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Alimentary Tract

Gluten-free diet does not influence the occurrence and the Th1/Th17-Th2 nature of immune-mediated diseases in patients with coeliac disease

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ABSTRACT

Introduction: Coeliac disease (CD) is the most common Th1-mediated enteropathy, frequently associated with other immune-mediated disorders (IMD).
Aims: To evaluate: (1) the prevalence of IMD at the time of and after CD diagnosis; (2) a possible change in immune response to gluten free diet (GFD); (3) the potential role of GFD in reducing and/or preventing IMD in CD.
Methods: Prospective study including all consecutive adult CD patients who underwent investigations for Th1-Th17/Th2-IMD at the time of CD diagnosis and after a 5-year follow-up period.
Results: 1255 CD were enrolled. Of these, 257 patients (20.5%) showed IMD at the time of CD diagnosis, with 58.4% presenting a Th1/Th17-IMD. After a 5-year follow-up period, 682 patients (54.3%) showed new IMD despite GFD. Of these, 57.3% presented a Th1/Th17-IMD and 42.7% a Th2-IMD ($p = 0.8$). When compared the prevalence of each type of IMD before and after CD diagnosis, we did not identify any significant "switch" from Th1/Th17- to Th2-IMD or vice versa. The number of patients with Th1/Th17- and/or Th2-IMD increased during the GFD period (20.5% vs 54.3%; $p < 0.01$; OR 1.9).
Conclusions: The prevalence of IMD at the time of CD diagnosis is high and it seems to increase in the follow-up period despite GFD.

1. Introduction

Coeliac disease (CD) is the most common enteropathy in Western genetically susceptible subjects (HLA DQ2/DQ8) [1] mediated by a T helper cell type (Th) 1 immune response to gluten, a complex of water insoluble proteins from wheat, barley and rye [2].

Indeed, as a chronic autoimmune disorder, both innate and adaptive immune responses are involved in the pathogenesis of CD [3].

Some studies have shown how the translocation of α2-gliadin-33mer may depend on an apical-basal transcytosis stimulated by INF-γ, a cytokine involved in immunopathogenesis of CD [4]. Once it has reached the lamina propria, the gliadin would react with the tissue transglutaminase (tTG) – i.e. the enzyme catalyzing glutamine's deamidation – thus creating a transglutaminase-gliadin deamidated complex [5]. The deamidated peptide would be picked up by HLA DQ2 or DQ8 molecules on the surface of antigen-presenting cells (APC), and would be "presented" to the CD4+ T helper 1 (Th1). These Th1-cells would produce high levels of pro-inflammatory cytokines (IL2, IL6, INFγ, TNF), which could promote an increased cytotoxicity of intraepithelial lymphocytes (IELs) and natural killer (NK) T cells, causing apoptosis of enterocytes and the production of Th2 cytokines activating B cells and favoring the differentiation of plasma cells, hence resulting in the release of antibodies: anti-gliadin and anti-transglutaminase [6–9].

Despite this pathogenesis, it seems that both Th1/Th17- and Th2-mediated diseases may co-exist in cases of CD [10]. It is well known that CD patients show a particular tendency for

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multiple autoimmune and/or allergic and/or immuno-mediated disorders (IMD) over their lifetime [11,12], which supports this possibility.

To date, only hypotheses exist to explain the observed split in the association between the type of T lymphocyte-mediated reaction and CD. CD is frequently associated to other Th1/Th17-IMD [13–15], such as type 1 diabetes mellitus [16], autoimmune thyroiditis [17], rheumatoid arthritis [18], psoriasis [19], multiple sclerosis [20]. More recently, several studies have also shown an association between CD and some Th2-mediated disorders [15]: allergies and asthma, eczema, rhinitis [21], urticaria [22], Grave's disease [17], Sjogren syndrome [23], lichen planus [24], systemic lupus erythematosus [25].

To date, a gluten free diet (GFD) is considered the only treatment for CD. Numerous papers have investigated the effects of CD therapy on the incidence and prognosis of coexisting or subsequent IMD but thus far they have reported contradictory results [26–36]. The aims of our study were: (1) to establish the prevalence of IMD at the time and after CD diagnosis in a large sample of adult individuals; (2) to identify any possible changes in immune response after commencement of GFD, in particular with regard to shifts from Th1/Th17- to Th2-immune response or vice versa; and (3) to investigate the potential role of GFD in reducing and/or preventing IMD in adult CD patients.

2. Methods

Between September 2011 and February 2015 we carried out a prospective study including all consecutive adult CD patients (age > 18 years) followed up at our Gastrointestinal Unit (Tertiary Centre for Food Intolerance and CD, "Federico II" University, Naples, Italy). In accordance with current guidelines, CD diagnosis was made in the presence of Marsh ≥ 2 histology associated with both anti-tissue Transglutaminase (a-tTG) IgA > 7 U/mL and positive anti-endomysial (EMA) antibodies [11]. All subjects with positive serology but negative histology (Marsh 0 or 1) underwent further investigations for genetic susceptibility and were diagnosed as "potential" CD in the presence of HLA DQ2/DQ8. The main demographic, clinical, serological, endoscopic and histological features were recorded for all CD patients. All patients were investigated for the presence of Th1/Th17 and/or Th2-IMD at the time of CD diagnosis. The diagnosis of pre-existent and new IMD was made by the evaluation of medical records with the confirmation of a specialist visit (i.e. rheumatologist, dermatologist, immunologist) and laboratory tests. The classification of Th1/Th17 and Th2-IMD was made in line with current guidance [13–15].

Information on the presence/absence of any allergic, atopic, or autoimmune disease preceding CD diagnosis was gathered at the time of the first consultation and during the study period each study participant underwent clinical examination and laboratory tests at least once a year.

In particular, we assessed the clinical and biochemical presence of: vitiligo, allergic rhinitis, ankylosing spondylitis, Sjogren syndrome, multiple sclerosis, scleroderma, nephrotic syndrome, ulcerative colitis, psoriasis, polymyalgia rheumatica, urticaria, IgA nephropathy, lichen planus, systemic lupus erythematosus, type 1 diabetes mellitus (or latent autoimmune diabetes in adults), Hashimoto's thyroiditis, eczema, atopic dermatitis, primary sclerosing cholangitis, Crohn's disease, allergic conjunctivitis, primary biliary cirrhosis, Grave's disease, asthma, alopecia, rheumatoid arthritis and other allergies. Table 1 summarizes the main Th1-, Th17- and Th2-IMD. The search for Th1/Th17 and/or Th2-IMD was reassessed after starting GFD in a 5-years follow-up period.

Table 1
Classification of main Th1/Th17 and Th-2 mediated disorders.

Th1/Th17-mediated diseases	Th2-mediated diseases
Coeliac disease	IgE related-allergies
Vitiligo	Sjogren syndrome
Ankylosing spondylitis	Allergic conjunctivitis
Multiple sclerosis	Atopic dermatitis
Nephrotic syndrome	Grave's disease
Crohn's disease	Ulcerative colitis
Psoriasis	Asthma
Polymyalgia rheumatic	Urticaria
Alopecia	Eczema
IgA nephropathy	Scleroderma
Type 1 diabetes mellitus	Systemic lupus erythematosus
Hashimoto's thyroiditis	Allergic rhinitis
Rheumatoid arthritis	Lichen planus
Primary biliary cirrhosis	Other allergies
Primary sclerosing cholangitis	

2.1. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS software v.15.0, Chicago IL, United States) for Windows. The descriptive statistics included determination of mean values and standard deviation (SD) of the continuous variables, and the percentages and proportions of the categorical variables. The chi-square (χ^2) test was used to assess the comparison of categorical variables, while the Mann–Whitney *U* test was used for unpaired data. Analysis of variance (ANOVA) was performed with and without adjustment for covariates. The odd ratio (OR) for quantifying the statistical difference between the dichotomous variables was also calculated. All results were considered statistically significant when p value < 0.05.

3. Results

During the study period, 1255 CD patients referred to our Centre were enrolled (males/females 258/997). At the time of CD diagnosis, mean age + SD was 28.1 + 15.7; mean a-tTG serum level was 98.7 + 108.2 U/mL; all patients were positive for EMA. Histological exam showed a Marsh 1 grade in 64 patients (5%), Marsh 2 grade in 50 (4%), Marsh 3A in 171 (13.7%), Marsh 3B in 311 (24.8%), and Marsh 3C in 659 (52.5%). Main patient characteristics and diagnostic results are reported in Table 2.

Data collected through medical history showed that 257 patients out of 1255 (20.5%) suffered from at least one immunological/allergic IMD before the diagnosis of CD. Of these, 150 (58.4%) suffered from Th1/Th17-mediated and 107 (41.6%) from

Table 2
Main patient characteristics and diagnostic results of 1255 coeliac patients.

		Number (%)
Demographic data	Male	258 (20.6)
	Female	997 (79.4)
	Mean age ± SD	28.1 ± 15.7
Symptoms (in accordance with Oslo) [11]	Classical CD	454 (36.2)
	Non Classical CD	627 (49.9)
	Asymptomatic CD	110 (8.7)
	Potential CD	64 (5)
Risk factors for CD	Down Syndrome	8 (0.6)
	Familial history of CD	406 (32.3)
Laboratory	a-tTG (U/mL)	98.7 ± 108.2
	EMA positive	100%
Histology	Marsh 1	64 (5)
	Marsh 2	50 (4)
	Marsh 3A	171 (13.7)
	Marsh 3B	311 (24.8)
	Marsh 3C	659 (52.5)