Phenotypic Analysis of Disease-Relevant T Cells in Dermatitis Herpetiformis

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TO THE EDITOR

Gluten-specific CD4+ T cells are central players in the pathogenesis of celiac disease (CeD), an inflammatory disease driven by exposure to dietary gluten proteins. Patients with CeD are treated with a lifelong gluten-free diet. When gluten is reintroduced to the diet of patients in remission, there is a wave of activated (CD38+) gluten-specific CD4+ T cells in the blood that peaks on days 6–8 after the first gluten exposure. This increase in the frequency of activated gluten-specific CD4+ effector-memory T cells (T_{EM}) in the blood can be detected by IFN-γ enzyme-linked immunospot assay (Anderson et al., 2000; Tye-Din et al., 2010) or by HLA-DQ:gluten tetramers (Ráki et al., 2007; Sarna et al., 2018b; Zühlke et al., 2019). The gluten-specific CD4+ T cells in the blood that increase in frequency also alter their phenotype to that of gluten-specific CD4+ T cells in the gut mucosa of untreated CeD (Christophersen et al., 2021, 2019). Parallel with the increase of gluten-specific CD4+ T cells in the blood, there is an increase in the frequency of gut-homing and activated CD8+ and γδ T cells (Christophersen et al., 2021; Han et al., 2013; Risnes et al., 2021).

Although dermatitis herpetiformis (DH) is considered an extraintestinal manifestation of CeD, little is known about the gluten-induced T-cell responses in patients with DH. Recently, Kalliokoski et al. (2020) performed IFN-γ enzyme-linked immunospot assay on PBMCs from patients with DH and CeD on day 6 after a 3-day gluten challenge as part of an oral challenge for a period of up to 1 year. Only 47% of the patients with DH displayed reactivity to deamidated gluten, leading to the conclusion that early IFN-γ response to selected gluten peptides does not predict clinical relapse in patients with DH on long-term gluten provocation. The study was approved by the Finnish Regional Ethics Committee of Tampere University Hospital (Tampere, Finland), and all patients gave written informed consent.

Using cryopreserved PBMCs that were available from 7 of the 19 patients from the original challenge study (Mansikka et al., 2019), in this study, we aimed to identify the phenotypic markers of disease-relevant T cells in patients with DH by performing multiparametric flow cytometric analysis of CD4+, CD8+, and γδ T cells. We sought to identify gluten-reactive T cells by use of HLA-DQ2.5:gluten tetramers (termed HLA-tetramers in the remaining part of this paper) representing a mixture of the immunodominant DQ2.5-glia-α1a, DQ2.5-glia-α2, DQ2.5-glia-ω1, DQ2.5-glia-ω2, and DQ2.5-hor3 epitopes (Tye-Din et al., 2010). We stained the T cells for CD45RA and CD62L as well for gut-homing marker integrin β7, cutaneous lymphocyte antigen (CLA), and CCR4 (for further details, see Supplementary Materials and Methods).

We detected CD4+ T_{EM} (CD45RA−CD62L−) cells that bound HLA tetramers in five of the seven patients investigated, but only two patients displayed a clear increase in the frequency of such cells on day 6 (Figure 1a and b). The number of cells retrieved from the cryopreserved PBMC samples ranged from 0.35 to 3.9 million cells (median = 1.1) with a viability of 78–97% (median = 93%). Ideally, more cells should have been analyzed. Although the tetramer enrichment method is highly sensitive and distinguishes patients with CeD from HLA-matched controls, precise cell number estimates require higher numbers (>10 million) of PBMCs, especially for the baseline samples because the method detects low-frequency cells (typically one cell per million CD4+ T cells) (Sarna et al., 2018a).

The majority of the HLA-tetramer–positive T_{EM} cells expressed integrin β7, suggesting that these are gut-homing cells. Curiously, 31% of the HLA-tetramer–positive T_{EM} cells in one of the patients (DH4) expressed the skin-homing marker CLA (Figure 1a and c). The CLA+ HLA-tetramer–positive T cells of this one patient also expressed high levels of CCR4. Whether additional patients with DH also have skin-homing gluten-specific CD4+ T cells in addition to gut-homing gluten-specific CD4+ T cells cannot be concluded from this single observation. More studies are warranted. Despite variation in the increase of numbers of HLA-tetramer–positive T_{EM} cells, in all the five patients, we observed a uniform increase in CD38 expression in HLA-tetramer–positive T_{EM} cells after gluten challenge (Figure 1d). We further showed that all the HLA-tetramer–positive T_{EM} cells clustered distinctly from the HLA-tetramer–negative cells (Figure 1e). In line with previous observations of patients with CeD (Christophersen et al., 2021, 2019), the HLA-tetramer–positive T_{EM} cells on gluten challenge upregulated CD38 and PD-1 and downregulated CD127 (Figure 1f).

To identify which epitope the gluten-specific T cells in patients with DH were reactive to, we sorted CD4+ HLA-tetramer–positive T_{EM} cells to generate T-cell clones and successfully established 10 T-cell clones from four patients. All the 10 T-cell clones displayed proliferative response against deamidated gluten and to at least one of the four immunodominant epitopes of wheat gluten that were represented in the HLA-tetramer cocktail used during sorting (Figure 1g). We also verified the HLA-tetramer binding of these T-

Abbreviations: CeD, celiac disease; CLA, cutaneous lymphocyte antigen; DH, dermatitis herpetiformis; T_{EM}, effector-memory T cell

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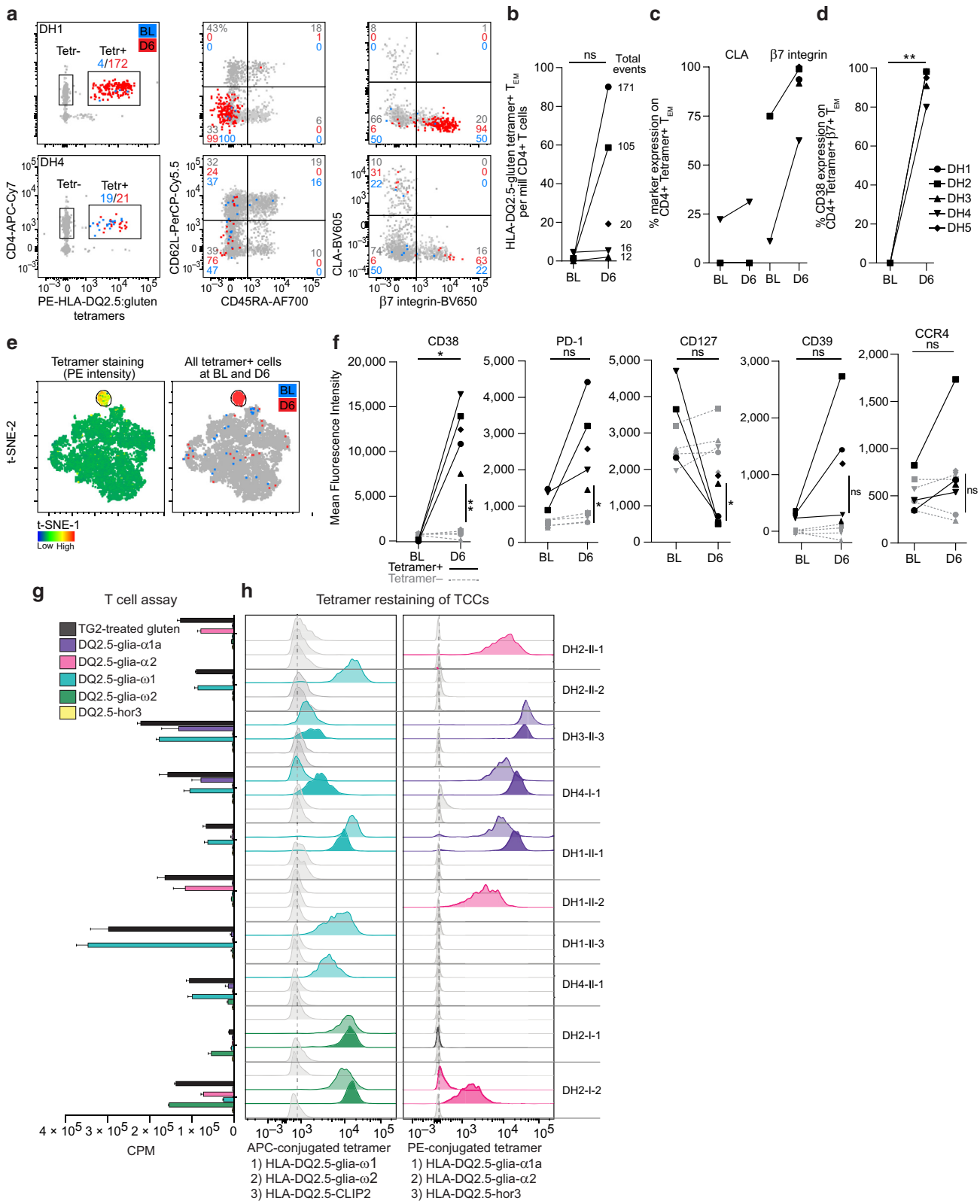

Figure 1. Analysis of CD4+ T cells in response to 3-day gluten challenge. (**a**) Staining of CD4+ T cells with HLA-DQ2.5:gluten tetramers. HLA-tetramer–positive cells at BL are in blue, and those on D6 are in red. HLA-tetramer–negative cells are in gray. (**b**) Frequency estimates of HLA-tetramer–positive T_{EM} per million CD4+ T cells of patients with DH (n = 5) before and after challenge. (**c**) Frequency of HLA-tetramer–positive T_{EM} cells expressing integrin β7 and CLA before and after challenge for samples with >5 cells. (**d**) CD38 expression on integrin β7+ HLA-tetramer–positive T_{EM} cells. (**e**) t-SNE plots of HLA-tetramer–positive cells (n = 366) and HLA-tetramer–negative cells (n = 9,750) from five patients. (**f**) Mean fluorescence intensity of HLA-tetramer–positive T_{EM} cells for different markers. (**g**) Reactivity of TCCs (n = 10) against gluten and five gluten peptides represented in HLA-DQ2.5:gluten tetramer mixture. (**h**) TCCs restained with five

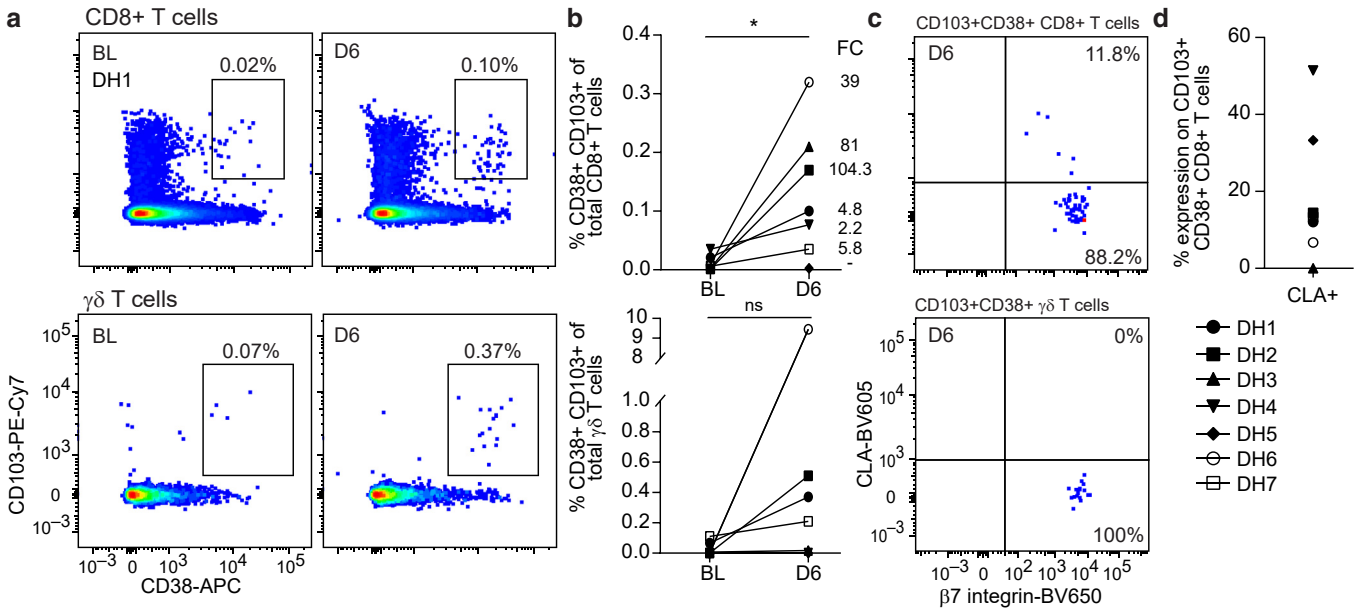

Figure 2. CD8+ and γδ T cell responses to 3-day gluten challenge. (**a**) Representative flow plot of one patient depicting CD103+CD38+ CD8+ T cells (top) and CD103+CD38+ γδ T cells (bottom) before (BL) and after (D6) gluten challenge. (**b**) Frequency of CD103+CD38+ cells among all CD8+ T cells (top) and all γδ T cells (bottom) for all patients (n = 7). The FC in the frequency of cells from D6 to BL is shown for CD8+ T cells. (**c**) Representative flow plot of one patient plot depicting coexpression of integrin β7 and CLA on CD8+ and γδ T cells. (**d**) Percentage of CD103+CD38+CD8+ T cells expressing integrin β7 and CLA plotted for all patients. *P*-values are obtained with paired *t*-test. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. APC, allophycocyanin; BL, baseline; CLA, cutaneous lymphocyte antigen; D6, day 6; DH, dermatitis herpetiformis; FC, fold change; ns, not significant; PE, phycoerythrin.

cell clones by restaining them with individual HLA-tetramers used for analysis and sorting (Figure 1h). The results indicate that epitope specificities of gluten-specific CD4+ T cells of patients with DH are similar to those of regular patients with CeD. Clinical parameters and T-cell measurements of the study subjects are summarized in Supplementary Table S1.

Furthermore, we investigated the response of CD8+ T cells and γδ T cells. For this purpose, we used the depleted PBMCs after enrichment for HLA-tetramer–binding cells. Similar to the flow panel used for CD4+ T cells, we stained for CLA and CCR4 to investigate for potential skin homing. After gluten challenge, we observed an increase of CD103+CD38+ CD8+ T cells in six of seven patients ($P = 0.02$) and an increase of CD103+CD38+ γδ T cells in four of seven patients (Figure 2a and b). Moreover, the increase in the frequency of CD8+ T cells as fold change ranged from 2.2 to 104. Interestingly, patient DH5 had the lowest frequency of CD103+CD38+ CD8+ T cells on day 6, and this was the only patient who continued for the entire 12-months challenge period. CLA was expressed by a substantial proportion of total CD8+ (9–76%, median = 32%) and γδ T cells (13–63%, median = 36%). Also among CD103+CD38+ CD8+ T cells, some expressed CLA (range = 0–51%, median = 13%) (Figure 2c and d). None of the CD103+CD38+ γδ T cells expressed the CLA skin-homing marker. Whether any of the activated CD8+ T cells found in the blood on day 6 after gluten challenge home to skin and exert effector functions there remains to be proven.

Although further studies with a higher number of patients and cells are warranted, our study suggests that patients with DH have gluten-induced T-cell responses with similar characteristics to regular patients with CeD. Furthermore, CD38 expression in gluten-specific integrin β7+ CD4+ T cells and CD103+CD38+ CD8+ T cells are promising markers to predict clinical relapse on a short gluten challenge in patients with DH.

Data availability statement

The data are not publicly available owing to Finnish legislation concerning patient-related data.

ORCIDs

Louise F. Risnes: http://orcid.org/0000-0002-2580-1678
Markéta Chlubnová: http://orcid.org/0000-0002-9612-3106
Elio Magistrelli: http://orcid.org/0000-0001-7434-5499
Esko Kemppainen: http://orcid.org/0000-0002-5491-5391
Kaisa Hervonen: http://orcid.org/0000-0002-6759-8399
Eriika Mansikka: http://orcid.org/0000-0002-3358-3922
Katri Lindfors: http://orcid.org/0000-0001-7417-5151
Teea Salmi: http://orcid.org/0000-0001-7459-4938
Shiva Dahal-Koirala: http://orcid.org/0000-0002-0165-5098
Ludvig M. Sollid: http://orcid.org/0000-0001-8860-704X

CONFLICT OF INTEREST

The authors state no conflict of interest.

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HLA-DQ2.5:gluten tetramers and HLA-DQ2.5-CLIP2–negative control tetramer. Darker color indicates cells treated with PKI dasatinib. *P*-values were obtained with paired *t*-test. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. BL, baseline; CLA, cutaneous lymphocyte antigen; D6, day 6; DH, dermatitis herpetiformis; ns, not significant; PKI, protein kinase inhibitor; TCC, T-cell clone; T_{EM}, effector-memory cell; t-SNE, t-distributed stochastic neighbor embedding.

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AUTHOR CONTRIBUTIONS

Conceptualization: LFR, LMS, KL, TS; Formal Analysis: LFR, MC, SDK, LMS; Funding Acquisition: LMS; Investigation: LFR, MC, EMag, SDK; Methodology: LFR, MC, EMag, SDK; Resources: LMS, TS; Supervision: LFR, SDK, LMS; Visualization: LFR, MC, SDK; Writing — Original Draft Preparation: LFR, SDK, LMS; Writing — Review and Editing: LFR, MC, EMag, EK, KH, EMan, KL, TS, SDK, LMS

***Louise F. Risnes*[1,2,*], *Markéta Chlubnová*[1,2,5], *Elio Magistrelli*[1,2,5], *Esko Kemppainen*[3], *Kaisa Hervonen*[3,4], *Eriika Mansikka*[3], *Katri Lindfors*[3], *Teea Salmi*[3,4], *Shiva Dahal-Koirala*[1,2] and *Ludvig M. Sollid*[1,2]**

[1]*KG Jebsen Coeliac Disease Research Centre, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway;*
[2]*Department of Immunology, Oslo University Hospital, Rikshospitalet, Oslo, Norway;*
[3]*Celiac Disease Research Center, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland; and*
[4]*Department of Dermatology, Tampere University Hospital, Tampere, Finland*
[5]*These authors contributed equally to this work*

[*]*Corresponding author e-mail: l.f.risnes@medisin.uio.no*

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org/, and at https://doi.org/10.1016/j.jid.2022.07.007

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