Digestive and Liver Disease 48 (2016) 745–752

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Alimentary Tract

Innate and adaptive immunity in self-reported nonceliac gluten sensitivity *versus* celiac disease

Antonio Di Sabatino[a,*,1], Paolo Giuffrida[a,1], Giulia Fornasa[d], Chiara Salvatore[a], Alessandro Vanoli[b], Samuele Naviglio[e], Luigina De Leo[f], Alessandra Pasini[a], Mara De Amici[c], Costanza Alvisi[a], Tarcisio Not[e,f], Maria Rescigno[d], Gino Roberto Corazza[a]

[a] *Department of Internal Medicine, San Matteo Hospital, University of Pavia, Pavia, Italy*
[b] *Department of Molecular Medicine, San Matteo Hospital, University of Pavia, Pavia, Italy*
[c] *Department of Pediatrics, San Matteo Hospital, University of Pavia, Pavia, Italy*
[d] *Department of Experimental Oncology, European Institute of Oncology, Milan, Italy*
[e] *Department of Medicine, Surgery, and Health Sciences, University of Trieste, Trieste, Italy*
[f] *Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy*

ARTICLE INFO

Article history:
Received 13 December 2015
Accepted 30 March 2016
Available online 7 April 2016

Keywords:
Chemokine
Cytokine
Duodenal mucosa
Tissue transglutaminase

ABSTRACT

Background: Immune mechanisms have been implicated in nonceliac gluten sensitivity (NCGS), a condition characterized by intestinal and/or extraintestinal symptoms caused by the ingestion of gluten in non-celiac/non-wheat allergic individuals.
Aims: We investigated innate and adaptive immunity in self-reported NCGS *versus* celiac disease (CD).
Methods: In the supernatants of *ex vivo*-cultured duodenal biopsies from 14 self-reported NCGS patients, 9 untreated and 10 treated CD patients, and 12 controls we detected innate cytokines – interleukin (IL)-15, tumor necrosis factor-α, IL-1β, IL-6, IL-12p70, IL-23, IL-27, IL-32α, thymic stromal lymphopoietin (TSLP), IFN-α-, adaptive cytokines – interferon (IFN)-γ, IL-17A, IL-4, IL-5, IL-10, IL-13-, chemokines – IL-8, CCL1, CCL2, CCL3, CCL4, CCL5, CXCL1, CXCL10-, granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF).
Results: Mucosal innate and adaptive cytokines, chemokines and growth factors did not differ between self-reported NCGS, treated CD and controls. On the contrary, IL-6, IL-15, IL-27, IFN-α, IFN-γ, IL-17A, IL-23, G-CSF, GM-CSF, IL-8, CCL1 and CCL4 were significantly higher in untreated CD than in self-reported NCGS, treated CD and controls, while TSLP was significantly lower in untreated CD than in self-reported NCGS, treated CD and controls.
Conclusion: In our hands, patients with self-reported NCGS showed no abnormalities of the mucosal immune response.

1. Introduction

Nonceliac gluten sensitivity (NCGS) is a clinical entity characterized by intestinal and/or extraintestinal symptoms due to the ingestion of gluten-containing foods in non-celiac/non-wheat allergic patients [1–3]. Unlike celiac disease (CD), which is induced in genetically susceptible individuals by both T helper cell type (Th)1/Th17-mediated adaptive[4,5] and innate immune mechanisms [6,7], NCGS has been presumed to be exclusively caused by abnormalities of the mucosal innate immune response (reviewed in Ref. [8]). The latter evidence was obtained in patients who did not undergo an oral double-blind, placebo-controlled gluten challenge test, and the concept of self-reported NCGS has been gaining international credibility [9,10]. Clinical features of patients who believe themselves to be gluten sensitive have been widely described in *ad hoc* studies [11,12].

On this basis, we aimed to perform a more in-depth investigation of a number of mucosal cytokines and chemokines, which are

* Corresponding author at: Clinica Medica I, Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Piazzale Golgi 19, 27100 Pavia, Italy. Tel.: +39 0382 501596; fax: +39 0382 502618.
E-mail address: a.disabatino@smatteo.pv.it (A. Di Sabatino).
[1] These authors contributed equally to this paper and should be considered joint first authors.

http://dx.doi.org/10.1016/j.dld.2016.03.024
1590-8658/

known to drive either the innate or the adaptive immune response in chronic intestinal inflammation, by detecting their levels in 24 h-cultured duodenal biopsy samples collected from patients with self-reported NCGS in comparison to those from CD patients.

2. Materials and methods

2.1. Patients and tissues

Well-oriented endoscopic biopsy specimens were collected from the second part of the duodenum of 14 subjects (mean age 37.5 years, range 20–62) referred to our Center because they were affected by self-reported NCGS, that is they complained of intestinal and/or extraintestinal symptoms which they themselves believed to be caused by gluten-containing food (Table 1). All 14 subjects had been under gluten-containing diet at the time of biopsy collection for at least two months. CD was ruled out on the basis of negative serum anti-endomysial and anti-tissue transglutaminase (tTG) antibodies and demonstration of normal duodenal histology, while they were under gluten-containing diet, whereas wheat allergy was excluded on the basis of negative serum specific IgE for wheat. Biopsies were also taken from nine consecutive patients with untreated CD (mean age 35.0 years, range 18–54), used as positive controls, and ten consecutive patients with CD after at least 12 months of gluten-free diet (GFD) (mean age 34.2 years, range 18–77), and from 12 control individuals (mean age 56.3 years, range 32–76) undergoing endoscopy for functional dyspepsia. All the control subjects did not complain of any intestinal and/or extraintestinal symptom related to the ingestion of gluten-containing foods, and they were all negative for anti-endomysial and anti-tTG antibodies and with normal histology. CD diagnosis was based on the positivity of serum anti-endomysial and anti-tTG antibodies associated with typical histopathological lesions, namely villous atrophy, increased intraepithelial lymphocyte (IEL) infiltration and crypt hyperplasia [13]. Among the nine untreated CD patients, seven showed a B2 lesion and two showed a B1 lesion [14]. Histological improvement was documented in all treated CD patients. After the perendoscopic collection of duodenal biopsies, 11 out of the 14 patients with self-reported NCGS underwent an oral double-blind, placebo-controlled, cross-over gluten challenge trial (for trial description, see Ref. [15]). Briefly, these patients belonged to a cohort of 61 patients randomly assigned to two groups given a 1-week treatment with 4.375 g/day of purified wheat gluten or rice starch (placebo), both administered *via* gastrosoluble capsules. Gluten capsules were free of fermentable, oligo-, di- and monosaccharides and polyols. After a 1-week wash-out period, participants crossed over to another week of placebo or gluten treatment, respectively. During the trial, patients filled in a daily questionnaire in order to evaluate a rating scale of both intestinal and extraintestinal symptoms. Hence, for each of these 11 patients with self-reported NCGS we calculated the overall (intestinal *plus* extraintestinal) score under gluten and the *delta* overall score, obtained by subtracting the weekly overall score under placebo from the weekly overall score under gluten. All the subjects included in the study were tested to rule out gastrointestinal infections, and they were not taking any kind of medication. Biopsies were processed for routine histology or organ culture. Each patient who took part in the study gave informed consent, and Ethics Committee approval was obtained in all cases.

2.2. Duodenal histology

Sections were taken from paraffin blocks of biopsies and stained with hematoxylin–eosin and with immunoperoxidase using anti-CD3 antibody (1:100 dilution, clone PS1, Novocastra, Newcastle,

Table 1
Clinical data of the 14 patients with self-reported nonceliac gluten sensitivity.

At baseline									Double-blind, placebo-controlled, cross-over trial		
Pt	Age	Sex	Duration of symptoms (months)	Previous self-prescribed GFD	Family history of CD	HLA-DQ2/DQ8	Serum IgG AGA	Anti-tTG2 IgA antibody deposits	IEL (%)	Overall score under gluten	Overall score under placebo	*Delta* overall score[a]
1	43	F	42	Yes	No	Negative	Negative	NA	24	15	20	−5
2	43	F	84	Yes	No	Negative	Negative	Absent	23	64	29	+35
3	47	F	14	Yes	No	Positive	Negative	Absent	34	56	85	−29
4	49	F	25	Yes	No	Negative	Positive	Absent	27	71	16	+55
5	42	F	15	Yes	No	Negative	Negative	Absent	45	107	65	+42
6	52	F	4	No	No	Positive	Negative	Absent	15	86	69	+17
7	20	F	38	Yes	No	Negative	Negative	Absent	38	NR	NR	NR
8	31	F	3	No	No	Positive	Negative	Absent	28	24	49	−25
9	37	F	12	No	No	Negative	Negative	Absent	30	18	8	+10
10	53	F	8	No	No	Negative	Negative	Absent	14	NR	NR	NR
11	21	F	33	No	No	Negative	Negative	Absent	21	58	154	−96
12	33	F	84	Yes	No	Negative	Negative	Absent	18	NR	NR	NR
13	22	F	11	Yes	Yes	Positive	Negative	NA	21	37	18	+19
14	62	M	8	No	No	Negative	Negative	NA	40	24	15	+9

[a] *Delta* overall score is calculated by subtracting the weekly overall score under placebo from the weekly overall score under gluten. AGA, anti-gliadin antibodies; CD, celiac disease; F, female; GFD, gluten-free diet; HLA, human leukocyte antigen; IEL, intraepithelial lymphocyte; M, male; NA, not available; NR, not randomized in the trial; Pt; patient; tTG, tissue transglutaminase.