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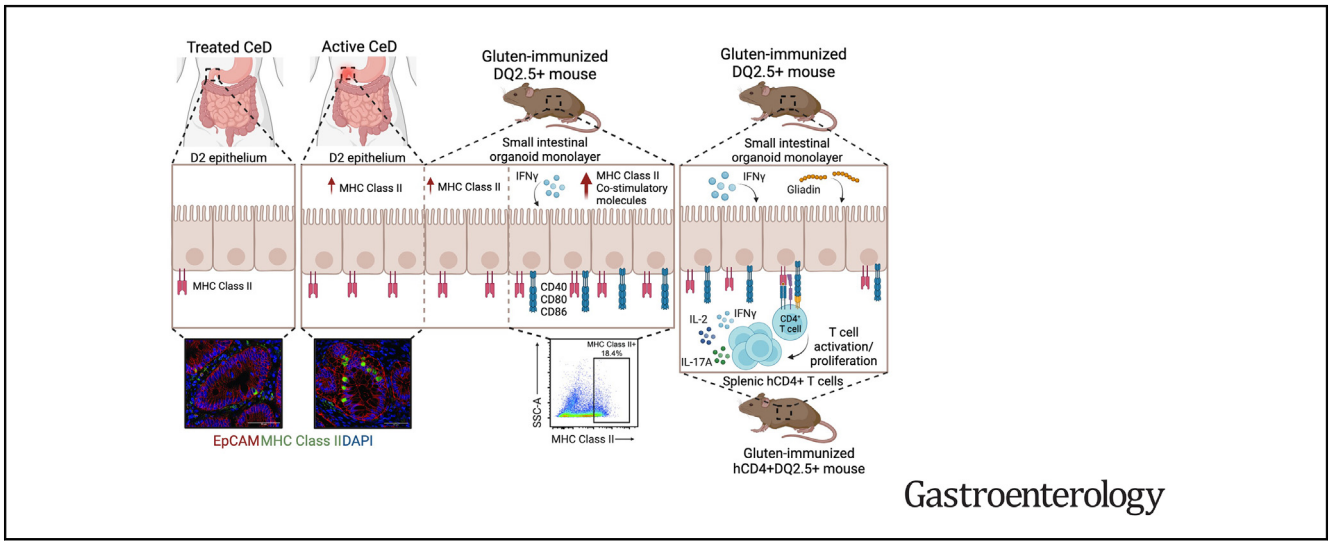
CELIAC DISEASE

Gluten-Dependent Activation of CD4$^+$ T Cells by MHC Class II–Expressing Epithelium

Sara Rahmani,[1,2,3] Heather J. Galipeau,[2,3] Alexandra V. Clarizio,[2,3] Xuanyu Wang,[2,3] Amber Hann,[2,3] Gaston H. Rueda,[2,3] Utkarshini N. Kirtikar,[2,3] Marco Constante,[2,3] Mark Wulczynski,[2,3] Hsuan-Ming Su,[4] Rebecca Burchett,[3] Jonathan L. Bramson,[3] Maria Ines Pinto-Sanchez,[2,3] Juan Pablo Stefanolo,[5] Sonia Niveloni,[5] Michael G. Surette,[2,3] Joseph A. Murray,[6] Robert P. Anderson,[7] Premysl Bercik,[2,3] Alberto Caminero,[2,3] Fernando G. Chirdo,[8] Tohid F. Didar,[1,4] and Elena F. Verdu[2,3]

[1]School of Biomedical Engineering, McMaster University, Hamilton, Ontario, Canada; [2]Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Ontario, Canada; [3]Department of Medicine, McMaster University, Hamilton, Ontario, Canada; [4]Department of Mechanical Engineering, McMaster University, Hamilton, Ontario, Canada; [5]C. Bonorino Udaondo Hospital, Buenos Aires, Argentina; [6]Division of Gastroenterology and Hepatology, Department of Immunology, Mayo Clinic College of Medicine, Rochester, Minnesota; [7]Mackay Base Hospital, Mackay, Queensland, Australia; and [8]Instituto de Estudios Inmunológicos y Fisiopatológicos - IIFP (UNLP-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina



BACKGROUND & AIMS: Intestinal epithelial cell (IEC) damage is a hallmark of celiac disease (CeD); however, its role in gluten-dependent T-cell activation is unknown. We investigated IEC-gluten-T-cell interactions in organoid monolayers expressing human major histocompatibility complex class II (HLA-DQ2.5), which facilitates gluten antigen recognition by CD4$^+$ T cells in CeD. **METHODS:** Epithelial major histocompatibility complex class II (MHCII) was determined in active and treated CeD, and in nonimmunized and gluten-immunized DR3-DQ2.5 transgenic mice, lacking mouse MHCII molecules. Organoid monolayers from DR3-DQ2.5 mice were treated with or without interferon (IFN)-γ, and MHCII expression was evaluated by flow cytometry. Organoid monolayers and CD4$^+$ T-cell co-cultures were incubated with gluten, predigested, or not by elastase-producing *Pseudomonas aeruginosa* or its *lasB* mutant. T-cell function was assessed based on proliferation, expression of activation markers, and cytokine release in the co-culture supernatants. **RESULTS:** Patients with active CeD and gluten-immunized DR3-DQ2.5 mice demonstrated epithelial MHCII expression. Organoid monolayers derived from gluten-immunized DR3-DQ2.5 mice expressed MHCII, which was upregulated by IFN-γ. In organoid monolayer T-cell co-cultures, gluten increased the proliferation of CD4$^+$ T cells, expression of T-cell activation markers, and the release of interleukin-2, IFN-γ, and interleukin-15 in co-culture supernatants. Gluten metabolized by *P aeruginosa*, but not the *lasB* mutant, enhanced CD4$^+$ T-cell proliferation and activation. **CONCLUSIONS:** Gluten antigens are efficiently presented by MHCII-expressing IECs, resulting in the activation of gluten-specific CD4$^+$ T cells, which is enhanced by gluten predigestion with microbial elastase. Therapeutics directed at IECs may offer a novel approach for modulating both adaptive and innate immunity in patients with CeD.

Keywords: Celiac Disease; Organoid Monolayers; MHC Class II; T-Cell Activation; Gluten; Microbial Metabolism.

Celiac disease (CeD) has a global prevalence of approximately 1% and is an immune-mediated systemic condition precipitated by gluten in genetically predisposed individuals.[1] CeD is characterized by gluten-dependent small intestinal villous atrophy, crypt hyperplasia, intraepithelial lymphocytosis,[2] gluten-dependent autoantibodies specific for transglutaminase 2 (TG2)[3] and a persistent adaptive immune response directed preferentially against deamidated gluten peptides.[4] Specific major histocompatibility complex (MHC) class II (MHCII) genes are necessary, but insufficient for CeD, and co-factors have been implicated.[5] Ninety percent of patients with CeD carry HLA-DQA1*05 and HLA-DQB1*02, which encode the protein HLA-DQ2.5, expressed by professional antigen-presenting cells (APCs), such as B cells and dendritic cells. HLA-DQ2.5 allows the presentation of a distinct set of deamidated, protease-resistant gluten peptides recognized by specific $CD4^+$ T cells found in HLA-DQ2.5$^+$ patients with CeD.[6] Patients negative for HLA-DQ2.5 are usually positive for HLA-DQ8,[7] which can also present a characteristic set of deamidated gluten peptides.

Gluten encompasses prolamin proteins in wheat, rye, and barley, of which wheat gliadins are the best-characterized fraction.[8,9] Prolamins are partially digested in the human gut by host[10] and microbial proteases,[11] allowing immunogenic, protease-resistant gluten peptides to undergo transcytosis mediated by secretory immunoglobulin (Ig)A linked to CD71 to cross the epithelium.[12] Selective deamidation mediated by TG2[13] enhances the avidity of immunogenic gluten peptides for binding to HLA-DQ2.5 molecules.[14,15]

IECs play key roles in CeD pathogenesis by expressing stress-induced markers secondary to inflammation and infections,[16–18] and by releasing TG2 into the gut lumen to generate TG2-gluten complexes.[19] A recent study concluded that TG2- and gliadin-specific B cells and plasma cells are the preferred APCs for gluten in the lamina propria.[20,21] IECs express MHCII, and proinflammatory stimuli can upregulate MHCII in IECs[22,23]; however, the functional consequences of this expression in CeD have never been demonstrated. Here, we investigated the epithelial expression of CeD-associated MHCII in patients with CeD and in gluten-immunized mice that lack all murine MHCII molecules and transgenically express human DR3-DQ2.5. We further dissected the conditions leading to the gluten-dependent activation of $CD4^+$ T cells.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Intestinal epithelial cells express major histocompatibility complex class II molecules, but evidence of their capacity to present gluten peptides to activate $CD4^+$ T cells in the context of celiac disease is lacking.

NEW FINDINGS

Patients with active celiac disease show enhanced epithelial major histocompatibility complex class II expression in the duodenum. Gluten stimulation of interferon-γ–treated organoid monolayers from immunized DR3-DQ2.5 mice activates T cells expressing the human CD4 receptor.

LIMITATIONS

The mechanism of gluten peptide- major histocompatibility complex class II and $CD4^+$ T-cell interactions remain to be determined.

CLINICAL RESEARCH RELEVANCE

Identification of the immune role of intestinal epithelial cells in activating $CD4^+$ T cells, which is central to celiac disease pathogenesis, will open new lines of drug development targeting this pathway and its environmental determinants.

BASIC RESEARCH RELEVANCE

Intestinal epithelial cells have been overlooked as functional antigen-presenting cells for gluten-specific $CD4^+$ T cells and may serve to localize and further increase injury to the epithelium caused by gluten-specific $CD4^+$ T cells in celiac disease.

Materials and Methods

Human Samples

Second-part duodenal (D2) biopsy samples were obtained during endoscopy from 5 active (elevated TG2 serology and histology reporting villous atrophy) and 4 treated (>2 years on gluten-free diet [GFD]) patients with CeD attending the Hospital Bonorino Udaondo, Argentina (Supplementary Table 1). D2 biopsies from 3 patients with CeD diagnosed by TG2 serology and histology (2F, symptomatic, mean age: 34; 1F, asymptomatic, 46 years old) were obtained at the McMaster University Celiac Disease Clinic and used for IEC isolation and flow cytometry. Patients had no concomitant autoimmune or chronic inflammatory diseases, such as type 1 diabetes or inflammatory bowel disease. This study was approved by the Research Ethics Committee of the Hospital Bonorino Udaondo (code #6005) or the Hamilton Integrated Research Ethics Board (HiREB #15311).

Mice

DR3-DQ2.5 mice,[24] lacking all murine MHCII and expressing only human HLA-DQ2.5 and DR3, were used to culture organoid monolayers. DR3-DQ2.5-hCD4[25] mice were used to isolate $CD4^+$ T cells and in some experiments, to culture organoid monolayers. HLA-DQ8 mice, which express only human DQ8,[24] were used to culture the monolayers. Mice were 8 to 12 weeks

Abbreviations used in this paper: **APC, antigen-presenting cell; CeD, celiac disease; D2, second-part duodenal; DAPT, deamidated pepsin-trypsin-digested; IEC, intestinal epithelial cell; IEL, intraepithelial lymphocyte; IFN-γ, interferon-γ; IL, interleukin; Ig, immunoglobulin; MHCII, major histocompatibility complex class II; NI, nonimmunized; NK, natural killer; TG2, transglutaminase 2; TNF, tumor necrosis factor; WT, wild type.**

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old and fed a GFD (Envigo Teklad, TD. 05620) for 2 generations and throughout the experiments, which were approved by the McMaster University Animal Care Committee and McMaster Animal Research Ethics Board (performed under Animal Utilization Protocol #210930). See Supplementary Methods for details.

Mouse Immunization

Mice were immunized using a validated protocol.[26] Briefly, mice were gavaged with pepsin-trypsin-digested gliadin (PT-gliadin) and cholera toxin once a week for 3 weeks, followed by gavages with gliadin 3 times a week for 3 weeks. Non-immunized (NI) mice received cholera toxin alone during the immunization phase and vehicle alone during the challenge phase. Naïve mice that did not receive any treatments were used as additional controls in some experiments. Analysis of anti-gliadin and anti-TG2 antibodies, small intestinal histology (villus height-to-crypt depth ratios and $CD3^+$ intraepithelial lymphocyte [IEL] counts), and gene expression were conducted as previously described.[26,27] In some experiments, gluten-immunized mice were intraperitoneally injected with 1×10^5 U of recombinant mouse (rm)IFN-γ (R&D Systems; 485-MI) (gluten-immunized + IFN-γ-injected). See Supplementary Methods for details.

IEC Isolation

IECs were freshly isolated from human biopsies or from the duodenum and proximal jejunum of the mice. See Supplementary Methods for details.

Organoid Monolayer Cultures

Organoid monolayers were developed from the duodenum and proximal jejunum of mice as previously described.[28,29] In some experiments, organoid monolayers were stimulated with 10 ng/mL IFN-γ (R&D Systems, 485-MI). See Supplementary Methods for details.

Immunofluorescence Staining

MHCII expression was assessed by immunofluorescence staining of human biopsies and sections of the duodenum and proximal jejunum of mice using an HLA-DR-DQ antibody (LS Bio, B5329). Immunofluorescence was also used to assess the cellular composition and polarity of murine organoid monolayers. See Supplementary Methods for details.

Gene Expression

To measure the expression of *Cd74* and *Ciita*, two MHCII-related genes, total RNA was extracted from isolated IECs and organoid monolayers using the RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. Complementary DNA was assayed using SsoFast EvaGreen mix (Bio-Rad) on a CFX Real-Time PCR System. See Supplementary Methods for details.

Organoid Monolayer-$CD4^+$ T-Cell Co-cultures

Organoid monolayers were derived from gluten-immunized DR3-DQ2.5, or in some experiments from gluten-immunized+IFN-γ-injected DR3-DQ2.5-hCD4 mice and treated with IFN-γ. The IFN-γ–containing medium was then removed, and the monolayers were treated with deamidated pepsin-trypsin-digested (DAPT)-gliadin, or wild-type (WT) *Pseudomonas aeruginosa* PA14–digested DAPT-gliadin. Controls were DAPT-zein, media alone, *P aeruginosa* PA14 supernatant, or DAPT-gliadin digested with an isogenic *P aeruginosa* nonfunctional *lasB* mutant ($lasB^{\triangle/\triangle}$). $CD4^+$ T cells were isolated from the spleens of gluten-immunized DR3-DQ2.5-hCD4 mice, labeled with CellTrace Violet, and introduced into the basolateral side of organoid monolayers. After 4 days, $CD4^+$ T-cell proliferation and activation were assessed using flow cytometry. See Supplementary Methods for details.

Flow Cytometry Analysis

IECs, organoid monolayers, or $CD4^+$ T cells were stained using commercially available antibodies. HLA-DQ expression in human samples was assessed using an HLA-DQ antibody (NBP3-08720; R&D Systems). MHCII expression in murine IECs or organoid monolayers was evaluated using an HLA-DR-DP-DQ antibody (Invitrogen; MA1-80678). MHCII expression in organoid monolayers from HLA-DQ8 mice was measured using an HLA-DQ antibody (BioLegend, 318104). Cytokine and chemokine levels in the supernatants were measured using a multiplex LEGENDplex immunoassay kit (BioLegend). See Supplementary Methods for details.

Statistical Analysis

The GraphPad Prism (version 9.0 for Mac IOS) was used for analysis. Data are shown as dot plots, where each dot represents an individual mouse or human and are presented as the mean ± standard error of the mean (SEM). Data distribution was assessed using the Shapiro-Wilk test and analyzed using a one-way analysis of variance test or unpaired Student *t* test, as appropriate. Tukey's post hoc test for multiple comparisons was used, where applicable. A P value $<.05$ was considered statistically significant. Samples in which technical issues were encountered were excluded from analysis. See Supplementary Methods for details.

Results

Patients With Active CeD Have a Higher Expression of MHCII in IECs

We determined the effect of CeD activity on IEC MHCII expression by evaluating its expression in D2-obtained biopsies (Figure 1*A*). Sections from both active and treated patients revealed MHCII immunostaining in the lamina propria. MHCII expression was prominently observed in IECs from patients with active CeD compared with treated CeD who achieved a Marsh $\leq$1 (Figure 1*B*), suggesting that increased inflammation is associated with higher epithelial MHCII expression. No staining was detected in isotype controls (data not shown). Using flow cytometry, IECs from patients with CeD demonstrated expression of the HLA-DQ isotype of MHCII (Figure 1*C*; Supplementary Figure 1), as well as CD40, CD80, and CD86 (Supplementary Figure 2*A*–*C*). Altogether, these data reveal that in CeD, the epithelium exhibits characteristics of APCs.

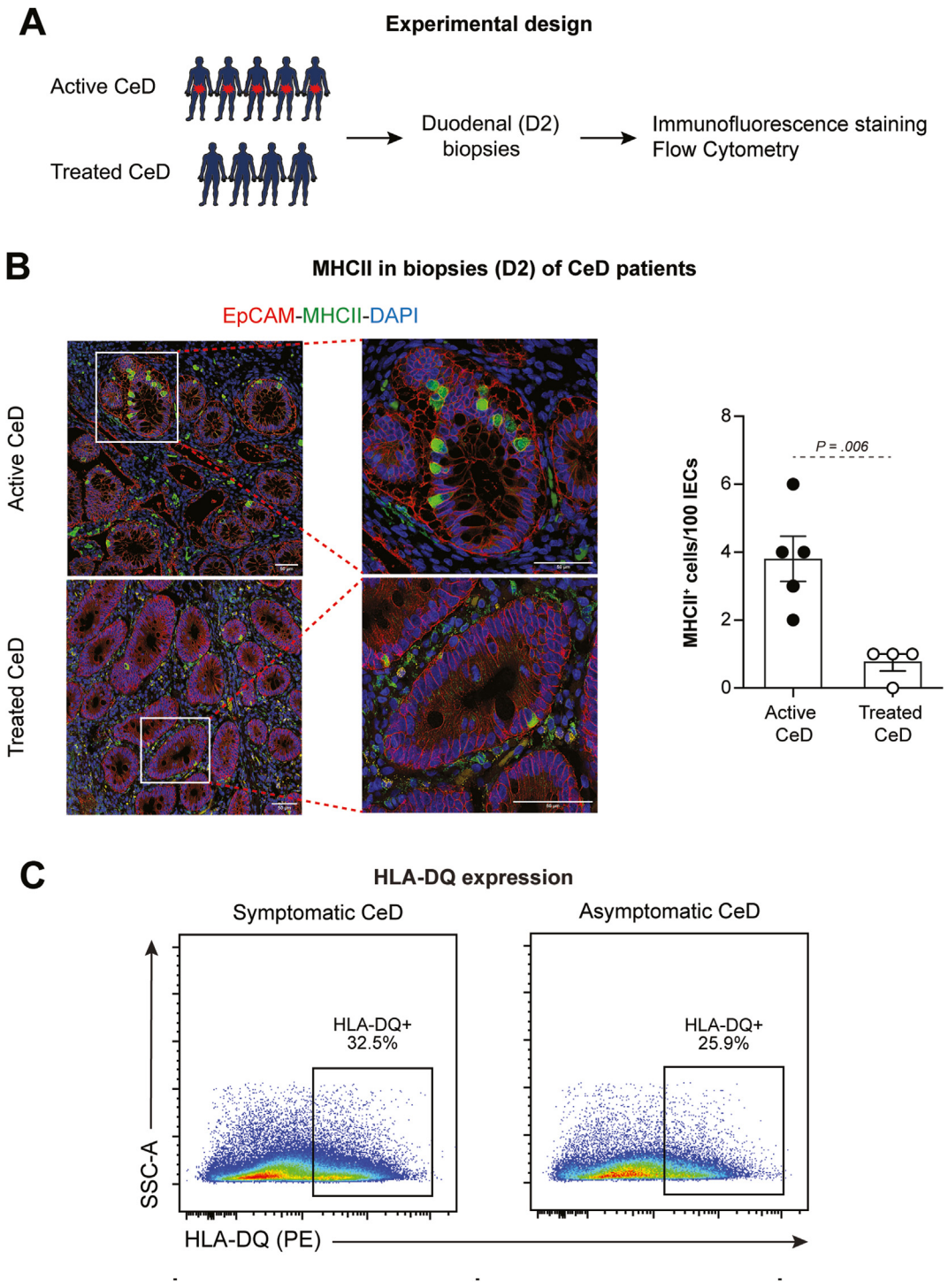


Figure 1. CeD activity demonstrates higher expression of MHCII molecules in IECs. (*A*) Duodenal biopsies (D2) were obtained from patients with active (n = 5) and treated CeD (n = 4), and MHCII or HLA-DQ expression was assessed by immunostaining or flow cytometry. (*B*) Representative immunofluorescence staining of D2 biopsies for $MHCII^+$ ($EpCAM^+$: *red*; $MHCII^+$: *green*; 4′,6-diamidino-2-phenylindole [DAPI]: *blue*; *scale bar*, 50 μm), and quantification of $MHCII^+$ cells per 100 IECs in D2 biopsies. (*C*) Percentage of $HLA\text{-}DQ^+$ cells, gated on live $CD45^-EpCAM^+$ cells using the HLA-DQ antibody from D2 biopsies from symptomatic and asymptomatic patients with CeD, assessed by flow cytometry. Representative dot plots are shown. Data are presented as mean ± SEM, with each *dot* representing an individual patient with CeD. *P* value was determined using a two-tailed unpaired Student *t* test.

Gluten Immunization Induces MHCII Expression in DR3-DQ2.5 Mice

To dissect the functional consequences of MHCII expression in IECs of patients with CeD, we used transgenic mice carrying the human CeD risk gene, DR3-DQ2.5, and evaluated small intestinal inflammation after gluten immunization (Figure 2*A*). Gluten-immunized mice had higher serum anti-TG2 IgG (Figure 2*B*), higher small intestinal anti-gliadin IgA (Figure 2C), reduced small intestinal villus-to-crypt ratios (Figure 2*D*), and higher $CD3^+$ IEL counts (Figure 2*E*) than NI mice. Gene expression analysis in the small intestine revealed 22 genes that were differentially expressed between gluten-immunized and NI mice (Figure 2*F*). Genes related to innate immune function, such as *Rhoa, Tollip*, and MAP kinases (MAPK), including *Mapk3*, *Mapk1,* and *Map3k1*, were overexpressed in gluten-immunized mice. The expression of other inflammation-related genes, such as *Il15, Il1a, Tlr3,* and *Cfb*, was higher in gluten-immunized than NI mice (Figure 2*F*). Gluten-immunized mice also had higher IEC MHCII expression, as assessed by immunofluorescence staining (Figure 2*G*) and flow cytometry (Figure 2*H*; Supplementary Figure 3), compared with NI mice. No staining was detected in isotype controls (data not shown). To investigate whether induced inflammation in vivo leads to stronger small intestinal MHCII expression, gluten-immunized mice were injected with IFN-γ. Expression of epithelial MHCII and *Cd74* mRNA, which encodes an invariant chain that facilitates the assembly and trafficking of MHCII,[30] were higher in gluten-immunized+IFN-γ-injected mice compared with controls, which displayed lamina propria $MHCII^+$ cells only (Figure 3*A*–*C*).

Qa-1, a stress-induced non-classical MHC class I (MHCI) marker and a murine homolog of HLA-E that is increased in patients with CeD,[31] was higher in gluten-immunized than in NI mice (Supplementary Figures 3 and 4). Therefore, gluten-immunized DR3-DQ2.5 mice develop small intestinal inflammation, which is associated with the upregulation of MHCII expression in IECs.

Gluten Immunization and IFN-γ Induce MHCII and Costimulatory Molecule Expression in Organoid Monolayers

We developed organoid monolayers from the duodenum and proximal jejunum of naïve mice and verified the expression of the main intestinal epithelial lineage markers. E-cadherin staining confirmed the presence of adherens junctions, indicating epithelial polarity. The monolayers from both DR3-DQ2.5 (Figure 4*A*) and HLA-DQ8 (Supplementary Figure 5*A*) mice were populated with $Mucin2^+$ goblet cells, $Chromogranin\ A^+$ enteroendocrine cells, $Villin1^+$ enterocytes, and $Lysozyme^+$ Paneth cells.

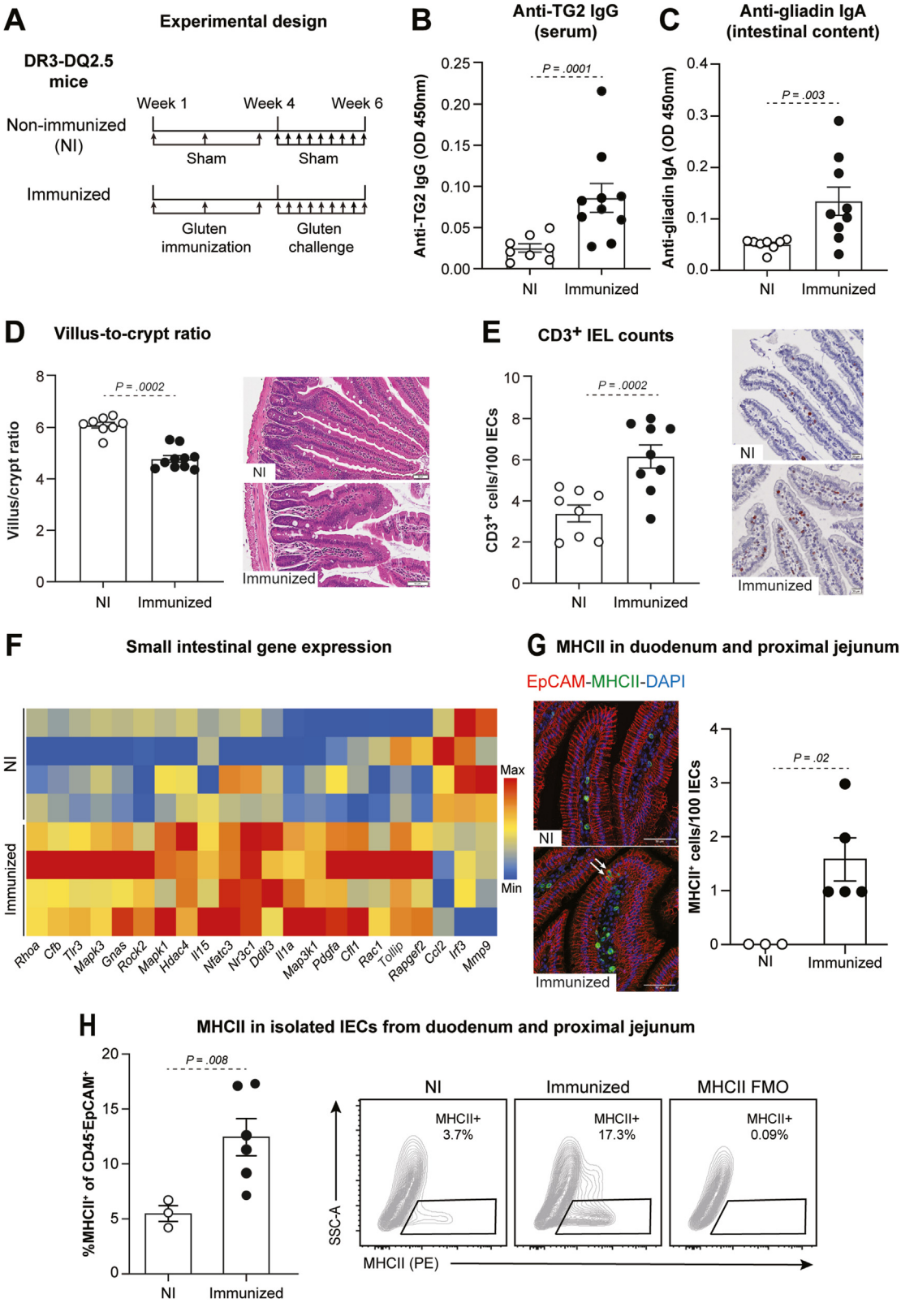
We then used organoid monolayers from gluten-immunized, NI and naïve DR3-DQ2.5 mice to investigate the expression of epithelial MHCII (Figure 4*B*). Organoid monolayers from gluten-immunized mice showed enhanced expression of MHCII molecules compared with monolayers from NI or naïve mice (Figure 4*C*; Supplementary Figure 6). We then determined the effect of IFN-γ, a cytokine secreted by gluten-specific $CD4^+$ T cells in CeD, on the expression of MHCII by IECs. In vitro IFN-γ treatment increased the expression of MHCII in organoid monolayers from NI mice, and this expression was further upregulated in monolayers from gluten-immunized DR3-DQ2.5 (Figure 4*C*) and HLA-DQ8 mice (Supplementary Figure 5*B* and *C*). Monolayers from WT (C57BL/6) mice did not show any MHCII expression, indicating that the human MHCII antibody did not cross-react with murine MHCII molecules (Supplementary Figure 7). Monolayers from gluten-immunized+IFN-γ-injected mice had higher expression of MHCII compared with monolayers from control mice, which was further upregulated by in vitro treatment with IFN-γ (Figure 5*A* and *B*). Expression of *Cd74* and *Ciita*, the main transcription factor regulating MHCII expression,[30] was higher in monolayers from gluten-immunized+IFN-γ-injected mice, and further increased after IFN-γ stimulation in vitro compared with controls (Figure 5*C*).

We next investigated the expression of the classical costimulatory molecules CD80, CD86, and CD40, which are required, in addition to antigens bound to MHCII, to activate $CD4^+$ T cells (Supplementary Figure 6). The expression of CD40 was higher in organoid monolayers from gluten-immunized mice compared with monolayers from NI or naïve mice (Figure 4*D*). IFN-γ treatment increased the expression of CD40, CD86, and CD80 in organoid monolayers from gluten-immunized, but not from NI or naïve mice (Figure 4*D*–*F*). Similar results were obtained using organoid monolayers from gluten-immunized HLA-DQ8 mice (Supplementary Figure 5*D*–*F*). These results indicate that the expression of costimulatory molecules requires several inflammatory stimuli, such as those provided by in vivo gluten immunization and a permissive IFN-γ milieu.

We also assessed the expression of Qa-1 and CD71, a receptor implicated in transepithelial gluten peptide transport,[12] in monolayers from DR3-DQ2.5 mice. Organoid monolayers from gluten-immunized mice stimulated with IFN-γ had higher CD71 and Qa-1 expression compared with monolayers from gluten-immunized mice treated with media, or monolayers from NI or naïve mice (Figure 4*G*; Supplementary Figure 8). Taken together, these data demonstrate that under induced in vivo or in vitro inflammatory conditions, organoid monolayers from DQ2.5 or DQ8 mice express MHCII and markers involved in IEL activation and transepithelial transport.

MHCII Organoid Monolayers Activate $hCD4^+$ T Cells in a Gluten-Dependent Manner

The immune response in CeD is $CD4^+$ T-cell dependent and HLA-DQ restricted.[24] To investigate epithelial MHCII-$CD4^+$ T-cell interactions, we used mice that, in addition to DQ2.5, carry functional human CD4 receptor on T cells (DR3-DQ2.5-hCD4).[25] We established a co-culture system using MHCII-expressing organoid monolayers from gluten-immunized DR3-DQ2.5 mice and splenic $CD4^+$ T cells from gluten-immunized DR3-DQ2.5-hCD4 mice. IFN-γ–treated MHCII-expressing organoid monolayers were


A
Experimental design
DR3-DQ2.5 mice
Week 1
Week 4
Week 6
Non-immunized (NI)
Sham
Sham
Immunized
Gluten immunization
Gluten challenge
B
Anti-TG2 IgG (serum)
Anti-TG2 IgG (OD 450nm)
P = .0001
NI
Immunized
C
Anti-gliadin IgA (intestinal content)
Anti-gliadin IgA (OD 450nm)
P = .003
NI
Immunized
D
Villus-to-crypt ratio
Villus/crypt ratio
P = .0002
NI
Immunized
E
CD3+ IEL counts
CD3+ cells/100 IECs
P = .0002
NI
Immunized
F
Small intestinal gene expression
NI
Immunized
Max
Min
Rhoa
Cfb
Tlr3
Mapk3
Gnas
Rock2
Mapk1
Hdac4
Il15
Nfatc3
Nr3c1
Ddit3
Il1a
Map3k1
Pdgfa
Cfl1
Rac1
Tollip
Rapgef2
Ccl2
Irf3
Mmp9
G
MHCII in duodenum and proximal jejunum
EpCAM-MHCII-DAPI
NI
Immunized
MHCII+ cells/100 IECs
P = .02
NI
Immunized
H
MHCII in isolated IECs from duodenum and proximal jejunum
%MHCII+ of CD45-EpCAM+
P = .008
NI
Immunized
NI
Immunized
MHCII FMO
MHCII+ 3.7%
MHCII+ 17.3%
MHCII+ 0.09%
SSC-A
MHCII (PE)

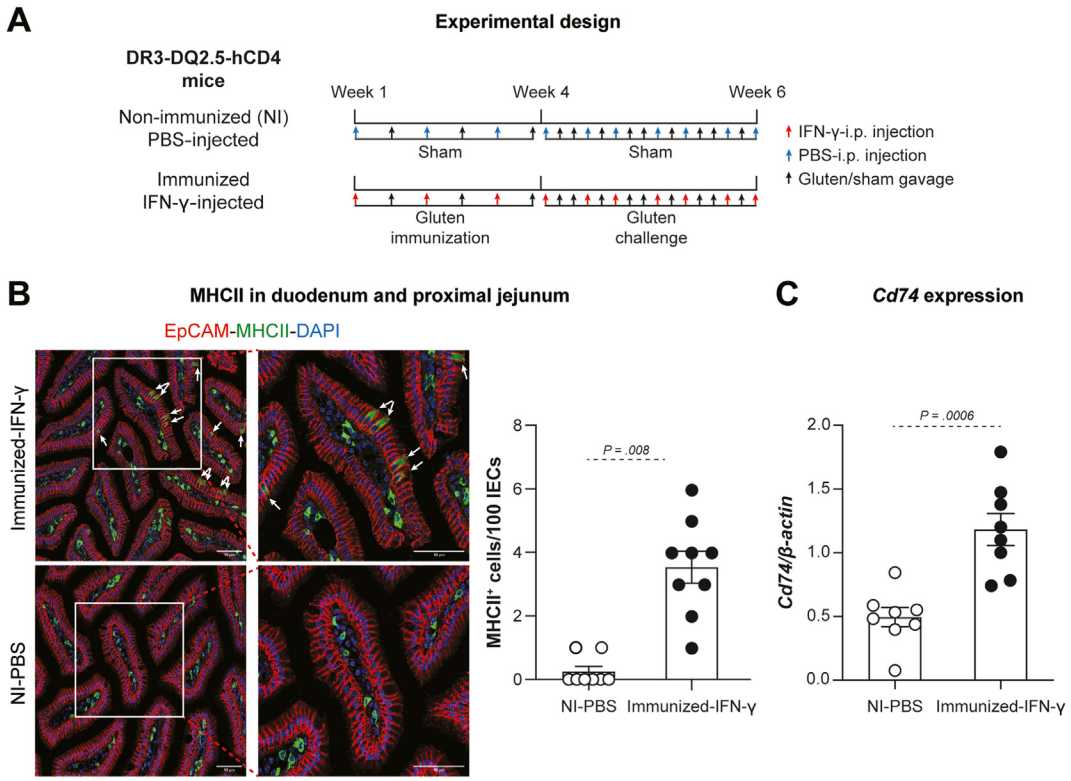


Figure 3. IFN-γ injection and gluten immunization enhance epithelial MHCII expression in DR3-DQ2.5-hCD4 mice. (*A*) NI DR3-DQ2.5-hCD4 mice received sham gavages and were injected with phosphate-buffered saline (NI-PBS; *open dots*). Gluten-immunized mice received PT-gliadin and cholera toxin, followed by gluten challenges. Gluten-immunized mice received intraperitoneal (i.p.) injections of rmIFN-γ throughout the experiment (immunized-IFN-γ; *black dots*). (*B*) Representative immunofluorescence staining of the duodenum and proximal jejunum for MHCII$^+$ (*white arrows*; EpCAM$^+$: *red*; MHCII$^+$: *green*; 4′,6-diamidino-2-phenylindole [DAPI]: *blue*; *scale bar*, 50 μm). Quantification of MHCII$^+$ cells per 100 IECs from NI-PBS (n = 8) and immunized-IFN-γ (n = 9) mice. (*C*) *Cd74* mRNA expression relative to the housekeeping gene β-*actin* in IECs isolated from NI-PBS (n = 8) and immunized-IFN-γ (n = 8) mice. Data are presented as mean ± SEM. Each *dot* represents an individual mouse. All *P* values were determined using a two-tailed unpaired Student *t* test.

apically stimulated with DAPT-gliadin, DAPT-zein, or media before introducing hCD4$^+$ T cells into the basolateral side of the organoid monolayers (Figure 6*A*). The proliferation of hCD4$^+$ T cells, which required the presence of the monolayer (Supplementary Figure 9*A* and *B*), increased by 2.5-fold in response to DAPT-gliadin stimulation compared with DAPT-zein treatment (Figure 6*B*). Concomitantly, hCD4$^+$ T cells co-cultured with DAPT-gliadin–treated monolayers, but not in the absence of monolayers (Supplementary Figure 9*C*–*E*), exhibited an activated phenotype with higher expression of known T-cell activation markers, including CD69, CD25, and CD44, compared with hCD4$^+$ T cells from DAPT-zein–treated monolayers (Figure 6*C*–*E*; Supplementary Figure 10). In addition, T-cell

Figure 2. Gluten immunization induces inflammation and MHCII expression in DR3-DQ2.5 mice. (*A*) NI DR3-DQ2.5 mice received sham gavages and were used as controls (*open dots*). Gluten-immunized mice received PT-gliadin and cholera toxin, followed by gluten challenges (*black dots*). (*B*) Serum anti-TG2 IgG levels in NI (n = 8) and immunized (n = 10) mice. (*C*) Anti-gliadin IgA levels in the intestinal contents in NI (n = 8) and immunized (n = 9) mice. (*D*) Quantification of the small intestinal villus height-to-crypt depth ratios in NI (n = 8) and immunized (n = 10) mice. Representative hematoxylin and eosin (H&E)-stained small intestinal sections are shown. *Scale bar*, 50 μm. (*E*) Quantification of CD3$^+$ IELs per 100 IECs from NI (n = 8), and immunized (n = 9) mice. Representative CD3$^+$ stained sections of the duodenum and proximal jejunum, where CD3$^+$ IELs are stained in *red*, are shown. *Scale bar*, 20 μm. (*F*) Heatmap of significantly altered genes in the small intestinal tissues of NI (n = 4), and immunized (n = 4) mice. (*G*) Representative immunofluorescence staining of the duodenum and proximal jejunum for MHCII$^+$ (*white arrows*; EpCAM$^+$: *red*; MHCII$^+$: *green*; 4′,6-diamidino-2-phenylindole [DAPI]: *blue*; *scale bar*, 50 μm). Quantification of MHCII$^+$ cells per 100 IECs from NI (n = 3) and immunized (n = 5) mice. (*H*) Percentage of MHCII-expressing cells gated on live CD45$^-$EpCAM$^+$ cells from IECs isolated from the duodenum and proximal jejunum of NI (n = 3) and immunized (n = 6) DR3-DQ2.5 mice. Representative dot plots showing MHCII$^+$ cells, gated on live CD45$^-$EpCAM$^+$ cells using an HLA-DR-DP-DQ antibody. Data are presented as mean ± SEM, with each *dot* representing an individual mouse. All *P* values were determined using a two-tailed unpaired Student *t* test. One sample from the immunized group in (*C*) and (*E*) was removed from the analysis because of technical issues. In the heatmap, *columns* represent each gene, and *rows* indicate individual mice. The *color* indicates the degree of expression.

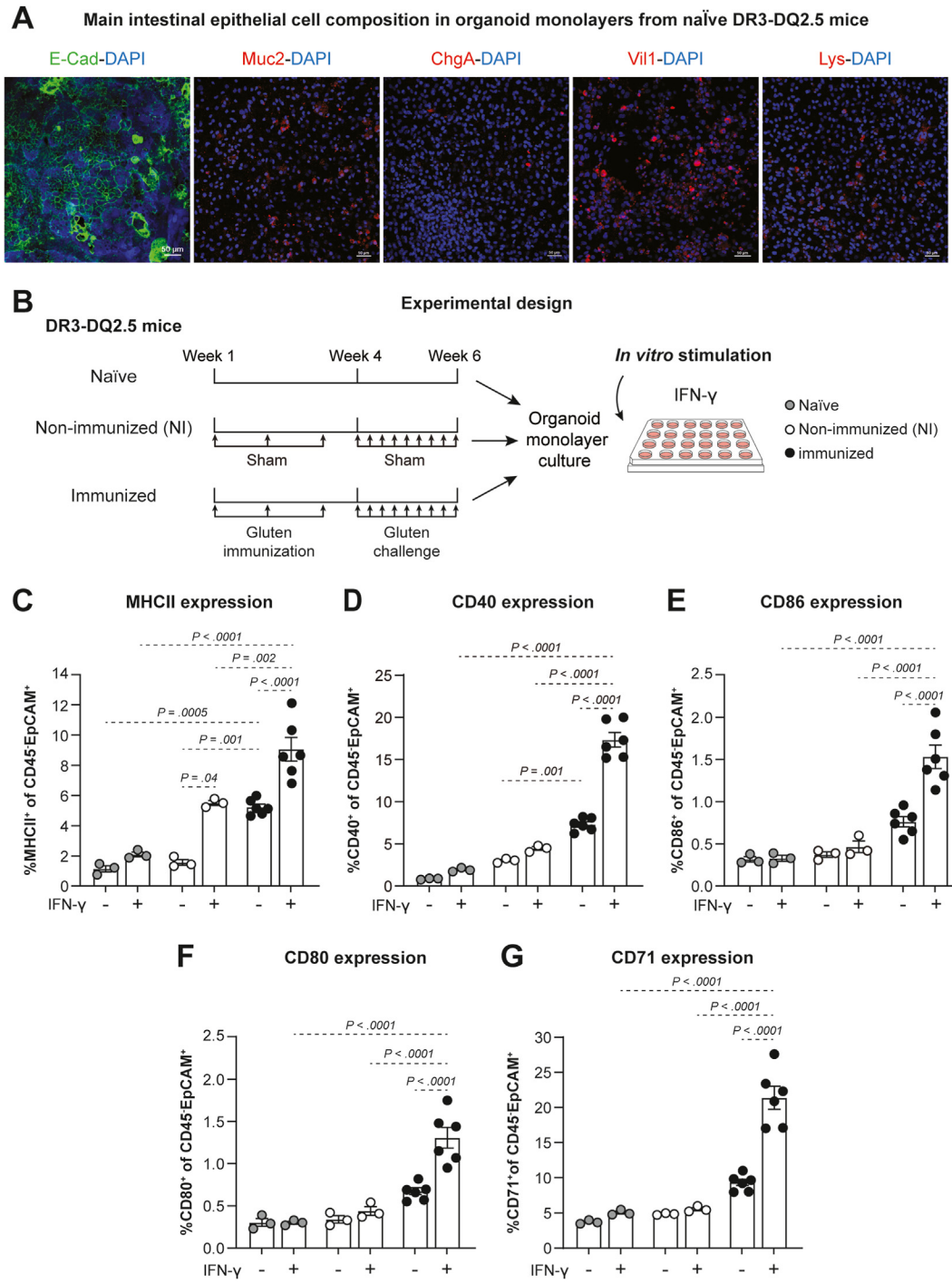


Figure 4. Gluten immunization and IFN-γ induce MHCII expression in organoid monolayers from DR3-DQ2.5 mice. (*A*) Representative immunofluorescence staining of organoid monolayers from naïve DR3-DQ2.5 mice for E-cadherin$^+$ (E-Cad$^+$: *green*; 4′,6-diamidino-2-phenylindole [DAPI]: *blue*); Mucin2$^+$ (Muc2$^+$: *red*; DAPI: *blue*); Chromogranin A$^+$ (ChgA$^+$: *red*; DAPI: *blue*); Villin1$^+$ (Vil1$^+$: *red*; DAPI: *blue*); and Lysozyme$^+$ (Lys$^+$: *red*; DAPI: *blue*). *Scale bar*, 50 μm. (*B*) Naïve DR3-DQ2.5 mice received no treatment and were used as controls (n = 3; *gray dots*). NI DR3-DQ2.5 mice received sham immunization and challenge and were used as controls (n = 3; *open dots*). Gluten-immunized DR3-DQ2.5 mice received PT-gliadin and cholera toxin, followed by gluten challenges (n = 6; *black dots*). Organoid monolayers were then stimulated in vitro with or without IFN-γ. (*C–G*) Percentage of (*C*) MHCII-expressing cells using an HLA-DR-DP-DQ antibody, (*D*) CD40-expressing cells, (*E*) CD86-expressing cells, (*F*) CD80-expressing cells, or (*G*) CD71-expressing cells, gated on live CD45$^-$EpCAM$^+$ cells from organoid monolayers from naïve, NI, and immunized DR3-DQ2.5 mice stimulated in vitro with or without IFN-γ. Data are presented as mean ± SEM. Each *dot* represents an individual mouse. All *P* values were determined using one-way analysis of variance with Tukey's post hoc test for multiple comparisons.

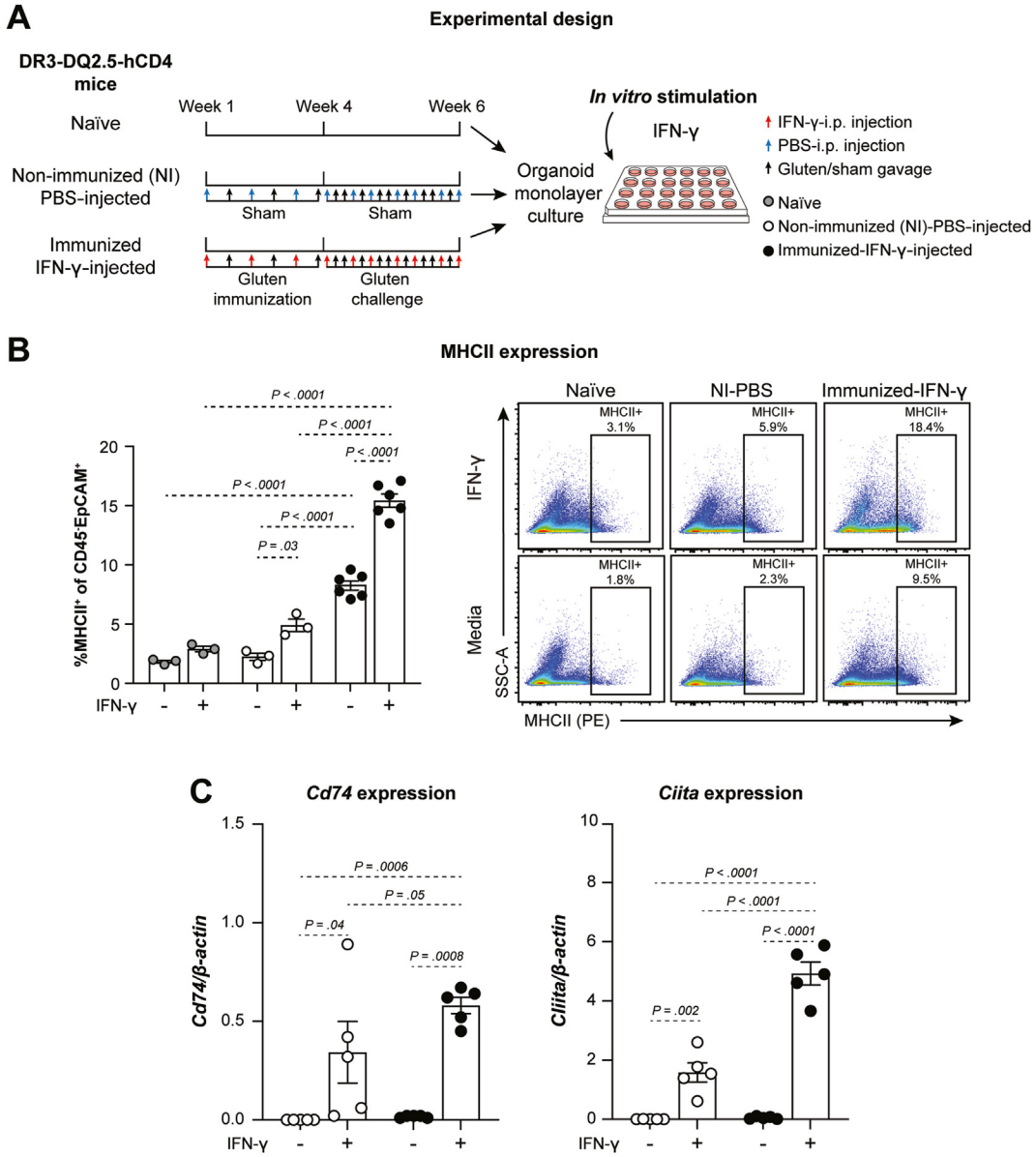
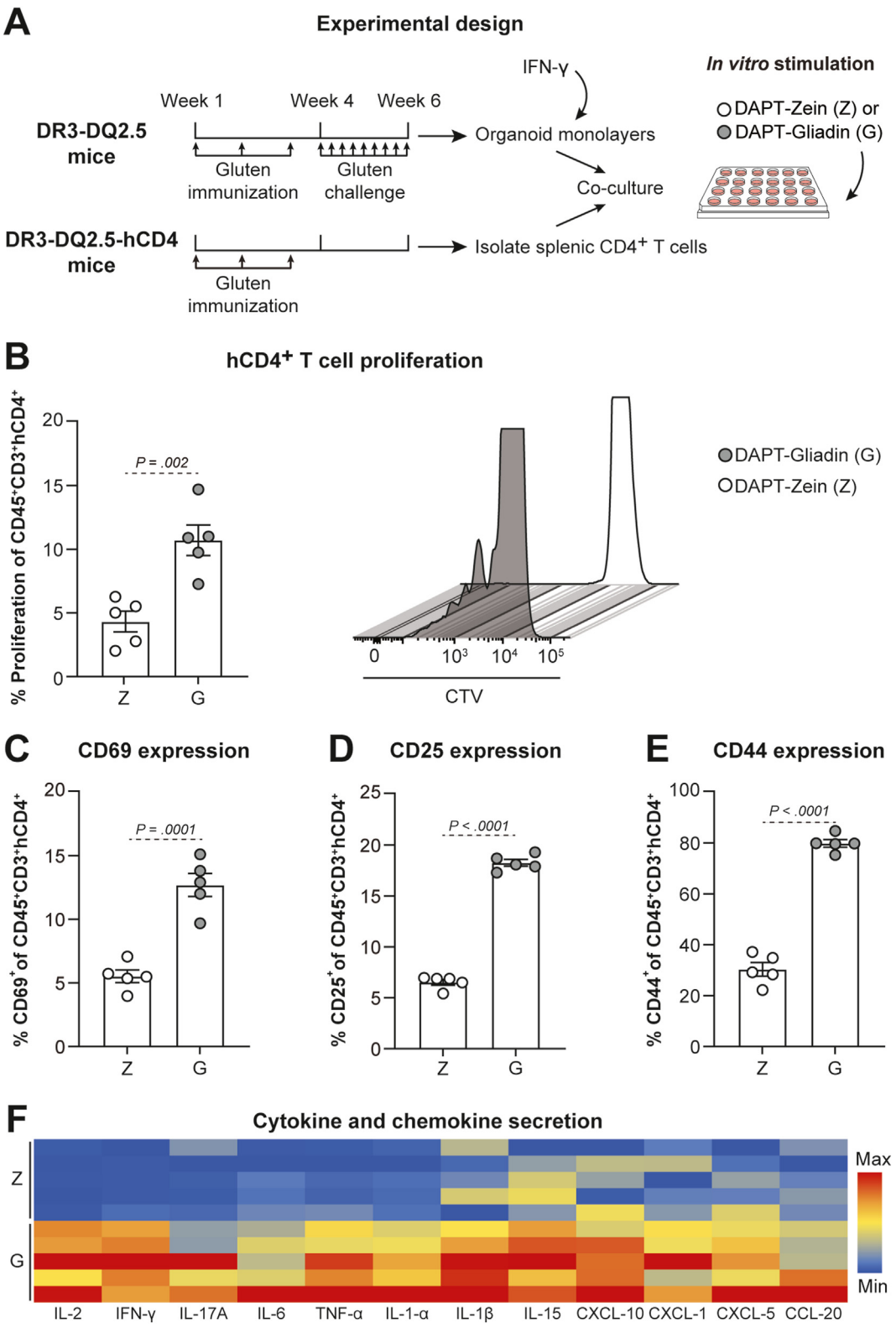


Figure 5. Expression of MHCII in organoid monolayers from gluten-immunized+IFN-γ-injected DR3-DQ2.5-hCD4 mice. (*A*) Organoid monolayers were developed from naïve (no treatment; n = 3; *gray dots*), NI+phosphate-buffered saline–injected (NI-PBS; n = 3; *open dots*), and gluten-immunized+IFN-γ-injected (immunized-IFN-γ; n = 6; *black dots*) DR3-DQ2.5-hCD4 mice. The organoid monolayers were then stimulated with or without IFN-γ. (*B*) Percentage of MHCII$^+$ expressing cells from organoid monolayers, gated on live CD45$^-$EpCAM$^+$ cells using an HLA-DR-DP-DQ antibody. Representative dot plots showing MHCII$^+$ cells, gated on live CD45$^-$EpCAM$^+$ cells using an HLA-DR-DP-DQ antibody. (*C*) *Cd74* and *Ciita* mRNA expression relative to the housekeeping gene *β-actin* in organoid monolayers from NI-PBS (n = 5; *open dots*) and immunized-IFN-γ (n = 5; *black dots*) treated in vitro with or without IFN-γ. Data are presented as mean ± SEM. Each *dot* represents an individual mouse. All *P* values were determined using one-way analysis of variance with Tukey's post hoc test for multiple comparisons.

activation markers were positively correlated with monolayer MHCII expression (Supplementary Figure 11).

Stimulation of monolayers with DAPT-gliadin, but not DAPT-zein, increased the secretion of several cytokines and chemokines in the co-culture supernatant (Figure 6*F*). The DAPT-gliadin–stimulated monolayers yielded a 37-fold elevation in interleukin (IL)-2 compared with DAPT-zein–treated monolayers (Supplementary Figure 12*A*). In accordance with clinical studies showing elevated gluten-mediated serum cytokines in patients with CeD,[32] we found a 28-fold and 4-fold increase in the secretion of IFN-γ (Supplementary Figure 12*B*) and IL-17A (Supplementary Figure 12*C*), respectively, after treatment of the monolayers with DAPT-gliadin compared with DAPT-zein. Notably, the T-cell–secreted cytokines IL-2 and IL-17A were not detectable in monolayer-only cultures, in which CD4$^+$ T cells were absent (Supplementary Figure 13).

The concentrations of proinflammatory cytokines, such as IL-6, tumor necrosis factor (TNF)-α, IL-1α, and IL-1β, were elevated in response to DAPT-gliadin treatment of co-


A
Experimental design
In vitro stimulation
IFN-γ
Week 1
Week 4
Week 6
DR3-DQ2.5 mice
Gluten immunization
Gluten challenge
Organoid monolayers
DAPT-Zein (Z) or
DAPT-Gliadin (G)
Co-culture
DR3-DQ2.5-hCD4 mice
Gluten immunization
Isolate splenic CD4+ T cells
B
hCD4+ T cell proliferation
% Proliferation of CD45+CD3+hCD4+
P = .002
Z
G
DAPT-Gliadin (G)
DAPT-Zein (Z)
CTV
C
CD69 expression
% CD69+ of CD45+CD3+hCD4+
P = .0001
D
CD25 expression
% CD25+ of CD45+CD3+hCD4+
P < .0001
E
CD44 expression
% CD44+ of CD45+CD3+hCD4+
P < .0001
F
Cytokine and chemokine secretion
Max
Min
IL-2
IFN-γ
IL-17A
IL-6
TNF-α
IL-1-α
IL-1β
IL-15
CXCL-10
CXCL-1
CXCL-5
CCL-20

culture compared with DAPT-zein treatment (Supplementary Figure 12*D*–*G*). In addition, an increase in IL-15, a major cytokine in the pathogenesis of CeD,[31] was observed on exposure to DAPT-gliadin vs DAPT-zein (Supplementary Figure 12*H*). The secretion of chemokines known to participate in immune and inflammatory cell recruitment and migration,[33,34] such as C-X-C motif chemokine ligand (CXCL)-10, CXCL-1, CXCL-5, and C-C motif chemokine ligand (CCL)-20, were higher in the supernatants from DAPT-gliadin–treated monolayers than in the supernatants from DAPT-zein–stimulated monolayers (Supplementary Figure 12*I*–*L*). When monolayers were stimulated with IFN-γ in the absence of CD4$^+$ T cells, higher levels of epithelial-associated innate cytokines and chemokines, including IL-6, IL-1α, IL-1β, IL-15, CXCL-10, and CCL-20 were detected (Supplementary Figure 13).

Finally, the expression of the stress-induced markers, Qa-1 and Rae-1, a ligand for NKG2D activating natural killer (NK) cell receptors, was higher in co-cultures treated with DAPT-gliadin (Supplementary Figure 14*A*–*C*).

Collectively, our data show that epithelial monolayers expressing MHCII induce the proliferation and activation of underlying hCD4$^+$ T cells in a gluten-dependent manner, leading to increased secretion of a panel of CeD-associated cytokines and chemokines.

Modulation of hCD4$^+$ T-Cell Activation by Opportunistic Pathogen-Derived Elastase

Microbial factors have emerged as modulators of inflammation in CeD.[5,35] We previously showed that gluten metabolized by bacterial elastase had increased immunogenicity.[36] To investigate whether bacterial metabolism influences IEC–T-cell interactions, organoid monolayer-CD4$^+$ T-cell co-cultures were stimulated with gliadin predigested with elastase-producing WT *P aeruginosa* PA14. DAPT-gliadin, gliadin predigested with the *P aeruginosa* isogenic *lasB* mutant strain lacking elastase activity, referred to as *lasB*$^{\triangle/\triangle}$, and the WT *P aeruginosa* PA14 supernatant were used as controls (Figure 7*A*). Compared with co-cultures treated with DAPT-gliadin predigested with *lasB*$^{\triangle/\triangle}$, DAPT-gliadin, or WT *P aeruginosa* PA14 supernatant, treatment with DAPT-gliadin predigested with WT *P aeruginosa* PA14 led to 1.7-, 1.4-, and 2.6-fold increases in hCD4$^+$ T-cell proliferation, respectively (Figure 7*B*). Increased expression of the activation markers CD69, CD25, and CD44 (Figure 7*C*–*E*) in hCD4$^+$ T cells confirmed a more robust activated phenotype of these cells in co-cultures stimulated with DAPT-gliadin predigested with WT *P aeruginosa* PA14 vs controls. This was associated with elevated levels of a panel of cytokines and chemokines (Figure 7*F*), including a 5.4-, 3.7-, and 676-fold increase in IL-2 levels in the supernatant when the organoid monolayers were treated with DAPT-gliadin predigested with WT *P aeruginosa* PA14 compared with DAPT-gliadin predigested with *lasB*$^{\triangle/\triangle}$, DAPT-gliadin, or WT *P aeruginosa* PA14 supernatant, respectively (Supplementary Figure 15*A*). The secretion of IFN-γ (Supplementary Figure 15*B*) and IL-17A (Supplementary Figure 15*C*) was the highest when organoid monolayers were treated with DAPT-gliadin predigested with WT *P aeruginosa* PA14 vs controls. In addition, organoid monolayers treated with DAPT-gliadin predigested with WT *P aeruginosa* PA14 exhibited elevated release of the proinflammatory cytokines IL-6, TNF-α, IL-1α, IL-1β, and IL-15 compared with controls (Supplementary Figure 15*D*–*H*). Stimulation of organoid monolayers with DAPT-gliadin predigested with WT *P aeruginosa* PA14 led to higher production of CXCL-10, CXCL-1, CXCL-5, and CCL-20 compared with controls (Supplementary Figure 15*I*–*L*). Taken together, these results demonstrate that MHCII-expressing organoid monolayers enable more robust proliferation and activation of the underlying hCD4$^+$ T cells when gliadin is partially metabolized by bacterial elastase.

Discussion

IECs express MHCII molecules in the small intestine,[37–39] implying that they possess an essential prerequisite for functioning as APCs. The functional consequences of this expression remain controversial, with some studies associating it with inflammation and others with immunosuppressive activities and epithelial differentiation or renewal.[40–43] These conflicting results are likely contextual, suggesting that a complete understanding will require investigation of specific diseases. IECs are central to the pathogenesis of CeD; however, whether they are involved in the presentation of gluten-derived peptides to MHCII-restricted CD4$^+$ T cells has never been demonstrated. Here, we show that IECs from patients with CeD have higher epithelial MHCII expression compared with IECs from patients compliant with a GFD that achieved histological remission. Using humanized organoid monolayers, we

Figure 6. MHCII-expressing organoid monolayers induce a gluten-dependent CD4$^+$ T-cell response. (*A*) Organoid monolayers were co-cultured with splenic CD4$^+$ T cells in the presence of DAPT-zein (Z; *open dots*) or DAPT-gliadin (G; *gray dots*). Organoid monolayers from gluten-immunized DR3-DQ2.5 mice (n = 5) were further stimulated with IFN-γ for 24 hours before co-culture. Splenic CD4$^+$ T cells were isolated from gluten-immunized DR3-DQ2.5-hCD4 mice (n = 5). (*B*) *Left panel*, percentage of proliferating CellTrace Violet (CTV)-labeled hCD4$^+$ T cells, gated on live CD45$^+$CD3$^+$hCD4$^+$ cells that were co-cultured with organoid monolayers in the presence of DAPT-zein (Z) or DAPT-gliadin (G). Right panel, representative histograms of CTV-labeled hCD4$^+$ T cells gated on live CD45$^+$CD3$^+$hCD4$^+$ T cells. (*C*–*E*) Percentage of (*C*) CD69-expressing cells, (*D*) CD25-expressing cells, or (*E*) CD44-expressing cells gated on live CD45$^+$CD3$^+$hCD4$^+$ T cells that were co-cultured with organoid monolayers in the presence of DAPT-zein (Z) or DAPT-gliadin (G). (*F*) Heatmap of cytokines and chemokines in co-culture supernatants that were significantly different between (DAPT)-zein (Z) and DAPT-gliadin (G). Data are presented as mean ± SEM. Each *dot* represents an individual mouse. All *P* values were determined using a two-tailed unpaired Student *t* test. In the heatmap, *columns* represent each cytokine or chemokine, and *rows* indicate individual mice. The *color* indicates the degree of expression.

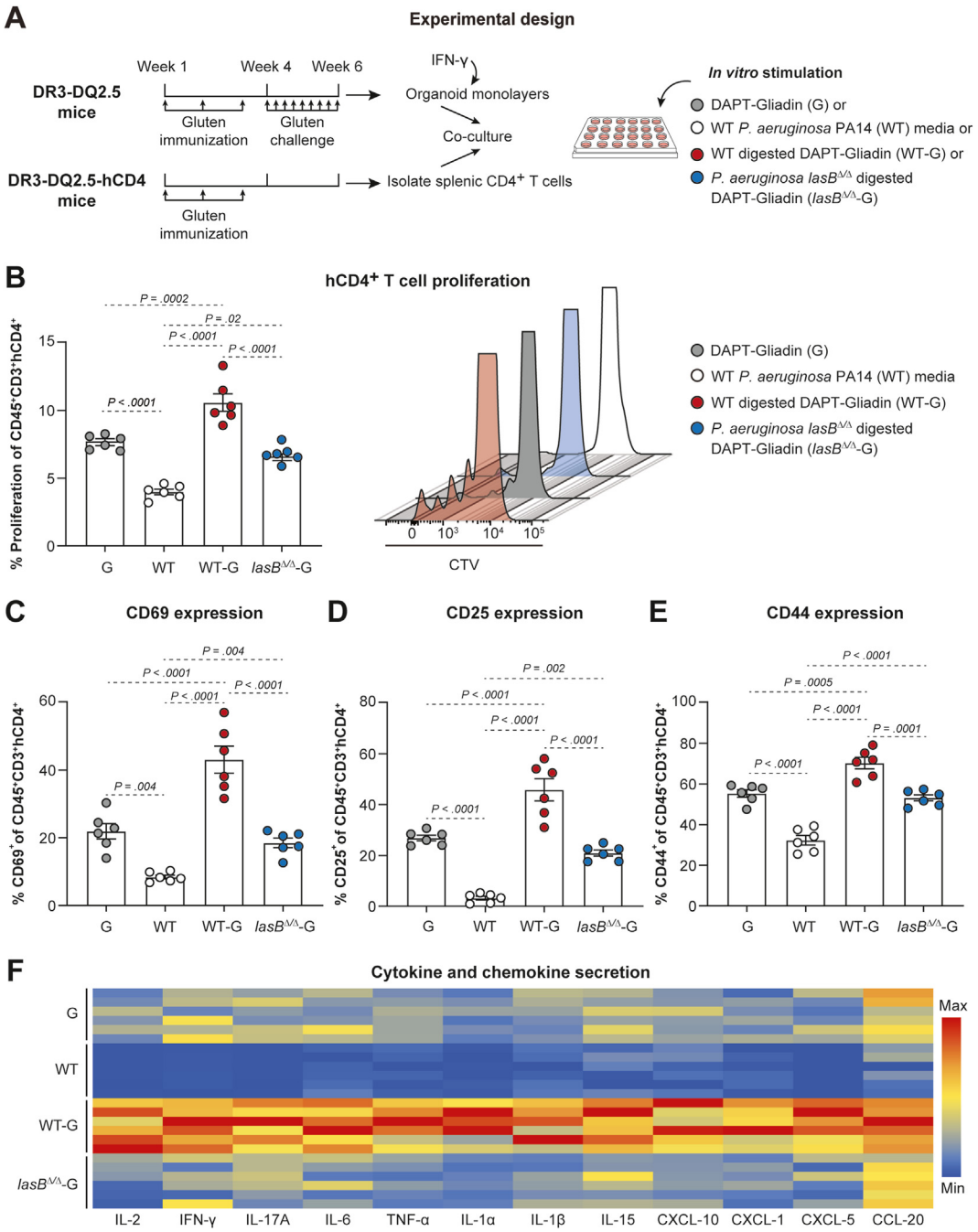


Figure 7. Gluten metabolized by opportunistic pathogen-derived elastase induces a robust CD4$^+$ T-cell response. (*A*) Organoid monolayers were co-cultured with splenic CD4$^+$ T cells in the presence of DAPT-gliadin (G; *gray dots*), WT *Pseudomonas aeruginosa* PA14 (WT; *open dots*) supernatant, WT digested DAPT-gliadin (WT-G; *red dots*), or *P aeruginosa lasB*$^{\Delta/\Delta}$ digested DAPT-gliadin (*lasB*$^{\Delta/\Delta}$-G; *blue dots*). Organoid monolayers derived from gluten-immunized DR3-DQ2.5 mice (n = 6) were stimulated with IFN-γ for 24 hours before co-culture. Splenic CD4$^+$ T cells were isolated from gluten-immunized DR3-DQ2.5-hCD4 mice (n = 6). (*B*) *Left panel*, percentage of proliferating CTV-labeled hCD4$^+$ T cells gated on live CD45$^+$CD3$^+$hCD4$^+$ cells that were co-cultured with organoid monolayers in the presence of DAPT-gliadin (G), *P aeruginosa* PA14 (WT) supernatant, WT digested DAPT-gliadin (WT-G), or *P aeruginosa lasB*$^{\Delta/\Delta}$ digested DAPT-gliadin (*lasB*$^{\Delta/\Delta}$-G). *Right panel*, representative histograms of CTV-labeled hCD4$^+$ T cells gated on live CD45$^+$CD3$^+$hCD4$^+$ T cells. (*C–E*) Percentage of (*C*) CD69-expressing cells, (*D*) CD25-expressing cells or (*E*) CD44-expressing cells, gated on live CD45$^+$CD3$^+$hCD4$^+$ T cells that were co-cultured with organoid monolayers in the presence of DAPT-gliadin (G), *P aeruginosa* PA14 (WT) supernatant, WT digested DAPT-gliadin (WT-G), or *P aeruginosa lasB*$^{\Delta/\Delta}$ digested DAPT-gliadin (*lasB*$^{\Delta/\Delta}$-G). (*F*) Heatmap of cytokines and chemokines that were significantly different between co-cultures stimulated with (DAPT)-gliadin (G), wild-type *P aeruginosa* PA14 (WT) supernatant, WT digested DAPT-gliadin (WT-G), and *P aeruginosa lasB*$^{\Delta/\Delta}$ digested DAPT-gliadin (*lasB*$^{\Delta/\Delta}$-G). Data are presented as mean ± SEM. Each *dot* represents an individual mouse. All *P* values were determined using one-way analysis of variance with Tukey's post hoc test for multiple comparisons. In the heatmap, *columns* represent each cytokine or chemokine, and *rows* indicate individual mice. The *color* indicates the degree of expression.

demonstrated that IECs mediate gluten presentation in the context of MHCII molecules and activate hCD4$^+$ T cells. Finally, predigestion of gluten by elastase-producing opportunistic pathogens, known to metabolize gluten peptides,[36] further modulated hCD4$^+$ T-cell activation in our model.

Approximately 90% of the CeD population carries the HLA-DQ2.5 allele,[6] and the remaining patients express HLA-DQ2.2 and/or -DQ8.[44] Gluten peptides are presented to CD4$^+$ T cells preferentially via these HLA-DQ molecules expressed in professional APCs such as dendritic cells, macrophages, and B cells.[21,45,46] Although MHCII expression and function are generally restricted to professional APCs, several studies have shown that non-hematopoietic cells, including IECs, can express MHCII.[23,37,38,40] More importantly, the expression of MHCII in enterocytes of patients with CeD has been previously reported.[22,39] Despite this, the role of epithelial MHCII in gluten presentation and T-cell activation in CeD has been overlooked. Thus, we first determined that patients with active CeD have enhanced duodenal epithelial MHCII expression compared with patients on a GFD in histological remission, implying that CeD activity is associated with upregulation of epithelial MHCII. We then confirmed the presence of CeD-associated MHCII, HLA-DQ, in IECs isolated from biopsies of patients with CeD using flow cytometry. These results suggest that the intestinal epithelium has the capacity for MHCII-mediated gluten antigen presentation and T-cell activation during CeD activity. To further investigate gluten-epithelial MHCII-CD4$^+$ T-cell interactions we used humanized organoid monolayers from mice carrying HLA-DQ2.5, the genotype expressed in *cis* or *trans* by most patients with CeD.[6,8,47] Gluten-immunized DR3-DQ2.5 mice developed proximal small intestinal inflammation and proinflammatory gene expression, including *Il15,* which encodes a key cytokine in CeD.[48] Gluten immunization also enhanced the expression of MHCII in isolated IECs from DR3-DQ2.5 mice, suggesting that in vivo inflammation upregulates MHCII, and this is recapitulated in organoid monolayers. This is supported by our data in gluten-immunized mice also injected with IFN-γ, which further upregulated the expression of epithelial MHCII and associated genes (*Cd74*). This is consistent with previous studies indicating that IECs express IFN-γ receptors, and that IFN-γ is a potent inducer of IEC MHCII upregulation,[23,43,49,50] suggesting that in vivo inflammation can prime the intestinal epithelium to further upregulate MHCII. The epithelial expression of costimulatory molecules and CD71 required inflammatory stimuli of in vivo gluten immunization in addition to in vitro stimulation with IFN-γ. Our model recapitulates findings that CD71, a transferrin receptor involved in gluten peptide transport across the intestinal epithelium, is overexpressed in patients with active but not potential CeD.[12]

A key finding of this study is that MHCII-bearing organoid monolayers are functional and can activate CD4$^+$ T cells in a gluten-dependent manner. This is demonstrated by multiple avenues, including the increased proliferation of hCD4$^+$ T cells, higher expression of T-cell activation markers, which correlated with monolayer MHCII expression, and elevated secretion of IL-2, a cytokine indicative of T-cell activation[51] that is increased in patients with CeD following gluten challenge.[32,34] The inability of CD4$^+$ cells to proliferate and activate in the absence of MHCII-expressing monolayers supports the functionality of our model, whereas the absence of IL-2 and IL-17A in monolayer-only cultures indicates that their release is dependent on the presence of activated T cells. The upregulation of CD25 (IL-2Rα[51]) on hCD4$^+$ T cells in the presence of gluten could be attributed to increased IL-2–mediated signaling in the co-culture system. The production of IFN-γ in the supernatant of co-culture exposed to gluten is in accordance with previous observations in patients with CeD.[34,52] Other inflammatory cytokines, such as IL-6, TNF-α, IL-1α, IL-1β, and IL-15, previously implicated in CeD,[34,53–55] were higher in the supernatants of co-cultures treated with gluten and monolayer-only cultures treated with IFN-γ. We also detected an increase in CXCL-10, CXCL-1, CXCL-5, and CCL-20 levels in gluten-treated co-cultures, which are chemotactic for effector/memory T cells, B cells, NK cells, dendritic cells, and neutrophils.[56] The Th1-associated chemokine CXCL-10 (IFN-γ-induced protein 10) was previously found to correlate with increased IFN-γ levels and is produced by epithelial and plasma cells in the lamina propria of patients with active CeD.[57] Serum CCL-20, a Th17-associated chemokine, was also found to be higher 4 hours after gluten challenge in patients with CeD.[32,34] Finally, MHCII-bearing organoid monolayers exposed to gluten and co-cultured with hCD4$^+$ T cells showed increased expression of the stress-induced markers, Qa-1 and Rae-1. This agrees with studies in which the expression of human analogs of these markers was associated with cellular stress in patients with active CeD.[16,17] Although recent studies have implicated epithelial MHCII in the regulation of acute graft-vs-host disease[43] and bacteria-specific effector CD4$^+$ T-cell responses,[49] we show for the first time, gluten-dependent hCD4$^+$ T-cell activation by IECs that express celiac-associated MHCII molecules.

Duodenal opportunistic pathogens are emerging as co-factors in CeD.[5] One mechanism involves modification of peptide antigenicity by the metabolism of gluten by microbial proteases.[5,58] Here, we investigated whether gluten partially metabolized by elastase-producing *P aeruginos*a PA14, a previously described duodenal pathobiont in CeD,[36] modified hCD4$^+$ T-cell activation. We observed higher proliferation and activation phenotypes of hCD4$^+$ T cells with increased secretion of IL-2 and several other proinflammatory cytokines. These results are in accordance with the previously demonstrated in vitro activation of gluten-specific T cells from patients with CeD by *P aeruginosa*–modified gluten peptides,[36] implying that gluten modified by microbial elastase can also influence epithelial MHCII-mediated activation of hCD4$^+$ T cells.

Recent studies using organoids from patients with CeD have reported phenotypic differences between CeD and controls, including gene expression,[59] permeability function,[60] and macrophage responses to gluten.[61] Our results advance the field by identifying a new immunological role for IECs in activating CD4$^+$ T cells in the context of CeD.

Nevertheless, our study has limitations. We provide evidence of higher MHCII expression in duodenal biopsies of patients with active CeD. However, gluten-IEC MHCII-T-cell activation was explored in organoid monolayers from a humanized murine model. Human organoid monolayer-$CD4^+$ T-cell co-cultures would require T cells to be isolated from the same patient to restrict T-cell receptors to HLA molecules expressed by IECs. Even in this case, we would not be able to determine which HLA molecules present gluten peptides that mediate T-cell activation. The humanized characteristics of our in vitro model, namely the expression of HLA-DQ2.5 and hCD4 in the absence of other murine MHCII molecules, make this model well suited to address the question explored in this study and pathogen-metabolized gluten-IEC-T-cell interactions. The mechanism of MHCII-$CD4^+$ T-cell interaction remains to be determined, but it could involve gluten-MHCII exosome release by IECs.[62] Further investigations are required to determine whether the mechanisms described here contribute to CeD onset, its maintenance, or both.

In conclusion, we demonstrated that MHCII-expressing IECs exposed to gluten mediate $hCD4^+$ T-cell activation, indicating a previously unknown role for the intestinal epithelium in the modulation of $CD4^+$ T-cell responses in CeD through antigen presentation that could be targeted therapeutically. Because IECs are in first contact with dietary antigens, our results may encourage the study of the regulation of immune responses by the epithelium in other MHCII-associated diseases.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2024.07.008.

References

1. Catassi C, Verdu EF, Bai JC, et al. Coeliac disease. Lancet 2022;399:2413–2426.
2. Lindfors K, Ciacci C, Kurppa K, et al. Coeliac disease. Nat Rev Dis Primers 2019;5:3.
3. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797–801.
4. Anderson RP. Emergence of an adaptive immune paradigm to explain celiac disease: a perspective on new evidence and implications for future interventions and diagnosis. Expert Rev Clin Immunol 2022;18:75–91.
5. Verdu EF, Schuppan D. Co-factors, microbes, and immunogenetics in celiac disease to guide novel approaches for diagnosis and treatment. Gastroenterology 2021;161:P1395–P1411.E4.
6. Sollid LM, Markussen G, Ek J, et al. Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. J Exp Med 1989;169:345–350.
7. Lundin KEA, Scott H, Fausa O, et al. T cells from the small intestinal mucosa of a DR4, DQ7/DR4. DQ8 celiac disease patient preferentially recognize gliadin when presented by DQ8. Hum Immunol 1994;41:285–291.
8. **Tye-Din JA, Stewart JA, Dromey JA**, et al. Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. Sci Transl Med 2010;2:41ra51.
9. Shan L, Qiao SW, Arentz-Hansen H, et al. Identification and analysis of multivalent proteolytically resistant peptides from gluten: implications for celiac sprue. J Proteome Res 2005;4:1732–1741.
10. Shan L, Molberg Ø, Parrot I, et al. Structural basis for gluten intolerance in celiac spru. Science (1979) 2002; 297:2275–2279.
11. Caminero A, Meisel M, Jabri B, et al. Mechanisms by which gut microorganisms influence food sensitivities. Nat Rev Gastroenterol Hepatol 2019;16:7–18.
12. Matysiak-Budnik T, Moura IC, Arcos-Fajardo M, et al. Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease. J Exp. Med 2008;205:143–154.
13. Molberg Ø, McAdam SN, Korner R, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat Med 1998;4:713–717.
14. Ráki M, Tollefsen S, Molberg Ø, et al. A unique dendritic cell subset accumulates in the celiac lesion and efficiently activates gluten-reactive T cells. Gastroenterology 2006;131:428–438.
15. Beitnes ACR, Ráki M, Brottveit M, et al. Rapid accumulation of CD14 +CD11c + dendritic cells in gut mucosa of celiac disease after in vivo gluten challenge. PLoS One 2012;7:1–10.
16. Hüe S, Mention JJ, Monteiro RC, et al. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. Immunity 2004;21:367–377.
17. Allegretti YL, Bondar C, Guzman L, et al. Broad MICA/B expression in the small bowel mucosa: a link between cellular stress and celiac disease. PLoS One 2013;8: e73658.
18. Jabri B, Abadie V. IL-15 functions as a danger signal to regulate tissue-resident T cells and tissue destruction. Nat Rev Immunol 2015;15:771–783.
19. **Iversen R, Amundsen SF**, Kleppa L, et al. Evidence that pathogenic transglutaminase 2 in celiac disease derives from enterocytes. Gastroenterology 2020;159:788–790.
20. Høydahl LS, Richter L, Frick R, et al. Plasma cells are the most abundant gluten peptide MHC-expressing cells in inflamed intestinal tissues from patients with celiac disease. Gastroenterology 2019;156:1428–1439.
21. Iversen R, Roy B, Stamnaes J, et al. Efficient T cell–B cell collaboration guides autoantibody epitope bias and onset of celiac disease. Proc Natl Acad Sci U S A 2019; 116:15134–15139.
22. Marley NJ, Macartney JC, Ciclitira PJ. HLA-DR, DP and DQ expression in the small intestine of patients with coeliac disease. Clin Exp Immunol 1987;70:386–393.
23. Wosen JE, Ilstad-Minnihan A, Co JY, et al. Human intestinal enteroids model MHC-II in the gut epithelium. Front Immunol 2019;10:1–8.
24. Black KE, Murray JA, David CS. HLA-DQ determines the response to exogenous wheat proteins: a model of

gluten sensitivity in transgenic knockout mice. J Immunol 2002;169:5595–5600.
25. **Kauwe AL de, Chen Z**, Anderson RP, et al. Resistance to celiac disease in humanized HLA-DR3-DQ2-transgenic mice expressing specific anti-gliadin CD4 + T cells. J Immunol 2009;182:7440–7450.
26. Galipeau HJ, Rulli NE, Jury J, et al. Sensitization to gliadin induces moderate enteropathy and insulitis in nonobese diabetic-DQ8 mice. J Immunol 2011; 187:4338–4346.
27. Biagi F, Luinetti O, Campanella J, et al. Intraepithelial lymphocytes in the villous tip: do they indicate potential coeliac disease? J Clin Pathol 2004;57:835–839.
28. Moon C, Vandussen KL, Miyoshi H, et al. Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. Mucosal Immunol 2014;7:818–828.
29. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 2011;141:1762–1772.
30. Neefjes J, Jongsma MLM, Paul P, et al. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 2011; 11:823–836.
31. Jabri B, Jian R, Desreumaux P, et al. Selective expansion of intraepithelial lymphocytes expressing the HLA-E–specific natural killer receptor CD94 in celiac disease. Gastroenterology 2000;118:867–879.
32. Goel G, Tye-Din JA, Qiao SW, et al. Cytokine release and gastrointestinal symptoms after gluten challenge in celiac disease. Sci Adv 2019;5:eaaw7756.
33. Oo YH, Shetty S, Adams DH. The role of chemokines in the recruitment of lymphocytes to the liver. Dig Dis 2010; 28:31–44.
34. Goel G, Daveson AJM, Hooi CE, et al. Serum cytokines elevated during gluten-mediated cytokine release in coeliac disease. Clin Exp Immunol 2019;199:68–78.
35. Caminero A, Verdu EF. Celiac disease: should we care about microbes? Am J Physiol Gastrointest Liver Physiol 2019;317:G161–G170.
36. Caminero A, Galipeau HJ, McCarville JL, et al. Duodenal bacteria from patients with celiac disease and healthy subjects distinctly affect gluten breakdown and immunogenicity. Gastroenterology 2016;151:670–783.
37. Wosen JE, Mukhopadhyay D, Macaubas C, et al. Epithelial MHC class II expression and its role in antigen presentation in the gastrointestinal and respiratory tracts. Front Immunol 2018;9:1–14.
38. Heuberger C, Pott J, Maloy KJ. Why do intestinal epithelial cells express MHC class II? Immunology 2021; 162:357–367.
39. Arnaud-Battandier F, Cerf-Bensussan N, Amsellem R, et al. Increased HLA-DR expression by enterocytes in children with celiac disease. Gastroenterology 1986;91:1206–1212.
40. **Biton M, Haber AL, Rogel N**, et al. T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell 2018;175:1307–1320.e22.
41. Jamwal DR, Laubitz D, Harrison CA, et al. Intestinal epithelial expression of MHCII determines severity of chemical, T-cell–induced, and infectious colitis in mice. Gastroenterology 2020;159:1342–1356.e6.
42. Thelemann C, Eren RO, Coutaz M, et al. Interferon-γ induces expression of MHC class II on intestinal epithelial cells and protects mice from colitis. PLoS One 2014;9:e86844.
43. Koyama M, Mukhopadhyay P, Schuster IS, et al. MHC class II antigen presentation by the intestinal epithelium initiates graft-versus-host disease and is influenced by the microbiota. Immunity 2019;51:885–898.e7.
44. Green PHR, Jabri B. Coeliac disease. Lancet 2003; 362:383–391.
45. Lundin KEA, Scott H, Hansen T, et al. Gliadin-specific, HLA-DQ(α1*0501, β1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. J Exp Med 1993;178:187–196.
46. Bodd M, Kim C, Lundin KEA, et al. T-cell response to gluten in patients with HLA-DQ2.2 reveals requirement of peptide-MHC stability in celiac disease. Gastroenterology 2012;142:552–561.
47. **Kim C-Y, Quarsten H**, Bergseng E, et al. Structural basis for HLA-DQ2-mediated presentation of gluten epitopes in celiac disease. Proc Natl Acad Sci U S A 2004;101:4175–4179.
48. **Abadie V, Kim SM, Lejeune T**, et al. IL-15, gluten and HLA-DQ8 drive tissue destruction in coeliac disease. Nature 2020;578:600–608.
49. Heuberger CE, Janney A, Ilott N, et al. MHC class II antigen presentation by intestinal epithelial cells fine-tunes bacteria-reactive CD4 T cell responses. Mucosal Immunol 2024;17:416–430.
50. Malik A, Sharma D, Aguirre-Gamboa R, et al. Epithelial IFNγ signalling and compartmentalized antigen presentation orchestrate gut immunity. Nature 2023; 623:1044–1052.
51. Malek TR. The biology of interleukin-2. Annu Rev Immunol 2008;26:453–479.
52. Nilsen EM, Jahnsen FL, Lundin KEA, et al. Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. Gastroenterology 1998;115:551–563.
53. Tye-Din JA, Skodje GI, Sarna VK, et al. Cytokine release after gluten ingestion differentiates coeliac disease from self-reported gluten sensitivity. United European Gastroenterol J 2020;8:108–118.
54. Manavalan JS, Hernandez L, Shah JG, et al. Serum cytokine elevations in celiac disease: association with disease presentation. Hum Immunol 2010;71:50–57.
55. Sabatino A Di, Ciccocioppo R, Cupelli F, et al. Epithelium derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease. Gut 2006;55:469–477.
56. Sokol CL, Luster AD. The chemokine system in innate immunity. Cold Spring Harb Perspect Biol 2015;7: a106303.
57. Bondar C, Araya RE, Guzman L, et al. Role of CXCR3/CXCL10 axis in immune cell recruitment into the small intestine in celiac disease. PLoS One 2014;9:1–12.
58. **Constante M, Libertucci J**, Galipeau HJ, et al. Biogeographic variation and functional pathways of the

gut microbiota in celiac disease. Gastroenterology 2022; 163:1351–1363.e15.
59. Dieterich W, Neurath MF, Zopf Y. Intestinal ex vivo organoid culture reveals altered programmed crypt stem cells in patients with celiac disease. Sci Rep 2020;10:1–10.
60. Freire R, Ingano L, Serena G, et al. Human gut derived-organoids provide model to study gluten response and effects of microbiota-derived molecules in celiac disease. Sci Rep 2019;9:7029.
61. Serena G, Huynh D, Lima RS, et al. Intestinal epithelium modulates macrophage response to gliadin in celiac disease. Front Nutr 2019;6:167.
62. Van Niel G, **Mallegol J, Bevilacqua C**, et al. Intestinal epithelial exosomes carry MHC class II/peptides able to inform the immune system in mice. Gut 2003;52:1690–1697.

Author names in bold designate shared co-first authorship.

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Correspondence
Address correspondence to: Elena F. Verdu, Health Sciences Centre, Rm 3N8, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada. e-mail: verdue@mcmaster.ca; or Tohid F. Didar, Department of Mechanical Engineering, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada. e-mail: didart@mcmaster.ca; or Fernando G. Chirdo, Instituto de Estudios Inmunológicos y Fisiopatológicos - IIFP (UNLP-CONICET), 1489, 120 Boulevard, La Plata 1900, Argentina. e-mail: fchirdo@biol.unlp.edu.ar.

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CRediT Authorship Contributions
Sara Rahmani, MASc (Conceptualization: Equal; Data curation: Lead; Formal analysis: Lead; Funding acquisition: Supporting; Investigation: Lead; Methodology: Lead; Validation: Equal; Visualization: Lead; Writing – original draft: Lead; Writing – review & editing: Lead)

Heather J. Galipeau, PhD (Conceptualization: Equal; Funding acquisition: Supporting; Methodology: Supporting; Writing – original draft: Equal; Writing – review & editing: Lead)

Alexandra V. Clarizio, MD (Data curation: Equal; Formal analysis: Equal; Investigation: Equal; Methodology: Equal; Writing – review & editing: Supporting)

Xuanyu Wang, MD (Formal analysis: Supporting; Investigation: Equal; Methodology: Equal; Visualization: Equal; Writing – review & editing: Supporting)

Amber Hann, BSc (Methodology: Supporting; Writing – review & editing: Supporting)

Gaston H. Rueda, MD (Resources: Supporting; Writing – review & editing: Supporting)

Utkarshini Nitin Kirtikar, MSc (Resources: Supporting; Writing – review & editing: Supporting)

Marco Constante, PhD (Formal analysis: Supporting; Methodology: Supporting; Writing – review & editing: Supporting)

Mark Wulczynski, MSc (Methodology: Supporting; Writing – review & editing: Supporting)

Hsuan-Ming Su, MD (Data curation: Supporting; Methodology: Supporting; Writing – review & editing: Supporting)

Rebecca Burchett, BSc (Methodology: Supporting; Writing – review & editing: Supporting)

Jonathan L. Bramson, PhD (Resources: Supporting; Writing – review & editing: Equal)

Maria Ines Pinto-Sanchez, MD, MSc (Resources: Lead; Writing – review & editing: Equal)

Juan Pablo Stefanolo, MD (Resources: Lead; Writing – review & editing: Equal)

Sonia Isabel Niveloni, MD (Resources: Lead; Writing – review & editing: Equal)

Michael G. Surette, PhD (Resources: Supporting; Writing – review & editing: Supporting)

Joseph A. Murray, MD (Resources: Lead; Writing – review & editing: Equal)

Robert P. Anderson, MD, PhD (Resources: Lead; Writing – review & editing: Equal)

Premysl Bercik, MD (Resources: Lead; Writing – review & editing: Equal)

Alberto Caminero, PhD (Formal analysis: Equal; Methodology: Equal; Resources: Lead; Writing – review & editing: Equal)

Fernando G. Chirdo, PhD (Conceptualization: Lead; Supervision: Lead; Writing – original draft: Equal; Writing – review & editing: Lead)

Tohid F. Didar, PhD (Conceptualization: Equal; Funding acquisition: Lead; Supervision: Lead; Writing – original draft: Supporting; Writing – review & editing: Equal)

Elena F. Verdu, MD, PhD (Conceptualization: Lead; Funding acquisition: Lead; Supervision: Lead; Writing – original draft: Equal; Writing – review & editing: Lead)

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Data Availability
NanoString raw data has been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE219286.