α, but the expression levels of IFN-γ did not change [21]. Consisting with this observation, we demonstrated in the current study that stimulation of Caco-2 cells with gluten enhances CCL28 expression, indicating that gluten may directly upregulate CCL28. However, we have to consider that Caco-2 cells are not the best cell line model for studying CD, but to our best knowledge these cells mimic many characteristics of the intestinal cells [22]. Consistent with this study, a recent study has shown that when the human duodenal garments were cultured in the presence of gluten, the expression levels of IL-21 were significantly upregulated, but the levels of IL-17A did not [21].

In conclusion, we demonstrate here that CCL28 levels are significantly higher in the serum of patients with CD than normal individuals and that CCL28 is decreased along with reduction levels of Anti-tTG IgA.

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Disclosure of conflict of interest

None.

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