Immunol Res
DOI 10.1007/s12026-013-8420-0

DIAGNOSIS OF AUTOIMMUNITY

A subset of anti-rotavirus antibodies directed against the viral protein VP7 predicts the onset of celiac disease and induces typical features of the disease in the intestinal epithelial cell line T84

Marzia Dolcino · Giovanna Zanoni · Caterina Bason · Elisa Tinazzi · Elisa Boccola · Enrico Valletta · Giovanna Contreas · Claudio Lunardi · Antonio Puccetti

Claudio Lunardi

Abstract Celiac disease (CD) is an autoimmune disorder of the small intestine triggered by environmental factors in genetically predisposed individuals. A strong association between type 1 diabetes (T1DM) and CD has been reported. We have previously shown that rotavirus infection may be involved in the pathogenesis of CD through a mechanism of molecular mimicry. Indeed, we identified a subset of anti-transglutaminase IgA antibodies that recognize the rotavirus viral protein VP7. In this study, we aimed at evaluating whether such antibodies may predict the onset of CD in children affected by T1DM. Moreover, to further analyze the link between rotavirus infection and pathogenesis of CD, we analyzed the effect of anti-rotavirus VP7 antibodies on T84 intestinal epithelial cells using the gene-array technique, complemented by the analysis of molecules secreted in the supernatant of stimulated cells. We found that anti-rotavirus VP7 antibodies are present in the vast majority (81 %) of T1DM-CD tested sera, but are detectable also in a fraction (27 %) of T1DM children without CD. Moreover, we found that anti-rotavirus VP7 antibodies are present before the CD onset, preceding the detection of anti-tTG and anti-endomysium antibodies. The gene-array analysis showed that purified anti-rotavirus VP7 antibodies modulate genes that are involved in apoptosis, inflammation, and alteration of the epithelial barrier integrity in intestinal epithelial cells, all typical features of CD. Taken together, these new data further support the involvement of rotavirus infection in the pathogenesis of CD and suggest a predictive role of anti-rotavirus VP7 antibodies.

Keywords Celiac disease · Autoantibodies · Rotavirus · Gene expression

Electronic supplementary material The online version of this article (doi:10.1007/s12026-013-8420-0) contains supplementary material, which is available to authorized users.

M. Dolcino · A. Puccetti
Institute Giannina Gaslini, Genoa, Italy

G. Zanoni · E. Boccola
Department of Pathology and Diagnostics, Section of Immunology, University of Verona, Verona, Italy

C. Bason · E. Tinazzi · C. Lunardi (✉)
Department of Medicine, Unit of Autoimmune Diseases, University of Verona, Verona, Italy
e-mail: claudio.lunardi@univr.it

E. Valletta · G. Contreas
Department of Pediatrics, University of Verona, Verona, Italy

A. Puccetti
Department of Experimental Medicine, Section of Histology, University of Genoa, Genoa, Italy

Introduction

Celiac disease (CD) is an inflammatory disorder of the small intestine characterized by a permanent intolerance to gluten-derived peptides that may affect as many as 1–3 % of the European and North American populations [1].

CD is a multifactorial disorder and both genetic and environmental factors are required for the disease phenotype to develop [2]. Among environmental factors, infections were thought to play a role in the etiopathogenesis of the disease; however, a direct link was still missing; in our attempt to clarify some aspects of the disease pathogenesis, we used a random peptide library approach that we had previously successfully applied to the study of other autoimmune diseases [3–6].

By means of this strategy, we were able to identify a peptide (called celiac peptide) recognized by serum

Published online: 10 April 2013

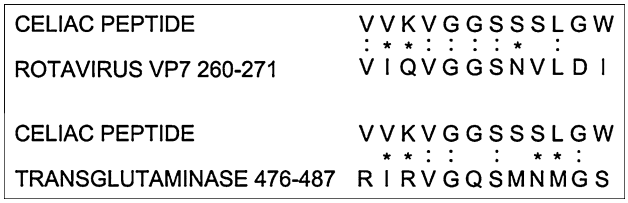

Fig. 1 Sequence homology between the celiac peptide and the rotavirus VP7 protein and between the celiac peptide and tissue transglutaminase. The peptide sequence was compared using the BLASTP via the NCBI BLAST network service (: indicates identity and asterisk indicates conservative substitutions)

immunoglobulins of patients with active disease [7]; this peptide shares homology with the rotavirus major neutralizing protein VP7 and with the self-antigen tissue transglutaminase (tTG) (Fig. 1). Surprisingly, antibodies directed against the celiac peptide, purified from the sera of patients with active disease, recognized rotavirus major neutralizing protein VP7 and the self-antigen tTG. We also demonstrated that in active CD, a subset of anti-transglutaminase IgA antibodies recognize the viral protein VP7, suggesting a possible involvement of rotavirus infection in the pathogenesis of the disease and thus identifying a potential link between rotavirus infection and the onset of CD: these antibodies seem to be a hallmark of active disease and have never been detected in healthy controls. Interestingly, it has been observed that rotavirus can induce the same mucosal villous atrophy that is induced by gluten in CD in small intestine [8].

The link between rotavirus infection and the risk to develop CD was supported by an epidemiological study conducted in a large population of children carrying the risk allele for CD [9]. The association between Type 1 diabetes (T1DM) and CD is frequent, and the two diseases share a common genetic background that may be explained by the combined presence of HLA-DQ transdimer termed HLA-DQ8 *trans* [10].

It has been shown that the prevalence of CD among T1DM children is 5–10 times higher than in the general population [11] ranging from 3 to 16 %, as described by Volta et al. [12].

In the present study, we aimed at further analyzing the relationship between rotavirus infection and the pathogenesis of CD using two different approaches: firstly, given the high prevalence of CD in children with T1DM [13, 14], we tested the sera of a group of children affected by T1DM, with or without CD, for the presence of anti-VP7 peptide antibodies. The sera of some of T1DM patients were available for the detection of antiviral antibodies, before and after the onset of CD.

Secondly, we used a gene-array technique to analyze the effects of purified anti-rotavirus VP7 peptide antibodies on intestinal epithelial cells T84 that we have used in our previous work [7] and that are commonly used to study the intestinal barrier function.

The results obtained indicate that anti-VP7 antibodies may have an important role in predicting the onset of CD and that such antibodies modulate clusters of genes typically involved in the pathogenesis of celiac disease, further confirming the link between rotavirus infection and CD.

Materials and methods

Patients

Three hundred and fifty-seven children affected by T1DM have been evaluated prospectively. During the follow-up, 32 of them developed CD diagnosed on the basis of the presence of anti-tTG and anti-endomysium (EMA) antibodies, detected by commercially available ELISA kits (EliA Celikey kit, Phadia, Uppsala, Sweden and Antiendomysium, Eurospital kit, Trieste, Italy, respectively) and by mucosal biopsy [15]. The presence of anti-VP7 antibodies was assessed in 26 of the 32 T1DM-CD patients and was compared with a group of 37 T1DM children who did not develop CD.

Sera from 8 of the T1DM-CD patients (Online Resource 1) were available before and after the onset of CD and were used for the detection of anti-VP7 rotavirus peptide, anti-celiac peptide, and anti-tTG (476-487) peptide IgA antibodies.

The time interval between the blood collection and the CD onset ranged from 6 to 120 months.

To perform the gene-array analysis, anti-VP7 peptide antibodies were isolated from sera of 20 patients with active disease, 12 males and 8 females, aged 1–38 (mean = 16) years, at diagnosis of biopsy confirmed CD, as previously described [7].

Briefly, the patients with CD included in the study showed no evidence of other autoimmune diseases; all the patients had serum anti-tTG and EMA IgA antibodies before gluten-free diet (GFD). The duodenal histological damage at diagnosis ranged from grade 3a to 3c, according to Marsh's classification [16].

Peptide synthesis

The rotavirus VP-7 peptide (VIQVGGSNVLDI), the celiac peptide (VVKGGSSSLGW), the tTG peptide (RIRVGQSMNMGS), and the irrelevant control peptide (VTLPKDSDVELP) were manually synthesized using the standard method of solid-phase peptide synthesis, which follows the 9-fluorenylmethoxycarbonyl (FMOC) strategy with minor modifications [17].

All the synthesized compounds were purified by reverse-phase high-performance liquid chromatography (RP-HPLC) and the molecular weights were finally confirmed by electrospray mass spectrometry.

Cell culture

T84 cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were grown with media containing a 1:1 mixture of Ham's F-12 nutrient mixture and Dulbecco's modified Eagle's medium supplemented with 6 % heat-inactivated fetal bovine serum, 15 mmol L^{-1}*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES), 14.3 mmol L^{-1} $NaHCO_3$, and antibiotics (penicillin and streptomycin)–antimycotics (pH 7.4). Cells were passaged weekly on reaching confluence. For experiments, cells were plated onto collagen-coated permeable supports, where they were fed every 3 days and used from days 7–14 (within fifth passage).

Cell monolayers were stimulated with antibodies affinity purified against the VP7 rotavirus peptide or an irrelevant control peptide (20 μg/ml) for 3 and 6 h. Cell pellets were used for gene-array experiments, while soluble mediators were measured in the cell supernatants.

Detection of antibodies anti-VP7, anti-celiac and anti-transglutaminase peptides

The ELISA test for antibody binding to the synthetic peptides has already been described elsewhere with minor modifications [3].

Briefly, the synthetic peptides were used at a concentration of 20 μg/ml in PBS to coat polystyrene plates (Immulon 2HB, Thermo). Plates were then blocked for 1 h with PBS 3 % BSA. Serum samples were diluted 1:100 in diluting buffer (PBS 1 % BSA) and incubated overnight at 4 °C; plates were then washed twice with PBS/Tween and twice with PBS.

The secondary reagent, alkaline phosphatase-conjugate anti-human IgA antiserum (1:1,000 in diluting buffer; Sigma), was incubated for 3 h at room temperature; plates were then washed as described. The alkaline phosphatase substrate (Sigma) was then added to the wells and the plates read after 30 min or 1 h at a spectrophotometer set at 405 nm.

For the detection of anti-rotavirus VP7 peptide, anti-celiac peptide, and anti-tTG peptide IgA antibodies, we considered positive only the sera whose OD readings were higher than 0.160, 0.105, and 0.110, respectively. OD values higher than the mean plus three standard deviations (SD) of each serum dilution of the control group were considered positive.

Affinity purification of anti-peptide antibodies

Synthetic peptides (5 mg peptide per gram of dried Sepharose powder) were coupled to Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Serum Igs diluted in PBS were applied to the columns. The columns were washed with PBS. Bound Igs were eluted with 0.1 M glycine (pH 2.5) and dialyzed against PBS. The purity of the preparations was assessed by SDS-PAGE followed by silver staining; sometimes, when albumin contamination was present in the purified preparation, an additional step was added using an Albumin Removal Kit (Pierce). Antibody concentration matching was performed using a densitometric analysis of a silver stain of the purified material.

Preparation of cRNA and array analysis

Preparation of cRNA, hybridization, and scanning of probe arrays were performed according to the protocols of the manufacturer (Affymetrix, Santa Clara, CA, USA) by IFOM-IEO Campus for Oncogenomics, Affymetrix Microarray Unit (Milano, Italy) using the Human Genome U133A 2.0 GeneChip (Affymetrix). This is a single array representing 14,500 well-characterized human genes and including more than 22,000 probe sets and 500, 000 distinct oligonucleotide features.

The different gene expression patterns were analyzed using Array Assist version 5.0 (Stratagene, La Jolla, CA, USA) which calculated background-adjusted, normalized, and log-transformed intensity values applying the PLIER algorithm [18]. The PLIER method uses quantile normalization and runs an optimization procedure which determines the best set of weights on the perfect match and mismatch for each probe pair. Finally, the normalized background-corrected data were transformed to the $\log_2$ scale. A signal $\log_2$ ratio of 1.0 indicates an increase in the transcript level by twofold change (2 F.C.) and −1.0 indicates a decrease by twofold (−2 F.C.). A signal $\log_2$ ratio of zero would indicate no change. We analyzed the gene expression profiles in T84 cells incubated with anti-VP7 peptide antibodies (test samples) or with anti-irrelevant peptide antibodies (control samples) at a concentration of 20 μg/ml, for 3 and 6 h. Genes were selected for final consideration when their expression was at least 1.5-fold different in the test sample versus control sample at each one time point.

Selected genes were submitted to a functional classification according to the Gene Ontology (GO) annotations (http://www.geneontology.org/).

Analysis of soluble mediators released in the supernatant of T84 cells

Aliquots of the supernatant of the T84 cell cultures used to perform the gene-array analysis, treated with affinity purified anti-VP7 antibodies or with affinity purified anti-control peptide antibodies, were filtered and kept frozen at −80 °C until assayed.

The presence of soluble mediators released in the supernatants was analyzed by BIOCLARMA (Turin, Italy) using Bio-Plex Human Cytokine Plex Panel (IL-1beta, IL-4, IL-6, IL-8, IL-13, CCL11/eotaxin, FGF2, G-CSF/CSF3, GM-CSF/CSF2, CXCL10/IP-10, and PDGF-B).

Statistical analysis

The Mann–Whitney U test was performed for comparison of the anti-VP7 rotavirus peptide antibodies detection between the T1DM and the T1DM-CD groups.

Student's t-test for independent paired data was used to compare the levels of molecules in the supernatant of cells incubated with antiviral peptide antibodies or with antibodies directed against an irrelevant peptide.

Results

Detection of anti-VP7 rotavirus, anti-celiac and anti-transglutaminase peptides antibodies

Sera from 26 of the 32 T1DM-CD patients were tested in ELISA assay for the presence of anti-VP7 rotavirus peptide antibodies; results were compared with 37 T1DM patients.

Anti-VP7 rotavirus peptide antibodies were present in 21 out of 26 (81 %) T1DM-CD patients and in 10 out of 37 (27 %) T1DM patients. Figure 2 shows that the levels of such antibodies, as detected by OD, are significantly different in the two populations ($p < 0.0001$).

We tested 8 sera of T1DM-CD patients before and after the CD onset for the presence of anti-VP7 rotavirus peptide, anti-celiac peptide, and anti-tTG (aa 476–487) peptide IgA antibodies. As shown in Table 1, six out of eight patients (patients 1, 2, 4, 5, 7, and 8) were positive for anti-VP7 rotavirus and anti-celiac peptides antibodies months before the CD onset, when the usual serological screening test was still negative. All these six patients maintained the positivity to both peptides after the onset of the disease.

Of the remaining two cases, serum from patient 3 remained negative for the presence of anti-celiac and VP7 peptides antibodies in the two samples, whereas serum from patient 6 became positive for the presence of the anti-VP7 rotavirus peptide antibodies after the onset of CD.

Among the eight patients, only three (patients 1, 2, and 8) showed a high response to the tTG peptide before the onset of the disease and before the appearance of anti-tTG and EMA positivity at the screening test; other three patients (patients 3, 4, and 5) showed a borderline response and two patients (patients 6 and 7) did not show any recognition of this peptide.

Finally, apart from patient 6, all the other 7 patients resulted positive for the presence of anti-tTG peptide antibodies after the onset of CD.

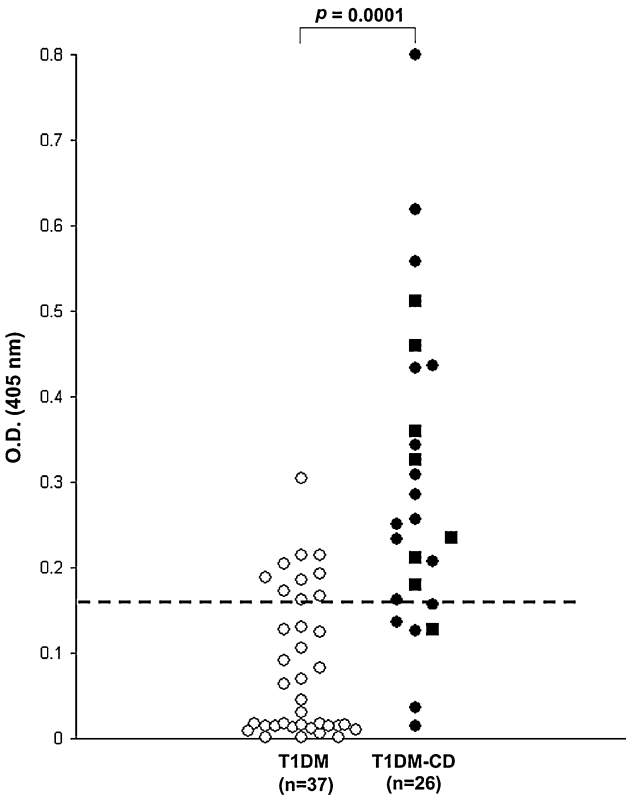

Fig. 2 Levels of anti-VP7 rotavirus peptide IgA antibodies tested by ELISA in sera of T1DM patients with or without CD. *Square dots*: indicate the 8 patients with T1DM who developed CD analyzed before and after the onset of CD. Here, the O.D. values obtained after the CD onset are reported. *Black line*: threshold for positivity (O.D. 0.160). *p* value is calculated by Mann–Whitney *U* test

Gene-array analysis

We analyzed the gene expression profiles in T84 intestinal cells treated with antibodies affinity purified against the rotavirus VP7 peptide in order to identify clusters of genes involved in the pathogenesis of intestinal damage in CD.

Indeed, the treatment of T84 cells with purified anti-rotavirus VP7 peptide antibodies had a profound impact on the gene expression pattern: such antibodies up-regulated (fold change ≥ 1.5) 1,574 and 1,506 probe sets after 3 and 6 h of incubation, respectively. Moreover, 3,279 probe sets after 3 h and 1,641 probe sets after 6 h were down-regulated (fold change ≤ -1.5). The complete list of the modulated genes (Online Resource 2) is deposited in the "ArrayExpress Archive database of functional genomics experiments" at http://www.ebi.ac.uk/arrayexpress/.

The distribution of selected genes signal intensity after 3 and 6 h of treatment is shown by scatter plot graphs in Fig. 3.

The Gene Ontology analysis of the corresponding regulated transcripts showed that the vast majority of them are

Table 1 Levels of anti-VP7, anti-celiac, and anti-transglutaminase peptide IgA antibodies tested by ELISA in sera of patients with celiac disease before and after the disease onset

Patients	Months before the onset	Before the disease onset [a]			After the disease onset		
		Celiac peptide	VP7 peptide	tTG peptide	Celiac peptide	VP7 peptide	tTG peptide
Patient 1	6	0.123	0.517	0.191	0.126	0.511	0.225
Patient 2	28	0.226	0.350	0.173	0.234	0.360	0.284
Patient 3	14	0.097	0.103	0.101	0.102	0.128	0.140
Patient 4	120	0.104	0.244	0.101	0.129	0.212	0.149
Patient 5	72	0.111	0.340	0.109	0.205	0.460	0.160
Patient 6	16	0.070	0.016	0.031	0.058	0.179	0.025
Patient 7	60	0.114	0.215	0.084	0.228	0.326	0.119
Patient 8	16	0.278	0.267	0.258	0.276	0.235	0.192

[a] Before the disease onset: anti-tTG and EMA negativity at the screening test

involved in several biological processes that may play a role in the pathogenesis of CD, including regulation of apoptosis, inflammatory and immune response, cell proliferation, cell differentiation, cell junctions, matrix metalloproteases, receptors and signal transducers, cytoskeleton components, ion transport and exchange, metabolism, EGF pathway.

Table 2 shows an overview of selected genes within the above-mentioned clusters.

Increased apoptosis is considered the main cause of villous atrophy [19]. Several pro-apoptotic transcripts were indeed up-regulated by the incubation of T84 cells with anti-VP7 antibodies, including caspase recruitment domain family, member 10, CARD10; suppressor of cytokine signaling 3, SOCS3; TIMP metallopeptidase inhibitor 3, TIMP3; caspase 10, apoptosis-related cysteine peptidase, CASP10; amyloid beta precursor protein-binding, family B, member 2, APBB2 and v-fos FBJ murine osteosarcoma viral oncogene homolog, FOS.

Moreover, various anti-apoptotic genes were strongly down-regulated such as the homeodomain interacting protein kinase 3, HIPK3; optic atrophy 1, OPA1; spindlin family, member 2, SPIN2, and Cas-Br-M ecotropic retroviral transforming sequence b, CBLB.

It has been proposed that an altered differentiation of epithelial cells in the small intestine plays a role in the generation of celiac lesions [19]. Several genes responsible for differentiation mechanisms were down-regulated by the treatment with anti-VP7 peptide antibodies including: meprin A, beta (MEP1B), and v-myb myeloblastosis viral oncogene homolog (MYB).

These molecules play an active role in the intestinal epithelial development and crypt formation: at the early stage, the expression of MEP1B in intestinal brush border membranes directs epithelial differentiation and cell migration and then MYB is required for fully functional normal crypt formation, while in its absence, columnar/enterocytes cells are reduced [20].

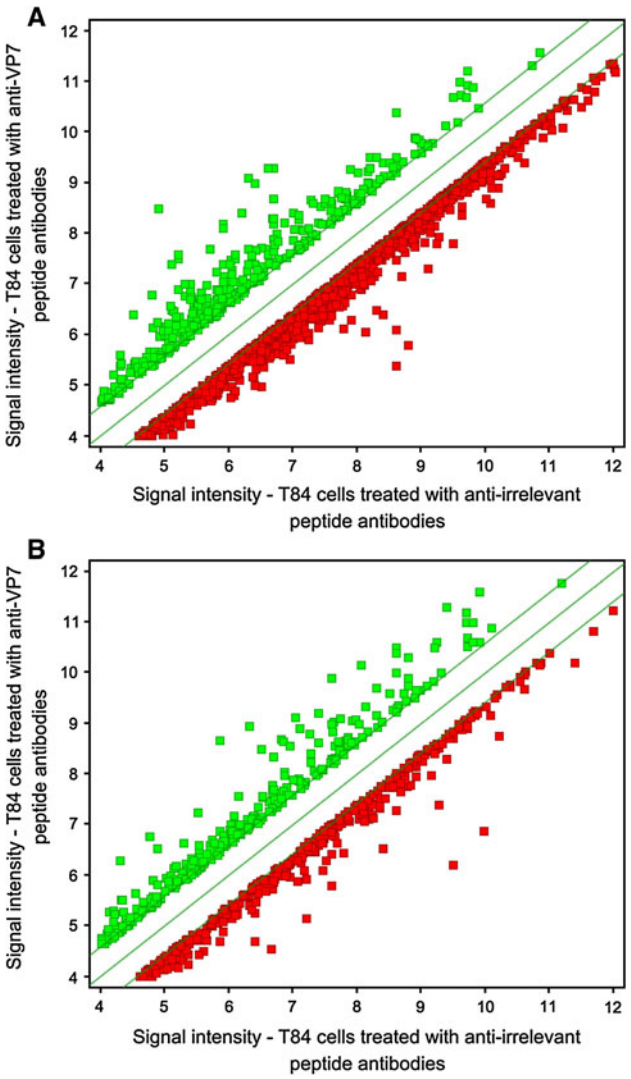

Fig. 3 Comparative scatter plot of normalized relative signal intensity ($\log_{10}$) between T84 cells treated with anti-VP7 peptide antibodies versus T84 cells treated with anti-irrelevant peptide antibodies. A: after 3 h, B: after 6 h of incubation. *Green squares*: up-regulated genes; *red squares*: down-regulated genes

Table 2 Gene expression in T84 cells after 3 and 6 h of stimulation with anti-VP7 rotavirus peptide antibodies

Probe set ID	Accession number	Gene title	Gene symbol	FC (3 h)	FC (6 h)
Apoptosis					
210025_s_at	AY028896	Caspase recruitment domain family, member 10	CARD10	4.74	3.54
206359_at	NM_003955	Suppressor of cytokine signaling 3	SOCS3	2.75	2.15
201148_s_at	NM_000362	TIMP metallopeptidase inhibitor 3	TIMP3	2.12	1.64
211888_x_at	AF111345	Caspase 10, apoptosis-related cysteine peptidase	CASP10	2.57	NC[a]
216747_at	U62325	Amyloid beta precursor protein-binding, family B, member 2	APBB2	3.09	1.60
209189_at	BC004490	v-fos FBJ murine osteosarcoma viral oncogene homolog	FOS	2.60	3.47
213697_at	NM_005734	Homeodomain interacting protein kinase 3	HIPK3	−2.08	−1.98
216287_at	AB011139	Optic atrophy 1 (autosomal dominant)	OPA1	−8.05	−3.42
207841_at	NM_019003	Spindlin family, member 2	SPIN2	−2.15	−1.92
208348_s_at	U26710	Cas-Br-M ecotropic retroviral transforming sequence b	CBLB	−9.29	−8.63
Inflammatory and immune response					
205082_s_at	AB046692	Aldehyde oxidase 1	AOX1	2.11	2.51
204748_at	NM_000963	Prostaglandin-endoperoxide synthase 2	PTGS2	1.76	2.26
202992_at	NM_000587	Complement component 7	C7	3.11	3.04
205098_at	NM_001295	Chemokine (C–C motif) receptor 1	CCR1	3.27	4.34
208304_at	NM_001837	Chemokine (C–C motif) receptor 3	CCR3	2.80	3.47
206380_s_at	NM_002621	Complement factor properdin	CFP	2.92	1.76
208548_at	NM_021002	Interferon, alpha 6	IFNA6	NC[a]	2.08
207932_at	NM_002170	Interferon, alpha 8	IFNA8	2.23	1.53
206569_at	NM_006850	Interleukin 24	IL24	2.19	1.65
217112_at	NM_002608	Platelet-derived growth factor beta	PDGFB	1.68	4.01
207442_at	NM_000759	Colony stimulating factor 3 (granulocyte)	CSF3	NC[a]	1.50
39402_at	M15330	Interleukin 1, beta	IL1B	1.80	1.50
207538_at	NM_000589	Interleukin 4	IL4	2.00	1.72
205207_at	NM_000600	Interleukin 6 (interferon, beta 2)	IL6	NC[a]	1.51
202859_x_at	NM_000584	Interleukin 8	IL8	1.86	1.62
204533_at	NM_001565	Chemokine (C–X–C motif) ligand 10	CXCL10/IP10	NC[a]	1.82
210133_at	D49372	Chemokine (C–C motif) ligand 11	CCL11	1.96	NC[a]
215078_at	BT006967	Superoxide dismutase 2, mitochondrial	SOD2	−2.06	NC[a]
209341_s_at	AF080158	Inhibitor of kappa light polypeptide gene enhancer kinase beta	IKBKB	−2.25	−1.78
207433_at	NM_000572	Interleukin 10	IL10	−3.56	−2.15
221470_s_at	NM_014439	Interleukin 1 family, member 7 (zeta)	IL1F7	−2.26	−1.67
Cell proliferation					
210237_at	AF120274	Artemin	ARTN	1.96	2.10
213586_at	U20498	Cyclin-dependent kinase inhibitor 2D	CDKN2D	2.40	1.95
204463_s_at	NM_001957	Endothelin receptor type A	EDNRA	4.40	2.55
204422_s_at	NM_002006	Fibroblast growth factor 2 (basic)	FGF2	2.68	2.34
208449_s_at	NM_006119	Fibroblast growth factor 8	FGF8	3.34	3.54
206742_at	NM_004469	Vascular endothelial growth factor D	FIGF	2.28	1.79
221722_x_at	AF123652	Leucine zipper, putative tumor suppressor 1	LZTS1	4.14	6.12
Cell differentiation					
215152_at	NM_005375	v-myb myeloblastosis viral oncogene homolog	MYB	−1.99	−3.62
207251_at	NM_005925	Meprin A, beta	MEP1B	−3.99	−4.31
Cell junctions					
207025_at	NM_020435	Gap junction protein, alpha 12, 47 kDa	GJA12	1.88	1.85
207324_s_at	NM_004948	Desmocollin 1	DSC1	−1.64	−2.22
221132_at	NM_016369	Claudin 18	CLDN18	2.03	NC[a]

Table 2 continued

Probe set ID	Accession number	Gene title	Gene symbol	FC (3 h)	FC (6 h)
Metalloproteases					
204943_at	NM_021641	ADAM metallopeptidase domain 12	ADAM12	2.11	1.50
202828_s_at	NM_004995	Matrix metallopeptidase 14	MMP14	2.29	2.26
222342_at	NM_197941	ADAM metallopeptidase with thrombospondin type 1 motif, 6	ADAMTS6	5.06	6.96
204580_at	NM_002426	Matrix metallopeptidase 12 (macrophage elastase)	MMP12	NC[a]	1.56
206234_s_at	NM_016155	Matrix metallopeptidase 17 (membrane-inserted)	MMP17	1.58	1.50
Receptor and signal transducer					
220821_at	NM_001480	Galanin receptor 1	GALR1	5.22	3.37
207072_at	NM_003853	Interleukin 18 receptor accessory protein	IL18RAP	2.26	3.06
211533_at	M22734	Platelet-derived growth factor receptor, alpha polypeptide	PDGFRA	5.87	4.27
208256_at	NM_001405	Ephrin-A2	EFNA2	1.87	2.17
205841_at	NM_004972	Janus kinase 2	JAK2	2.20	2.64
211083_s_at	NM_004721	Mitogen-activated protein kinase kinase kinase 13	MAP3K13	1.87	2.97
211437_at	NM_006724	Mitogen-activated protein kinase kinase kinase 4	MAP3K4	2.79	2.80
204936_at	NM_004579	Mitogen-activated protein kinase kinase kinase kinase 2	MAP4K2	2.07	3.14
206040_s_at	NM_002751	Mitogen-activated protein kinase 11	MAPK11	4.01	2.08
216933_x_at	S67788	Adenomatosis polyposis coli	APC	−2.06	−1.50
Cytoskeleton					
205373_at	NM_004389	Catenin (cadherin-associated protein), alpha 2	CTNNA2	2.25	3.43
216947_at	NM_001927	Desmin	DES	3.86	2.33
200600_at	NM_002444	Moesin	MSN	−1.96	−2.58
209209_s_at	NM_006832	Pleckstrin homology domain containing, family C, member 1	PLEKHC1	−2.44	−1.67
Ion transport					
205410_s_at	NM_001684	ATPase, Ca ++ transporting, plasma membrane 4	ATP2B4	2.41	2.15
212292_at	NM_003045	Solute carrier family 7	SLC7A1	2.19	NC[a]
221106_at	NM_016609	Solute carrier family 22 member 17	SLC22A17	3.26	3.30
208589_at	NM_020389	Transient receptor potential cation channel, subfamily C, 7	TRPC7	1.50	2.64
220552_at	NM_012471	Transient receptor potential cation channel, subfamily C, 5	TRPC5	2.73	2.63
208432_s_at	NM_000721	Calcium channel, voltage-dependent, alpha 1E subunit	CACNA1E	NC[a]	2.12
211830_s_at	AF211189	Calcium channel, voltage-dependent, alpha 1I subunit	CACNA1I	2.60	1.50
208479_at	NM_000217	Potassium voltage-gated channel, member 1	KCNA1	1.50	2.31
206765_at	AF153820	Potassium inwardly rectifying channel, subfamily J, member 2	KCNJ2	2.20	2.70
207141_s_at	NM_002239	Potassium inwardly rectifying channel, subfamily J, member 3	KCNJ3	6.19	3.78
214647_s_at	NM_000410	Hemochromatosis	HFE	NC[a]	1.60
207212_at	NM_004174	Solute carrier family 9 (sodium/hydrogen exchanger), member 3	SLC9A3/NHE3	−3.04	NC[a]
Metabolism					
207262_at	NM_001638	Apolipoprotein F	APOF	4.69	4.14
216418_at	AL133173	ATP-binding cassette, sub-family D (ALD), member 1	ABCD1	11.54	4.52
209601_at	S69189	Acyl-coenzyme A oxidase 1, palmitoyl	ACOX1	2.43	2.82
207015_s_at	NM_003888	Aldehyde dehydrogenase 1 family, member A2	ALDH1A2	4.04	3.58
214315_x_at	NM_004343	Calreticulin	CALR	2.04	NC[a]
207316_at	NM_001523	Hyaluronan synthase 1	HAS1	2.10	2.42
213723_s_at	NM_000203	Iduronidase, alpha-L-	IDUA	4.38	3.21
216529_at	NM_005732	RAD50 homolog	RAD50	6.45	3.44
214490_at	NM_004042	Arylsulfatase F	ARSF	−3.20	−1.51
203178_at	NM_001482	Glycine amidinotransferase	GATM	−5.70	−1.60
208019_at	NM_003446	Zinc finger protein 157 (HZF22)	ZNF157	−3.51	−9.46
202588_at	NM_000476	Adenylate kinase 1	AK1	NC[a]	−2.12

Table 2 continued

Probe set ID	Accession number	Gene title	Gene symbol	FC (3 h)	FC (6 h)
205939_at	NM_000765	Cytochrome P450, family 3, subfamily A, polypeptide 7	CYP3A7	−2.12	−2.16
221561_at	L21934	Sterol O-acyltransferase	SOAT1	−1.98	−2.33
214970_s_at	X17247	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1	ST6GAL1	−2.44	−1.83
206624_at	NM_004654	Ubiquitin specific peptidase 9, Y-linked	USP9Y	−2.36	−3.28
205978_at	NM_004795	Klotho	KL	7.15	3.94
EGFR pathway					
211607_x_at	U48722	Epidermal growth factor receptor	EGFR	2.11	NC[a]
211453_s_at	M77198	v-akt murine thymoma viral oncogene homolog 2	AKT2	2.19	1.72
206370_at	NM_002649	Phosphoinositide-3-kinase, catalytic, gamma polypeptide	PIK3CG	2.89	2.52
202668_at	NM_004093	Ephrin-B2	EFNB2	1.68	NC[a]
201533_at	NM_001904	Catenin (cadherin-associated protein), beta 1, 88 kDa	CTNNB1	1.71	1.65
205106_at	NM_001018025	Mature T cell proliferation 1	MTCP1	1.52	NC[a]
201130_s_at	NM_004360	Cadherin 1, type 1, E-cadherin (epithelial)	CDH1	1.66	1.50

[a] Not significantly changed

In celiac crypt cell population, an increased division rate has been observed, leading to crypt hyperplasia [19]. Consistently with this finding, in T84 cells, the anti-VP7 antibodies activate the transcription of several genes involved in cell proliferation; up-regulated genes include the following: artemin (ARTN), cyclin-dependent kinase inhibitor 2D (CDKN2D), endothelin receptor type A (EDNRA), fibroblast growth factor 2 (FGF2) and 8 (FGF8), vascular endothelial growth factor D (FIGF), and leucine zipper, putative tumor suppressor 1 (LZTS1).

Some of the genes up-regulated by the antibody treatment are involved in the epidermal growth factor receptor (EGFR) pathway including EGFR, v-akt murine thymoma viral oncogene homolog 2 (AKT2), phosphoinositide-3-kinase, catalytic, gamma polypeptide (PIK3CG), catenin beta 1 (CTNNB1), ephrin-B2 (EFNB2), and ephrin-A2 (EFNA2).

Ephrin receptors and their ligands, ephrins, are involved in developmental processes as they regulate cell migration and cell adhesion; moreover, they influence the normal as well as the pathological architecture of tissues, including intestinal epithelium [21]. Interestingly, several genes involved in the EGFR signaling pathway have been found up-regulated in epithelial cells derived from active CD patients, and EGFR expression is more pronounced in the crypt regions of CD patients with active disease [22].

The EGFR signaling pathway members are thought to regulate also cell proliferation and cytoskeleton reorganization. Interestingly, some genes associated with cytoskeleton organization are modulated in T84 cells exposed to anti-VP7 antibodies including moesin (MSN), pleckstrin homology domain containing, family C member 1 (PLEKHC1), desmin (DES), and catenin alpha 2 (CTNNA2). MSN acts as cross-linker between plasma membranes and actin-based cytoskeletons, PLEKHC1 defines a connection between ECM adhesions and the actin cytoskeleton and participates in the orchestration of actin assembly and cell shape modulation, CTNNA2 is able to regulate microtubule dynamics since it is directly involved in actin organization and therefore acts as a central player of cytoskeleton rearrangements in response to extracellular interactions.

As the epithelial layer architecture is damaged in active celiac disease, the physiological functions of intestinal cells are altered, and among these functions, cell adhesion and transport are dysregulated [23].

The antibodies treatment also modulated several genes involved in cell–cell adhesion, in particular tight junctions proteins, as claudin 18 (CLDN18) and gap junctions proteins as the gap junction protein alpha 12 (GJA12) were up-regulated, while desmosomes protein desmocollin 1 was down-modulated. CLDN18 has been found to be up-regulated during experimental colitis and in patients with ulcerative colitis [24]. GJA12 is a gap junction protein and it was postulated that an increased number of these junctions may facilitate the diffusion of gluten peptides between cells along the epithelial layer [23].

Desmocollin is a desmosomal protein required for cell to cell adhesion and its down-regulation may contribute to increased epithelial cell permeability, a key feature of celiac disease.

Moreover, several genes coding for ion channels and transporters such as ATPase, Ca ++ transport, plasma membrane 4 (ATP2B4), solute carrier family 22 member 17 (SLC22A17), potassium voltage-gated channel, shaker-related subfamily, member 1 (KCNA1), and potassium

inwardly rectifying channel, subfamily J, member 3 (KCNJ3) were overexpressed in T84 cells treated with anti-VP7 rotavirus antibodies. Noteworthy, an up-regulation of genes coding for ion channels has been described in intestinal epithelial cells [23], and ion transport is known to be increased in celiac disease [25]. Indeed, intestinal epithelial cells chronically exposed to IFN-gamma down-regulate the Na(+)/H(+) exchangers NHE2 and NHE3 expression leading to inflammation-associated diarrhea [26]. Moreover, increased levels of serotonin, which has been implicated in the pathophysiology of diarrhea associated with CD, are known to down-regulate NHE2 and NHE3 expression [27]. Interestingly, NHE3 transcription is down-regulated in T84 cells after treatment with anti-rotavirus VP7 antibodies.

The gene encoding for the iron transporter hemochromatosis (HFE) was up-regulated in treated T84 cells. The overexpression of this molecule has been associated with the reduction in iron uptake typical of CD [28].

Several genes up-regulated in T84 cells treated with anti-VP7 antibodies encode for matrix metallopoteases (MMPs), including MMP-12. An increased expression of several MMPs and in particular of MMP-12 has been observed in biopsies of patients with active CD [19, 23].

The gene expression profiles of T84 cells show that the anti-VP7 antibodies exert a proinflammatory effect since they induced an overexpression of proinflammatory genes such as prostaglandin-endoperoxide synthase 2, PTGS2; chemokine (C–C motif) receptor 1, CCR1; chemokine (C–C motif) receptor 3, CCR3; and the proinflammatory interleukin 8, IL-8; they decreased the expression of anti-inflammatory genes such as mitochondrial superoxide dismutase 2, (SOD2) and the protective interleukin 10 (IL-10).

Another cluster of differentially expressed genes were genes encoding for receptors and signal transducers molecules. Several of these genes such as galanin receptor 1 (GALR1), interleukin 18 receptor accessory protein (IL18RAP), platelet-derived growth factor receptor, alpha polypeptide (PDGFRA), and mitogen-activated protein kinases (such as MAP3K13, MAP3K4, MAP4K2, MAPK11) showed an increased expression in T84 cells treated with anti-VP7 antibodies.

Galanin receptors are expressed by smooth muscle cells lining the gastro-intestinal tract and mediate the effect of galanin in altering intestinal motility. In particular, Gal1-R is also expressed by the human T84 epithelial cell line and, when stimulated, this receptor cause Cl^- secretion. Moreover, Gal1-R expression is transcriptionally up-regulated under inflammatory conditions and an increased receptor expression was observed in the intestinal epithelium of patients with inflammatory bowel disease and infectious diarrhea. Therefore, this receptor plays an important role in diarrhea associated with a variety of inflammatory disorders [29].

Analysis of soluble mediators released in the supernatant of T84 cells

The analysis of gene expression profiles was complemented by the analysis of some of the corresponding soluble mediators released by treated T84 cells.

Figure 4 shows the concentration (pg/ml) of the molecules tested in the supernatants of T84 cells incubated with antibodies against the irrelevant peptide and with anti-VP7 peptide antibodies.

A fulfilling correspondence was found for 9 transcripts (CSF3, PDGFB, CXCL10, FGF2, IL1B, IL8, IL6, CCL11, IL4) whose overexpression was paralleled by increased secretion of the corresponding molecules in the supernatant of T84 cells incubated with antiviral antibodies, after 3 and/or 6 h.

Other two molecules (IL13 and CSF2) did not show a significantly difference in their concentration between treated and control supernatants and the gene expression levels was also similar in T84 cells treated with anti-VP7 peptide and anti-irrelevant peptide antibodies (1.19, 1.20 and 1.2, 1.3 F.C. respectively at 3 and 6 h of treatment; data not shown).

Discussion

The aim of this study was to further analyze the link between rotavirus infection and pathogenesis of CD. First of all, we wanted to evaluate whether the presence of anti-VP7 peptide antibodies precedes the onset of CD and therefore whether the detection of such antibodies might be used as a predictive marker for the onset of CD when anti-tTG and anti-endomysium antibodies are still not detectable. To this aim, we tested the sera of patients with T1DM, who present a high prevalence of CD.

Secondly, we wanted to study the effects of anti-viral peptide antibodies on intestinal epithelial cells by means of genes modulation complemented by the analysis of molecules secreted in the supernatant, to confirm that such antibodies are able to induce in vitro some of the features observed in celiac epithelium in vivo.

A strong association between T1DM and CD has been reported. Indeed, a CD prevalence as high as 7 % has been observed in T1DM north Italian population [15].

Among our T1DM patients, nearly 9 % developed CD (32 of 357), a percentage slightly higher than the one already reported only a few years ago. These results raise the question whether the prevalence of CD is increasing among T1DM patients and whether this could

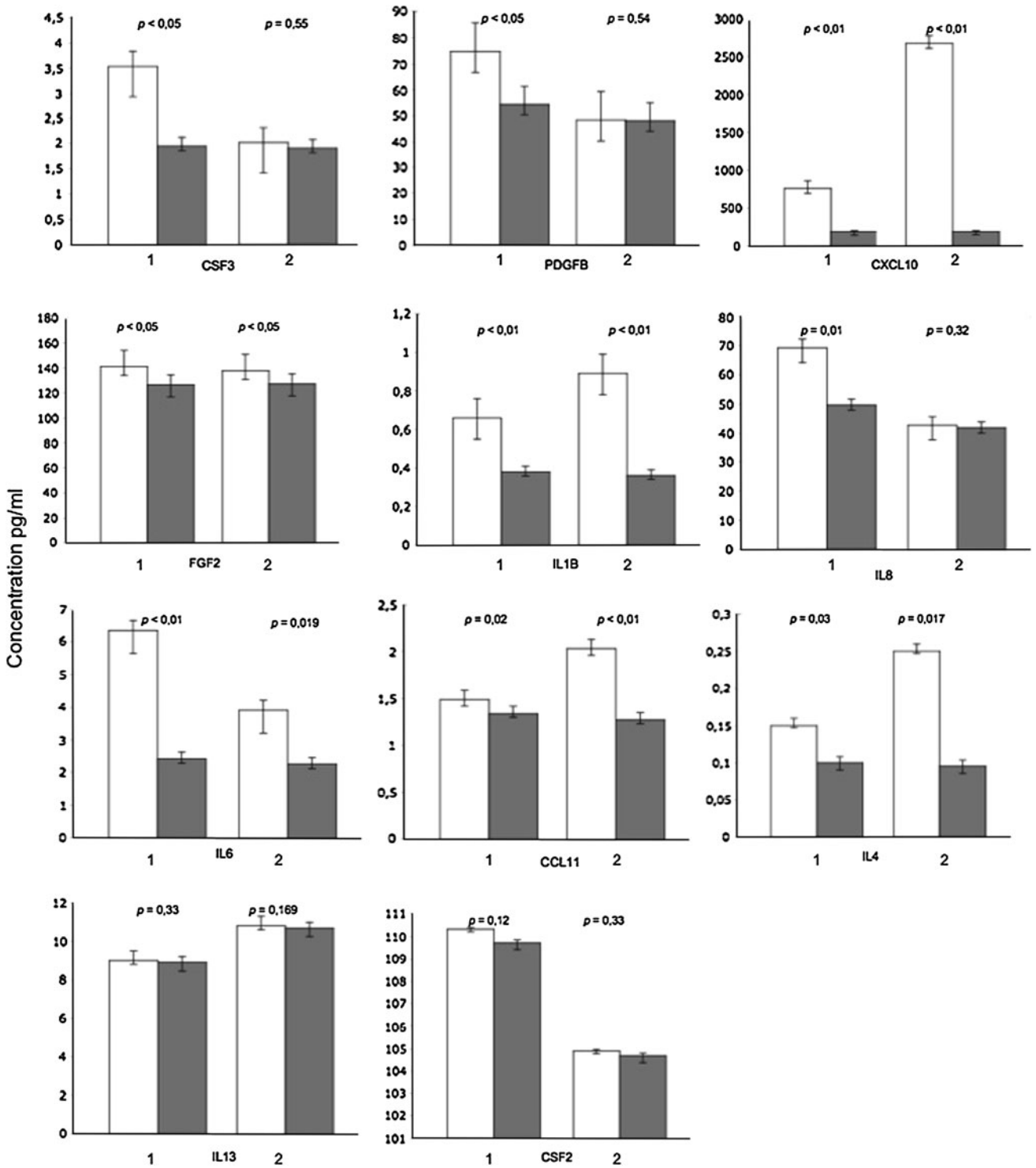

Fig. 4 Soluble mediators released in cell culture supernatants of T84 cells. T84 cells were treated with: anti-VP7 peptide antibodies (*white bar*) and anti-irrelevant peptide antibodies (*gray bar*) at 3 and 6 h. Results are expressed in pg/mL as mean of three independent experiments. *p* value is calculated using the Student's *t*-test for paired independent samples

be due to environmental factors such as food and viral infections [13].

When we analyzed the levels of anti-rotavirus VP7 peptide antibodies in T1DM patients with CD compared to T1DM patients without CD, we found statistically significant higher level of antibodies in the first group, suggesting that such antibodies are possibly related to the onset of CD. Moreover, anti-VP7 peptide antibodies appeared before the

onset of CD in 6 of the 8 T1DM patients analyzed, in particular one patient had such antibodies 120 months before evidence, both humoral and histological, of the disease.

The VP7 rotavirus peptide shares a high degree of sequence homology with the identified celiac peptide [7] which is homologous also to the tTG 476–487 peptide. Interestingly, before CD onset, all the six sera that recognized the viral peptide recognized also the celiac peptide and three of them recognized also the tTG peptide and another one (patient 5) presented a borderline recognition. These findings confirm our previous data on the ability of anti-celiac peptide antibodies to cross-recognize the rotavirus-derived peptide and, to a minor extent, the tTG peptide.

Altogether, the results of this first part of our work further support a possible etiological link between rotavirus infection and pathogenesis of CD.

Anti-rotavirus VP7 antibodies seem to be also directly involved in the celiac disease pathogenesis as shown by the gene-array results.

Indeed, the large majority of genes that are modulated by the anti-VP7 antibodies in T84 cells have been reported to play a role in the biological processes that lead to the most important features of the disease.

Some of the modulated genes are involved in triggering the apoptotic process and in the regulation of cell proliferation and differentiation. Therefore, the treatment of T84 cells with the antiviral peptide antibodies induces the typical alterations seen in CD since the gluten-induced small-intestinal lesions are characterized by mucosal villous atrophy and crypt hyperplasia, associated with decreased differentiation and increased proliferation of epithelial cells.

A gene-array analysis previously performed by Diosdado et al. [30] suggested that, in the celiac villous crypt, stem cells normally proliferate but fail to completely differentiate with consequent formation of hyperplastic crypts leading to villous atrophy.

Moreover, it has been shown that gliadin peptides induce actin cytoskeleton rearrangements in a wide range of cell types [31]. The gene expression profiles of the array analysis reported in this work show a modulation of several genes involved in cytoskeleton organization.

The gene-array results also reflect the dysregulation of cell–cell adhesion molecules seen in CD, since genes encoding for tight junctions and gap junctions proteins are up-regulated, while desmosomes proteins are down-modulated by the treatment. These alterations are preceding events of the decreased transepithelial resistance and of the increased permeability that are typical features of the celiac intestinal epithelium.

Moreover, the antibody treatment was also able to modulate cellular ion transport in T84 cells. This modulation includes the up-regulation of several ion channels and transporter [23, 25] and the down-regulation of the Na(+)/H(+) exchanger NHE3 that is present in celiac intestinal epithelial cells. In particular, it has been supposed that the reduction in NHE3 expression may play a role in the onset of the celiac-associated diarrhea, one of the major clinical features of the disease [27].

The gene-array data are also consistent with the increased expression of several MMPs observed in the biopsies of patients with active disease [19], and among these, we want to point out the increased expression of MMP-12 since it has been considered crucial for the immunological mechanisms that lead to tissue injury [32].

Besides their classical role in connective tissue protein turnover and degradation, MMPs may promote inflammation acting on chemokines and other inflammatory mediators [33].

Moreover, many genes involved in inflammation resulted up-regulated by exposure of cells to the antibodies indicating that such antibodies may induce a proinflammatory effect on T84 intestinal cells. This effect is increased by the down-regulation of some anti-inflammatory molecules such as IL-10.

Finally, the anti-VP7 antibodies up-regulated the expression of several receptors such as Galanin receptor, involved in inflammation-associated diarrhea, EGFR, EGFR-pathway-associated molecules, involved in the orchestration of epithelial cells proliferation, and signal transducer molecules such as ephrin-A2 that acts as a key regulator of developmental process in many epithelial cells.

In conclusion, we describe here two important characteristics of the previously identified anti-rotavirus VP7 antibodies that further support the link between rotavirus infection and CD: first, the possibility that they may identify subjects who will develop CD and second, the ability of these antibodies to modulate sets of genes in intestinal epithelial cells. Such genes are involved in apoptosis, in alteration of the epithelial barrier integrity and in inflammation, which are typical features of CD.

Early diagnosis of CD is necessary and is based on periodical autoantibody screening following the diagnosis of T1DM. In this regard, the evaluation of anti-VP7 rotavirus antibodies may represent an additional and useful marker with important predictive value.

It will be important to confirm the predictive value of such antiviral antibodies in further studies including subjects with a family history of CD and with genetic predisposition.

Conflict of interest The authors have no conflicting financial interests.

References

1. Rewers M. Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease? Gastroenterology. 2005;128:S47–51.
2. Sollid LM. Celiac disease: dissecting a complex inflammatory disorder. Nat Rev Immunol. 2002;2:647–55.
3. Lunardi C, Bason C, Navone R, Millo E, Damonte G, Corrocher R, et al. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. Nat Med. 2000;6:1183–6.
4. Lunardi C, Bason C, Leandri M, Navone R, Lestani M, Millo E, et al. Autoantibodies to inner ear and endothelial antigens in Cogan's syndrome. Lancet. 2002;360:915–21.
5. Navone R, Lunardi C, Gerli R, Tinazzi E, Peterlana D, Bason C, et al. Identification of tear lipocalin as a novel autoantigen target in Sjögren's syndrome. J Autoimmun. 2005;25:229–34.
6. Puccetti A, Bason C, Simeoni S, Millo E, Tinazzi E, Beri R, et al. In chronic idiopathic urticaria autoantibodies against Fc epsilonRII/CD23 induce histamine release via eosinophil activation. Clin Exp Allergy. 2005;35:1599–607.
7. Zanoni G, Navone R, Lunardi C, Tridente G, Bason C, Sivori S, et al. In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes. PLoS Med. 2006;3:e358.
8. Lindfors K, Mäki M, Kaukinen K. Transglutaminase 2-targeted autoantibodies in celiac disease: pathogenetic players in addition to diagnostic tools? Autoimmun Rev. 2010;9:744–9.
9. Stene LC, Honeyman MC, Hoffenberg EJ, Haas JE, Sokol RJ, Emery L, et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. 2006;101:2333–40.
10. Kooy-Winkelaar Y, van Lummel M, Moustakas AK, Schweizer J, Mearin ML, Mulder CJ, et al. Gluten-specific T cells cross-react between HLA-DQ8 and the HLA-DQ2α/DQ8β transdimer. J Immunol. 2011;187:5123–9.
11. Waisbourd-Zinman O, Hojsak I, Rosenbach Y, Mozer-Glassberg Y, Shalitin S, Phillip M, Shamir R. Spontaneous normalization of anti-tissue transglutaminase antibody levels is common in children with type 1 diabetes mellitus. Dig Dis Sci. 2012;57:1314–20.
12. Volta U, Tovoli F, Caio G. Clinical and immunological features of celiac disease in patients with Type 1 diabetes mellitus. Expert Rev Gastroenterol Hepatol. 2011;5:479–87.
13. Salardi S, Volta U, Zucchini S, Fiorini E, Maltoni G, Vaira B, et al. Prevalence of celiac disease in children with type 1 diabetes mellitus increased in the mid-1990s: an 18-year longitudinal study based on anti-endomysial antibodies. J Pediatr Gastroenterol Nutr. 2008;46:612–4.
14. Gabriel S, Mihaela I, Angela B, Mariana A, Doru D. Prevalence of IgA antitissue transglutaminase antibodies in children with type 1 diabetes mellitus. J Clin Lab Anal. 2011;25:156–61.
15. Contreas G, Valletta E, Ulmi D, Cantoni S, Pinelli L. Screening of coeliac disease in North Italian children with type I diabetes: limited usefulness of HLA-DQ typing. Acta Paediatr. 2004;93:628–32.
16. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999;11:1185–94.
17. Wellings DA, Atherton E. Standard FMOC protocols. Meth Enzymol. 1997;289:44–67.
18. Katz S, Irizarry RA, Lin X, Tripputi M, Porter MW. A summarization approach for Affymetrix GeneChip data using a reference training set from a large, biologically diverse database. BMC Bioinformatics. 2006;. doi:10.1186/1471-2105-7-464.
19. Diosdado B, van Oort E, Wijmenga C. "Coelionomics": towards understanding the molecular pathology of coeliac disease. Clin Chem Lab Med. 2005;43:685–95.
20. Ramsay RG, Malaterre J. Insights into c-Myb functions through investigating colonic crypts. Blood Cells Mol Dis. 2007;39:287–91.
21. Merlos-Suàrez A, Batlle E. Eph-ephrin signalling in adult tissue and cancer. Curr Opin Cell Biol. 2008;20:194–200.
22. Juuti-Uusitalo K, Mäki M, Kainulainen H, Isola J, Kaukinen K. Gluten affects epithelial differentiation-associated genes in small intestinal mucosa of coeliac patients. Clin Exp Immunol. 2007;150:294–305.
23. Bracken S, Byrne G, Kelly J, Jackson J, Feighery C. Altered gene expression in highly purified enterocytes from patients with active coeliac disease. BMC Genomics. 2008;. doi:10.1186/1471-2164-9-377.
24. Zwiers A, Fuss IJ, Leijen S, Mulder CJ, Kraal G, Bouma G. Increased expression of the tight junction molecule claudin-18 A1 in both experimental colitis and ulcerative colitis. Inflamm Bowel Dis. 2008;14:1652–9.
25. Schulzke JD, Schulzke I, Fromm M, Riecken EO. Epithelial barrier and ion transport in coeliac sprue: electrical measurements on intestinal aspiration biopsy specimens. Gut. 1995;37:777–82.
26. Rocha F, Musch MW, Lishanskiy L, Bookstein C, Sugi K, Xie Y, et al. IFN-gamma downregulates expression of Na(+)/H(+) exchangers NHE2 and NHE3 in rat intestine and human Caco-2/bbe cells. Am J Physiol Cell Physiol. 2001;280:C1224–32.
27. Gill RK, Saksena S, Tyagi S, Alrefai WA, Malakooti J, Sarwar Z, et al. Serotonin inhibits Na +/H + exchange activity via 5-HT4 receptors and activation of PKC alpha in human intestinal epithelial cells. Gastroenterology. 2005;128:962–74.
28. Juuti-Uusitalo K, Mäki M, Kaukinen K, Collin P, Visakorpi T, Vihinen M, Kainulainen H. cDNA microarray analysis of gene expression in coeliac disease jejunal biopsy samples. J Autoimmun. 2004;22:249–65.
29. Benya RV, Matkowskyj KA, Danilkovich A, Hecht G. Galanin causes Cl- secretion in the human colon. Potential significance of inflammation-associated NF-kappa B activation on galanin-1 receptor expression and function. Ann N Y Acad Sci. 1998;863:64–77.
30. Diosdado B, Wapenaar MC, Franke L, Duran KJ, Goerres MJ, Hadithi M, et al. A microarray screen for novel candidate genes in coeliac disease pathogenesis. Gut. 2004;53:944–51.
31. Barone MV, Gimigliano A, Castoria G, Paolella G, Maurano F, Paparo F, et al. Growth factor-like activity of gliadin, an alimentary protein: implications for coeliac disease. Gut. 2007;56:480–8.
32. Ciccocioppo R, Di Sabatino A, Bauer M, Della Riccia DN, Bizzini F, Biagi F, et al. Matrix metalloproteinase pattern in celiac duodenal mucosa. Lab Invest. 2005;85:397–407.
33. Parks WC, Wilson CL, Lopez-Boado YS. Matrix metallo-proteinases as modulators of inflammation and innate immunity. Nat Rev Immunol. 2004;4:617–29.