T CELLS IN COELIAC DISEASE

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Background

An immune reactivity to gluten proteins is responsible of celiac disease (CD), a chronic inflammatory process mainly targeting the proximal part of the small intestine. This important dietary component is highly resistant to gastrointestinal digestion, resulting in the release of long peptides highly immunogenic in CD individuals. Several studies, also from our group, have demonstrated the key role of CD4+ T lymphocytes restricted by HLA DQ molecules in CD pathogenesis. These mucosal T cells proliferate upon gluten peptide recognition, and release several inflammatory cytokines, mainly as interferon-γ (IFNγ) and interleukin(IL)-21. Albeit the central role of CD4+ T cells in gluten intolerance, it has been clearer that the CD8+ T lymphocytes, massively infiltrating the celiac inflamed gut mucosa, have a crucial role in the induction of intestinal villous atrophy, typical of acute CD. Our studies have demonstrated that CD8+ T lymphocytes, reactive to certain gluten peptides restricted by HLA class I molecules efficiently lyse enterocytes and contribute to the villous atrophy. However, it can be envisaged that a break of homeostatic mechanisms has to contribute to the inflammatory cascade leading to the enteropathy. In the healthy gut, the IL10 and regulatory T cells secreting IL10, namely the Tr1 cells, have a central role in controlling the inflammatory response to microbial and dietary antigens, and in mucosal homeostasis.

There is a general consensus that the large spectrum of clinical manifestations and mucosa histological lesions in CD reflects the multifactorial nature of this immune disease, and that regulatory immune mechanisms mediate prevention or delay the progression from the potential CD, characterized by a mild mucosa inflammation, towards the acute CD with a total villous atrophy. In fact, high expression of IL10 has

been detected in the gut mucosa of potential CD, whilst IFNγ production is strongly decreased by IL10 and TGFβ regulatory cytokines.

Main achievements

A significant improvement in the understanding of CD immune pathogenesis came from the isolation of gluten-reactive T lymphocytes from the intestinal mucosa of CD patients. These T cells have been expanded in highly specific T cell lines and clones, thus representing a sensitive tool to dissect CD pathogenesis, or to validate alternative drug therapies and gluten detoxifying strategies.

Taking advantages from these T cell lines and clones, we have contributed to the identification of the gluten peptides activating the CD4+ T cells in celiac mucosa. We have demonstrated that a large number of CD4+ T cell epitopes are contained in all gliadin families, in particular in the, so far overlooked, γ-gliadins (1-3). Notably, T cells reactive to gliadin have been detected also in the intestine of children at early disease, or even with a normal mucosa and negative CD-associated antibodies, but at high risk of developing CD(4). Furthermore, we found that children at a very early stage of CD recognize the same gliadin peptides that are active in adult CD patients. The tissue-transglutaminase (TGase), the CD autoantigen, strongly enhances the gluten T cell immunogenicity in early CD phase, thus providing a further evidence of the inflammatory role of TGase in CD (4).

The immunological mediators involved in the progression of histological mucosa lesion from potential to the acute CD are still unknown. We have investigated the phenotype and the cytokine production patterns of T cells infiltrating the gut mucosa of children with potential-CD or overt-CD (5). By a multiparametric flow cytometric analysis we have demonstrated that the transition from mild mucosa inflammation to villous atrophy in CD patients is characterized by an expansion of TCRγδ+ T cells, and a concomitant disappearance of IL4+ T cells. These findings, along with the indirect correlation between the anti-TGase titer and density of IL4+ T cells, suggest a shift from Th2 to Th1 phenotype in the atrophic mucosa. Further studies are required to validate the use of IL4+ and TCRγδ+ T cells as biomarkers of the different CD forms (5). It could be interesting to evaluate if these IL4+ T cells have a regulatory function. Previous studies from our research group have demonstrated an enhanced expression of IL10 in intestinal mucosa of celiac subjects with villous atrophy. Notably, IL10+ Tr1, of CD4+ T cell lineage, are gluten-specific and are active in untreated mucosa, most likely for a compensatory mechanism to suppress the excessive inflammatory responses to ingested gluten (6).
Notwithstanding the central role of CD4+ T cells in CD pathogenesis, and in the

transition from mild to acute phase, we have provided consistent evidences that gliadin contains peptides that activate T lymphocytes of adaptive CD8+ lineage. So far, several peptides have been identified to stimulate CD8+ T cells presented by HLA class I molecules. These CD8+ T cells, upon activated, release IFNγ and induce the enterocyte lysis, typical features of acute CD (7).

Future perspectives

One of the unresolved question in CD pathogenesis is what arms the inflammatory pathways responsible of the switch from normal and poorly inflamed mucosa, typical of potential CD to the villous atrophyin acute CD.

It is very current the need of further studies to elucidate the role of: i) CD4+ Th1 cells producing IFNγ and IL21;ii)CD4+ Th2 cells producing IL4; iii) CD4+ T cells of regulatory phenotype; iv) the role of CD8+ T cells in the villous atrophy of acute CD.

The future of research of T cells in CD will be focused on the characterization on IL4+ CD4+ T cells, in particular we will analyse the profile of cytokine production, the function (effector o regulatory) and the gliadin specificity. In particular, we will dissect the diagnostic relevance of the infiltration of IL4+ cells ands TCRγδ+ T cells to discriminate between the potential and acute CD, being these two cell subsets inversely correlated, and differentially expanded in the main CD forms.

Concomitantly the analysis of IL4+ cells ands TCRγδ+ T cells, we will expand the study to Tr1 Tregs in controlling the immune tolerance to gluten, an particularly to dissect their role in preserving mucosal integrity in those individuals that have already developed the tTG-autoantibodies, but have a normal mucosa as potential-CD. The frequency and the function of T regulatory cells will be compared among patients with villous atrophy, with disease remission, and those with potential CD. We will take advantage by the availability of Tr1 cells specific markers, such as the CD38, CD49b and LAG-3 surface molecules. The availability of these new Tr1 markers will allow us to monitor the adaptive regulatory T cells in peripheral blood of treated CD patients who undergo a brief gluten consumption.

Studies aimed to elucidate most of CD pathogenic players, in particular the identification of repertoire of gluten T cell epitopes, cytokines released by mucosa T cells upon gluten exposure, the balance between effector and regulatory pathways have a translation relevance. Dissecting these unresolved questions would be useful for the design of pharmacological therapies alternative to the gluten-exclusion diet targeting the pathogenic T cells. In fact, the definition of the repertoire CD4+ T gluten epitopes immunodominant in DQ2positive celiacs have been included in a

desensitized immunotherapeutic peptide-based strategy (Nexvax2) (8). Results of a phase I trial of an intradermalNexvax2formulation of have showed a good safety profile. However, the efficacy of NexVax2 to protect from gluten toxicity still needs to be demonstrated (8).

Finally, a goal of our research is also to provide a proof of concept to prevent CD (4). The understanding of the minimal gluten daily intake, necessary to mount the adverse immune reaction in genetically predisposed subjects, is pivotal for a preventive strategy (9). According to our recent studies, an in vitro threshold model to achieve the inflammatory T cell response has been provided (9). We have demonstrated that the pathogenic response is strictly dependent on the amount of gluten immunogenic peptides and independent from the HLA genetic background. The availability of ancient wheat variety with a reduced amount of gluten toxic sequence would be very promising to this respect (10).

Publications

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