Antibodies specific for peptides bound to human leukocyte antigen (HLA) molecules are valuable tools for studies of antigen presentation and may have therapeutic potential. Here, we generated human T cell receptor (TCR)–like antibodies toward the immunodominant signature gluten epitope DQ2.5-glia-α2 in celiac disease (CeD). Phage display selection combined with secondary targeted engineering was used to obtain highly specific antibodies with picomolar affinity. The crystal structure of a Fab fragment of the lead antibody 3.C11 in complex with HLA-DQ2.5:DQ2.5-glia-α2 revealed a binding geometry and interaction mode highly similar to prototypic TCRs specific for the same complex. Assessment of CeD biopsy material confirmed disease specificity and reinforced the notion that abundant plasma cells present antigen in the inflamed CeD gut. Furthermore, 3.C11 specifically inhibited activation and proliferation of gluten-specific CD4⁺ T cells in vitro and in HLA-DQ2.5 humanized mice, suggesting a potential for targeted intervention without compromising systemic immunity.

INTRODUCTION

Antibodies (Abs) with specificity for peptide-MHC (pMHC), so-called T cell receptor (TCR)–like Abs, have been successfully used to detect and quantify peptide presentation on cells, and several studies suggest therapeutic potential by different modes of action, including inhibition of pathogenic T cells and killing mechanisms to delete antigen-presenting cells (APCs) (1–3). As soluble reagents, Abs are often preferred over TCRs because of their increased stability and higher affinity (4–8). We have previously and in this study generated TCR-like Abs specific for pMHC complexes implicated in celiac disease (CeD) (9).

CeD is an inflammatory autoimmune-like condition of the small intestine caused by immune reactions to dietary gluten proteins (10). The disease is driven by CD4⁺ T cells that recognize deamidated gluten peptides in the context of the disease-associated human leukocyte antigen (HLA)–DQ molecules HLA-DQ2.5 (DQA1*05/DQB1*02), HLA-DQ2.2 (DQA1*02:01/DQB1*02:02), and HLA-DQ8 (DQA1*03/DQB1*03:02) (11). Out of these HLA variants, HLA-DQ2.5 is the most strongly associated with CeD and is detected in around 90% of patients with CeD compared with about 20% in healthy controls (11). Deamidation of gluten peptides is mediated by the enzyme transglutaminase 2 (TG2) and results in conversion of Glu to negatively charged Gln at specific sites in polypeptides transforming proteolytically stable, but immunologically inert, peptides into pathogenic T cell epitopes (12, 13). A range of gluten T cell epitopes has been characterized, but four immunodominant epitopes derived from α-gliadin and α-gliadin are particularly prominent in the context of HLA-DQ2.5, namely, DQ2.5-glia-α1 (PFPQPELPY), DQ2.5-glia-α2 (PQPPELPQ), DQ2.5-glia-α1 (PFPQPELPQ), and DQ2.5-glia-α2 (PQPPELPQFW). T cell responses against these epitopes are found in most HLA-DQ2.5⁺ patients and are considered to orchestrate tissue destruction in the small intestine and autoantibody production (14–16). The only currently available treatment for CeD is lifelong adherence to a gluten-free diet. Development of alternative treatments is sought after because of poor patient compliance with the dietary restrictions, unavailability of strictly gluten-free food, and the feared transformation of uncomplicated CeD to refractory CeD (17, 18). Although it is still unclear how the destructive T cells emerge in CeD, recent findings have suggested a role for environmental factors including possible T cell cross-reactivity with microbial antigens (19, 20) and highlighted a need for further investigations into the roles of different MHC-presented antigens in CeD.

Recently, we reported the generation and use of Abs selected on HLA-DQ2.5:DQ2.5-glia-α1a. We unexpectedly found that gut plasma cells (PCs) are the most abundant gluten peptide–presenting cells in the inflamed small intestine of patients with CeD and that they express both HLA class II and T cell costimulatory molecules (9). In the current study, we describe the generation and affinity maturation of human TCR-like Abs specific for HLA-DQ2.5 in...
complex with DQ2.5-glia-α2. We have previously shown that this complex elicits a prototypic signature CeD T cell response, which appears to be shared across all patients (21, 22). Our strategy was based on phage display selection of a fully human naïve Ab library combined with secondary complementarity determining region (CDR)–targeted optimization and gave rise to highly specific binders with picomolar monomeric affinities toward HLA-DQ2.5: DQ2.5-glia-α2. The cocystal structure of a Fab fragment of the lead Ab 3.C11 in complex with HLA-DQ2.5-glia-α2 revealed an about diagonal docking geometry with respect to the peptide and a footprint highly similar to prototypic CeD patient–derived TCRs with the same specificity. The 3.C11 Ab readily stained a population of PCs from inflamed CeD lamina propria biopsy material, whereas the scarce CD11c+ and CD14+ dendritic cells (DCs) and macrophages (Mfs) from the corresponding material stained less prominently. These results confirm and extend our previous observation on the central role of the B cell compartment in this tissue (9). We further observed potent and strictly HLA- and epitope-dependent inhibition of T cell activation, both using CeD-derived CD4+ T cells in vitro, as well as primary CD4+ T cells in vivo upon oral administration of gluten peptide in HLA-DQ2.5 humanized mice, suggesting that the lead Ab 3.C11 has potential to be used for CeD-specific immunotherapy.

RESULTS

Primary selection of Abs specific for HLA-DQ2.5 with bound DQ2.5-glia-α2

To generate human Abs specific for HLA-DQ2.5 in complex with the CeD epitope DQ2.5-glia-α2, a human naïve single-chain fragment variable (scFv) phage display library (23) was panned against soluble, recombinant pMHC. The method used to select the primary lead was based on our previous selection of a HLA-DQ2.5:DQ2.5-glia-α1–specific Ab (9). After three rounds of selection, we assessed antigen reactivity of the selection output in a polyclonal enzyme-linked immunosorbent assay (ELISA) and observed increased and preferential binding to the target (fig. S1A). We then reformatted the selection output from the phagemid to a vector for soluble scFv expression and screened 70 single clones for target binding by ELISA (Fig. 1A). A total of 49 single clones bound target preferentially, and sequence analysis identified 14 unique sequences (Fig. 1A and fig. S1B). Five of the clones were enriched in the selection output. The V gene segment usage of the single clones was dominated by IGHV1-69, which paired with a diverse set of IGKV/LV segments (fig. S1B and table S1). To characterize the selected scFvs and choose a lead clone, we expressed all unique clones in *Escherichia coli* and directly compared periplasmic fractions in ELISA for target binding (Fig. 1B). Next, we performed pilot surface plasmon resonance (SPR) measurements...
using purified scFv (fig. S1C). On the basis of reactivity profiles and apparent affinities, we chose a lead clone, termed 206. When reformatted to full-length human immunoglobulin G1 (hIgG1), 206 retained binding to HLA-DQ2.5:DK2.5-glia-α2, albeit weakly (Fig. 1C and fig. S1D). No binding to the highly similar epitope HLA-DQ2.5:DK2.5-glia-α2 was observed (Fig. 1D). To accurately determine the monomeric affinity, a Fab version of 206 was constructed and SPR analysis estimated the monomeric affinity to $K_D = 240 \pm 20 \text{nM}$ with a high off-rate ($2.4 \times 10^{-1} \text{s}^{-1}$) (Fig. 1E).

**CDR-targeted secondary library design and selection of high-affinity gluten pMHC–specific Abs**

Given the low affinity of the 206 clone that was inadequate, for example, in detecting peptide on pulsed model APC (Fig. 6A), we sought to improve the Ab-pMHC interaction by targeted engineering. We generated focused libraries with sequence randomization and increased lengths of either the CDR H1 or H3 loop based on structural models assuming a TCR-like binding mode and the anticipated importance of the CDR H3 loop in target binding. We have previously found high valence (HV) pIX phage display to be very efficient at isolating Ab clones with high affinity and stability (23). Thus, the libraries were prepared accordingly and selected using a stringent strategy aimed at parallel identification of specific binders with high affinity and high thermostability in two separate arms (Fig. 2A). After an initial low-stringency round (R1), the libraries were split into a competition branch (CDR H1 and H3 selected separately, libraries denoted H1C and H3C) and a thermostability branch (CDR H1 and H3 pooled for selection, library denoted “H1/H3 T”) for a highly stringent R2 with low target concentration followed by a nonstringent R3 loosely based on the hammer-hug selection protocol (24). In R2 of the competition branch, scFvs were displayed at low valence (LV). In the thermal branch, scFvs were displayed at HV and subjected to a heat challenge at temperatures that induced unfolding of the parent clone (Fig. S2A). This was done before selection to aggregate and remove unstable library members (6, 25). In R3, we aimed at recovering and amplifying selected binders and therefore increased antigen concentration. For the thermal branch, we included a second heat challenge and displayed scFvs at LV to favor clones with high monomeric affinities. The stringent competition in R2 of the competition branch resulted in close to no selection output in the CDR H3-LV library, and it was therefore discontinued. To determine antigen reactivity of the output, we performed a polyclonal phage ELISA (fig. S2B). The libraries showed signs of enrichment of binders, and we continued to screen single clones from the R3 output as soluble scFv (Fig. 2B) and scFv displayed on phage (Fig. 2C) by ELISA. Clones with preferential binding to the target were present in both selection branches, and sequence analysis revealed that 66 of 73 were unique DNA sequences, and 64 were unique at the amino acid level (fig. S2C). All positive clones came from the CDR H1 library only, and most of the unique DNA sequences (56 of 66) were derived from the library with a loop length increased by two residues with the remaining clones (10 of 66) stemming from the pool with a length increase of three residues.

On the basis of target binding in screening and enrichment of sequence features, we chose six clones for large-scale Fab expression in human embryonic kidney 293E cells. These were analyzed regarding their peptide specificity in ELISA, and all were found to bind their HLA-DQ2.5:DK2.5-glia-α2 target specifically (Fig. 2D and fig. S2D). The Fabs cross-reacted neither with the homologous HLA-DQ2.5:DK2.5-glia-α2 complex, which differs at three peptide positions only (p5, p7, and p9), nor with HLA-DQ2.5 with any of DK2.5-glia-α1, DK2.5-glia-α3a, or class II–associated invariant chain peptide 2 (CLIP2). Thus, the chosen candidates all bound specifically to their target despite the relatively broad selection of CDR H1 phenotypes (Fig. 2E).

**Biophysical characterization of affinity-matured pMHC-specific Abs**

We then performed binding analysis by SPR and ranked the six Abs on the basis of their off-rates (Figs. 2E and 3A and table S2). Strongly reduced off-rates were observed for all clones tested with clone containing pooled CDR H1 and H3 libraries was selected in the thermostability branch, whereas the H1-H3 library containing pooled CDR H1 and H3 libraries was selected in the competition branch. CDR H1 only was selected in the competition branch (C). R3 selection outputs were screened in phage format and are represented as in (B). (D) Purified, monomeric Fab fragments were tested for binding to a panel of HLA-DQ2.5:peptide complexes in ELISA. Error bars illustrate means ± SD of duplicates ($n = 2$). (E) Sequence alignment of the mother clone 206 and the high-affinity offspring containing CDR H1 mutations (bold red). *IGHV* gene segment usage and numbering according to the international ImMunoGeneTics information system (IMGT). The *IGHV*-69 V gene of clone 206 is in germine configuration.

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Fig. 2. Selection and screening of Ab libraries. (A) Schematic overview of the selection strategy. LV and HV displays were achieved by packaging with the helper phage M13KO7 or DeltaPhage, respectively. After R1, the libraries were split into a competition branch and a thermostability branch. (B) Selection outputs after R3 were screened as soluble scFvs to assess binding to target pMHC and HLA-DQ2.5:CLIP2 (background) in ELISA, and ratios were calculated. Each dot represents one clone. Gray dots denote unknown sequences, black dots denote unique single sequences, and colors represent enriched amino acid sequences. The H1/H3 library containing pooled CDR H1 and H3 libraries was selected in the thermostability branch, whereas the H1 library (CDR H1 only) was selected in the competition branch. (C) R3 selection outputs were screened in phage format and are represented as in (B). (D) Purified, monomeric Fab fragments were tested for binding to a panel of HLA-DQ2.5:peptide complexes in ELISA. Error bars illustrate means ± SD of duplicates ($n = 2$). (E) Sequence alignment of the mother clone 206 and the high-affinity offspring containing CDR H1 mutations (bold red). *IGHV* gene segment usage and numbering according to the international ImMunoGeneTics information system (IMGT). The *IGHV*-69 V gene of clone 206 is in germine configuration.
3.C11 exhibiting the lowest off-rate. In concordance with the improved (lower) off-rates, 3.C11 had a strong improvement in affinity with a $K_D = 88 \pm 8$ pM (Fig. 3B and table S2). This is a 2700-fold improvement compared with the parent clone. 3.C11 was then expressed as full-length hIgG1 and tested for specific binding in ELISA (Fig. 3C). In agreement with previous results, 3.C11 bound exclusively to HLA-DQ2.5:DQ2.5-glia-2. Thus, the high-affinity Abs maintained the high specificity of the mother clone.

We next assessed the thermostability of all Fab fragments by determining their melting temperatures using nanoscale differential scanning fluorimetry (nanoDSF) (Fig. 3D). The high-affinity variants unexpectedly had slightly lower melting temperatures than their mother clone. The lead clone 3.C11 had the highest thermostabilities out of the engineered clones. Because of its favorable biophysical properties, we selected 3.C11 as a lead candidate for further characterization.

**Structural basis for epitope specificity of 3.C11**

To understand the molecular basis for the specificity and affinity of 3.C11, we crystallized the complex of the 3.C11 Fab fragment and HLA-DQ2.5:DQ2.5-glia-2 and solved its structure to 2.4-Å resolution (table S3). The structure revealed that the 3.C11 Fab bound HLA-DQ2.5:DQ2.5-glia-2 with a “TCR-like” docking mode (Fig. 4, A and B) (26), which was unexpectedly similar to that observed for the prototypic HLA-DQ2.5:DQ2.5-glia-2–specific TRAV26-1/TRBV7-2 TCRs D2, S16, and JR5.1 (27). To characterize the TCR-like features of 3.C11, we compared the structure of the 3.C11 ternary complex with that of the S16 TCR (Fig. 4, C and D). The layout of the CDR loops relative to the pMHC and the footprint of 3.C11 (Fig. 4B) also broadly resembled that of the S16 TCR (Fig. 4D), such that 3.C11 and S16 shared a large number of pMHC contact residues.

The total buried surface area (BSA) of 3.C11 and HLA-DQ2.5:DQ2.5-glia-2 was 2385 Å², which is moderately larger than that of the prototypic TCRs S16 (2130 Å²), D2 (2145 Å²), and JR5.1 (1765 Å²). The increase was mainly attributable to additional pMHC contacts made by the 3.C11 CDR H1 and CDR H2. With a BSA contribution...
of 76.2%, the 3.C11 H chain dominated the pMHC interface with relatively even contributions made by CDR H3 (27.5%), CDR H2 (22.7%), and CDR H1 (22.4%). The smaller interface of the L chain (23.8%) featured CDR L1 (11.2%) as the largest contributor (Fig. 4, B and D). The dominant role of the H chain in the 3.C11 pMHC interface broadly reflected the relative contributions of the β chains observed for TCRs S16 (59.7%; Fig. 4D), D2 (62.8%), and JR5.1 (73.6%). Accordingly, the moderately larger BSA of 3.C11 compared with the TCRs contributes to the vastly higher affinity of 3.C11. The 3.C11 Fab-pMHC interface comprises 5 H-bonds and three salt bridges, which is numerically comparable to the 12 H-bonds present in the pMHC interface of S16 TCR. To further investigate the basis for the vastly higher affinity of 3.C11 compared with the S16 TCR, we calculated the geometric surface complementarity of the interfaces using shape complementarity (SC) (28), which resulted in nearly identical SC values for 3.C11 (SC = 0.691) and S16 (SC = 0.684). To investigate the charge complementarity of the complexes, we calculated the electrostatic surface potentials of the 3.C11 (fig. S3A) and S16 (fig. S3B) complexes using the Adaptive Poisson-Boltzmann Solver (29). The results revealed an overall similar polarity of the surface potential on 3.C11 and S16. 3.C11 showed an additional positively charged patch in the area of CDR H1, which was opposed by an overall weakly charged area on the pMHC (fig. S3A) and is therefore unlikely to drive the vast difference in affinities. In accordance with the positional shift toward the HLA-DQ2.5 α chain, compared with the S16 TCR, 3.C11 formed an extensive interface with the HLA-DQ2.5 α chain (48.3%) and comparatively smaller interfaces with the HLA-DQ2.5 β chain (35.3%) and the peptide (16.5%) (Fig. 4, B and D). The CDR H2 and CDR H3 loops made the largest contribution to the interface with the HLA-DQ2.5 α chain. Here, the CDR H3 loop contacted the inner side of the peptide binding cleft, whereas the CDR H2 cupped the top and outer side of the HLA-DQ2.5 α chain helix (Fig. 5A). The hydrophobic core of the CDR H2–HLA-DQ2.5 α chain interface was composed of germline
residues unique to IGHV1-69 (Ile^57, Ile^59, and Phe^62), which suggests that the preferential IGHV1-69 selection as observed is in part driven by these interactions. Moreover, key elements of the 3.C11 Fab-pMHC interface were unexpectedly similar to features observed for the S16 TCR–HLA-DQ2.5:DQ2.5-glia-α2 interface. Namely, the peptide residues directly contacted by 3.C11 (p2, p5, p6, p7, p8, p9, and p11) (Fig. 5B) included all peptide residues contacted by S16 (p2, p5, p6, p7, and p8) (Fig. 5C), and the interaction pattern of 3.C11 with the peptide (CDR L1 → p2; CDR H3 → p5, p6, p7; CDR H1 → p8, p9, p11) closely resembled that observed in the S16 complex (CDR α and CDR α → p2; CDR β → p5, p6, p7; CDR β1 → p8). Both the CDR H3 loop of 3.C11 (Fig. 4B) and the CDR β3 loop of S16 (Fig. 4D) formed brace-like structure across the central portion of the peptide and bridge across the peptide binding cleft (Fig. 5A).

The prototypic TRAV26-I/TRBV7-2 TCRs, such as S16, are preferentially selected by HLA-DQ2.5:DQ2.5-glia-α2 and generally contain a nongermline-encoded Arg residue in CDR β3 (Fig. 5C), which is critical for pMHC recognition (27, 30). In 3.C11, Gln^109 at the tip of the CDR H3 loop took up the position of this Arg residue (Fig. 5A).

To determine the impact of each peptide amino acid position on 3.C11 binding, we performed an alanine scan of the DQ2.5-glia-α2 peptide. Compared with TCRs 364 and S16 (fig. S4A and B), 3.C11 binding was affected by mutagenesis over the entire peptide length with a >50% reduction in binding observed for mutation of p4-Glu, p5-Leu, and p10-Pro (fig. S4C) consistent with the 3.C11 footprint covering peptide residues p2 to p11, whereas the S16 footprint covered peptide residues p2 to p8 and was highly sensitive to substitution of residues p3 to p8 (fig. S4B).

**Structural basis for affinity enhancement of 3.C11**

The differences between the parental 206 and the affinity-matured leads reside within the CDR H1 loop, which, in the case of 3.C11, replaced a four-residue segment (GGTVRSVHA) with a new six-residue segment (GGTVYRSSY). The CDR H1 loop of 3.C11 formed a short helical segment that sat atop of p8-Pro and interacted with the backbone of the C-terminal peptide residues p9 and p10, as well as with both the HLA-DQ2.5 α and β chains (Fig. 5A). The interface between CDR H1 and HLA-DQ2.5:DQ2.5-glia-α2 was mediated by interdigitating interactions of the helix backbone, the side chains of Val^30 and Val^36 at either end of the CDR H1 helical turn and Arg^34, which further formed a salt bridge with HLA-DQ2.5-α-Asp^66, and a H-bond with Q64 (Fig. 5A). To better understand the basis for the affinity enhancement of 3.C11 over the parental clone 206 containing the canonical IGHV1-69 CDR H1, we compared the structures of four published IGHV1-69 Fabs with canonical IGHV1-69 CDR H1 loops to 3.C11 (Fig. 5A, box inset). Alignment of IGHV1-69 revealed a consensus positioning at the N-terminal end of the CDR H1 loops, which suggested that the shorter CDR H1 loop of 206 was likely to interact with the HLA-DQ2.5 α chain and p8-Pro of the peptide but was clearly too short to reach the HLA-DQ2.5 β chain. Accordingly, the affinity maturation of the CDR H1 loop in 3.C11 created an extension that allowed a short helical segment to form multiangle peptide contacts and effectively bridged the peptide binding cleft and added a salt bridge and a H-bond with the HLA-DQ2.5 β chain. All of the affinity enhanced clones featured an extended CDR H1 loop (Fig. 2E), suggesting that the affinity gain in each clone is based on the introduction of additional BSA and discrete binding interactions with both the peptide and the HLA-DQ2.5 β chain.

**Detection of gluten peptide presentation on cell lines and human small intestinal biopsy material**

Having demonstrated specificity and improved affinity of the Abs to soluble, recombinant pMHC molecules, we tested whether the Abs would bind pMHC complexes on the surface of cells. We loaded HLA-DQ2.5+ Raji B lymphoma cells (31) with soluble gluten peptides. To exclude species-dependent cross-reactivity, the Abs were reformatted and expressed as mouse IgG2b (mIgG2b) (fig. S5A). Whereas the mother clone 206 failed to show detectable binding to peptide-loaded Raji cells, the high-affinity variant 3.C11 exhibited specific binding to cells loaded with DQ2.5-glia-α2 or 33-mer peptides (Fig. 6A). The 33-mer contains three overlapping copies of the DQ2.5-glia-α2 epitope and is thought to be presented on APCs in the celiac lesion (32). Flow cytometric analysis using A20 B cells engineered to express HLA-DQ2.5 with covalently linked DQ2.5-glia-α2 peptide improved baseline separation compared with the staining of peptide-loaded Raji cells, and 3.C11 binding was comparable with a pan-HLA-DQ2 Ab, indicating that loading only results in a fraction of MHC molecules being occupied with specific peptide (Fig. 6A and fig. S5B).

To further assess the HLA restriction of the Abs, human Epstein-Barr virus (EBV)–transduced B cell lines of different HLA-DQ allo-types were loaded with gluten peptides and immediately stained (Fig. 6B and fig. S5C). HLA-DQ2.2 binds DQ2.5-glia-α2 less stably than HLA-DQ2.5, and HLA-DQ8 is not expected to bind the peptide at all (33). The flow cytometry experiments confirmed that 3.C11 is pMHC specific, binding to peptide presented on either HLA-DQ2.5 or the closely related HLA-DQ2.2.

We recently showed that an HLA-DQ2.5:DQ2.5-glia-α1a-specific Ab termed 107 specifically stained B cells and PCs in single-cell suspensions made from inflamed intestinal biopsies derived from HLA-DQ2.5+ patients with CeD who consumed gluten (9). Here, we again generated fresh single-cell suspensions from such HLA-DQ2.5+ patients with CeD that were on a gluten-containing diet [untreated CeD (UCEd)] or control participants with normal gut histology (ctrl). We then stained with the mlgG2b version of 107 or 3.C11, as well as Abs with different APC surface marker specificities (Fig. 6, C to F, and fig. S6, A to D). We confirmed the previous observation using the more sensitive high-affinity Ab. The two Abs stained PCs from patients with UCeD with roughly the same staining pattern and level (Fig. 6C and fig. S6, C and D). The results of the staining of PCs from five control participants suggested that there was little background staining with both Abs (Fig. 6D). Two of eight patient samples were seemingly negative for the targeted pMHC using both Abs. We further extended the analysis to pMHC expressed on DCs and Mfs (Fig. 6, E and F). The pMHC-specific Abs did not stain cells in control participants, but they appeared to detect low levels of peptide presentation in HLA-DQ2.5+ patients with UCeD. The total number of DCs/Mfs was low in the biopsy material.

**Potent inhibition of T cell activation in vitro by high-affinity Abs**

Having confirmed that cell-surface pMHC was specifically detected both in vitro peptide-loaded cells and on patient-derived APCs loaded in vivo, we wondered whether we could block the interaction...
between T cells and APCs using the high-affinity Ab. DQ2.5-glia-α2 is the most extensively characterized gluten T cell epitope to which all HLA-DQ2.5+ patients with CeD appear to mount a T cell response (22, 34).

To determine the inhibitory capacity of 3.C11, human SKW3 T cells devoid of endogenous TCR were engineered to express TCRs that were cloned from patients with CeD and that were specific for HLA-DQ2.5 with DQ2.5-glia-α1a [TCR 380 (21)] or DQ2.5-glia-α2 [TCR S16 (27) and TCR 364 (30)]. We loaded Raji B cells with tritiated amounts of stimulatory DQ2.5-glia-α2 12-mer gluten peptide and cocultured them with the SKW3 T cells. T cell activation was measured by determining up-regulation of CD69 expression on the SKW3 T cells using flow cytometry (fig. S7, A and B). A peptide concentration inducing 60% T cell activation was chosen for subsequent experiments, where the peptide-loaded Raji B cells were incubated with 3.C11 or control Abs upon addition of SKW3 T cells (Fig. 7A). 3.C11 specifically and completely inhibited T cell activation of the SKW3 364 T cells specific for HLA-DQ2.5:DQ2.5-glia-α2, whereas the HLA-DQ2.5:DQ2.5-glia-α1a–specific SKW3 380 T cells were unaffected. The effect was HLA-DQ specific because inhibition was observed using a pan-HLA-DQ Ab (SPV-L3), whereas no effect was seen with a pan-HLA-DR Ab (L243). This strong inhibitory capacity was not seen with the low-affinity 206 counterpart (Fig. 7A). To quantify the inhibitory efficacy, we did a titration of 3.C11 in the same T cell activation assay using the 33-mer as stimulating antigen (fig. S7, C and D). In line with the very high affinity of the Ab, the IC50 (median inhibitory concentration) indexes were found to be in the low picomolar range. The inhibition was markedly more sensitive on the effector property of the T cells on the basis of interleukin-2 (IL-2) cytokine production than the corresponding effect on the early activation marker CD69 (IC50s of 39 pM versus 400 pM for SKW3 364 T cells and 38 pM versus 130 pM...
for SKW3 S16 T cells, respectively). We next assessed the specificity of this inhibitory capacity by loading Raji B cells separately with DQ2.5-glia-α1a or DQ2.5-glia-α2 or a mix of both peptides. We further included the 33-mer gliadin peptide. The peptide-loaded Raji B cells were cocultured with a blend of SKW3 380 and 364 T cells [(B), n = 2], or K562-CIITA cells were loaded with stimulatory peptides or combinations of them and cocultured with a blend of the SKW3 364 and R12C9 T cells [(C), n = 3]. In both panels, SKW3 364 T cells were labeled with carboxyfluorescein diacetate succinimidyl ester to guide T cell separation in flow analysis. The presence or absence of Abs is indicated. Signals were normalized to antigen-specific activation in the absence of Abs, and the heatmap represents means of duplicates. (D) The primary TCC-specific for HLA-DQ2.5:DQ2.5-glia-α2, TCC820.250, was cocultured with peptide-loaded Raji B cells in the presence of Abs as annotated. T cell proliferation was assessed in a 3H-thymidine incorporation assay. Error bars represent means ± SD of triplicates (n = 2). (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.)
proliferation for both the minimal epitope and the highly stimulatory 33-mer peptide. We then performed a similar experiment using four different HLA-DQ2.5:DQ2.5-glia-α2–specific TCCs and added titrated amounts of Abs to determine the inhibitory effect of monoclonal Ab (mAb) 3.C11 on T cell proliferation as well as secretion of interferon-γ (IFN-γ) (Fig. 7E and fig. S8A to C). Ab 3.C11 inhibited T cell proliferation (Fig. 7E and fig. S8B) and IFN-γ secretion (fig. S8C) in a concentration-dependent manner, whereas control Abs had minimal to no effect.

**Inhibition by mAb 3.C11 of gliadin-dependent primary CD4+ T cell proliferation in HLA-DQ2.5 humanized mice**

Although the in vitro inhibitory capacity of mAb 3.C11 even on antigen experienced human primary TCCs was highly encouraging, it is difficult to translate the impact to the more complex environment of the human gut. Currently, there is no HLA-DQ2.5 humanized preclinical model that recapitulates the gluten–dependent immune-mediated enteropathy of CeD. As an approximation, we therefore turned to a mouse model relying on adoptive transfer of naive CD4+ T cells isolated from HLA-DQ2.5 knock-in (KI)/TCR-glia-α2 transgenic (tg) mice (35) into HLA-DQ2.5 KI recipient mice (36). This allowed us to study antigen-specific T cell proliferation in the gut-associated lymphoid tissue and draining mesenteric lymph nodes (MLN) upon oral administration of the antigen (Fig. 8A). The day after adoptive cell transfer, 200 μg of Abs [or phosphate-buffered saline (PBS)] were given by intraperitoneal injection followed by intragastric administration of 100 μg of deamidated gluten 33-mer peptide. A second dose of Abs (or PBS) was given the next day. Mice were euthanized 48 hours after peptide administration, and T cell proliferation in the gut-draining MLN, gut Peyer’s patches (PPs), the spleen, and control nondraining inguinal lymph nodes (ILN) was determined. Whereas about 60% of recovered T cells proliferated in the MLN and PPs of mice receiving intraperitoneal PBS or the isotype control Ab, administration of 3.C11 efficiently blocked T cell proliferation in these lymphoid tissues (Fig. 8, B and C). In contrast to the complete blocking effect of 3.C11, the pan-HLA-DQ mAb SPV-L3 was less efficient, inhibiting proliferation to an intermediate extent of 34 and 23% in the MLN and PPs, respectively. Oral gavage...
of gluten peptide resulted in a weak peripheral T cell proliferation in the ILN from 3 of the 10 mice possibly due to systemic distribution of the peptide antigen (Fig. 8C). No such T cell proliferation was seen in the ILN of the mice receiving 3.C11 or the pan-DQ Ab SPV-L3.

**DISCUSSION**

We here report the generation of a human high-affinity TCR-like Ab termed 3.C11 specific for the pMHC complex HLA-DQ2.5:DQ2.5-glia-α2 by use of phage display. This engineered Ab allowed us to extend the results from a previous study that focused on the HLA-DQ2.5:DQ2.5-glia-α1 complex only (9) and enabled detection of cells with low levels of peptide presentation. 3.C11 potently inhibited T cell activation in vitro and in vivo and may thus have therapeutic potential.

The primary lead 206, as well as our previously reported Ab 107 specific for the HLA-DQ2.5:DQ2.5-glia-α1 complex (9), was selected from a human naïve scFv library that contains light- and heavy-chain gene segments from diverse endogenous repertoires (23, 37). Whereas the three HLA-DQ2.5:DQ2.5-glia-α1 lead candidates used the germ-line segments IGHV6-1 and IGKV1-9 (table S1), the eight lead candidates against the HLA-DQ2.5:DQ2.5-glia-α2 complex all used the IGHV1-69 segment and diverse IGKV segments. Thus, our data suggest a preference primarily for VH germ lines to acquire specificity, and the fact that we used an Ab library based on a diverse, endogenously derived gene repertoire may have been crucial for the success of the primary selections.

In a previous study, we displayed scFv at HV on pIX and observed efficient enrichment of full-length functional scFvs during selection. We retrieved clones with higher affinity and thermostability in direct comparison with both LV and HV display on pIIII (23), suggesting that HV display on pIX could be particularly valuable for affinity engineering. Thus, we chose pIX as a display scaffold for the second-generation libraries to select for high-affinity variants, which turned out to be a successful approach.

The crystal structure of Fab 3.C11 bound to HLA-DQ2.5:DQ2.5-glia-α2 showed that the Fab used a docking mode similar to that of TCRs, sharing several contact residues with prototypic DQ2.5-glia-α2-specific TRAV26-1/TRA2V7-2′ TCRs (27). This contrasts the pMHC binding mode of other TCR-like Abs, which either deviated from the TCR diagonal binding mode or focused on peptide hotspots (38). 3.C11 interacts with the pMHC using all six CDR loops. Seven of nine amino acids of the peptide core are contacted, a feature found indicative of high peptide specificity (38). 3.C11 also engages several residues of both the MHC α and β chains. In particular, the strong selection of IGHV1-69 in the candidate clones after the primary selection can in part be explained by the hydrophobic CDR H2 of this germ-line segment forming a strong interface with the MHC α chain. A signature feature of the semipublic TCRs is the presence of a conserved Arg residue in the apex of CDRL3, which invariably interacts with p5-Leu, p6-Pro of DQ2.5-glia-α2 and Thr31, Asn32, and Val65 of the HLA-DQ2.5-α chain (27, 30). In 3.C11, CDR H3-Gln109 appears to play an equivalent role. The high complexity of the CDR H3 layout and interactions may explain the lack of binders enriched from the CDR H3 library, because CDR H3 engineering would disrupt the delicate arrangement of interdependent interactions for most library members. In contrast to the CDR H3 library, the selection of the CDR H1 libraries resulted in high-affinity binders. The libraries were designed to contain only CDR H1 loops elongated by two or three amino acids, which likely contributed to the large BSA contribution made by this loop. In the case of the 3.C11 complex, the CDR H1 loop formed a short α helix that results in a rigid CDR H1 conformation and extended interactions with the peptide C terminus. This suggests that the substantial increase in binding affinity may in part be driven by stabilizing intraloop interactions in addition to an increased BSA. Affinity enhanced Fabs may therefore, in principle, be engineered from initial binders via addition and selection of self-stabilizing elements in peripheral CDR loops.

We observed staining of patient biopsies using both 107 and 3.C11 and primarily detected PCs, which are characteristically expanded in celiac lesions. We detected only minimal staining of the few CD14+ or CD11c+ Mfs and DCs using our TCR-like Abs despite a high HLA class II expression (9). MF and DC subsets were previously shown to occur at altered frequencies in the lamina propria of patients with CeD and were therefore suggested to contribute as an important tissue APC in CeD pathogenesis (39–42). Thus, detection of peptide-presenting DCs may, for example, have been affected by migratory behavior to the gut-draining MLN upon antigen encounter (42).

Our previous and current data strongly indicate a peptide-presentation pattern where the B cell compartment dominates as APCs at least in the inflamed tissue with high disease activity (9). The recent observation that B cells are also required for development of villous atrophy in the DQ8/IL15 transgenic mouse model of CeD further points to the importance of the T-B cell axis in CeD pathogenesis and suggests this as a therapeutic focus (43). Despite the high affinity of 3.C11, we did not, however, detect peptide presentation in all patient samples independent of APC type alluding to the complexity of the epitope landscape in this heterogeneous disease. The reason for lack of detection in peptide presentation seen for some patients is unknown. However, detection would require that the patients have maintained a gluten-containing diet until the time of gastroendoscopy. In addition, differences in wheat strains and α-gliadin gene content used for food (44), as well as individual variation in ability to proteolytically digest gluten (45), are factors that might underpin the lack of staining in some of the CeD patient samples.

mAb 3.C11 specifically inhibited T cell activation and proliferation in vitro and in vivo. This clearly demonstrates the benefit of targeting specific epitopes on MHC complexes to avoid a large in vivo antigenic sink effect presumably seen using the pan-HLA-DQ mAb, even in this targeted HLA-DQ2.5 KI mouse expressing MHC class II (MHCIi) exclusively on APCs. The effect in vivo was observed in mice that represent the critical role of TCR and HLA-DQ2.5 seen in CeD (44). Thus, detection of peptide-presenting PCs may, for example, have been affected by migratory behavior to the gut-draining MLN upon antigen encounter (42).

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peptide bound to the murine MHC variant IA\(^{\alpha}\) delayed disease onset and progression in nonobese diabetic mice, which could be attributed to a decrease in lymphocytes infiltrating the pancreatic islets. This observation was disease specific but not restricted to insulin-specific T cells and seen for both CD4\(^+\) and CD8\(^+\) T cells, as well as B cells (48). Recently, a high-affinity Ab against the B9-23-IA\(^{\alpha}\) complex was isolated and was found more effective in preventing T cell activation and protecting against T1D compared with the low-affinity Ab. Treatment with the high-affinity Ab against the B9-23 insulin epitope also had an effect when administered at a disease stage where epitope-spreading had already occurred (52). Although it is unclear whether targeting the immunodominant DQ2.5–glia-\(\alpha\)-2 epitope alone is sufficient for treatment of CeD, these studies with TCR-like Abs in T1D are promising because they have shown that a high-affinity Ab inhibiting a single T cell epitope may be sufficient to induce a more tolerogenic immune response and delay disease onset or progression in a preclinical model of a complex autoimmune disease. Thus, the ability of mAb 3.C11 to efficiently prevent T cell activation encourages further studies to assess the therapeutic potential in inhibition, cytotoxicity, or induction of infectious tolerance for treatment of CeD.

**MATERIALS AND METHODS**

**Study design**

The primary objective of this study was to generate and engineer high-affinity human Abs with specificity for a pMHC complex characteristic of CeD and to use this Ab for detection of peptide presentation in patient samples. A secondary objective was to determine the therapeutic potential of the lead Ab by performing proof-of-concept experiments. We used a naïve fully human Ab phage library for a primary selection to identify binding clones, followed by a secondary CDR-targeted library to obtain Abs with high specificity and affinity. The sample size for single-clone screening after phage selections was determined on the basis of previous experience to achieve a sufficient number of antigen-reactive clones. Single clones were chosen randomly, and screening experiments were performed once. Binding studies (ELISA and SPR) and cellular studies (flow cytometry and T cell assays) were performed two or three independent times (unless stated otherwise) with duplicate or triplicate samples where appropriate and as specified in the figure legends. For in vivo mouse experiments, animals were randomly assigned into treatment groups, and the sample size was determined on the basis of previous experience and is specified in the figure legend. Because of a technical issue, data from the spleen of one mouse were included after analysis. The in vivo experiments were approved by the Norwegian Food Safety Authority (Mattilsynet). All results shown are either representative of or the mean of independent experiments.

**Human material**

Research protocols and use of human duodenal biopsy material were approved by the Regional Ethics Committee of South-Eastern Norway (REK Sør-Øst approval ID 6544). All participants gave informed written consent to donate biological material to a REK Sør-Øst–approved biobank (ID 20521). Participants with suspected CeD were referred for biopsy sampling at Oslo University Hospital or Akershus University Hospital. Disease status (CeD diagnosis or not) was unknown to the investigator at the time of performing and analyzing the experiment. CeD diagnosis was based on the guidelines from the British Society for Gastroenterology including clinical history, anti-TG2 serological testing, HLA typing, and histological analysis of small intestinal biopsies obtained by esophagogastroduodenoscopy and forceps sampling from the duodenum (53). Small intestinal resections (duodenum-proximal jejunum tissue) were obtained from nonpathological small intestine during Whipple procedure (pancreatoduodenectomy) of patients with pancreatic cancer who also donated material to the biobank (number 2012/341) and gave informed written consent. Normal histology of the duodenal mucosa was confirmed in these samples, thus serving as a noninflammatory control sample.

**Ab engineering, characterization, and functional studies**

Methods for Ab engineering are described in detail in Supplementary Materials and Methods. Briefly, first-generation HLA-DQ2.5:DP2.5–glia-\(\alpha\)-2–specific Abs were isolated from a human naïve scFv phage library (37) selected on recombinant pMHC essentially as described before (9, 23). Targeted phage libraries were constructed using degenerate oligonucleotides, and scFv were displayed on phage coat protein pIX (54) and selected for high affinity and stability using a protocol based on (24). Candidate clones were assessed in nanoDSF, ELISA, SPR, and flow cytometry experiments.

**Recombinant pMHC expression and purification**

Recombinant, soluble pMHC with the peptides DQ2.5–glia-\(\alpha\)-1a (QLQFPFPQELPY), DQ2.5–glia-\(\alpha\)-2 (PQPELPYQPE), CLIP2 (MATPLMLQALPGMAL), DQ2.5–glia-\(\alpha\)-1i (QQPPFPQEPQFPFP), DQ2.5–glia-\(\alpha\)-2 (FPQPELPQFPQPQWPQ), DQ2.5–glia-\(\gamma\)-2 (QGIQQEPQPAQL), and DQ2.5–hor-3 (EQPIQEPQQPQ) covalently linked to the MHC \(\beta\) chain (linker GAGSLVPGRSGGGGS) was produced in insect cells and affinity-purified using the mAb 2.12.E11 as previously described (54). The recombinant pMHC molecules were biotinylated in a site-specific manner using the enzyme BirA. If used for SPR, monomeric proteins were isolated by size exclusion chromatography using a Superdex 200. For crystallization and structure determination of the 3.C11 DQ2.5–glia-\(\alpha\)-2 complex, pMHC with a modified DQ2.5–glia-\(\alpha\)-2 peptide (APQPELPYQPY) and linker (GSGGSIEGRGSGGGAS) was expressed in Hi5 insect cells and purified as previously described (27). Briefly, media were buffer-exchanged and pMHC-purified via Ni-affinity and S200 gel filtration chromatography, and the C-terminal fos/jun dimerization domain was removed via enterokinase cleavage followed by HiTrap Q anion exchange chromatography. Subsequently, HLA-DQ2.5:DP2.5–glia-\(\alpha\)-2 was incubated with an equimolar amount of 3.C11, and the complex was concentrated to 8 mg/ml after a final S200 gel filtration step.

**Crystallization data collection and structure determination**

Crystals of the 3.C11–HLA-DQ2.5:DP2.5–glia-\(\alpha\)-2 complex were grown via the sitting drop vapor diffusion method in mother liquor composed of equal volumes of protein and reservoir solution containing 10% (w/v) polyethylene glycol 4000, 20% (w/v) 2-propanol, and 0.1 M HEPES (pH 7.5). Plate-like crystals were obtained after several weeks, and crystals were transferred into reservoir solution supplemented with 20% (w/v) glycerol before freezing in liquid \(N_2\) for data collection. X-ray diffraction data were collected at the MX2 beamline of the Australian Synchrotron and merged using the local implementation of XDS (56) and AIMLESS of the CCP4 package (57). The structure was solved via molecular replacement in Phaser using single protoners from Protein Data Bank (PDB) files 5JO4 and

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6MFG. Model building and structure refinement were performed using Coot (58) and the PHENIX package (59), respectively. Structural figures were prepared using PyMOL.

**Ab modeling**

Structural models of the Fv fragment were generated as described (9, 60). CDR loops and framework region templates were selected by sequence homology and combined to produce a single “grafted” model using RosettaAntibody. We used 10 templates for the VL/VH relative orientation (61) resulting in 10 grafted models. The grafted models were then energy-minimized (relaxed) in the Rosetta score function (62, 63) and further improved by de novo CDR H3 loop modeling and VL/VH docking. The CDR H3 loop was constrained to a kinked conformation (64), and a total of 2800 Fv models were generated. The 10 final models were selected on the basis of low Rosetta score and VL/VH orientations within the natural distribution. Models were taken from at least three initial grafted templates to maintain diversity.

**Ab docking to pMHC**

We used the cocrystal structure of a TCR (4OZI) (27) as a template for an initial orientation of the Fv model relative to the pMHC. A crystal structure of HLA-DQ2.5:DQ2.5-glia-α2 (4OZF) (27) was retrieved from the PDB and relaxed in the Rosetta score function. Combining the HLA structure with an ensemble of 10 final Fv models, we used SnugDock to generate 1000 complex models, as described in (65). The final models were ranked and selected on the basis of low Rosetta interface score, occurrence of “energy funnels” (i.e., structurally similar models separated from the bulk of models by a decrease in score), and an agreement with the experimental observations.

**B cell lines**

The murine A20 B cell lymphoma cell line had previously been engineered to express HLA-DQ2.5 with different peptide variants covalently linked to the MHC β chain (9, 21). The ectodomains are identical as in the soluble pMHC molecules used for selection, screening, and characterization of Ab binding by SPR and ELISA. The human B cell lymphoma Raji cells express HLA-DQ2.5. The human EBV–transduced B cell line VAVY is homozygous for HLA-DQ2.2 and HLA-DQ8, respectively. All cells were cultured under standard conditions in RPMI 1640 supplemented with 10% fetal calf serum (FCS), streptomycin (0.1 μg/ml), and penicillin (100 U/ml).

**Flow cytometric analyses of stained B cells**

Raji and EBV B cells were loaded with peptide by culturing in the presence of 50 μM peptide ON. The following peptides were used (9-mer core epitope sequences are underlined): 33-mer α-gliadin LQLQFQPQELPYQPYELPYQPYELPYQPYELPYQPYQPF, 12-mer DQ2.5-glia-α1a QLQFPQELPYPE or 12-mer DQ2.5-glia-α2 POQPQPQPQPE or 12-mer DQ2.5-glia-α2 POQPQPQPQPL. The cell lines were stained with pMHC-specific mlgG2b Abs (5 μg/ml) directly conjugated to phycoerythrin (PE) (Abcam) in the presence of human Fc receptor (FcR) blocking reagent (1:50; Miltenyi Biotech). For staining of A20 B cells, 3.C11 hlgG1 Ab was used at 5 μg/ml together with rat anti-mouse CD16/CD32 block (1:200; BD Biosciences). Bound hlgG1 was detected with biotinylated goat F(ab’)2 anti-hlgG (2 μg/ml; Southern Biotech) followed by streptavidin R-PE (2 μg/ml; Invitrogen). Alternatively, A20 cells were stained with biotinylated 2.12.E11 mlgG1 (5 μg/ml) followed by streptavidin R-PE as before. All stainings were performed on ice using V-bottom shaped 96-well plates, and an equal number of cells were used in each staining (at least 100,000). PBS supplemented with 2% FCS was used to wash cells and for dilution of Abs and streptavidin. Data were acquired using an Attune NxT Flow Cytometer (Thermo Fisher Scientific). For the alanine scan experiments, Raji B cells were loaded with the 12-mer DQ2.5-glia-α2 YPQPELPYPQPE [wild-type (WT)] or single alanine substitutions. The loaded cells were stained with pMHC-specific hlgG1 (5 μg/ml) in the presence of human FcR blocking reagent (1:100; Miltenyi Biotech) followed by goat F(ab’)2–anti-hlgG-biotin (2 μg/ml; Southern Biotech) and streptavidin-PE (2 μg/ml; Thermo Fisher Scientific). Cells were washed and resuspended in autoMACS buffer with 2% FCS, and data were acquired on an Accuri C6 flow cytometer. Data analysis was performed using FlowJo software (Tree Star).

**Isolation of single-cell suspensions from duodenal biopsies and small intestinal resections and flow cytometry**

Single-cell suspensions from duodenal biopsies or small intestinal resection were prepared as described (66) and blocked with human FcR Blocking Reagent (Miltenyi Biotec) and stained with the following Abs specific for CD markers: CD3-FITC (fluorescein isothiocyanate) (OKT3, BioLegend), CD11c-APC (S-HCI-3, BD Biosciences), CD14-APC (HCD14, BioLegend), CD19-PE-Cy7 (HIB19, BioLegend), CD27-BV421 (O232, BioLegend), CD38-APC-Cy7 (HIT2, BioLegend), and CD45-BV510 (H130, BioLegend), all at 1:20. Alternatively, CD3-Pacific Blue (OKT3, BioLegend), CD11c-APC (S-HCI-3, BD Biosciences), CD14-APC (HCD14, BioLegend), CD19-PerCP-Cy5.5 (HIB19, BioLegend), CD27-Per-Cy7 (LG-7F9, BioLegend), CD38-APC-Cy7 (HIT2, BioLegend), and CD45-BV510 (H130, BioLegend), all at 1:50, were used. Peptide presentation was analyzed using mlgG2b 107, mlgG2b 3.C11, or mlgG2b anti-outer membrane vesicle (OMV) (all in-house generated, 10 μg/ml) followed by detection using anti-mlgG2b conjugated to PE (1 μg/ml; BioLegend). Propidium iodide or LIVE/DEAD Fixable Violet Dead Cell Stain (Invitrogen) was used for exclusion of dead cells, and samples were immediately acquired on an LSRFortessa cytometer (BD Biosciences). Data analysis was performed using FlowJo software (Tree Star).

**Retroviral transduction of human SKW3 T cells and K562 cells and flow cytometry**

TCR sequences encoding the variable domains of TCRs 380 (21), 364 (30), S16 (27), and R12C9 (67) were reconstructed by gene synthesis as human/mouse chimeric TCRs as described (22) and cloned into pMSCV (Clontech Laboratories). Retroviral transduction of the SKW3 human T cells (CLS Cell Lines Service GmbH) was performed using the Retro-X Universal Packaging System (Clontech Laboratories) according to the manufacturer’s instructions. Stable, homogeneous TCR-expressing SKW3 T cells were obtained by standard cell expansion and fluorescence-activated cell sorting (FACS) using a FACSARia II cytometer (BD Biosciences) based on their TCR expression levels assessed by H57-Alexa Fluor 647 (Thermo Fisher Scientific) Ab staining. The TCR-transduced SKW3 cells were validated for peptide-specific activation using a panel of known agonistic and antagonistic peptides, essentially as described (21). T cell activation was measured by CD69 up-regulation assessed by anti-human CD69-APC (BD Biosciences) Ab staining. Data were acquired on an Accuri C6 cytometer (BD Biosciences) and
analyzed using FlowJo software V10 (Tree Star). The human myeloid leukemia cell line K562 (ATCC-CCL-243) was transduced with the hCITTA:eGFP dual open reading frame encoding retroviral vector pMMLV (VectorBuilder GmbH) to induce endogenous HLA class II expression. Stable, homogeneous HLA class II–positive cells were obtained by standard cell expansion and FACS using a FACSaria II cytometer (BD Biosciences) based on their dual enhanced green fluorescent protein and HLA expression levels assessed by pan–HLA class II (clone CR3/43-Alexa647) Ab staining.

**T cell activation and inhibition assays**

For T cell activation assays, 50,000 Raji B cells were incubated in RPMI 1640/10% FCS at 37°C/ON with titrated amounts of peptide followed by washing to remove remaining free peptide and addition of 40,000 SKW3 T cells. Cells were cultured at 37°C/ON before they were analyzed in flow cytometry. The following peptides were used (9-mer core epitopes are underlined): DQ2.5-glia-α1a (QLQPFPQPELPY) and DQ2.5-glia-α2 (QPPELPYPQPQPE). For alanine scan experiments, Raji B cells were loaded with 5 μM of the 12-mer DQ2.5-glia-α2 YPPELPYPQPQPE (WT) or single alanine substitutions. As a control, cell stimulation cocktail containing phorbol 12-myristate 13-acetate and ionomycin (1:500; eBioscience) was added to wells containing SKW3 T cells only. On the basis of the established dose response in T cell activation, a peptide concentration estimated to result in about 60% T cell activation (measured as CD69 up-regulation on the CD19− population) was chosen for the inhibitory assays. After ON incubation with peptide as above and washing, 1 μM (final concentration) of either 3.C11 or an isotype control was added to the Raji cells before T cells were added and incubation continued ON. The resulting T cell activation was measured as above. As control Abs, either 0.1 μM (final concentration) of pan–anti-DR (clone L243: Thermo Fisher Scientific) or pan–anti-DQ (clone SPV-L3: DiaTec) was added in parallel. As an alternative readout, IL-2 secretion in supernatants was assayed in ELISA using the Human IL-2 ELISA MAX Deluxe Set (BioLegend) according to the manufacturer’s protocol.

The TCCs reactive to the DQ2.5-glia-α2 epitope were established from intestinal biopsies of patients with CD (CD412, CD436, and CD820) and have been described before. Briefly, TCC820.250 was generated by direct cloning from intestinal biopsies (68), whereas TCC412.5.28, TCC436.5.3, and TCC436.5.6 were generated by limiting dilution of a T cell line established from antigen-challenged intestinal biopsy specimens (12, 30). For T cell activation experiments, Raji B cells in RPMI supplemented with 10% heat-inactivated, pooled human serum, 0.1 mM 2-mercaptoethanol, and antibiotics were irradiated with 75 Gy (gray) before 75,000 cells per well were incubated overnight with titrated amounts of peptide at 37°C. The plates were centrifuged to remove remaining free peptide before addition of 50,000 T cells. 3H-thymidine (1 μCi per well) was added 2 days after addition of T cells, and cells were harvested after an additional 16 to 20 hours using an automated harvester. 3H-thymidine incorporation was measured by liquid scintillation counting. For T cell inhibition experiments, Raji B cells were incubated overnight with 10 μM DQ2.5-glia-α2 (QPPELPYPQ) or 33-mer (final concentration) of either 3.C11 or an isotype control was added on day 2. As a control Ab, either 0.1 μM for mAbs SPV-L3 (pan–HLA-DQ, DiaTec) or the pan–anti-DR Ab B8-11 (DiaTec) was added in parallel. As an alternative readout, IL-2 secretion in supernatants was assayed in ELISA using the Human IL-2 ELISA MAX Deluxe Set (BioLegend) according to the manufacturer’s protocol.

**Mice**

All mice were on C57BL/6 background. HLA-DQ2.5 KI mice express a chimeric MHCII protein where the peptide-binding extracellular domains of H2-Aa and H2-Ab1 have been replaced with those of HLA-DQA1*05:01 and HLA-DQB1*02:01 (36). When homozygous, these mice do not express endogenous MHCII molecules because C57BL/6 lacks expression of H2-Ea because of a deletion of the H2-Ea gene (70). TCR tg mice expressing the rearranged TCRα and TCRβ chains of TCC 364.1.014 specific for the DQ2.5-glia-α2 epitope were previously described (35). TCR-glia-α2 tg mice were crossed to CD45.1 mice (B6.SJ-L-Ptpcr-Pepcb/BoyCrl; Charles River Laboratories, Calco, Italy) and HLA-DQ2.5 KI mice and bred to homozygosity for HLA-DQ2.5. Mice were maintained on a gluten-free chow (RDI OpenStandard Diet, Research Diets) and bred at the Department of Comparative Medicine, Oslo University Hospital-Rikshospitalet (Oslo, Norway) under specific pathogen–free conditions. The experiments were approved by the Norwegian Food Safety Authority (Mattilsynet).

**In vivo T cell proliferation assay**

Single-cell suspensions from spleens and lymph nodes were prepared by carefully grinding and filtering tissues through a 70-μm nylon mesh (BD Biosciences) or using the gentleMACS Dissociator (Miltenyi Biotec). Erythrocytes were removed from spleen samples using ammonium–chloride potassium lysis buffer. CD4+ T cells were isolated from spleens and lymph nodes of HLA-DQ2.5 TCR-glia-α2 double tg mice by negative selection using the EasySep Mouse CD4+ T cell isolation kit (STEMCELL Technologies). The purity of CD4+ population was >90%. Isolated CD4+ T cells were labeled with Cell Trace Violet (Molecular Probes) and about 2.5 × 10^6 cells were adoptively transferred to homozygous HLA-DQ2.5 KI recipient mice by intravenous injection. The following day, recipient mice were administered intraperitoneally with 200 μg of either of the mAbs 3.C11 (mlg2b), anti-OMV (mlg2b), or anti–HLA-DQ (SPV-L3, mlg2a) in 200 μl of PBS or PBS alone. Thirty minutes after Ab administration, mice received 100 μg of deamidated 33-mer (LQLQPPQPPELPYPQPELPYPQPQPP) in 200 μl of PBS by intragastric gavage. PPs, gut-draining MLN, nondraining ILN, and the spleen were isolated 48 hours after peptide antigen administration and were analyzed for T cell proliferation.

**Flow cytometry**

Cells were resuspended in PBS containing 2% FCS (v/v), pretreated with anti-CD16/32 (93; BioLegend), and then stained with optimal amounts of FITC-conjugated anti-CD45.1, PerCP-Cy5.5–conjugated
antti-CD4, and PE-conjugated anti-CD3 (all BioLegend). Cells were analyzed on FACSCalibur (BD Biosciences) or Attune NxT Flow Cytometer (Thermo Fisher Scientific). Data analysis was performed using FlowJo software (Tree Star).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.1 or FlowJo software (Tree Star). Statistical analysis was performed using Biacore T200 Evaluation Software v1.0. For visual comparison of off-rates in SPR, the individual curves were normalized to a maximum binding of 100% using Biacore T200 Evaluation Software v1.0.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S8

Materials and Methods

legends.

Statistical analysis was performed using GraphPad Prism 8.0.1 or FlowJo software (Tree Star).

Cytometer (Thermo Fisher Scientific). Data analysis was performed 100% using Biacore T200 Evaluation Software v1.0.


and the staff at the Monash Macromolecular Crystallization Facility. Parts of some figures were created with BioRender.com. Funding: This work received funding from the South-Eastern Norway Regional Health Authority (grant nos. 2016039 and 2018067) and the Research Council of Norway through its Centers of Excellence funding scheme, project number 179573/V40, and from the Stiftelsen Kristian Gerhard Jebsen (project SKGJ-MED-017). J.R.J. and J.J.G. were supported by the U.S. NIH grants R01-GM078221 and F31-GM078221. The computations were performed on resources provided by the Maryland Advanced Research Computing Center (MARCC) and UNINETT Sigma2—the National Infrastructure for High Performance Computing and Data Storage in Norway. J.R. is supported by an ARC Laureate Fellowship and ARC Centre of Excellence in Advanced Molecular Imaging. Author contributions: R.F., L.S.H., J.P., M.F.d.P., S.K., G.B., A.E.D., J.R.J., K.S.G., T.F., E.S.V., and C.L. designed and performed research and analyzed data. K.E.A.L. and J.J. performed endoscopic examination and provided biopsies. S.Y. provided intestinal resections. J.J.G., J.R., L.M.S., I.S., and G.Å.L. designed research, analyzed data, and supervised the study. R.F., L.S.H., J.P., I.S., and G.Å.L. wrote the manuscript. All authors critically reviewed the manuscript. Competing interests: R.F., L.S.H., L.M.S., I.S., and G.Å.L. are inventors on a patent application describing the pMHC-specific antibodies. G.Å.L. is affiliated with Nextera AS. G.Å.L. and I.S. hold shares in Nextera AS. L.M.S., privately or via his employer, has been a consultant during the last 2 years for Amagma Therapeutics, Intrexon Actobiotics, Bioniz Therapeutics, Chugai Pharmaceutical, Merck, Topas Therapeutics, and GSK. K.E.A.L., privately or via his employer, has been a consultant during the last 2 years for Amyra GmbH, Intrexon Actobiotics, Bioniz Therapeutics, Chugai Pharmaceutical, and Takeda. The other authors declare that they have no competing interests. Data and materials availability: The crystal structure of Fab 3.C11 in complex with HLA-DQ2.5:DQ2.5-glia-α2 was deposited with the PDB under PDB ID 6XP6. All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials. The 3.C11 antibody may be requested from the corresponding author (G.Å.L.) under a material transfer agreement with Nextera AS.

Submitted 11 January 2021
Accepted 22 July 2021
Published 20 August 2021
10.1126/sciimmunol.abg4925

A high-affinity human TCR-like antibody detects celiac disease gluten peptide–MHC complexes and inhibits T cell activation


Sci. Immunol. 6, eabg4925.
DOI: 10.1126/sciimmunol.abg4925

TCR-like antibodies tackle celiac disease
Ingestion of gluten-containing food triggers the gastrointestinal symptoms of celiac disease in patients with CD4+ T cells specific for deamidated gluten peptides presented by disease-associated HLA-DQ class II MHC molecules. Frick et al. used phage display technology to screen for TCR-like antibodies specific for an immunodominant gluten peptide bound by HLA-DQ2.5. Antibody engineering optimized affinity and binding stability, yielding an improved TCR-like antibody that structurally mimicked the TCR interface with gluten peptide–MHC complexes. These TCR-like antibodies blocked activation and proliferation of gluten-responsive human CD4+ T cells both in vitro and in DQ2.5 transgenic mice. TCR-like antibodies that block immunodominant epitope recognition have potential as personalized medicine treatments for blunting gluten-activated T cell responses without compromising effector functions provided by other T cells.