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A bispecific antibody targeting HLA-DQ2.5-gluten peptides potently blocks gluten-specific T cells induced by gluten ingestion in patients with celiac disease

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ABSTRACT

The gluten-free diet for celiac disease (CeD) is restrictive and often fails to induce complete symptom and/or mucosal disease remission. Central to CeD pathogenesis is the gluten-specific CD4+ T cell that is restricted by HLA-DQ2.5 in over 85% of CeD patients, making HLA-DQ2.5 an attractive target for suppressing gluten-dependent immunity. Recently, a novel anti-HLA-DQ2.5 antibody that specifically recognizes the complexes of HLA-DQ2.5 and multiple gluten epitopes was developed (DONQ52).

Objective: To assess the ability of DONQ52 to inhibit CeD patient-derived T-cell responses to the most immunogenic gluten peptides that encompass immunodominant T cell epitopes.

Methods: We employed an in vivo gluten challenge model in patients with CeD that affords a quantitative readout of disease-relevant gluten-specific T-cell responses. HLA-DQ2.5+ CeD patients consumed food containing wheat, barley, or rye for 3 days with collection of blood before (D1) and 6 days after (D6) commencing the challenge. Peripheral blood mononuclear cells were isolated and assessed in an interferon (IFN)-γ enzyme-linked immunosorbent spot assay (ELISpot) testing responses to gluten peptides encompassing a series of immunodominant T cell epitopes. The inhibitory effect of DONQ52 (4 or 40 μg/mL) was assessed and compared to pan-HLA-DQ blockade (SPVL3 antibody).

Results: In HLA-DQ2.5+ CeD patients, DONQ52 reduced T cell responses to all wheat gluten peptides to an equivalent or more effective degree than pan-HLA-DQ antibody blockade. It reduced T cell responses to a cocktail of the most immunodominant wheat epitopes by a median of 87% (IQR 72–92). Notably, DONQ52 also substantially reduced T-cell responses to dominant barley hordein and rye secalin derived peptides. DONQ52 had no effect on T-cell responses to non-gluten antigens.

Conclusion: DONQ52 can significantly block HLA-DQ2.5-restricted T cell responses to the most highly immunogenic gluten peptides in CeD. Our findings support in vitro data that DONQ52 displays selectivity and broad cross-reactivity against multiple gluten peptide:HLA-DQ2.5 complexes. This work provides proof-of-concept multi-specific antibody blockade has the potential to meaningfully inhibit pathogenic gluten-specific T-cell responses in CeD and supports ongoing therapeutic development.

1. Introduction

Celiac disease (CeD) is a prevalent chronic immune illness characterized by dietary gluten induced enteropathy and symptoms [1]. Central to its pathogenesis are CD4+ T cells that react to specific gluten peptides derived from wheat, rye and barley that have been deamidated

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(post-translationally modified) and bound to disease-relevant HLA molecules [2]. Strikingly, over 99% of CeD patients carry specific HLA-DQ allotypes, with 80–90% carrying HLA-DQ2.5 (encoded by *DQA1*05* and *DQB1*02*) and the remainder split between HLA-DQ2.2 (encoded by *DQA1*02:01* and *DQB1*02*) and HLA-DQ8 (encoded by *DQA1*03* and *DQB1*03:02*) [3]. This strong class II HLA association highlights the importance of the CD4+ T cell response to CeD pathogenesis but also indicates that pathogenic T cell epitopes will differ between HLA types.

There is increasing recognition that current treatment with a strict gluten-free diet is suboptimal, as many patients fail to achieve mucosal healing or symptom resolution and most will struggle with the burden of an onerous and restrictive lifelong dietary regimen. Whilst several therapies are in pre-clinical and clinical development, none have yet successfully progressed beyond Phase 3 trials [4].

Given the central role for gluten-specific CD4+ T cells in CeD pathogenesis, an attractive therapeutic approach is blockade of antigen-specific responses. Unfortunately, indiscriminant antibody blockade of disease-associated HLA is likely to lead to unacceptable safety risks by suppressing responses to non-gluten antigens. Further, given the abundant expression of HLA-DQ2.5 systemically, antibodies targeting HLA-DQ2.5 would be very rapidly cleared and treatment regimens would require unrealistically large doses and frequent intravenous administration. Thus, a targeted approach that combines disease associated HLA with pathogenic TCR(s) is considerably more attractive. Such a strategy is supported by comprehensive knowledge of the dominant T cell epitopes that drive CeD, particularly for the most common HLA variant, HLA-DQ2.5, but greatly tempered by the finding there are multiple T cell epitopes from wheat, rye and barley gluten that can drive discrete T-cell responses that are non-redundant [5,6].

Advances in engineering TCR-like antibodies have provided novel methods of targeting HLA:autoantigen. TCR-like antibodies specific for the immunodominant gluten T cell epitopes DQ2.5-glia-α1a and DQ2.5-glia-α2 have been reported [7,8]. These TCR-like antibodies effectively block activation and proliferation of gluten-specific CD4+ T cells in vitro and in HLA-DQ2.5 humanized mice [7]. However, other immunodominant T cell epitopes pathogenic in CeD, such as those in wheat ω-gliadin or from rye and barley, would not be blocked by this approach.

Recently, a TCR-like antibody in bi-specific format, which reacts to multiple immunodominant gluten T cell epitopes in complex with HLA-DQ2.5 was developed [9]. This antibody, termed DONQ52, was produced through a series of immunization, selection, and engineering processes. Initially, rabbits were immunized with recombinant HLA-DQ2.5:33mer gliadin peptide. Through rabbit B-cell screening, two lead antibodies were identified and engineered into a bi-specific format to cover multiple gluten epitopes. These antibodies were then humanized and underwent extensive protein engineering to improve their binding, selectivity, and other properties. DONQ52 recognizes over 25 distinct gluten peptides including the five most immunodominant T cell epitopes HLA-DQ2.5-glia-α1a/α2 and HLA-DQ2.5-glia ω1/ω2 from wheat and HLA-DQ2.5-hor-3a from barley. While it demonstrated selective binding to gluten peptides in complex with HLA-DQ2.5 (pHLA-DQ2.5), it did not show substantial binding to irrelevant peptides presented on HLA-DQ2.5 or to non-HLA-DQ2.5 dimers. In HLA-DQ2.5 transgenic mice, DONQ52 blocked T cell induction to peptides encompassing dominant gluten epitopes without affecting systemic immunity.

To support the feasibility of DONQ52 as a potential therapeutic for CeD it is important to establish ex vivo proof-of-concept using primary human samples to show that DONQ52 can specifically target pathogenic T cells restricted by CeD-relevant HLA. To achieve this, we utilized a well-established CeD-relevant model that leverages wheat, rye or barley ingestion in CeD patients to induce expansion of gut-homing, HLA-restricted gluten-specific CD4+ T cells in the circulation that can be employed in in vitro functional T cell assays [5,10,11]. Here we report the use of this approach to assess the effect of DONQ52 on gluten-specific T cell immunity.

2. Materials and methods

2.1. Study participants and ethics

The study was approved by the Melbourne Health Human Research Ethics Committee (2020.162) and the Walter and Eliza Hall Institute Ethics Committee (03/04). All participants provided written informed consent. CeD was diagnosed based on evidence of past duodenal histology showing characteristic villous atrophy in conjunction with positive CeD serology. Participants were following a gluten-free diet for at least 12 months prior to recruitment. Blood was collected at baseline for CeD serology (tissue transglutaminase (tTG)-IgA and deamidated gliadin peptide (DGP)-IgG; Melbourne Pathology or Dorevitch Pathology) and HLA typing (in house following previously described methods [12] or typed by Melbourne Pathology).

2.2. Oral grain challenges

Short-term (3-day) grain challenge and blood collection on day 6 (D6) was performed as previously described [5]. Wheat challenge consisted of 4 slices of white bread daily cut to toasting thickness (Bakers Delight, Victoria). Barley challenge consisted of 150 g/day pearl barley (McKenzies, Altona, Victoria) and cooked into muffins, pancakes, cookies, porridge, or risotto. Rye challenge consisted of 100 g/day rye flour (Four Leaf Milling, South Australia) cooked into muffins. Challenges delivered an approximate daily dose of 12 g wheat gluten, 6 g barley hordein, or 5.8 g rye secalin.

2.3. Peptides

High-quality peptides (>95%) were purchased from GL Biochem (Minhang, China) with LC-MS analysis performed (Table 1). Peptides were blocked at the N- and C-terminal ends with *N*-acetyl and C-amide groups, respectively.

2.4. IFN-γ ELISpot

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood using Ficoll-Paque™ Plus density-gradient centrifugation (GE Healthcare) and Leucosep™ tubes (Greiner Labortechnik, Kresmuster, Austria). Pre-coated IFN-γ ELISpot assays (Mabtech) were performed following manufacturer's recommendations with slight modifications. Following PBMC isolation, cells were divided and some incubated with 10 μg/mL SPVL3 (Beckman Coulter), 4 μg/mL DONQ52, or 40 μg/mL DONQ52. Cells were incubated at 37 °C for 30 min prior to adding into the ELISpot plates. Each CeD patient PBMC was tested against two concentrations of selected gluten peptides (1 or 10 μg/mL), and the positive controls tetanus toxoid (5 μg/mL, Enzo Life Sciences, NY, USA) and phytohemagglutinin-L (2.5 μg/mL; Sigma). Spot-forming units (SFU) were counted using an automated ELISpot reader (ELISpot Reader System, Autoimmun Diagnostika; Strassberg, Germany). A response cut-off was determined as the mean SFU for the nil control +3 standard deviations. SFU counts were adjusted to 10^6 PBMC/well for each patient when performing direct comparisons. Patients were classified as non-responders (NR) if they had no significant response to gluten antigen but did have a significant positive control response. Fold-changes were calculated by dividing the antigen response by the nil PBS response for each antigen.

2.5. DONQ52

The selection of DONQ52 concentrations were based on prior data [9]. Amongst multiple T cell epitopes screened in vitro, DQ2.5-glia-γ2 showed the weakest neutralization by DONQ52, but murine in vivo efficacy studies indicated 40 μg/mL of DONQ52 would likely be a sufficient dose to inhibit it. This plasma concentration would be readily

Table 1
In silico deamidated peptide sequences.

Peptide source - peptide name	Peptide sequence	Epitope name	Epitope sequence	Peptide cocktail
Gliadin - 33-mer	LQLQPFPQPELPYPQPELPYPQPELPYPQPQPF	DQ2.5-glia-α1a/α1b/α2	PFPQPELPY PYPQPELPY PQPELPYPQ	
Gliadin - α-gliadin	LQPFPQPELPYPQPQ	DQ2.5-glia-α1a DQ2.5-glia-α2	PFPQPELPY PQPELPYPQ	Wheat DQ2.5
Gliadin - ω-gliadin	QPFPQPEQPFPWQP	DQ2.5-glia-ω1 DQ2.5-glia-ω2	PFPQPEQPF PQPEQPFPW	Wheat DQ2.5
Gliadin - γ-gliadin	PQQPQQSFPEQEQPA	DQ2.5-glia-γ1	PQQSFPEQE	Wheat DQ2.5
Hordein - Hor3a	PEQPIPEQPQPYPQQ	DQ2.5-hor-3a	PIPEQPQPYP	Barley DQ2.5
Hordein - Hor3b	QPQPYPEQPQPYP	DQ2.5-hor-3b	PYPEQPQPY	Barley DQ2.5
Hordein - Hor1/Hor2	QPFPQPEQPIPYQ		PFPQPEQPIPY	Barley DQ2.5
Hordein - Barley 03	QPFPQPEQPFPWQP	DQ2.5-hor-1/2	PFPQPEQPF PQPEQPFPW	Barley DQ2.5
Secalin - Sec1/Sec2	QPFPQPEQPFPQS	DQ2.5-sec-1/2	PFPQPEQPF PQPEQPFPQ	Rye DQ2.5
Secalin - Sec3	QPFPEQPEQIIPQQP	DQ2.5-sec-3	PFPEQPEQI	Rye DQ2.5
Secalin - Rye 01	QPFPQPEQPIPQQ		PFPQPEQPI PQPEQPIPQ	Rye DQ2.5
Secalin - Rye 03	QPFPQPEQPTPIQ		PFPQPEQPT PQPEQPTPI	Rye DQ2.5

achievable in humans. 4 μg/mL was set as one-tenth of 40 μg/mL and was predicted to show some suppression but not complete inhibition.

2.6. Statistical analysis

Statistical analyses were performed using Prism software version 9 (Graphpad). Descriptive statistics were calculated and comparisons between two matched groups were performed using Wilcoxon matched pairs signed rank tests. *P* values of <0.05 were considered significant.

3. Results

3.1. Participant details

The study included 44 CeD individuals (31 females; 70.5%) with a median age of 47.5 years (range 26–72) as shown in Table 2. 37 of 44 (84%) participants had negative tTG-IgA and/or DGP-IgG at baseline consistent with their gluten-free status. 20 underwent wheat gluten challenge (median age 47.5; 27–68). Ten undertook barley challenge (median age 44 years; 26–63) and 14 undertook rye challenge (median 49 years; 30–72). All wheat, barley and rye challenges were performed in HLA-DQ2.5 positive individuals. All but two participants completed the 3-day challenge. Following the grain challenge 49% reported short-term adverse symptoms, mostly gastrointestinal, that resolved in 83% by day 7.

T cell response ranking was classified as follows: mild 10–50 SFU/million PBMC; moderate >50–100 SFU/million PBMC, and strong >100 SFU/million PBMC; NR = non-responder.

3.2. DONQ52 inhibits wheat gluten-specific T-cell responses in CeD

A significant T cell response to wheat-derived T cell epitopes was induced by oral wheat challenge in 15/20 (75%) CeD participants, consistent with previous studies [5,13,14]. DONQ52 significantly reduced gluten responses to peptides encompassing DQ2.5-glia-α1a/α2 and DQ2.5-glia-ω1/ω2, as effectively or more effectively than pan-HLA-DQ blocking antibody (Fig. 1A-B; $p < 0.05$, Wilcoxon signed rank test). T-cell responses against the α-gliadin-derived 33-mer peptide that encompasses five of the most immunodominant wheat gluten epitopes were significantly reduced in the presence of DONQ52 (Fig. 1C; $p < 0.0001$, Wilcoxon signed rank test at either concentration). DONQ52 also significantly blocked T-cell responses to a cocktail of peptides encompassing DQ2.5-glia-α1a/α2, DQ2.5-glia-ω1/ω2, and DQ2.5-glia-γ1 by a median of 87% (IQR 72–92) at the highest DONQ52 dose (Fig. 1D; $p < 0.0001$, Wilcoxon signed rank test). There was a dose-response effect of blocking by DONQ52 however even at 4 μg/mL T-cell responses could be abolished. DONQ52 did not block T-cell responses to tetanus toxoid (Fig. 1E). DONQ52 induced a significant fold-change reduction of T-cell responses for all tested immunogenic wheat peptides (Fig. 1F). A single CeD participant generated responses to the γ-gliadin peptide, consistent with it being a sub-dominant epitope [6], with DONQ52 blocking observed (Fig. 1F). Each antigen and the highest concentration of DONQ52 was also tested in matched baseline Day 1 PBMC isolated prior to gluten challenge. Consistent with prior reports, gluten-specific T-cell responses prior to gluten challenge were generally not detectable by ELISpot. No T-cell activation was seen in the presence of DONQ52 (Fig. 1G).

3.3. DONQ52 reduces barley hordein T-cell responses in CeD

Following barley challenge, significant T-cell responses to barley-derived T cell epitopes were seen in 8/10 (80%) of CeD participants. In responding patients, DONQ52 reduced responses to peptides encompassing the immunodominant T cell epitopes DQ2.5-hor-3a and 3b even though the induced responses were of high magnitude, in some cases over 100 SFU/million PBMC (Fig. 2A and B). The reduction in response was significant at the highest dose of DONQ52 ($p < 0.05$, Wilcoxon signed rank test). Neither DONQ52 nor SPVL3 antibodies completely blocked these strong responses. There was near complete blocking of responses induced by the peptide encompassing DQ2.5-hor-1/2 and its homolog B03 by the highest concentration of DONQ52 (Fig. 2C and D). Responses to the peptide cocktail containing both Hor3a and Hor3b peptides were reduced by a median of 55% (IQR 38–75) at the highest DONQ52 dose (Fig. 2E). DONQ52 blockade was also shown when the fold-change above the background response was calculated (Fig. 2F). Similar to wheat, no IFN-γ ELISpot responses were observed using the baseline PBMC prior to barley challenge (Fig. 2G).

3.4. DONQ52 reduces rye secalin T-cell responses in CeD

Following rye challenge, positive T-cell responses to secalin or rye-derived T cell epitopes were seen in 4/14 (28%) CeD participants and were generally of low magnitude. DONQ52 induced concentration-dependent blocking of the DQ2.5-sec-1/2 peptide and homologs (Fig. 3A-E). SPVL3 did not block T-cell responses in one patient. Responses to the peptide cocktail containing a mixture of the dominant rye secalin peptides were reduced by a median of 76% (IQR 72–82) at the highest DONQ52 dose (Fig. 3E). The low rate of response but effective

Table 2
Participant details.

ID	Age	Gender	HLA-DQ[#]	tTG-IgA[*]	DGP-IgG[*]	Grain challenge	T-cell response
CD1	49	F	2.5/X	18 (<20)	<3 (<20)	Wheat	Moderate
CD2	63	F	2.5/2.5	4 (<20)	<3 (<20)	Wheat	Strong
CD3	39	F	2.5/X	5 (<20)	<3 (<20)	Wheat	Strong
CD4	27	F	2.5/X	1 (<7)	<1 (<7)	Wheat	Mild
CD5	58	F	2.5/X	3 (<20)	<3 (<20)	Wheat	NR
CD6	41	F	2.5/2.5	11 (<20)	71 (<20)	Wheat	Moderate
CD7	35	F	2.5/X	<2 (<20)	<3 (<20)	Wheat	Mild
CD8	39	F	2.5/2.5	11 (<20)	<3 (<20)	Wheat	Mild
CD9	46	F	2.5/X	2 (<20)	13 (<20)	Wheat	NR
CD10	45	M	2.5/X	3 (<20)	<3 (<20)	Wheat	NR
CD11	60	F	2.5/X	5 (<20)	<3 (<20)	Wheat	Mild
CD12	41	M	2.5/2.5	7 (<20)	<3 (<20)	Wheat	NR
CD13	52	F	2.5/2.5	<2 (<20)	<3 (<20)	Wheat	NR
CD14	49	F	2.5/X	8 (<20)	<3 (<20)	Wheat	Moderate
CD15	63	F	2.5/X	4 (<20)	<3 (<20)	Wheat	Mild
CD16	32	M	2.5/X	2 (<7)	<1 (<7)	Wheat	Moderate
CD17	38	M	2.5/X	3 (<7)	1 (<7)	Wheat	Mild
CD18	68	M	2.5/X	4 (<20)	<3 (<20)	Wheat	Moderate
CD19	59	F	2.5/X	5 (<20)	64 (<20)	Wheat	Moderate
CD20	51	F	2.5/X	14 (<20)	<3 (<20)	Wheat	Mild
CD21	30	F	2.5/2.5	33 (<20)	<3 (<20)	Barley	NR
CD22	45	M	2.5/X	3 (<20)	<3 (<20)	Barley	Strong
CD23	63	M	2.5/X	7 (<20)	<3 (<20)	Barley	Strong
CD24	60	F	2.5/X	26 (<20)	37 (<20)	Barley	Mild
CD25	42	F	2.5/X	28 (<20)	5 (<20)	Barley	Mild
CD26	26	F	2.5/X	4 (<20)	4 (<20)	Barley	NR
CD27	54	M	2.5/X	2 (<7)	<1 (<7)	Barley	Mild
CD28	41	M	2.5/X	9 (<20)	<3 (<20)	Barley	Strong
CD29	46	F	2.5/2.5	5 (<20)	12 (<20)	Barley	Mild
CD30	40	F	2.5/X	9 (<20)	33 (<20)	Barley	Moderate
CD31	44	M	2.5/X	3 (<20)	<3 (<20)	Rye	Mild
CD32	48	F	2.5/X	3 (<20)	<3 (<20)	Rye	Moderate
CD33	54	F	2.5/X	6 (<20)	<3 (<20)	Rye	NR
CD34	49	F	2.5/2.5	6 (<20)	<3 (<20)	Rye	NR
CD35	52	F	2.5/X	8 (<20)	<3 (<20)	Rye	NR
CD36	58	M	2.5/X	3 (<20)	<3 (<20)	Rye	NR
CD37	47	F	2.5/X	3 (<20)	<3 (<20)	Rye	NR
CD38	38	M	2.5/X	9 (<20)	18 (<20)	Rye	NR
CD39	63	M	2.5/X	7 (<20)	<3 (<20)	Rye	Mild
CD40	72	F	2.5/X	2 (<20)	<3 (<20)	Rye	NR
CD41	49	F	2.5/X	2 (<20)	4 (<20)	Rye	Mild
CD42	68	F	2.5/X	5 (<20)	<3 (<20)	Rye	NR
CD43	30	F	2.5/X	30 (<20)	<3 (<20)	Rye	NR
CD44	41	F	2.5/X	<1 (<7)	<1 (<7)	Rye	NR

NR = non-responder
[#] X = another haplotype, not including DQ2.2 or DQ2.5.
[*] Serology normal cut-off shown in brackets (Units).

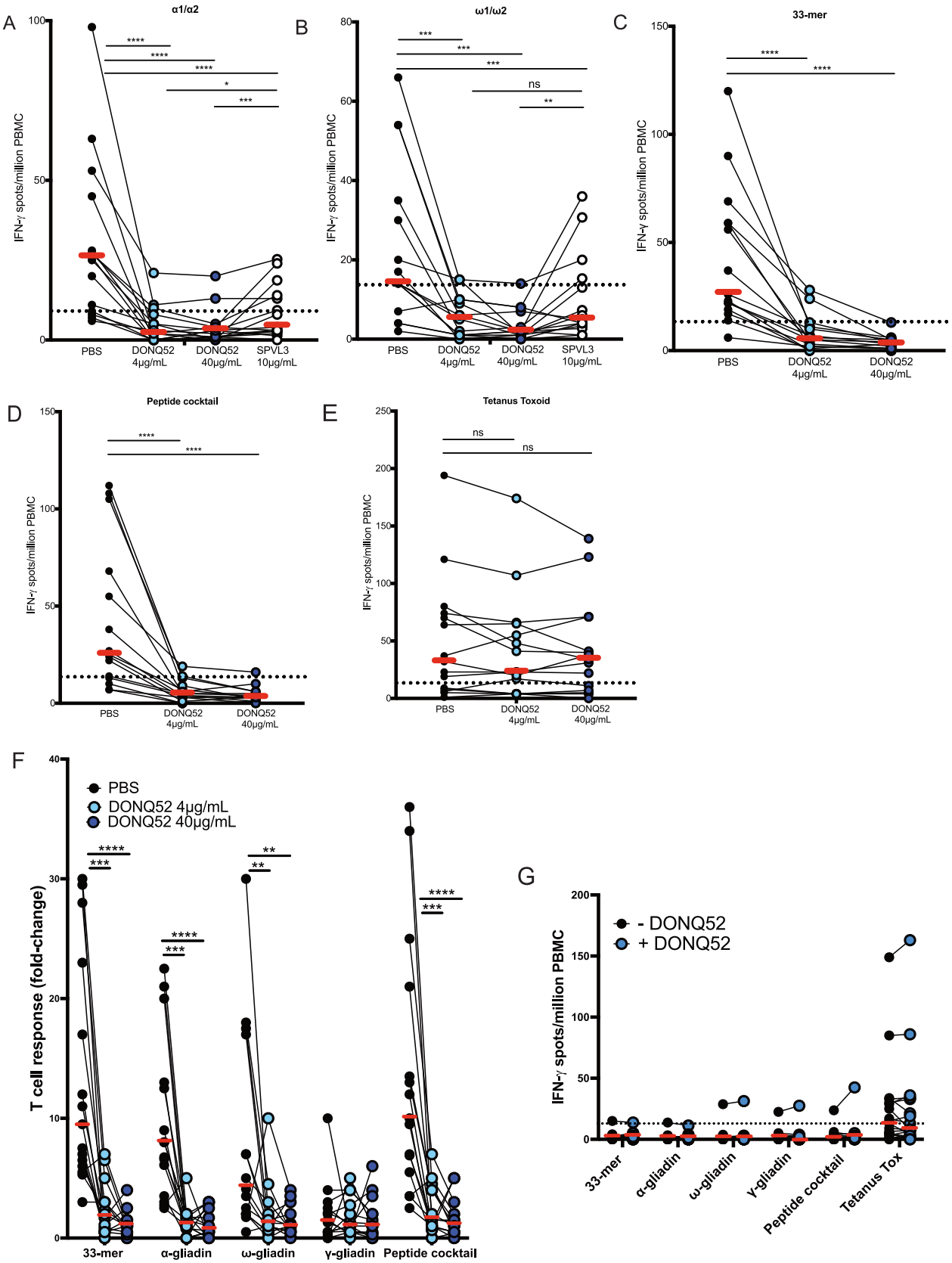

Fig. 1. DONQ52 potently blocks the IFN-γ response to gluten peptides in CeD after wheat ingestion. T cell responses measured by IFN-γ ELISpot were assessed to a series of immunogenic wheat gluten peptides after wheat ingestion in the presence or absence of DONQ52 or pan-HLA-DQ blocking antibody. $N = 15$ patients had a measurable immune response to peptide(s). Shown are responses to: A) peptide containing DQ2.5-glia-α1a/α2, B) peptide containing DQ2.5-glia-ω1/ω2, C) α-gliadin 33-mer peptide, D) a cocktail of α-gliadin, ω-gliadin, and γ-gliadin peptides, E) Tetanus toxoid. F) Fold-changes to all wheat gluten antigens. G) Baseline IFN-γ responses ($n = 15$). Dotted line denotes the assay cut-off for a positive response. Red lines show median response. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns $p > 0.05$ (Wilcoxon signed rank test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

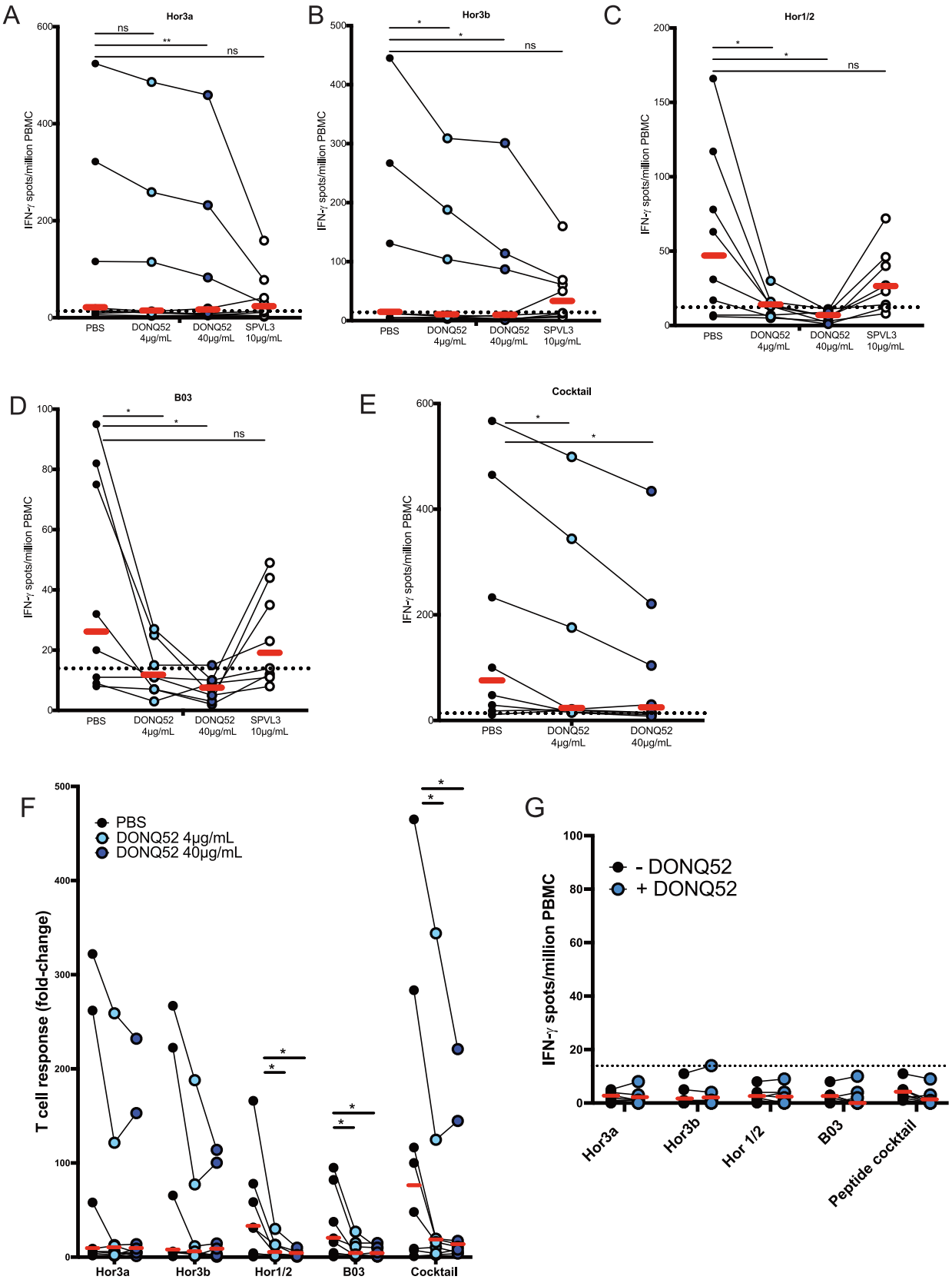

Fig. 2. DONQ52 efficiently reduces the IFN-γ response to barley hordein in CeD after barley ingestion. T cell responses measured by IFN-γ ELISpot were assessed to a series of immunogenic barley hordein peptides after barley ingestion in the presence or absence of DONQ52 or pan-HLA-DQ blocking antibody. $N = 8$ patients had measurable responses. Shown are responses to: A) HLA-DQ2.5-hor3a, B) DQ2.5-hor3b, C) DQ2.5-hor-1/2, D) B03 peptide containing DQ2.5-hor-1/2, E) a cocktail containing a mix of the 4 peptides. F) Fold-changes to all barley hordein antigens. G) Baseline (pre-challenge) IFN-γ responses ($n = 8$). Dotted line denotes the assay cut-off for a positive response. Red lines show median response. * $p < 0.05$, ** $p < 0.01$, and ns $p > 0.05$ (Wilcoxon signed rank test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

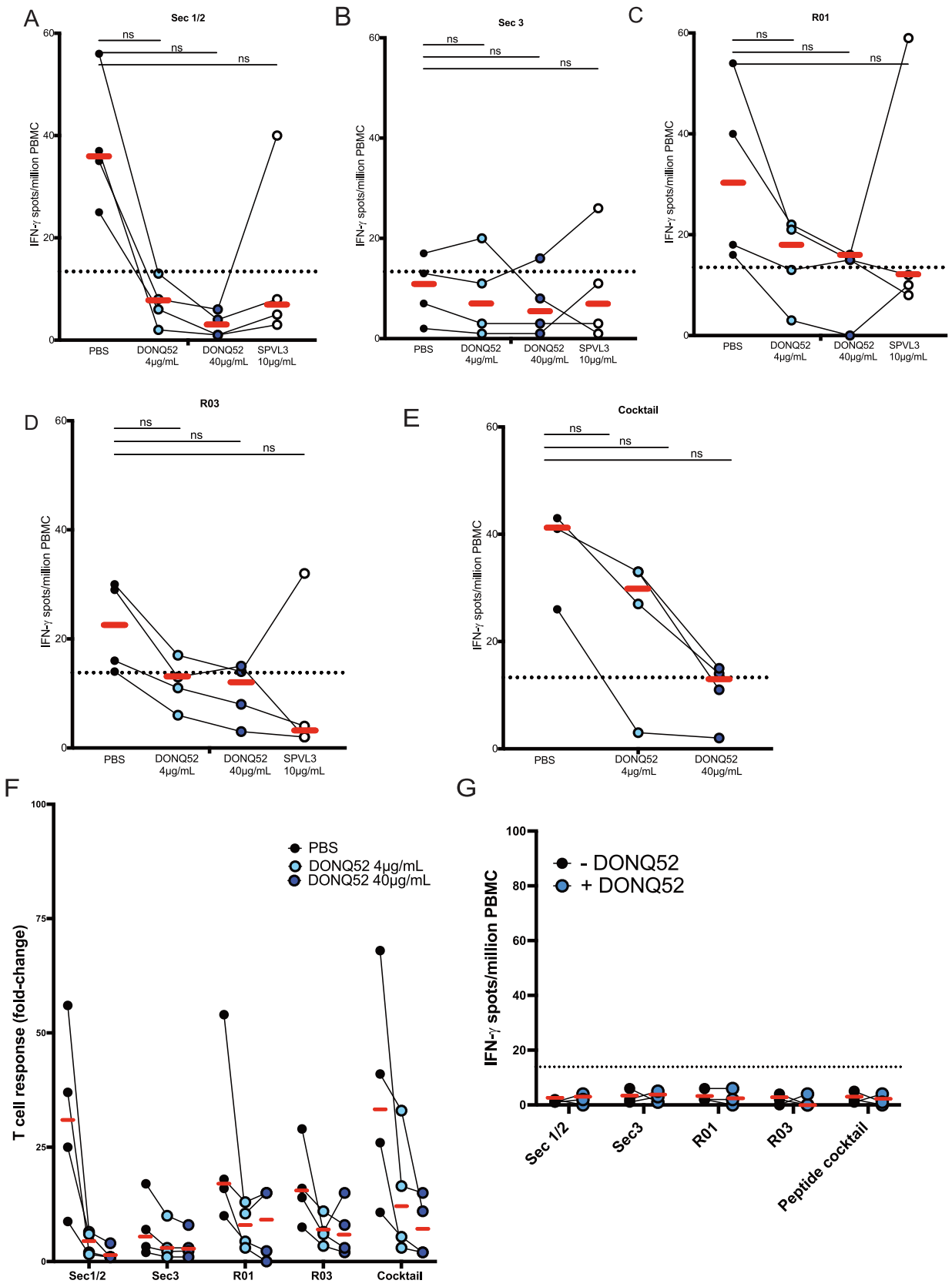

Fig. 3. DONQ52 reduces the IFN-γ response to rye secalin peptides in CeD after rye ingestion. T cell responses measured by IFN-γ ELISpot were assessed to a series of immunogenic rye secalin peptides after rye ingestion in the presence or absence of DONQ52 or pan-HLA-DQ blocking antibody SPVL3. $N = 4$ patients had measurable responses. Shown are responses to: A) peptide containing HLA-DQ2.5-s-1/2, B) peptide containing DQ2.5-s-3, C) R01 peptide, D) R03 peptide, E) a cocktail containing a mix of the 4 peptides. F) Fold-changes to all rye secalin antigens. G) Baseline (pre-challenge) IFN-γ responses ($n = 3$). Dotted line denotes the assay cut-off for a positive response. Red lines show median response. ns $p > 0.05$ (Wilcoxon signed rank test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

blocking was also seen when the fold-change in T cell response was calculated, although this did not reach statistical significance (Fig. 3F; $p = 0.125$, Wilcoxon signed rank test). No responses were observed in the baseline samples in the presence or absence of DONQ52 (Fig. 3G).

4. Discussion

Novel therapies for CeD that offer greater efficacy than the gluten-free diet remain an unmet need [4]. Targeted antigen-specific approaches are highly attractive as they could produce focused on-target effects without carrying the risk of broader immunosuppression. The comprehensive definition of the T cell hierarchy of pathogenic epitopes driving CeD and the ability to employ a disease-specific T cell model to support pre-clinical evaluation makes CeD an ideal disease to support the development and testing of TCR-HLA targeted antibody approaches.

We found that DONQ52 can potently block the activation of gluten-specific T cells by several known T cell epitopes, including the most immunodominant ones reported from wheat, rye and barley. Notably, in a potent cocktail containing the immunodominant wheat epitopes HLA-DQ2.5-glia-α1a/α2 and HLA-DQ2.5-glia-ω1/ω2, DONQ52 blocked over 85% of the induced immune response. The blocking of dominant wheat epitope responses by DONQ52 was often equivalent or superior to pan-HLA-DQ blockade, demonstrating the potency of targeted HLA-TCR blockade in reducing pathogenic T cell activation.

Our findings confirm the broad gluten blocking capacity of DONQ52 for the most immunogenic wheat epitopes, and importantly, show an effect on immunodominant barley hordein and rye secalin epitopes. This supports the notion that the cross-reactive nature of DONQ52 is capable of blocking numerous pathogenic TCRs specific to multiple distinct gluten epitopes that share in common proline and glutamine-rich motifs. Following barley ingestion, we noted that neither DONQ52 or SPVL3 completely blocked strong responses to hordein epitopes. This may suggest that greater amounts of DONQ52 are required for high magnitude responses, as indicated by Okura et al. [9]. Notably, most current antigen-specific therapies under development for CeD focus primarily on wheat and the field have generally not considered the pathogenic role for barley or rye [4]. However, as each of these cereals contain distinct epitopes [5,6], it reasons that broad coverage of these sequences will improve the efficacy and proportion of patients who might benefit from a blocking or tolerogenic therapy. Less is known about the identity of the driving epitopes in HLA-DQ8 and HLA-DQ2.2-associated CeD and it is likely many epitopes have not been yet reported [6]. A greater understanding of the immunodominant T cell epitopes in HLA-DQ8 and HLA-DQ2.2 CeD will enable design of antibody therapies relevant to CeD patients expressing these haplotypes.

Off-target effects of DONQ52 on non-gluten antigens like tetanus toxoid were not observed and DONQ52 did not non-specifically induce T-cell responses. These findings are important with respect to future safety considerations of DONQ52 administration in patients. Future work should include known HLA-DQ2.5 binding peptides from non-gluten proteins that induce an IFN-γ response to provide additional confirmatory evidence of the absence of off-target effects.

The 3-day gluten challenge approach to induce gluten-specific T cells in the blood of CeD patients has been widely employed [11,14–16] and has served as an informative tool to facilitate T cell epitope mapping [5,11,13,17], design an ultra-low gluten barley [18], pre-clinical drug development [5,19], and also as a clinical trial readout and monitoring tool [20–22]. Although 25–30% of CeD participants do not have a significant inducible gluten-specific T cell response after oral challenge on day 6, the current study underscores the utility of this tool in providing a disease-relevant and accessible readout.

This study establishes in vivo proof-of-concept that a HLA-gluten peptide targeting multi-specific antibody can potently and broadly inhibit T-cell responses to pathogenic gluten peptides but not unrelated antigens. The findings indicate that multi-specific antibody blockade of the gluten-specific T cell response may have therapeutic value in CeD and supports further clinical testing of DONQ52 [23].

Disclosures

JT-D has privately or via his institute been a consultant or advisory board member for Anatara, Anokion, Barinthus Biotherapeutics, Chugai Pharmaceuticals, Equillium, IM Therapeutics, Janssen, Kallyope, Mozart Therapeutics, TEVA and Topas, has received research funding from Barinthus Biotherapeutics, Chugai Pharmaceuticals, Codexis, DBV Technologies, EVOQ Therapeutics, Immunic, Kallyope, Novoviah Pharmaceuticals and Tillotts Pharmaceuticals and received Honoraria from Takeda. He is an inventor on patents relating to the use of gluten peptides in coeliac disease diagnosis and treatment. MYH is a consultant for Takeda.

CRediT authorship contribution statement

M.Y. Hardy: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **L.M. Henneken:** Writing – review & editing, Investigation, Data curation. **A.K. Russell:** Writing – review & editing, Project administration, Investigation. **Y. Okura:** Writing – review & editing, Resources. **A. Mizoroki:** Writing – review & editing, Resources. **Y. Ozono:** Writing – review & editing, Resources. **S. Kobayashi:** Writing – review & editing, Resources. **Y. Murakami:** Writing – review & editing, Resources. **J.A. Tye-Din:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability

The data that has been used is confidential.

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