A TRAV26-1-encoded recognition motif focuses the biased T cell response in celiac disease

Biased antigen-specific TCR repertoires are frequently observed in autoimmunity and infection. The T cell response against the immunodominant gluten epitope DQ2.5-glia-a2 presented on the MHC variant HLA-DQ2.5 in celiac disease (CeD) is characterized by overrepresentation and preferential pairing of V gene segments TRAV26-1 and TRBV7-2 and a canonical CDR3β loop sequence [1]. The molecular features driving this biased semi-public response are not well understood.

We have previously identified a framework-resident recognition motif centered on Y40TCRα encoded by TRAV26-1, which is crucial for the response to DQ2.5-glia-a2 of the prototypic TCR 364 [2]. The co-crystal structure of the similar TCR WT and Y40TCRα variants (Supporting Information Fig. S1) and assessed T cell activation by pMHC-expressing A20 B cells as APC. In line with our previous observations from the BW 364 T cells, residue Y40TCRα was indeed crucial for activation of the BW S16 T cells (Fig. 1B and C), suggesting the Y40TCRα-centered recognition motif is likely a general feature of these prototypic TCRs.

To assess whether alternative TRAV germline segments can form a similar Y40TCRα-centered recognition motif and substitute for TRAV26-1, we re-analyzed with an improved processing pipeline a TCR sequence data set [2] (Supporting Information Table S1) generated by single-cell paired αβ-chain sequencing of gliadin-specific CD4+ effector memory T cells sorted from CeD patients using a blend of four HLA-DQ2.5:gluten tetramers. We filtered out clones with the canonical CDR3β loop typical for DQ2.5-glia-a2-reactive TCRs and identified two private clonotypes using TRAV gene segments other than TRAV26-1. We chose the clonotype using TRAV5, as this gene segment is slightly overrepresented in the gluten-reactive repertoire compared to the naïve repertoire [4] (Supporting Information Table S1). Furthermore, TRAV5 is the only other human germline that contains residues Y38TCRα and Y40TCRα (Supporting Information Fig. S2).

To determine the contribution of Y40TCRα in a non-prototypic TCR, we reconstructed the TRAV5-encoded private clonotype on BW T cells (herein denoted TCR AV5/BW AV5 T cells) (Supporting Information Fig. S1 and Table S2). We confirmed DQ2.5-glia-a2-specificity using plate-bound recombinant pMHC complexes identical to the ones used for tetramer sorting (Fig. 1D) as well as peptide-loaded HLA-DQ2.5+ Raji B lymphoma cells (Fig. 1E). The BW AV5 T cells reacted specifically and with sensitivity similar to the prototypic TCRs, but exhibited slightly elevated baseline signaling in coculture with the Raji cells independent of peptide (Supporting Information Fig. S3). Unlike T cells expressing the prototypic TCRs, BW AV5 T cell activation was not affected by introduction of the Y40HTCRα mutation (Fig. 1F).

Thus, the TRAV5-encoded TCR appears to use a different recognition mode largely independent of Y40TCRα. However, both the prototypic TCR 364 and TCR AV5 depend on contacting residue R70MHC (Fig. 2A), which is a central residue in most TCR:pMHC interactions [5]. Unique TCRs employing such distinct yet convergent binding modes to the same pMHC have previously been described for the response to HLA-DQ8 presented gliadin epitopes in celiac disease [6].

Next, we sought to fine-map the recognition motif of the prototypic TCR 364 by generating BW T cell variants with mutations of residues predicted to participate in the interaction network centered on Y40TCRα guided by the TCR S16 structure [3] (Fig. 2B, Supporting Information Fig. S1). We found that single mutations of Y38ATCRα and H55ATCRα, as well as T115TCRα abrogated activation of BW 364 T cells (Fig. 2C and D). T115TCRα does not directly contact the pMHC, but rather seems important for the CDR3β loop to be recruited or stabilized through the TRAV26-1-encoded framework residues [3]. Thus, the recognition motif identified in the prototypic TCRs does not exclusively rely on residue
Figure 1. The Y40TCRα-centered recognition motif. Co-crystal structure of TCR S16 (grey) bound to HLA-DQ2.5 (blue, green) with bound DQ2.5-glia-α2 (orange). The prototypic CDR3β loop (cyan) and the sequence differences in the TCR α-chain between TCRs 364 and S16 (red) are highlighted (A). The IL-2 response of BW T cells expressing variants of TCRs 364, S16, and AV5 to pMHC-expressing A20 B cells (B, C, F), plate-bound pMHC (D), or peptide pulsed Raji cells (E) was assayed by ELISA. A20 B cells expressing HLA-DQ2.5:CLIP2, APC alone and T cells alone were used as negative controls (B, C, F). Anti-CD3ε antibody was used as a positive control (D). Data is shown for representative experiments (n = 2). Error bars indicate mean ± SD of triplicates (B, C, E, F) or duplicates (D) and dotted lines indicate detection limits.

Y40TCRα but also requires nearby residues in both TCR chains and the MHC β-chain.

To rationalize the observed differences in pMHC recognition between the prototypic TCRs S16 and 364, and the private TCR AV5, we generated a structural model of TCR AV5 bound to pMHC (Fig. 2E), assuming its overall docking orientation would closely resemble that of TCR S16. The S16 co-crystal structure and previous interaction studies where a Y40F mutation in a soluble TCR abrogated binding to pMHC [3], suggest a hydrogen bond network where residues Y38TCRα and H55TCRα interact with E69MHCβ, while Y40TCRα interacts with the CDR3β loop residue T115TCRβ and R70MHCβ (Fig. 2E). Direct interaction between T115TCRβ and R70MHCβ seems impossible due to the large distance. TCR AV5 shares residues Y38TCRα and Y40TCRα with the prototypic TCRs, but has a Tyr residue in position 55TCRα rather than a His. The model of TCR AV5 suggests slightly altered side chain conformations where T115TCRβ may be in closer proximity to R70MHCβ allowing for direct interaction with this residue without bridging via Y40TCRα. Thus, this model offers a structural hypothesis for the independence of Y40TCRα observed in TCR AV5. It is also in line with the observation that an R70MHCβ mutation abrogated activation of both WT and Y40HTCRA variants of TCR AV5.
Figure 2. Fine-mapping the recognition motif used by TCR 364. The IL-2 response of BW T cells to pMHC-expressing A20 B cells was assayed by ELISA (A). Co-crystal structure of TCR S16 (grey) with HLA-DQ2.5:DQ2.5-glia-α2 (MHC β-chain: green). Interacting residues are labeled and represented as sticks (B). The IL-2 response of BW T cells expressing variants of TCR 364 to pMHC-expressing A20 B cells was measured in ELISA (C–D). A model of TCR AV5 bound to pMHC was generated using the TCRmodel webserver, superimposed with TCR S16, minimized into the Rosetta score function, and compared to the co-crystal structure of TCR S16 (E). Possible hydrogen bonds are visualized in green if the distance is ≤3.5 Å. Data is shown for representative experiments (n = 2), error bars indicate mean ± SD of triplicates (A, C, D).

The clonotype expressing a TRAV5-encoded TCR using the prototypic TCR β-chain was private, while the prototypic TCRs were semi-public and frequent. Since all three BW T cells tested were specifically and efficiently activated by HLA-DQ2.5:DQ2.5-glia-α2 in vitro, there is no obvious explanation for differential recruitment into the antigen-experienced repertoire. Clonotype frequencies in antigen-specific repertoires are linked to precursor frequencies. The frequency of TRAV5 paired with TRBV7-2 or TRBV7-3 in the naïve repertoire differs across datasets and may be low [7, 8]. Prevalence of TRAV26-1, on the other hand, was found to be associated with usage of the HLA-DQ2.5 β-chain encoded by HLA-DQB1*02 [9]. Thus, differences in precursor frequencies may contribute to the biased repertoire.

In summary, we observed that the Y40TCRα-centered recognition motif is sensitive and likely unique to the prototypic TCRs using TRAV26-1. This recognition motif may well contribute to the formation of a biased gluten-specific T cell repertoire in CeD patients.

Rahel Frick1,2, Kristin Støen Gunnarsen1,2, Shiva Dahal-Koirala1,3, Louise Fremgaard Risnes1,3, Ludvig M. Sollid1,3, Inger Sandlie1,2, Lene Støkken Høydahl1,2,3, and Geir Age Løset2,4.* Shared senior authorship

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Abbreviations: CeD: celiac disease  pMHC: peptide:major histocompatibility complex

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Full correspondence: Geir Åge Løset, Department of Biosciences, University of Oslo, Oslo, Norway
  e-mail: g.a.loset@ibv.uio.no

Additional correspondence: Lene Støkken Høydahl, Department of Immunology, University of Oslo and Oslo University Hospital, Norway
  e-mail: l.s.hoydahl@medisin.uio.no

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