See What You Have Been Missing

Over 2600 Validated ELISA Kits
GMP & ISO Certified
100% Guaranteed
Manufactured in the USA
Browse ELISAs

Gliadin-Specific CD8$^+$ T Cell Responses Restricted by HLA Class I A*0101 and B*0801 Molecules in Celiac Disease Patients

This information is current as of February 10, 2017.

Stefania Picascia, John Sidney, Alessandra Camarca, Giuseppe Mazzarella, Nicola Giardullo, Luigi Greco, Renata Auricchio, Salvatore Auricchio, Riccardo Troncone, Alessandro Sette and Carmen Gianfrani

J Immunol published online 1 February 2017
http://www.jimmunol.org/content/early/2017/02/01/jimmunol.1601208

Supplementary Material **http://www.jimmunol.org/content/suppl/2017/02/01/jimmunol.1601208.DCSupplemental**

Subscriptions Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscriptions

Permissions Submit copyright permission requests at: http://www.aai.org/ji/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/cgi/alerts/etoc

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.
Copyright © 2017 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

Published February 1, 2017, doi:10.4049/jimmunol.1601208

Gliadin-Specific CD8+ T Cell Responses Restricted by HLA Class I A*0101 and B*0801 Molecules in Celiac Disease Patients

Stefania Picascia,* John Sidney,[†] Alessandra Camarca,[‡,§] Giuseppe Mazzarella,[‡] Nicola Giardullo,[¶] Luigi Greco,[§] Renata Auricchio,[§] Salvatore Auricchio,[§] Riccardo Troncone,[§] Alessandro Sette,[†] and Carmen Gianfrani*[,§]

Initial studies associated the HLA class I A*01 and B*08 alleles with celiac disease (CD) susceptibility. Subsequent analyses showed a primary association with HLA class II alleles encoding for the HLA DQ2.5 molecule. Because of the strong linkage disequilibrium of A*01 and B*08 alleles with the DR3-DQ2.5 haplotype and a recent genome-wide association study indicating that B*08 and B*39 are predisposing genes, the etiologic role of HLA class I in CD pathogenesis needs to be addressed. We screened gliadin proteins (2α-, 2ω-, and 2γ-gliadin) using bioinformatic algorithms for the presence of peptides predicted to bind A*0101 and B*0801 molecules. The top 1% scoring 9- and 10-mer peptides (*N* = 97, total) were synthesized and tested in binding assays using purified A*0101 and B*0801 molecules. Twenty of ninety-seven peptides bound B*0801 and only 3 of 97 bound A*0101 with high affinity (IC_{50} < 500 nM). These 23 gliadin peptides were next assayed by IFN-γ ELISPOT for recognition in peripheral blood cells of CD patients and healthy controls carrying the A*0101 and/or B*0801 genes and in A*0101/B*0801⁻ CD patients. Ten of the twenty-three peptides assayed recalled IFN-γ responses mediated by CD8+ T cells in A*0101/B*0801+ patients with CD. Two peptides were restricted by A*0101, and eight were restricted by B*0801. Of note, 50% (5/10) of CD8+ T cell epitopes mapped within the γ-gliadins. Our results highlight the value of predicted binding to HLA molecules for identifying gliadin epitopes and demonstrate that HLA class I molecules restrict the anti-gluten T cell response in CD patients. ***The Journal of Immunology*, 2017, 198: 000–000.**

Celiac disease (CD) is a T cell–mediated enteropathy that affects almost 1% of the worldwide population carrying the HLA genes coding for DQ2.5 (DQA1*05/DQB1*02) or DQ8 (DQA1*03/DQB1*03) heterodimers (1). CD arises in genetically susceptible individuals upon exposure to dietary wheat gluten or similar prolamins from rye and barley (2).

The strong genetic association with HLA class II genes and the identification of several DQ2.5/DQ8-restricted gluten epitopes, recognized specifically by CD patients, underscore the key role of adaptive immunity mediated by CD4+ T lymphocytes in CD pathogenesis (3, 4). However, one of the main features of all forms of CD, from silent to active, or even refractory CD, is a massive infiltration of CD8+ T lymphocytes in the epithelium and lamina propria of small intestinal mucosa (5). Several studies investigated the phenotype and function of CD8+ T cells resident in the epithelium of CD intestinal mucosa (5, 6). These cells display a pattern typical of cytotoxic CD8+ lymphocytes (CTLs), with large perforin granules and high Fas ligand surface expression, and are involved in enterocyte apoptosis and villous atrophy (7–9). When exposed to gluten, these CTLs become activated and express the markers of NK cells, such as NKG2C/D (10).

Despite these findings, evidence of a gluten-specific, TCR-dependent activation of CD8+ T lymphocytes is still lacking. We demonstrated previously that a gliadin peptide specifically stimulated CD8+ CTL responses in CD patients (9, 11). These CD8+ T cells are resident in the lamina propria, release IFN-γ, and lyse enterocytes upon recognition of the gliadin peptide presented by HLA class I A*02 molecules (9, 11). A further study demonstrated that brief consumption of gluten-containing food mobilized CD8+ T lymphocytes in the peripheral blood of patients with CD, along with the well-documented recruitment of CD4+ T cells (12). The expression of gut-homing markers and a focused TCR repertoire suggested a gluten-driven expansion of these CD8+ T cells (12). However, the lack of a genetic association of the HLA A*02 gene with CD cast doubt on the direct relevance of HLA class I–restricted CD8+ T cell responses in CD pathogenesis.

The primary genetic link of HLA with CD is with class II DQA1*05/DQB1*02 genes, carried either in *cis* configuration, as in the DR3-DQ2.5 haplotype, or in *trans* configuration, as in DR5-DQ7/DR7-DQ2.2 haplotypes (13, 14). However, the earliest genetic studies, performed in the 1970s, reported an association of CD with HLA class I A*01 and B*08 genes (15, 16). A more recent genome-wide association study (GWAS) finely mapped the MHC region and identified additional risk regions independent of

*Institute of Protein Biochemistry–National Research Council, 80131 Naples, Italy; [†]La Jolla Institute for Allergy and Immunology, La Jolla, CA 92130; [‡]Institute of Food Sciences–National Research Council, 83100 Avellino, Italy; [§]Department of Translational Medical Science and European Laboratory for Investigation and Food-Induced Diseases, University Federico II, 80131 Naples, Italy; and [¶]Gastroenterology Unit, Hospital Moscati, 83100 Avellino, Italy

ORCIDs: 0000-0002-7294-8277 (G.M.); 0000-0001-7013-2250 (A.S.).

Received for publication July 13, 2016. Accepted for publication January 3, 2017.

This work was supported by Grant 2009LC892E from the Italian Ministry of Research: PRIN 2009 - Interaction between Innate and Adaptive Immunity in the Pathogenesis of CD.

Address correspondence and reprint requests to Carmen Gianfrani, Institute of Protein Biochemistry–National Research Council, Via Pietro Castellino, 111, 80131 Naples, Italy. E-mail address: c.gianfrani@ibp.cnr.it

The online version of this article contains supplemental material.

Abbreviations used in this article: CD, celiac disease; GWAS, genome-wide association study; PT-gliadin, peptic-tryptic digest of gliadin; SFC, spot-forming cell; tTG, tissue transglutaminase.

Copyright © 2017 by The American Association of Immunologists, Inc. 0022-1767/17/$30.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1601208

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

the HLA DQA1*05 and DQB1*02 genes (17). Among them, the HLA B*0801 allele, in strong linkage disequilibrium with DR3-DQ2.5 genes, contributes to genetic susceptibility to CD (17).

In this study, we investigated whether A*0101 and B*0801 class I alleles restrict the adaptive CD8+ T cell responses to gluten in subjects with CD. A library of gliadin peptides predicted to bind to A*0101 or B*0801 were screened for in vitro binding to purified A*0101 or B*0801 molecules. High-binding peptides were then assayed for immunogenicity in CD subjects.

Materials and Methods

Study population and HLA class I and II gene typing

To recruit a study population carrying the A*0101 and/or B*0801 alleles, we genotyped 103 subjects (children and adults) with CD for HLA class I A and B genes, as well as 27 adult non-CD controls (Table I). The adult subjects were enrolled at Moscati Hospital of Avellino, and children were enrolled at the Department of Translational Medical Science, Section of Pediatrics, University of Naples Federico II. All subjects, or their parents in the case of children under 12 y old, gave full informed consent to the experiments. The study was approved by the Ethical Committees "Carlo Romano" of the University of Naples, "Federico II," register number 7/12 on April 11, 2012 and by Campania Nord, register number CECN/314 on October 21, 2015. Patients were diagnosed with CD in accordance with the 1990 European Society for Pediatric Gastroenterology Hepatology and Nutrition guidelines. All CD volunteers were on gluten-free diet for ≥2 y and were serum negative for anti-tissue transglutaminase (tTG) and anti-endomysial Abs at the time of enrollment. Non-CD adult controls were recruited from the blood donor volunteers at Moscati Hospital (Supplemental Table I).

Pure genomic DNA was extracted using commercial kits (Genomic DNA Miniprep Kit; Sigma-Aldrich, St. Louis, MO) from T cell blasts generated by stimulating PBMCs with PHA (Roche, Basel, Switzerland). CD patients and controls were genotyped for HLA A and B class I loci, as well as for DQA1, DQB1, DRB1 class II loci, using AllSet+ Gold SSP commercial typing kits (Life Technologies, distributed by EsseMedical, Milan, Italy).

*Identification of potential HLA A*0101- and B*0801-restricted gliadin peptides*

The amino acid sequences of six gliadin proteins (two α-gliadin, two γ-gliadin, and two ω-gliadin) (Swiss-Prot accession numbers Q9M4L6-1 and P18573-1 for α-gliadin, P08453-1 and P08079-1 for γ-gliadin, and AAG17702 and Q402I5-1 for ω-gliadin) were screened using bioinformatic algorithms to identify peptides predicted to bind HLA A*0101 and/or B*0801 molecules (Supplemental Table II). Predictions were performed using the consensus algorithm available from the Immune Epitope Database (http://www.iedb.org) (18). Peptides were synthesized (A&A Labs, San Diego, CA) and tested in classical competition assays, using purified MHC molecules, for their capacity to bind A*0101 and B*0801 molecules. MHC purification and quantitative competition assays using radiolabeled probe peptides were performed as previously described (19). Each competitor peptide was tested at six concentrations covering a 100,000-fold dose range and in three or more independent experiments. Under the conditions used, if the concentration of the radiolabeled ligand is less than the concentration of purified MHC (i.e., the probe is the limiting reagent) and the measured IC_{50} values are greater than the concentration of purified MHC (i.e., [label] < [MHC] and IC50 ≥ [MHC]), the measured IC_{50} values are reasonable approximations of the true kiloDalton values (20, 21). High-affinity binding peptides (IC_{50} < 500 nM) were subsequently synthesized as purified (>95%) material for use in T cell assays.

IFN-γ ELISPOT assay

The immunogenicity of gliadin peptides selected on the basis of their affinity for A*0101 or B*0801 molecules was assessed with IFN-γ ELISPOT assays on fresh PBMCs collected from CD patients and healthy volunteers, as previously reported (11). The study population for the immunogenicity experiments consisted of 24 CD subjects (mean age 25.4 y, range 5–60 y), either positive or negative for A*0101/B*0801 (Table II), and 10 non-CD controls carrying the A*0101 and/or B*0801 genes (Supplemental Table I).

Known immunogenic peptides from influenza A virus PB1 and NP proteins restricted by HLA A*0101 (CTELKLSDY and VSDGGPNLY) or B*0801 (ELRSYWAI) were used as intra-assay control peptides (Supplemental Fig. 1). Gliadin (Fig. 3) and control peptides (Supplemental Fig. 1) were assayed at a concentration of 10 μg/ml. A peptic-tryptic digest of gliadin (PT-gliadin) was also used to stimulate PBMCs at a concentration of 50 μg/ml. Cells (2×10^5 per 200 μl) were suspended in complete medium (X-VIVO 15 supplemented with 5% pooled AB human serum and antibiotics; all provided by Lonza, Basel, Switzerland), plated in 96-well nitrocellulose-backed plates (MAHAS4510; Millipore, Bedford, MA) coated with 10 μg/ml of anti–IFN-γ mAb (Mabtech, Stockholm, Sweden), and incubated for 36–40 h at 37°C in the presence or absence of gliadin/peptides. Plates were washed extensively with PBS/0.05% Tween-20 and incubated with 10 μg/ml of secondary anti–IFN-γ biotinylated Ab for 2 h and with streptavidin-HRP (BD Pharmingen, San Diego, CA) for 1 h. Spots were developed by adding aminoethyl carbazole (Sigma-Aldrich) solution and counted using an ImmunoSpot image analyzer (A.EL.VIS, Hannover, Germany). Data are expressed as the net IFN-γ−spot-forming cells (SFC) per 10^6 cells (i.e., SFC per 10^6 cells in the presence of gliadin/peptides minus the SFC per 10^6 cells with medium alone). A1/B8-binding peptides are considered immunogenic when IFN-γ SFC responses exceed the mean (+ 2 SD) of IFN-γ SFC in at least three A1/B8− CD patients.

Generation of short-term CD8+ T cell lines

Short-term CTL lines were derived against the most immunogenic gliadin peptides with high binding affinity for the B*0801 molecule, as previously reported (11). PBMCs (4×10^6) from three B*0801+ CD patients (CD#5, CD#14, CD#15) were stimulated with autologous monocytes (2×10^6) prepulsed with a pool of ω-gliadin$_{11–19}$ (AMAMKIATA) and γ-gliadin$_{224–232}$ (QGMHILLPL) peptides (6 μg/ml of each) in the presence of 3 μg/ml β2-microglobulin (Calbiochem, San Diego, CA). At days 19–21 from the culture set-up, CD8+ T cells were isolated from bulk lines using MACS immunomagnetic separation, according to the manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). Both bulk culture and purified CD8+ T cells were assayed by IFN-γ ELISPOT against each single peptide. Autologous PBMCs or HLA-matched immortalized B cells (1×10^5 cells per well) were used as APCs. APCs were pulsed overnight with each gliadin peptide (10 μg/ml) in the presence of β2-microglobulin (3 μg/ml) and then added to T cells.

Results

*HLA A*0101 and B*0801 are frequently represented in the study CD population*

To select a study cohort carrying HLA class I A*0101 and B*0801 alleles, we oligotyped 103 subjects with a diagnosis of CD for HLA class I A and B genes, as well as for DQA1 and DQB1 class II genes (Tables I, II). As reported in Table I, our CD cohort consisted totally of HLA DQ2.5 subjects. Among them, 18.4% were A*0101+ (B*0801−), 8.7% were B*0801+ (A*0101−), and 14.6% carried both A*0101 and B*0801 alleles. Overall, in our study population, the phenotypic frequencies of A*0101 and B*0801 were 33 and 23%, respectively, higher compared with 13 and 8% found in the general worldwide population (22, 23). Because the A*0101 and B*0801 alleles are in linkage with DR3-DQ2.5 genes in almost 60% of cases, resulting in the DR3-DQ2.5–A1–B8 extended haplotype (24, 25), we also oligotyped a subgroup of CD patients (61 of 103) for the DRB1 locus. A total of 24 subjects (39.3%) was DR3+, thus carrying the DQ2.5 coding alleles in *cis* configuration (Table I). Among these DR3+ individuals, 17 (70.1%) were A*0101 and/or B*0801 carriers, confirming the linkage disequilibrium between A*0101/B*0801 alleles and the DR3-DQ2.5 haplotype found in the general population (26).

*Screening of gliadin proteins for peptides binding to A*0101 and B*0801 molecules*

Bioinformatic algorithms available online (http://www.iedb.org) (18) were used to identify gliadin peptides predicted to bind HLA A*0101 and B*0801 molecules. The entire amino acid sequences of two α-gliadins, two γ-gliadins, and 2 ω-gliadins contain 2492 unique 9- and 10-mer peptides. The 97 peptides representing the top 1% scoring sequences for each size and each allele were selected for in vitro binding studies (Supplemental Table II). More specifically, 47 of 97 (48.5%) were predicted to bind A*0101, 46 of 97 (47.4%) were predicted to bind B*0801, and the remaining 4 of 97 (4.1%) were predicted to bind both molecules.

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

Table I. HLA class I and II frequency in the CD cohort

HLA Alleles	Cases	Frequency (%)
A*01	34/103	33.0
B*08	24/103	23.3
Total of A*01 and/or B*08	43/103	41.7
DQA1*05 and DQB1*02 (DQ2.5)	103/103	100.00
DRB1*03 (DR3)	24/61	39.3
DR3-DQ2.5-A1/B8	17/61	27.9

Interestingly, almost 50% of the peptides mapped to γ-gliadins (48/97), whereas the remaining peptides derived from ω-gliadins (24/97) or α-gliadins (25/97) (Supplemental Table II).

The selected peptides were tested for their capacity to bind purified A*0101 and B*0801 in in vitro quantitative competition binding assays, as described in *Materials and Methods*. As shown in Table III, 23 peptides bound the cognate HLA class I molecule with a high affinity ($IC_{50} < 500$ nM), with the great majority of these peptides (20/23, 87%) binding B*0801. Of note, two of the three gliadin peptides binding A*0101 mapped to α-gliadin, whereas the majority (12/20, 60%) of the B*0801 binders were γ-gliadin peptides.

*Immunogenicity of HLA A*0101- and B*0801-binding gliadin peptides*

Up to one half of HLA class I high-affinity binding peptides of viral protein origin are immunogenic in humans carrying the appropriate HLA class I genotype and in HLA class I–transgenic mice (25, 27–30). Accordingly, we next investigated the 23 gliadin peptides that bound A*0101 or B*0801 for their immunostimulatory properties in CD patients. CD subjects either positive or negative for A*0101/B*0801 (CD#1–24, Table II) and HLA A*01/B*08$^+$ healthy controls (CTR#1–10, Supplemental Table I) were selected for the functional assay.

IFN-γ production in PBMCs stimulated with each single peptide, according to a protocol that allows the detection of memory T cell responses in peripheral blood, was measured by ELISPOT assay (11). ELISPOT data are shown in Fig. 1 and are presented as net IFN-γ SFC per 10^6 cells (SFC per 10^6 cells in the presence of gliadin peptides minus the SFC per 10^6 cells with medium alone). A1/B8-binding peptides that elicited responses exceeding a specific cut-off value (see *Materials and Methods*) in at least three HLA A*0101/B*0801 patients were considered immunogenic. Two of the three HLA A*0101–binding peptides, α-gliadin$_{158–167}$ (SSQVLQQSTY) and ω-gliadin$_{414–423}$ (SEEPSPYQQY), were recognized by three or more A*0101$^+$ CD patients (Fig. 1). More specifically, α-gliadin$_{158–167}$ stimulated the activation of IFN-γ–secreting cells in four of eight (50%) patients, whereas ω-gliadin$_{414–423}$ was active in three of eight (37.5%) patients. In contrast, no IFN-γ responses were detected in HLA A*0101 healthy controls against gliadin peptides binding the A*0101 molecule.

The overall IFN-γ responses of PBMCs from patients with CD and healthy controls toward the B*0801 gliadin peptides are shown in Fig. 2. Eight peptides, derived from all three gliadin families, recalled significant immune reactivity in B*0801$^+$ CD patients. The γ-gliadin$_{286–294}$ (QLEAIRSLV) peptide recalled IFN-γ responses in 62% (8/13) of B*0801$^+$ patients with CD. For the A*0101$^+$ or B*0801$^+$ nonceliac controls, if excluding one subject who had a response to γ-gliadin$_{224–232}$ (QGMHILLPL) slightly exceeding the cut-off value, no responses were observed to any gliadin peptides. Consistent with the gliadin peptide results, we observed an IFN-γ response to known influenza A virus CTL epitopes, restricted by HLA A*0101 or B*0801, in a subgroup of A*0101/B*0801$^+$ subjects with a range of SFC per 10^6 cells comparable with that observed in response to gliadin peptides (Supplemental Fig. 1).

A large body of studies demonstrated that gluten peptides acquire T cell immunostimulatory properties after the tTG-mediated deamidation of specific glutamine residues (31, 32). Nevertheless,

Table II. CD patients enrolled for immunogenicity assays

Patients	Age (y)/Sex	HLA Class I	HLA Class II
HLA A*0101$^+$			
CD#1	38/F	A1$^+$, B8$^-$	DR5/DR7
CD#2	26/F	A1$^+$, B8$^-$	DR7/DR14
CD#3	5/F	A1$^+$, B8$^-$	DR5/DR7
CD#4	18/F	A1$^+$, B8$^-$	DR3/DRX
HLA B*0801$^+$			
CD#5	41/F	A1$^-$, B8$^+$	DR5/DR7
CD#6	45/F	A1$^-$, B8$^+$	DR3/DR5
CD#7	47/F	A1$^-$, B8$^+$	DR4/DR8
CD#8	8/F	A1$^-$, B8$^+$	DR3/DRX
CD#9	17/M	A1$^-$,B8$^+$	DR3/DR5
CD#10	10/M	A1$^-$, B8$^+$	DR3/DRX
CD#11	11/F	A1$^-$, B8$^+$	DR3/DRX
CD#12	11/F	A1$^-$,B8$^+$	DR3/DRX
CD#13	8/M	A1$^-$, B8$^+$	DR3/DRX
HLA A*0101 and B*0801$^+$			
CD#14	41/F	A1$^+$, B8$^+$	DR3/DR1
CD#15	38/F	A1$^+$, B8$^+$	DR3/DR7
CD#16	60/F	A1$^+$, B8$^+$	DR3/DR7
CD#17	40/F	A1$^+$, B8$^+$	DR3/DR5
HLA A*0101 and B*0801$^-$			
CD#18	27/M	A1$^-$, B8$^-$	DR3/DRX
CD#19	42/F	A1$^-$, B8$^-$	DR3/DRX
CD#20	13/F	A1$^-$, B8$^-$	DR3/DR3
CD#21	16/M	A1$^-$, B8$^-$	DR3/DRX
CD#22	12/F	A1$^-$, B8$^-$	DR5/DR7
CD#23	15/M	A1$^-$, B8$^-$	DR3/DR5
CD#24	20/M	A1$^-$, B8$^-$	DR5/DR7

F, female; M, male.

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

Table III. Binding affinity to HLA class I A*0101 and B*0801 molecules

Protein	Position	Sequence	IC_{50} (nM)[a]		Binder
			A*0101	B*0801	
α-Gliadin	158	SSQVLQQSTY	103	—[b]	A*0101
ω-Gliadin	414	SEEPSPYQQY	110	—	A*0101
α-Gliadin	217	VSFQQPQQQY	365	—	A*0101
ω-Gliadin	12	MAMKIATAA	—	5.2	B*0801
γ-Gliadin	159	FLLQQCKPV	—	7.8	B*0801
γ-Gliadin	160	LLQQCKPVSL	—	14	B*0801
γ-Gliadin	188	LLQQSKPASL	33,513	25	B*0801
γ-Gliadin	189	LQQSKPASL	58,779	35	B*0801
α-Gliadin	254	FEEIRNLAL	—	46	B*0801
ω-Gliadin	11	AMAMKIATA	—	48	B*0801
γ-Gliadin	161	LQQCKPVSL	—	72	B*0801
γ-Gliadin	224	QGMHILLPL	—	73	B*0801
ω-Gliadin	22	LLSPRGKEL	—	91	B*0801
γ-Gliadin	165	FPQQQRPFI	—	112	B*0801
ω-Gliadin	10	LAMAMKIATA	—	161	B*0801
γ-Gliadin	213	VMRQQCCQQL	—	162	B*0801
α-Gliadin	1	MKTFLILAL	—	223	B*0801
α-Gliadin	141	LIPCRDVVL	—	246	B*0801
γ-Gliadin	31	WLQQQLVPQL	—	286	B*0801
γ-Gliadin	287	LEAIRSLVL	—	303	B*0801
γ-Gliadin	286	QLEAIRSLV	17,070	353	B*0801
ω-Gliadin	14	MNIASASRL	—	368	B*0801
γ-Gliadin	203	CAAIHTIIH	—	465	B*0801

[a]Binder threshold (IC_{50} < 500 nM).
[b]Dash (—) indicates IC_{50} > 100,000 nM.

peptides able to stimulate CD T cells in their native form also were described (9, 33, 34). To confirm that gliadin contains peptides able to stimulate T cell–mediated responses in a deamidation-independent manner, we performed additional experiments in our cohort of A*0101/B*0801+ CD patients using partially digested gliadin extract (PT-gliadin) as Ag. Gliadin-specific cells can be detected in peripheral blood cells from treated CD patients, but not in healthy controls (Fig. 3).

Overall, these data demonstrate that gliadins contain short peptides that are able to recall, in their wild-type form, T cell responses in subjects with CD in the context of A*0101- and B*0801-restriction molecules.

*The reactivity to HLA class I A*0101/B*0801-restricted gliadin peptides is heterogeneous*

The frequency of IFN-γ responses in the A*0101/B*0801+ CD patients against the HLA-matched stimulatory peptides is summarized in Table IV. Five of eight (63%) A*0101 CD patients responded to at least one A*0101 gliadin peptide. The α-gliadin$_{158–167}$ SSQVLQQSTY peptide was the most prominent A*0101-restricted epitope; it was recognized by four of eight (50%) patients with CD.

A higher number of responding subjects was observed in B*0801+ patients with CD; 10 of the 13 (77%) screened patients were reactive to at least one B*0801 gliadin peptide. Interestingly, three patients (#7, #9, and #12) were the most responsive, either in terms of the number of peptides recognized (four of eight, seven of eight, and eight of eight, respectively) or the frequency of specific T cells. γ-Gliadin$_{286–294}$ (QLEAIRSLV) was the most active peptide; it was recognized by 8 of 13 (62%) patients with CD. Collectively, the profile of responses summarized in Table IV indicates a rather heterogeneous recognition pattern of A*0101- and B*0801-binding gliadin peptides.

*The immune response to HLA A*0101- and B*0801-binding peptides is mediated by CD8+ T cells*

Next, we investigated the nature of T cells responding to the newly identified HLA class I–restricted gliadin epitopes. Because T cells reactive to A*0101/B*0801-restricted gliadin peptides have a low

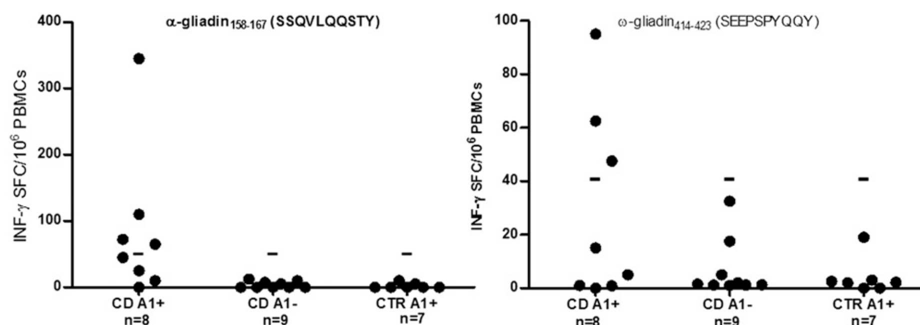

FIGURE 1. Immunogenicity of HLA A*0101 binder gliadin peptides. IFN-γ–releasing cells in response to gliadin peptides that bind to HLA A*0101 with high affinity were detected by ELISPOT in PBMCs from HLA A*0101+ CD patients and healthy controls, as well as in HLA A*0101− CD patients. Each peptide was assayed at 10 μg/ml, and results are shown as net IFN-γ SFC per 10^6 cells (SFC in the presence of peptide − SFC in the absence of peptide). Each circle represents the number of IFN-γ–releasing cells monitored in a single subject. Horizontal lines indicate the mean value + 2 SD of responses obtained in A*0101− CD subjects (cut-off value).

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

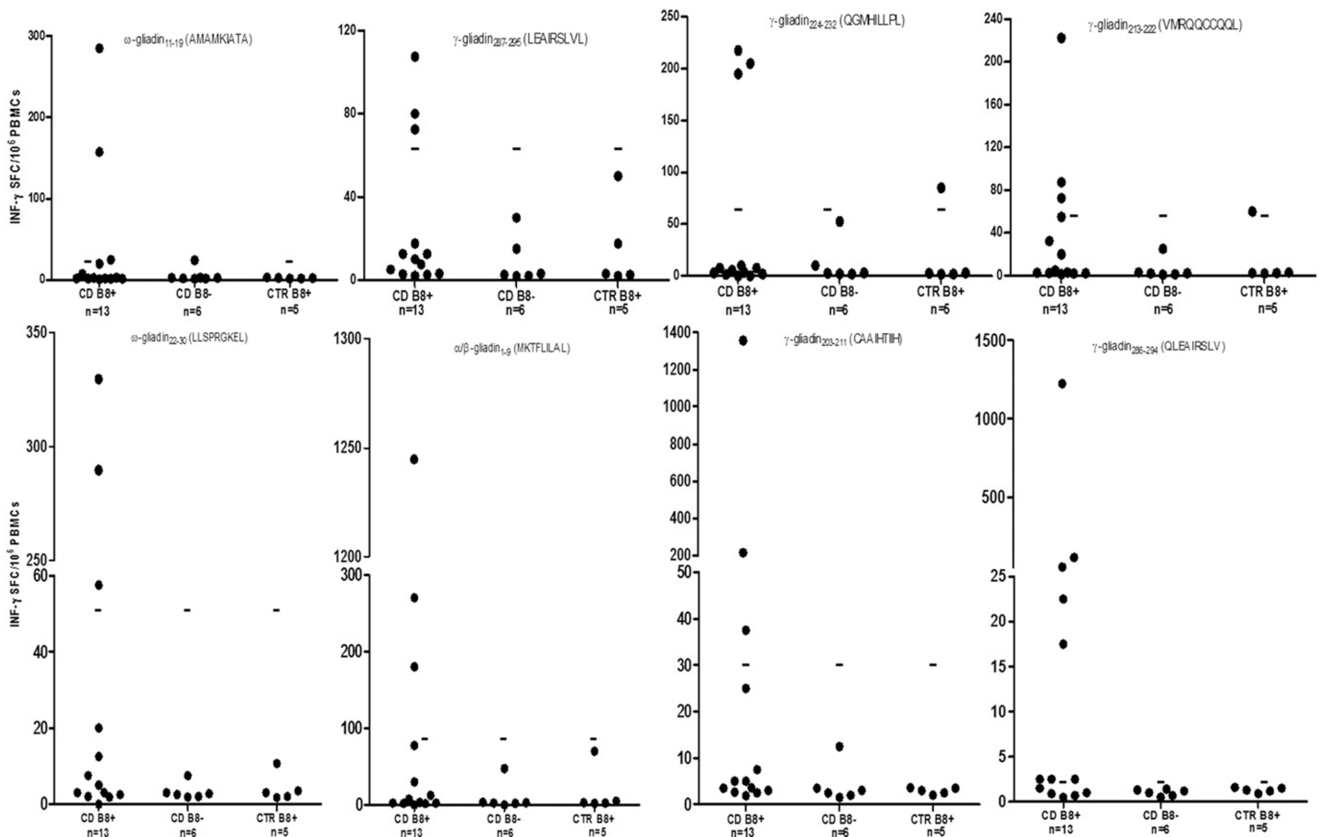

FIGURE 2. Immunogenicity of HLA B*0801 binder gliadin peptides. IFN-γ–releasing cells in response to gliadin peptides that bind HLA B*0801 with high affinity were detected by ELISPOT in PBMCs from HLA B*0801$^+$ CD patients and healthy controls, as well as in HLA B*0801$^-$ CD patients. Each peptide was assayed at 10 μg/ml, and results are shown as net IFN-γ SFC per 10^6 cells. Each circle represents the number of IFN-γ–releasing cells monitored in a single subject. Horizontal lines indicate the mean value + 2 SD of responses obtained in B*0801$^-$ CD subjects (cut-off value).

frequency in the peripheral blood of treated CD patients compliant with a gluten-free diet, we generated in vitro peptide-specific T cell cultures. T cells reactive toward two active B*0801 peptides, ω-gliadin$_{11-19}$ (AMAMKIATA) and γ-gliadin$_{224-232}$ (QGMHILLPL), were expanded to generate cytotoxic short-term T cell lines, as described in *Materials and Methods* (35). At the end of the expansion period, the bulk T cell cultures were enriched in CD8$^+$ T cells by immunomagnetic separation. FACS analysis determined that, in the bulk culture, an average of 52% of the cells were CD3$^+$CD8$^+$, whereas, upon immunomagnetic separation, 93.9% of the cells were CD3$^+$CD8$^+$. In the bulk culture, the frequency of cells secreting IFN-γ in response to ω-gliadin$_{11-19}$ peptide was 110–166.5 per 10^6 cells (mean 133 ± 47, ∼0.01%) (Fig. 4), whereas responses to γ-gliadin$_{224-232}$ were in the range of 399–3496 per 10^6 cells (mean 1948 ± 2189, ∼0.2%) (Fig. 2). Following immunomagnetic separation to enrich CD8$^+$ T cells, the IFN-γ–secreting cells were markedly enhanced in response to ω-gliadin$_{11-19}$ (4,628–6,327 per 10^6 cells, mean 5,477.85 ± 1200, ∼0.55%) and γ-gliadin$_{224-232}$ (8,891–11,255 per 10^6 cells, mean 10,073.25 ± 1671, ∼1%). Of note, an increase in the IFN-γ response to gliadin also was observed upon CD8$^+$ T cell enrichment.

Taken together, these data indicate that CD8$^+$ T cells specific for HLA class I–restricted gliadin peptides can be detected in the peripheral blood of CD patients.

Discussion

We report that several gliadin peptides with high binding affinity for HLA class I A*0101 or B*0801 molecules are able to stimulate CD8$^+$ T cell responses in CD patients. To identify CD8$^+$ T cell epitopes derived from gliadin, we took advantage of a strategy that combines bioinformatic analyses, in vitro MHC-peptide binding assays, and functional assays in patients with CD.

The sequences of two α-, γ-, and ω-gliadins were screened for the presence of peptides predicted to bind A*0101 and B*0801 molecules using algorithms available from the Immune Epitope Database (http://www.iedb.org) (18). Although all families of gliadin (α-, γ-, and ω-gliadins) contained potential binders to HLA A*0101 and B*0801, in vitro binding analysis identified 20 peptides with high affinity for B*0801 but only three A*0101 high binder peptides. This remarkable difference in repertoire is consistent with a previous study by Paul et al. (36) that highlighted how A*0101 is associated with a very narrow repertoire and relatively few binder peptides compared with other common HLA A and B molecules.

Almost half of the HLA class I–binding gliadin peptides were able to specifically stimulate IFN-γ responses in peripheral blood of CD patients. In particular, we found that 2 of 3 A*0101 peptides (α-gliadin$_{158-167}$ and ω-gliadin$_{414-423}$) and 8 of 20 B*0801 peptides (ω-gliadin$_{11-19}$, γ-gliadin$_{224-232}$, ω-gliadin$_{22-30}$, γ-gliadin$_{213-222}$, α-gliadin$_{1-9}$, γ-gliadin$_{287-295}$, γ-gliadin$_{286-294}$, and γ-gliadin$_{203-211}$) were selectively recognized by CD patients. Furthermore, we determined that the newly identified gliadin epitopes, selected based on their high affinity for HLA class I A*0101/B*0801 molecules, stimulated CD8$^+$ T cells in subjects with CD.

CD is an autoimmune disorder in which adaptive immunity, mediated by CD4$^+$ T cells, has a central pathogenic role. Several comprehensive studies characterized the phenotype, function, and peptide repertoire of gluten-specific CD4$^+$ T lymphocytes in CD patients (13, 34, 37, 38). However, several lines of evidence indicated that the massive infiltration of CD8$^+$ T lymphocytes into

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

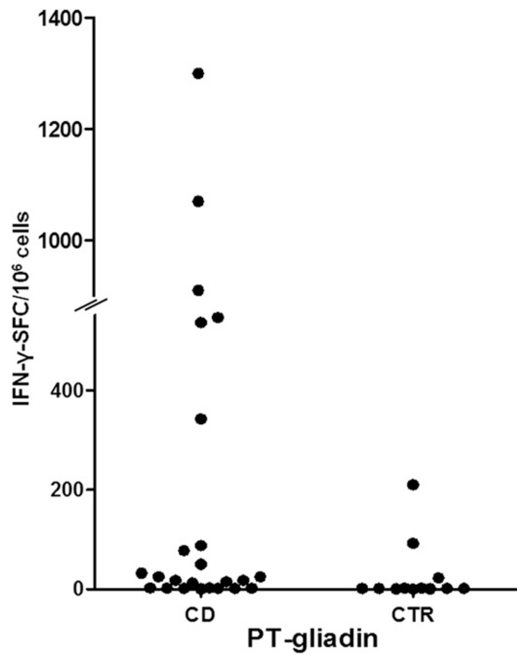

FIGURE 3. T cells reactive to native PT-gliadin in peripheral blood of CD patients. PBMCs from treated CD patients and healthy controls were stimulated PT-gliadin (50 μg/ml). Specific IFN-γ–releasing cells were detected by ELISPOT, as indicated in Fig. 1. Results are shown as net IFN-γ SFC per 10^6 cells. Each circle represents the number of IFN-γ–releasing cells monitored in the individual subject.

the intestinal mucosa of CD patients has a prominent role in the generation of intestinal villous atrophy. In particular, the pathogenic function was primarily attributed to the intraepithelial $CD8^+$ T lymphocytes associated with innate immunity (5, 6). These lymphocytes are armed to kill enterocytes after gluten exposure, albeit through a TCR-independent mechanism (5). Our data suggest that gliadin-specific adaptive $CD8^+$ T lymphocytes restricted by HLA class I molecules may also participate in the inflammatory cascade triggered by gluten.

We reported previously that gliadin contains a peptide capable of activating adaptive $CD8^+$ T lymphocytes exclusively in CD patients. This epitope, corresponding to the 123–132 residues of α-gliadin, is restricted by HLA A*02 class I molecule and is specifically recognized by $HLA*0201^+$ CD subjects (11). Because there is no strict genetic association with the HLA A*0201 gene [carried by 30–40% of CD subjects and the general white population (39)], we have now expanded our analysis to A*0101 and B*0801, two frequent HLA class I alleles in the general worldwide population and in linkage with the DQ2.5 CD-risk alleles.

Similar to findings on gliadin peptides activating $CD4^+$ T cells (34), we observed a heterogeneous recognition profile of $CD8^+$ T cell peptides, in particular for B*0801-restricted epitopes. Notwithstanding a large response heterogeneity, two peptides, α-gliadin$_{158–167}$ (restricted by HLA A*0101) and γ-gliadin$_{286–294}$ (restricted by HLA B*0801), were highly stimulatory; they induced T cell responses in 50 and in 62% of CD patients, respectively. Interestingly, we found that five of eight B*0801-restricted gliadin epitopes mapped to the γ-gliadins. This finding indicated that γ-gliadins are an important trigger factor in CD pathogenesis, in accordance with our previous studies describing several $CD4^+$ T cell epitopes in this prolamin family (40, 41). Notably, the HLA class I gliadin epitopes described in our study were active in their wild-type form. Although it is well known that the enzymatic deamidation of specific glutamines is key in en-

Table IV. Recognition frequency of HLA class I–restricted gliadin epitopes in CD patients

Code	Protein	Sequence	Volunteers with CD													Responders (%)
A*01-restricted CD8 epitopes			CD#1	CD#2	CD#3	CD#4	CD#14	CD#15	CD#16	CD#17						
p158–167	α-Gliadin	SSQVLQQSTY	+[a]			+			+	+						4/8(50)
p414–423	ω-Gliadin	SEEPSPYQQY			+	+			+							3/8(37.5)
B*08-restricted CD8 epitopes			CD#5	CD#6	CD#7	CD#8	CD#9	CD#10	CD#11	CD#12	CD#13	CD#14	CD#15	CD#16	CD#17	
p11–19	ω-Gliadin	AMAMKIATA				+	+			+						3/13(23)
p224–232	γ-Gliadin	QGMHILLPL			+		+			+						3/13(23)
p22–30	ω-Gliadin	LLSPRGKEL					+			+					+	3/13 (23)
p213–222	γ-Gliadin	VMRQQCCQQL					+			+				+		3/13 (23)
p1–9	α-Gliadin	MKTFLILAL			+		+			+						3/13 (23)
p287–295	γ-Gliadin	LEAIRSLVL			+					+				+		3/13 (23)
p286–294	γ-Gliadin	QLEAIRSLV	+		+	+	+		+	+		+			+	8/13 (62)
p203−211	γ-Gliadin	CAAIHTIIH					+			+				+		3/13 (23)

[a]+ indicates a positive peptide response.

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

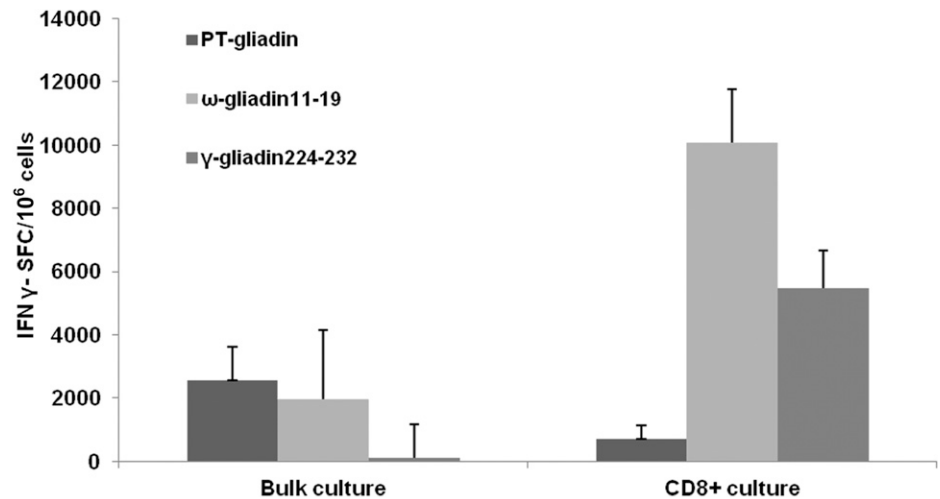

FIGURE 4. HLA class I B*0801–binding peptides are recognized by $CD8^+$ T cells in CD patients. Short-term T cell lines (CTLs) were obtained by stimulating PBMCs from HLA $B*0801^+$ CD patients with gliadin peptides ω-$gliadin_{11–19}$ and γ-$gliadin_{224–232}$. Peptide-specific IFN-γ–releasing cells were detected by ELISPOT in bulk CTL culture and in $CD8^+$ CTLs (magnetic depletion). Net IFN-γ SFC per 10^6 cells is shown. Each peptide was assayed at 10 μg/ml; PT-gliadin was assayed at 50 μg/ml as a control Ag. CTLs were generated from three CD patients. Results from one representative patient are shown.

hancing the $CD4^+$ T cell immunogenicity of gliadin in CD patients (31, 32), our data provide evidence of gliadin peptides that can elicit adaptive T cell responses independently of posttranscriptional modifications mediated by tTG.

Our findings are in agreement with previous studies demonstrating a strong correlation between HLA binding affinity and peptide stimulatory capability (30, 42, 43). It was shown that 50% of all high-affinity binders are immunogenic in an HLA A*02 mouse system (27). We found that, among the 23 gliadin peptides selected in this study on the basis of high binding affinity to HLA class I molecules, ∼43% were immunogenic in subjects with CD. These data confirm that binding analyses provide a useful tool to facilitate the identification of peptides that are potential $CD8^+$ T cell epitopes.

The PBMCs reacting to HLA A*0101/B*0801 gliadin epitopes are $CD8^+$ T cells, as demonstrated by analysis of short-term T cell lines specific for two of the most active epitopes. These results confirm previous findings from our group showing that gliadin contains a 10-mer epitope that is able to activate adaptive $CD8^+$ T cells in HLA A*0201 CD patients but not in healthy controls (9, 11). Recently, Han et al. (12) reported an increase (from 0.02 to 1.11%) in the proportion of total $CD8^+$ T cells of intestinal origin ($CD38^+$ and $αEβ7^+$) in the peripheral blood of CD patients after 3 d of gluten challenge, along with a concomitant increase (from 0.03 to 1.52%) in $CD38^+αEβ7^+$ TCRγδ T cells (12). These findings strongly suggest that gluten triggers the activation and mobilization of intestinal $CD8^+$ T cells.

Several studies demonstrated that $CD8^+$ T cells have a cytotoxic function (5–7, 9, 11) in CD intestinal mucosa. In addition, we previously demonstrated the apoptosis of $CaCO_2$ cells presenting a gluten peptide to HLA class I–restricted $CD8^+$ T lymphocytes isolated from CD gut mucosa (9). These observations parallel those pertaining to the involvement of cytotoxic $CD8^+$ T cells in the pathogenesis of several other autoimmune diseases. Islet-infiltrating pancreatic T cells are made predominantly by $CD8^+$ T cells (44) and further in vitro evidence established their role in pancreatic β-cell destruction (45). Of note, Coppieters et al. (46) showed the presence of islet-reactive $CD8^+$ T cells in insulitic lesions from patients with recent-onset or longstanding type 1 diabetes, suggesting a role for these cells in the onset and progression of autoimmune diabetes.

Early genetic studies reported an association of CD with the HLA A*0101 and B*0801 class I alleles (15, 16), although further studies demonstrated that primary genetic susceptibility is provided by HLA class II DQ genes (47). Of relevance, a recent fine mapping of the MHC locus identified the HLA B*0801 gene as an additional risk factor for CD patients (17). Interestingly, A*0101 and B*0801 genes are part of the extended DR3-DQ2.5–A1–B8 (COX) haplotype. COX is a highly conserved haplotype and is associated with several autoimmune diseases (24). Specifically, COX is expressed in CD with high frequency (48) and is associated with more severe clinical manifestations and with complicated CD (49). Although we found that approximately one third of our CD patients were DR3-DQ2.5-A*0101/$B*0801^+$, further studies are needed to define the exact penetrance of the extended DR3-DQ2.5–A1–B8 haplotype in larger Italian CD cohorts. Such studies could be important to allow for more complete risk stratification. It will be interesting to correlate the clinical onset, disease severity, and the magnitude of the antigluten T cell response with the HLA class I and II genotypes. These studies would help us to understand CD immunopathogenesis and may also define strategies for treating, or even preventing, this dietary disorder (50).

In conclusion, we demonstrate that gliadins contain epitopes that elicit $CD8^+$ T cell responses restricted by HLA class I A*0101 and B*0801 molecules. Our immunologic findings are corroborated by recent GWAS analyses showing a genetic association of CD with the HLA class I B*0801 gene (17), strongly suggesting a role for $CD8^+$ T lymphocytes in CD pathogenesis.

Disclosures

The authors have no financial conflicts of interest.

References

1. Sollid, L. M. 2000. Molecular basis of celiac disease. *Annu. Rev. Immunol.* 18: 53–81.
2. Lundin, K. E., S. W. Qiao, O. Snir, and L. M. Sollid. 2015. Coeliac disease - from genetic and immunological studies to clinical applications. *Scand. J. Gastroenterol.* 50: 708–717.
3. Sollid, L. M., R. Iversen, Ø. Steinsbø, S. W. Qiao, E. Bergseng, S. Dørum, M. F. du Pré, J. Stamnaes, A. Christophersen, I. Cardoso, et al. 2015. Small bowel, celiac disease and adaptive immunity. *Dig. Dis.* 33: 115–121.
4. Qiao, S. W., R. Iversen, M. Ráki, and L. M. Sollid. 2012. The adaptive immune response in celiac disease. *Semin. Immunopathol.* 34: 523–540.
5. Abadie, V., V. Discepolo, and B. Jabri. 2012. Intraepithelial lymphocytes in celiac disease immunopathology. *Semin. Immunopathol.* 34: 551–566.
6. Abadie, V., L. M. Sollid, L. B. Barreiro, and B. Jabri. 2011. Integration of genetic and immunological insights into a model of celiac disease pathogenesis. *Annu. Rev. Immunol.* 29: 493–525.
7. Ciccocioppo, R., A. Di Sabatino, R. Parroni, P. Muzi, S. D'Alò, T. Ventura, M. A. Pistoia, M. G. Cifone, and G. R. Corazza. 2001. Increased enterocyte apoptosis and Fas-Fas ligand system in celiac disease. *Am. J. Clin. Pathol.* 115: 494–503.
8. Maiuri, L., C. Ciacci, V. Raia, L. Vacca, I. Ricciardelli, F. Raimondi, S. Auricchio, S. Quaratino, and M. Londei. 2001. FAS engagement drives apoptosis of enterocytes of coeliac patients. *Gut* 48: 418–424.

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017

9. Mazzarella, G., R. Stefanile, A. Camarca, P. Giliberti, E. Cosentini, C. Marano, G. Iaquinto, N. Giardullo, S. Auricchio, A. Sette, et al. 2008. Gliadin activates HLA class I-restricted CD8+ T cells in celiac disease intestinal mucosa and induces the enterocyte apoptosis. *Gastroenterology* 134: 1017–1027.
10. Hüe, S., J. J. Mention, R. C. Monteiro, S. Zhang, C. Cellier, J. Schmitz, V. Verkarre, N. Fodil, S. Bahram, N. Cerf-Bensussan, and S. Caillat-Zucman. 2004. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* 21: 367–377.
11. Gianfrani, C., R. Troncone, P. Mugione, E. Cosentini, M. De Pascale, C. Faruolo, S. Senger, G. Terrazzano, S. Southwood, S. Auricchio, and A. Sette. 2003. Celiac disease association with CD8+ T cell responses: identification of a novel gliadin-derived HLA-A2-restricted epitope. *J. Immunol.* 170: 2719–2726.
12. Han, A., E. W. Newell, J. Glanville, N. Fernandez-Becker, C. Khosla, Y. H. Chien, and M. M. Davis. 2013. Dietary gluten triggers concomitant activation of CD4+ and CD8+ αβ T cells and γδ T cells in celiac disease. *Proc. Natl. Acad. Sci. USA* 110: 13073–13078.
13. Louka, A. S., and L. M. Sollid. 2003. HLA in coeliac disease: unravelling the complex genetics of a complex disorder. *Tissue Antigens* 61: 105–117.
14. Margaritte-Jeannin, P., M. C. Babron, M. Bourgey, A. S. Louka, F. Clot, S. Percopo, I. Coto, J. P. Hugot, H. Ascher, L. M. Sollid, et al. 2004. HLA-DQ relative risks for coeliac disease in European populations: a study of the European genetics cluster on coeliac disease. *Tissue Antigens* 63: 562–567.
15. Falchuk, Z. M., and W. Strober. 1972. HL-A antigens and adult coeliac disease. *Lancet* 2: 1310.
16. Stokes, P. L., P. Asquith, G. K. Holmes, P. Mackintosh, and W. T. Cooke. 1972. Histocompatibility antigens associated with adult coeliac disease. *Lancet* 2: 162–164.
17. Gutierrez-Achury, J., A. Zhernakova, S. L. Pulit, G. Trynka, K. A. Hunt, J. Romanos, S. Raychaudhuri, D. A. van Heel, C. Wijmenga, and P. I. de Bakker. 2015. Fine mapping in the MHC region accounts for 18% additional genetic risk for celiac disease. *Nat. Genet.* 47: 577–578.
18. Vita, R., J. A. Overton, J. A. Greenbaum, J. Ponomarenko, J. D. Clark, J. R. Cantrell, D. K. Wheeler, J. L. Gabbard, D. Hix, A. Sette, and B. Peters. 2015. The immune epitope database (IEDB) 3.0. *Nucleic Acids Res.* 43: D405–D412.
19. Sidney, J., S. Southwood, C. Moore, C. Oseroff, C. Pinilla, H. M. Grey, and A. Sette. 2013. Measurement of MHC/peptide interactions by gel filtration or monoclonal antibody capture. *Curr. Protoc. Immunol.* Chapter 18: Unit 18.3.
20. Cheng, Y., and W. H. Prusoff. 1973. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* 22: 3099–3108.
21. Gulukota, K., J. Sidney, A. Sette, and C. DeLisi. 1997. Two complementary methods for predicting peptides binding major histocompatibility complex molecules. *J. Mol. Biol.* 267: 1258–1267.
22. Middleton, D., L. Menchaca, H. Rood, and R. Komerofsky. 2003. New allele frequency database: http://www.allelefrequencies.net. *Tissue Antigens* 61: 403–407.
23. González-Galarza, F. F., L. Y. Takeshita, E. J. Santos, F. Kempson, M. H. Maia, A. L. da Silva, A. L. Teles e Silva, G. S. Ghattaoraya, A. Alfirevic, A. R. Jones, and D. Middleton. 2015. Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. *Nucleic Acids Res.* 43: D784–D788.
24. Price, P., C. Witt, R. Allcock, D. Sayer, M. Garlepp, C. C. Kok, M. French, S. Mallal, and F. Christiansen. 1999. The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases. *Immunol. Rev.* 167: 257–274.
25. Gough, S. C., and M. J. Simmonds. 2007. The HLA region and autoimmune disease: associations and mechanisms of action. *Curr. Genomics* 8: 453–465.
26. Aly, T. A., E. Eller, A. Ide, K. Gowan, S. R. Babu, H. A. Erlich, M. J. Rewers, G. S. Eisenbarth, and P. R. Fain. 2006. Multi-SNP analysis of MHC region: remarkable conservation of HLA-A1-B8-DR3 haplotype. *Diabetes* 55: 1265–1269.
27. Assarsson, E., J. Sidney, C. Oseroff, V. Pasquetto, H. H. Bui, N. Frahm, C. Brander, B. Peters, H. Grey, and A. Sette. 2007. A quantitative analysis of the variables affecting the repertoire of T cell specificities recognized after vaccinia virus infection. *J. Immunol.* 178: 7890–7901.
28. Gianfrani, C., C. Oseroff, J. Sidney, R. W. Chesnut, and A. Sette. 2000. Human memory CTL response specific for influenza A virus is broad and multispecific. *Hum. Immunol.* 61: 438–452.
29. Yewdell, J. W. 2006. Confronting complexity: real-world immunodominance in antiviral CD8+ T cell responses. *Immunity* 25: 533–543.
30. Sette, A., A. Vitiello, B. Reherman, P. Fowler, R. Nayersina, W. M. Kast, C. J. Melief, C. Oseroff, L. Yuan, J. Ruppert, et al. 1994. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *J. Immunol.* 153: 5586–5592.
31. van de Wal, Y., Y. Kooy, P. van Veelen, S. Peña, L. Mearin, G. Papadopoulos, and F. Koning. 1998. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. *J. Immunol.* 161: 1585–1588.
32. Arentz-Hansen, H., R. Körner, O. Molberg, H. Quarsten, W. Vader, Y. M. Kooy, K. E. Lundin, F. Koning, P. Roepstorff, L. M. Sollid, and S. N. McAdam. 2000. The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J. Exp. Med.* 191: 603–612.
33. Vader, W., Y. Kooy, P. Van Veelen, A. De Ru, D. Harris, W. Benckhuijsen, S. Peña, L. Mearin, J. W. Drijfhout, and F. Koning. 2002. The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology* 122: 1729–1737.
34. Hardy, M. Y., A. Girardin, C. Pizzey, D. J. Cameron, K. A. Watson, S. Picascia, R. Auricchio, L. Greco, C. Gianfrani, N. L. La Gruta, et al. 2015. Consistency in polyclonal T-cell responses to gluten between children and adults with celiac disease. *Gastroenterology* 149: 1541–1552.e2.
35. Wells, A. D., M. C. Walsh, D. Sankaran, and L. A. Turka. 2000. T cell effector function and anergy avoidance are quantitatively linked to cell division. *J. Immunol.* 165: 2432–2443.
36. Paul, S., D. Weiskopf, M. A. Angelo, J. Sidney, B. Peters, and A. Sette. 2013. HLA class I alleles are associated with peptide-binding repertoires of different size, affinity, and immunogenicity. *J. Immunol.* 191: 5831–5839.
37. Tye-Din, J. A., J. A. Stewart, J. A. Dromey, T. Beissbarth, D. A. van Heel, A. Tatham, K. Henderson, S. I. Mannering, C. Gianfrani, D. P. Jewell, et al. 2010. Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. *Sci. Transl. Med.* 2: 41ra51.
38. Camarca, A., A. Del Mastro, and C. Gianfrani. 2012. Repertoire of gluten peptides active in celiac disease patients: perspectives for translational therapeutic applications. *Endocr. Metab. Immune Disord. Drug Targets* 12: 207–219.
39. Ellis, J. M., V. Henson, R. Slack, J. Ng, R. J. Hartzman, and C. Katovich Hurley. 2000. Frequencies of HLA-A2 alleles in five U.S. population groups. Predominance of A*02011 and identification of HLA-A*0231. *Hum. Immunol.* 61: 334–340.
40. Camarca, A., R. P. Anderson, G. Mamone, O. Fierro, A. Facchiano, S. Costantini, D. Zanzi, J. Sidney, S. Auricchio, A. Sette, et al. 2009. Intestinal T cell responses to gluten peptides are largely heterogeneous: implications for a peptide-based therapy in celiac disease. *J. Immunol.* 182: 4158–4166.
41. Gianfrani, C., A. Camarca, G. Mazzarella, L. Di Stasio, N. Giardullo, P. Ferranti, G. Picariello, V. Rotondi Aufiero, S. Picascia, R. Troncone, et al. 2015. Extensive in vitro gastrointestinal digestion markedly reduces the immune-toxicity of *Triticum monococcum* wheat: implication for celiac disease. *Mol. Nutr. Food Res.* 59: 1844–1854.
42. Chen, Y., J. Sidney, S. Southwood, A. L. Cox, K. Sakaguchi, R. A. Henderson, E. Appella, D. F. Hunt, A. Sette, and V. H. Engelhard. 1994. Naturally processed peptides longer than nine amino acid residues bind to the class I MHC molecule HLA-A2.1 with high affinity and in different conformations. *J. Immunol.* 152: 2874–2881.
43. van Leeuwen, M. A., D. J. Lindenbergh-Kortleve, H. C. Raatgeep, L. F. de Ruiter, R. R. de Krijger, M. Groeneweg, J. C. Escher, and J. N. Samsom. 2013. Increased production of interleukin-21, but not interleukin-17A, in the small intestine characterizes pediatric celiac disease. *Mucosal Immunol.* 6: 1202–1213.
44. Foulis, A. K., and M. A. Farquharson. 1986. Aberrant expression of HLA-DR antigens by insulin-containing beta-cells in recent-onset type I diabetes mellitus. *Diabetes* 35: 1215–1224.
45. Campbell, P. D., E. Estella, N. L. Dudek, G. Jhala, H. E. Thomas, T. W. Kay, and S. I. Mannering. 2008. Cytotoxic T-lymphocyte-mediated killing of human pancreatic islet cells in vitro. *Hum. Immunol.* 69: 543–551.
46. Coppieters, K. T., F. Dotta, N. Amirian, P. D. Campbell, T. W. Kay, M. A. Atkinson, B. O. Roep, and M. G. von Herrath. 2012. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J. Exp. Med.* 209: 51–60.
47. Sollid, L. M., G. Markussen, J. Ek, H. Gjerde, F. Vartdal, and E. Thorsby. 1989. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J. Exp. Med.* 169: 345–350.
48. Bolognesi, E., K. Karell, S. Percopo, I. Coto, L. Greco, V. Mantovani, E. Suoraniemi, J. Partanen, K. Mustalahti, M. Mäki, and P. Momigliano-Richiardi. 2003. Additional factor in some HLA DR3/DQ2 haplotypes confers a fourfold increased genetic risk of celiac disease. *Tissue Antigens* 61: 308–316.
49. López-Vázquez, A., D. Fuentes, L. Rodrigo, S. González, M. Moreno, E. Fernández, J. Martínez-Borra, and C. López-Larrea. 2004. MHC class I region plays a role in the development of diverse clinical forms of celiac disease in a Saharawi population. *Am. J. Gastroenterol.* 99: 662–667.
50. Pisapia, L., A. Camarca, S. Picascia, V. Bassi, P. Barba, G. Del Pozzo, and C. Gianfrani. 2016. HLA-DQ2.5 genes associated with celiac disease risk are preferentially expressed with respect to non-predisposing HLA genes: implication for anti-gluten T cell response. *J. Autoimmun.* 70: 63–72.

Downloaded from http://www.jimmunol.org/ by guest on February 10, 2017