DEFECTIVE ANGIOGENESIS IN CELIAC DISEASE

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Background

Coeliac disease (CD) is an immune-mediated disease characterized by the presence of specific IgA class autoantibodies targed against transglutaminase 2 (TG2), a multifunctional enzyme also implicated in the angiogenesis process (Jones et al., Death Differ 2006). It has been demonstrated that, in untreated CD, the small bowel vasculature is altered and that autoantibodies against TG2 inhibit several steps of angiogenesis (Myrsky et al., Clin Exp Immunol 2008). Moreover in CD gliadin peptides exert damaging effects through various mechanisms. Some gliadin peptides are toxic and others are immunogenic for CD patients. The prototype of peptides effective on innate response peptide 31-43/49, which has been shown to be toxic for CD patients both in vitro and in vivo. Unlike p31-43 which is not immunogenic for T cells, peptide 57-68 (p57-68), which binds to HLA-DQ2/8 molecules, is one of the dominant epitopes recognized by T cells isolated from the intestine of CD patients. Since some

effects evoked by anti-TG2 antibodies is similar to the effect evoked by some gliadin peptides (Barone et al., Gut 2007) and based on the previous data demonstrating that angiogenesis and vascular biology in general might be an important player in the pathogenesis of CD, the major aim of the current study is to analize the effect of gliadin peptides in angiogenesis and vascular biology.

Main achievements

Previous studies demonstrated that CD patient-derived anti-TG2 antibodies disturb several steps in angiogenesis (Myrsky et al., Clin Exp Immunol 2008) and increase vascular permeability in vitro (Myrsky et al., Cell Mol Life Sci 2009). In particular, using the in vitro 3D matrigel system, ex vivo murine aorta-ring and the in vivo mouse matrigel-plug assays (Kalliokoski et al., PlosOne 2013), it has been demonstrated that angiogenesis is impaired as a result of CD antibodies on endothelial cells. We further showed that the celiac patients antibodies against TG2 can increase endothelial cell TG2 enzymatic activity and that the effects of celiac disease-specific anti-TG2 antibodies on endothelial cell behavior can be ameliorated by inhibiting the enzymatic activity of TG2. To investigated the molecular mechanism behind this anti-angiogenic effect, by using microarray technology the expression of 116 different genes related to angiogenesis were analyzed after treatment with CD autoantibodies against TG2 in endothelial cells. Of these genes the upregulated RhoB was selected for further studies. RhoB expression was found to be up-regulated at both mRNA and protein level in response to CD total IgA as well as anti-TG2-specific antibody derived from a celiac patient suggesting that RhoB plays a key role in the response of endothelial cells to celiac disease-specific anti-TG2 autoantibodies (Martucciello et al. J Mol Med (Berl) 2012).

Future perspectives

To study wether gliadin peptides inhibit angiogenesis, we will use a 3D in vitro system of HUVECs cultured inside matrigel. After the 24h of cultured period, endothelial length or tubule length inside matrigel will be measured using Image J software. Branch points number will be also calculated. We will also test effect of gliadin peptides using ex vivo angiogenesis assay. The aorta rings will be cultured in endothelial medium with or without gliadin peptides; after ten days of culture, images of endothelial sprouts and interconnected capillary tubes will randomly taken and quantified. If gliadin peptides will inhibit angiogenesis we expect reduction of angiogenesis parameters (tubule length, branch points). If possible, we will test the effect of gliadin peptides in angiogenesis in vivo, too. Then we will analize whether endothelial cells (ECs) motility is affected by gliadin peptides treatment. ECs migration is essential for angiogenesis. We will analyze the migratory capacity of treated ECs

analyzing basal random migration and directional migration. We expect that gliadin peptides will impair migration and response to chemoattractants. The mechanism behind the putative inhibitory action of the peptides will also be investigated. In particular Rho family GTPases are crucials regulators of cytoskeletal and adhesion dynamics, thereby stimulating cell migration and invasion. Although there are at least 20 human Rho GTPases, only a few have been rigorously tested for their involvement in the modulation of endothelial cell behavior. Thus we will try to identify which Rho GTPases is able to mediate gliadin effects on angiogenesis.
Finally we will study the relationship between TG2 extracellular transamidating activity and the effects evoked by gliadin peptides. To this end, we will inhibit the enzymatic activity of TG2 and we will study whether gliadin peptides can rescue the ECs behavior.

Publications

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External collaborations

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