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Characterisation of tissue transglutaminase-reactive T cells from patients with coeliac disease and healthy controls

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Abstract Previous studies have shown evidence for T lymphocytes specific for tissue transglutaminase (tTG) in the periphery of coeliac disease (CD) patients. These cells could play a role in disease pathogenesis and may be involved in providing help for the production of anti-tTG autoantibodies. The objective of this study was to further investigate the presence of tTG-specific T cells in patients with treated and untreated CD, and normal controls. Positive proliferative responses to three different commercial tTG antigens were detected in all groups tested, occurring more frequently and at higher levels in untreated CD patients. The addition of antibodies to HLA-DQ and HLA-DR caused a significant reduction in the proliferative response to tTG. T cell lines specific for tTG and composed predominantly of CD4-positive T cells were generated from responsive CD and control individuals, and were found to produce large amounts of interferon-γ, as well as interleukins 10, 17A, and 21.

1. Introduction

Coeliac disease (CD) is a common inflammatory disease of the small intestine caused by an inappropriate immune response to wheat gluten in genetically predisposed individuals. Investigation into the pathogenesis of coeliac disease

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reveals a complex interplay between environmental factors, genetics, the adaptive and innate immune systems, and the presence of autoantigens, in a process which has still not been fully elucidated. The principal autoantigen of coeliac disease is the ubiquitously expressed, multifunctional enzyme tissue transglutaminase (tTG) [1]. The demonstration of IgA autoantibodies directed to this self-protein is an integral component in the diagnosis of coeliac disease [2]. Tissue transglutaminase is also intimately involved in coeliac disease pathogenesis through the modification of gluten peptides by deamidation, which facilitates their presentation to the immune system via HLA-DQ2 or DQ8 molecules [3,4].

Due to the lymphocytic infiltration observed in the active CD intestinal lesion [5], and the strong association of the disease with HLA molecules [6], the concept of T-cell involvement in the pathogenesis of CD had been long-established prior to the isolation of gliadin-specific T cells from the CD intestinal mucosa [7]. In the time since this important observation, the CD4+ gliadin-specific T-cell response has been dissected and extensively characterised in relation to antigen recognition, phenotype, and function. The hallmark of gliadin-specific T-cell lines and clones is a mucosal-homing phenotype [8,9], and production of large amounts of IFN-γ, a cytokine that contributes substantially to the intestinal destruction seen in CD [10–12].

Apart from their role in the immune reaction that leads to the formation of the CD lesion [13], gliadin-specific T cells have been speculated to provide T cell help to tTG-specific B cells, resulting in the anti-tTG response characteristic of CD. This 'hapten-carrier' theory, proposed by Sollid, was based upon the fact that T cells specific for tTG had never been isolated, and the gliadin-dependant nature of the anti-tTG response [14]. However, preliminary findings from our group indicated that T cells specific for tTG could be detected in CD patients and in some control individuals, in a HLA-DQ and HLA-DR restricted manner [15]. The existence of tTG-specific T cells was confirmed in a recent paper by Ciccocioppo et al., who described the presence of T cells that were mostly CD4+ and proliferated to tTG in a HLA-DQ2-restricted manner in the periphery of untreated CD patients [16].

The aim of this study was to further investigate the presence of tTG-specific T cells in the periphery of CD patients and healthy controls, as it is known that T cells specific for other autoantigens have been detected in healthy subjects [17,18]. Peripheral blood samples were challenged with tTG and proliferative responses measured. tTG-reactive T cell lines were generated from responsive individuals and both the intracellular and secreted cytokines IFN-γ, IL-10, IL-17A, and IL-21 were measured, in tandem with the measurement of proliferation of these cells.

2. Materials and methods

2.1. Patients and controls

CD patients and controls were recruited from the Departments of Gastroenterology and Immunology, St James's Hospital Dublin. Ethical approval for this study was granted from the hospital ethics committee. The diagnosis of CD was based on a typical histological lesion, positive serology (IgA anti-tTG and endomysial (EMA) antibodies) and positive histological, serological and clinical responses to a gluten free diet. Patients with CD were sub-divided according to their treatment status. This included: 33 patients with untreated CD who were taking a normal diet and with positive serological tests; 65 patients with treated CD, taking a gluten free diet and with negative or low positive serological tests. In addition, 54 healthy control subjects, with negative coeliac serology, were investigated. The demographic information of each study group is summarised in Table 1.

2.2. Proliferation assays

Fresh peripheral blood mononuclear cells (PBMCs) from CD patients or control individuals were separated by density gradient centrifugation using Lymphoprep™ solution (Axis-Shield), and adjusted to a concentration of 5×10^5/ml in T cell medium (TCM) which contained RPMI containing HEPES, sodium pyruvate, non-essential amino acids, essential amino acids, penicillin, streptomycin, fungizone, L-glutathione, β-mercaptoethanol, and 5% autologous serum. Two hundred microlitres per well of this suspension was then cultured in triplicate with the appropriate antigen in a round-bottomed 96-well microtitre plate. Wells containing medium and cells only were added in order to measure background proliferation, and used to calculate the stimulation index (SI). The tTG antigens used were guinea pig tTG (gp tTG, Sigma), erythrocyte tTG (tTG^{ery}, Inova Diagnostics), recombinant human tTG (rh tTG, produced in SF9 insect cells, Zedira), at a concentration of 10 μg/ml. Phytohaemagglutinin (PHA) and purified protein

Table 1 Characteristics of the groups studied in proliferation assays using various commercial sources of tissue transglutaminase (tTG), with resulting responses and stimulation indices.

Antigen	Study group	Number	Sex (M/F)	Mean age (years) (range)	No. positive (%)	Mean SI (range)	Mean SI responders (range)
Guinea-pig tTG	CD untreated	15	2/13	45 (20–70)	12 (80)	4.7 (1.1–38.1)	8.5 (3.0–38.1)
	CD treated	30	7/23	59 (19–68)	12 (40)	3.1 (0.8–13.9)	4.8 (2.1–13.9)
	Controls	29	11/18	29 (18–54)	11 (38)	2.4 (0.7–20.6)	5.9 (2.1–20.6)
Erythrocyte tTG	CD untreated	11	4/7	48 (22–65)	5 (45)	5.0 (0.4–24.8)	9.3 (2.1–24.8)
	CD treated	35	14/21	58 (29–83)	11 (31)	1.6 (0.5–9.2)	2.8 (2.2–9.2)
	Controls	25	12/13	25 (24–65)	6 (24)	2.1 (0.7–7.6)	5.0 (3.4–7.6)
Recombinant htTG	CD untreated	7	2/5	42 (20–59)	6 (86)	19.1 (0.2–57)	22.8 (4.2–57)
	CD treated	15	4/11	52 (29–77)	6 (40)	2.2 (0.1–6.2)	4.0 (2.1–6.2)
	Controls	10	3/7	29 (24–57)	1 (10)	1.0 (0.4–2.1)	2.0 (n/a)