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Peculiar antibody reactivity to human connexin 37 and its microbial mimics in patients with Crohn's disease☆

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Abstract

Background/aims: We found that pooled Crohn's disease (CD) sera strongly react with a human gap-junction connexin 37 (Cx37) peptide and tested for anti-Cx37 antibody reactivity in sera from CD patients and controls. We also investigated whether peptide-recognition is due to Cx37/microbial molecular mimicry.
Methods: The *PSI-BLAST* program was used for $Cx37_{121-135}$/microbial alignment. Reactivity to biotinylated human $Cx37_{121-135}$ and its microbial mimics was determined by ELISA using sera from 44 CD, 30 ulcerative colitis and 28 healthy individuals.
Results: Anti-$Cx37_{121-135}$ reactivity (1/200 dilution) was present in 30/44 (68%) CD cases and persisted at 1/1000 dilution. Database search shows that $Cx37_{121-135}$ contains the –ALTAV– motif

Abbreviations: aa, amino acid; CAV24, coxsackievirus A24; CAV9, coxsackievirus A9; CBV4, coxsackievirus B4; Cx37, connexin 37; CD, Crohn's disease; ECV, enterovirus C; IBD, inflammatory bowel disease; LACLA, *Lactococcus lactis*; MTUB, *Mycobacterium tuberculosis*; OD, optical density; PLV, poliovirus; RBL, rubella virus.

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which is cross-recognized by diabetes-specific phogrin and enteroviral immunity. Testing of 9 $Cx37_{121-135}$-microbial mimics revealed 57–68% reactivity against human enterovirus C, *Lactococcus lactis*, coxsackie virus A24 and B4. Anti-$Cx37_{121-135}$ was inhibited by itself or the microbial mimics. No reactivity was found against the poliovirus, rubella, and *Mycobacterium tuberculosis* mimics, or the beta cell phogrin autoantigen. Microbial/Cx37 reactivity was not able to differentiate CD patients from UC or healthy controls, in terms of overall prevalence and antibody titres, but microbial mimics were unable to inhibit reactivity to human Cx37 in the majority of the controls.

Conclusions: Sera from CD patients react with connexin 37 and cross-react with specific Cx37-mimicking enteroviral peptides. Microbial/self reactivity can be seen in UC and healthy controls. The lack of responses to other $Cx37_{121-135}$ microbial mimics and the inability of the reactive microbes to inhibit reactivity to self is intriguing and warrants further investigation.

1. Introduction

Epidemiological, clinical, serological and experimental studies support the notion that immune-mediated intestinal destruction in Crohn's disease (CD) commences when genetically susceptible individuals are exposed to microbial stimulants, and that the microbial/host immune encounter is essential in triggering, initiating or modulating the course or outcome of this inflammatory bowel disease (IBD).[1–9] Accumulating evidence points to a role for microbial/self molecular mimicry as a mechanism to explain the involvement of microbial triggers in the immunopathogenesis of CD, but also as a tool to identify CD-specific autoantigens.[1,6,10,11] We have previously reported that *Mycobacterium avium* subsp. *paratuberculosis* antigenic sequences and its mimicking intestinal antigens, such as gastrointestinal glutathione peroxidase, are specific targets of cross-reactive antibody responses in approximately 40% of patients with CD.[11] These findings have prompted us to apply a similar approach in an attempt to identify other self and microbial cross-reactive targets in patients with CD.[11] In a preliminary set of experiments, we have tested a pool of 5 CD sera to screen for antibody binding using as an antigenic source a large library of approximately 800 peptide spanning sequences from human and microbial peptidyl sequences, never tested before in CD sera. These peptides have been constructed over the last 12 years, and tested as microbial/self cross-reactive targets in the Molecular Mimicry Project of Liver Immunopathology at King's College London.[12–24] These studies included patients with various gastrointestinal, liver and extrahepatic autoimmune diseases such as insulin-dependent diabetes, autoimmune thyroiditis and multiple sclerosis.[12–24] We have found a strong reactivity of the pooled CD sera against a peptide corresponding to connexin 37 kDa (Cx37), also known as gap junction alpha-4 protein.[25] Emerging data suggests that connexins contribute to the generation of diarrhoea during infectious enteric disease.[26] In addition, recent studies indicate that Cx37 and other connexins are expressed by various immune cell types, supporting an important role of these proteins in gap junction-mediated antigen transport and intercellular communication in the immune system.[27–29]

With regard to Cx37, there is no published data to suggest that this connexin is a target of an autoimmune attack, or that it is involved in the immunopathogenesis of CD. Nevertheless, a recent study demonstrated increased gene expression and altered protein expression of Cx37 in highly purified enterocytes with active coeliac disease, suggesting that this connexin may be involved in enterocyte-specific immunopathological processes.[30] On the basis of these emerging data, we have considered that Cx37's antibody recognition by pooled CD sera was worthy of a more detailed study. Intriguingly enough, the diabetes-specific phogrin peptide with a striking similarity (*see later discussion*) to Cx37 peptide, and included in the same peptide library, was totally unreactive, suggesting a Cx37-specific reactivity of the pooled CD sera.

The goal of the present study was to test for anti-Cx37-specific antibody responses in a well-defined population of CD patients. Having found that the great majority of these patients contain high-titres of anti-Cx37 antibodies, and that this peptide is highly homologous to phogrin and the phogrin-mimicking microbial peptides previously implicated in the pathogenesis of insulin-dependent diabetes mellitus[31–37], we have also assessed whether microbial and Cx37 peptides are targets of cross-reactivity antibody recognition in patients with CD.

2. Material and methods

2.1. Patients

Serum samples from 44 patients with CD (mean age 41, range 21–76 years, 23 female), attending the out-patient clinic of Hepatogastroenterology Unit, Attikon Hospital, University of Athens, Greece, were tested. The diagnosis of CD was based on clinical, endoscopic, radiological, and histological criteria. Table 1 summarizes the demographical and clinical characteristics of the patients enrolled in the study. Thirty five patients had ileocolonic disease, 8 had colonic disease and 1 had isolated small bowel disease. At the time of serum collection, 41 patients were on treatment (Table 1), and 7 patients had undergone at least 1 surgery for CD. With regard to disease severity, 21 patients had quiescent disease, 2 had mild disease, 13 had moderate and 8 had severe disease as classified using the Harvey–Bradshaw activity index.[38]

Demographically matched sera from 30 patients with well characterized UC (mean age 39, 39 ± 15.4 years, range, 19–79 years, 22 men) were included as pathologic controls.