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Research Article

Research Article

DNAM1 and TIGIT balance the T cell response, with low T cell TIGIT expression corresponding to inflammation in psoriatic disease

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Summary

Objectives Signals at the contact site of antigen-presenting cells (APCs) and T cells help orchestrate the adaptive immune response. CD155 on APCs can interact with the stimulatory receptor DNAM1 or inhibitory receptor TIGIT on T cells. The CD155/DNAM1/TIGIT axis is under extensive investigation as immunotherapy target in inflammatory diseases including cancer, chronic infection and autoimmune diseases. We investigated a possible role for CD155/DNAM1/TIGIT signaling in psoriatic disease.

Methods By flow cytometry, we analyzed peripheral blood mononuclear cells of patients with psoriasis ($n = 20$) or psoriatic arthritis ($n = 21$), and healthy individuals ($n = 7$). We measured CD155, TIGIT, and DNAM1 expression on leukocyte subsets and compared activation-induced cytokine production between CD155-positive and CD155-negative APCs. We assessed the effects of TIGIT and DNAM1

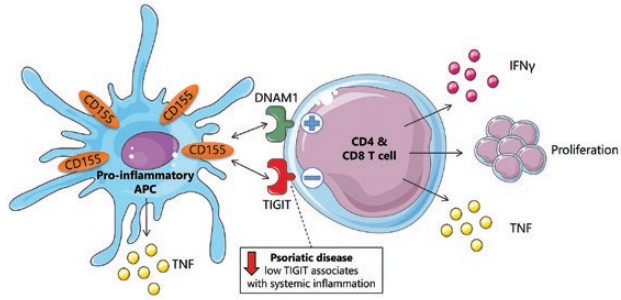
Abbreviations: APC: Antigen-presenting cell; APR: Acute phase reactant; CI: Confidence interval; cM: Classical monocyte; CRP: C-reactive protein; DC: Dendritic cell; DNAM1: DNAX-accessory molecule-1; ESR: Erythrocyte sedimentation rate; FSC: Forward scatter; HC: Healthy control; HLA-DR: Human leukocyte antigen – DR isotype; IFNγ: Interferon gamma; iM: Intermediate monocytes; LPS: Lipopolysaccharide; mAbs: Monoclonal antibodies; mDC: Myeloid dendritic cell; MFI: Median fluorescence intensity; MWU: Mann–Whitney *U* test; ncM: Non-classical monocytes; PASI: Psoriasis Area and Severity Index; PBMC: Peripheral blood mononuclear cell; pDC: Plasmacytoid dendritic cell; PsA: Psoriatic arthritis; PsD: Psoriatic disease; PsO: Psoriasis; r_s: Spearman's rank correlation coefficient; SSC: Sideward scatter; TIGIT: T-cell immunoglobin and ITIM domain; TNF: Tumor necrosis factor; Treg: Regulatory T cell; Th: Helper T cell.

blockade on T cell activation, and related the expression of CD155/DNAM1/TIGIT axis molecules to measures of disease activity.

Results High CD155 expression associates with tumor necrosis factor (TNF) production in myeloid and plasmacytoid dendritic cells (DC). In CD1c+ myeloid DC, activation-induced CD155 expression associates with increased HLA-DR expression. CD8 T cells – but not CD4 T cells – express high levels of TIGIT. DNAM1 blockade decreases T cell pro-inflammatory cytokine production, while TIGIT blockade increased T cell proliferation. Finally, T cell TIGIT expression shows an inverse correlation with inflammation biomarkers in psoriatic disease.

Conclusion CD155 is increased on pro-inflammatory APCs, while the receptors DNAM1 and TIGIT expressed on T cells balance the inflammatory response by T cells. In psoriatic disease, low TIGIT expression on T cells is associated with systemic inflammation.

Graphical Abstract



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Keywords: psoriatic disease, inflammation, CD155, TIGIT, DNAM1

Introduction

Integrated signals from antigen-presenting cells (APCs), among those most notably dendritic cells (DC), can orchestrate effector T cell responses [1]. Accordingly, activated APCs produce cytokines and upregulate the display of peptide/human leukocyte antigen (HLA) complexes and co-stimulatory molecules, to support ensuing adaptive immune responses [2]. Important molecules at the APC-T-cell contact site include co-inhibitory receptor T cell immunoreceptor with Ig and ITIM domains (TIGIT) and co-stimulatory receptor DNAX accessory molecule-1 (DNAM1, also known as CD226) on T cells. These receptors bind the same ligand on APCs, but have opposite effects [3]. Their ligand CD155 (also known as Poliovirus Receptor or NECL5) is mainly expressed by myeloid cells and interacts with effector T cells [4, 5]. While DNAM1 binding on T cells induces cytokine release and cytotoxicity, TIGIT evokes an immunosuppressive and non-cytotoxic profile [6, 7]. Binding of CD155 in DCs induces a rather tolerogenic profile as part of a negative feedback signal to prevent ongoing inflammation [6].

Dysregulation of the CD155/DNAM1/TIGIT axis plays an important role in the pathogenesis of various diseases. In cancer CD155 is upregulated in multiple tumor cell types and CD155 can modify tumor mass infiltration by lymphocytes, which may explain the association of CD155 upregulation with an unfavorable prognosis of solid tumors [8, 9]. Furthermore, aberrant expression of CD155, DNAM1, and TIGIT are suggested to be involved in the pathogenesis of non-malignant diseases, including primary Sjögren's syndrome [10], psoriasis [11], HIV infection [12–14], and in mouse models for rheumatoid arthritis [15], and sepsis [16]. In theory, depending on disease- and cell-specific DNAM1 and TIGIT expression, blockade of CD155 could either improve immune response or increase immunosuppression [6]. Moreover, targeting TIGIT with antagonistic monoclonal antibodies (mAbs) appears a logical immunotherapeutic strategy for solid tumors. Therefore, the CD155/DNAM1/TIGIT axis is under investigation as immunotherapy target.

Psoriasis is a common inflammatory disease that mainly affects the skin. Up to 30% of psoriasis patients develop musculoskeletal inflammation, termed psoriatic arthritis (PsA) [17]. The pathophysiology of psoriatic disease is characterized by increased release of pro-inflammatory cytokines – such as tumor necrosis factor (TNF), IL-17, and IL-23 – and chronic

activation of the innate and adaptive immune system, in which DC and T cells have a central role [17]. In psoriasis, one previous study observed decreased expression of TIGIT on CD4 T cells, which associated with an increased Psoriasis Area and Severity Index (PASI) – a tool to measure skin disease activity [11]. Stimulation of TIGIT using recombinant CD155/Fc protein inhibited proliferation of these CD4 T cells *in vitro* [11]. In psoriatic disease, both CD4 and CD8 T cells communicate with APCs to direct the adaptive immune response. CD4 T cells contribute importantly in the chronic phase of psoriatic disease, and CD8 T cells appear particularly important during the initiation of inflammation [18–20]. Variation in CD155/DNAM1/TIGIT expression levels under inflammatory conditions may lead to altered interactions at the APC-T cell contact site in psoriatic disease and offer potential targets for immunotherapy.

We first examined CD155 expression on six APC subsets, and quantified the expression of DNAM1 and TIGIT on CD4 and CD8 T cells. Next, in functional assays, we blocked DNAM1 or TIGIT specifically to monitor the effect of these molecules on T cell responses. Lastly, we examined whether the DNAM1 and TIGIT balance was disturbed in patients with the psoriatic diseases psoriasis and psoriatic arthritis.

Materials and Methods

Subjects

We used blood samples of two cohorts, that included a total of 7 healthy controls (HC), 20 psoriasis patients, and 21 PsA patients. We collected baseline characteristics and disease severity parameters, including disease-modifying anti-rheumatic drug use, PASI, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), swollen joint count, and tender joint count (Supplementary Tables S1 and S2). We used cohort 1 (HC n = 7, psoriasis n = 7, and PsA n = 7) to assess *ex vivo* CD155, TIGIT and DNAM1 expression, and for DNAM1/TIGIT blocking assays (Figs 1 and 4–6; Supplementary Figs S1A, S2, S3, and S5). We used cohort 2 (psoriasis n = 13 and PsA n = 15) to quantify TNF production by CD155-positive and CD155-negative APCs (Figs 2 and 3; Supplementary Figs S1B and S4). We obtained approval of the medical research ethics committee Utrecht for both study cohorts (Cohort 1 source ID 13/696, Trial NL4508; Cohort 2 source ID 15/429, Trial NL53860.041.15). Informed consent was obtained for experimentation with human samples. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Samples

Blood was drawn into BD Vacutainer™ Plastic Blood Collection Tubes with Lithium Heparin. Peripheral blood mononuclear cells (PBMC) were isolated using density centrifugation (Ficoll-Paque).

Ex vivo CD155, TIGIT, and DNAM1 expression

PBMCs *ex vivo* were used for the quantification of CD155 expression by APC subsets and TIGIT/DNAM1 expression by CD4 and CD8 T cells.

APC activation

To compare activation-induced TNF production between CD155-positive and CD155-negative APCs, we cultured PBMCs in medium (RPMI 1640 + 10% fetal bovine serum). Cells were left untreated (negative control) or stimulated with 100 ng/ml TLR-4 ligand (lipopolysaccharide [LPS]-EB Ultrapure) (tlrl-3pelps, Invivogen) for 4 hours, while inhibiting protein transport with 1:1000 BD GolgiStop (10716676, BD Bioscience).

TIGIT/DNAM1 blocking assays

To assess to assess the effect of TIGIT and DNAM1 blockade on T cell activation and proliferation, we cultured PBMCs in complete medium (RPMI 1640 + 10% fetal bovine serum + 1% Penicillin-Streptomycin) with 10 µg/ml TIGIT blocking antibody (16-9500-82, Invitrogen), 10 µg/ml DNAM1 blocking antibody (559787, BD Pharmingen), or 10 µg/ml isotype control for TIGIT (16-4714-82, Invitrogen) and DNAM1 (555746, BD Pharmingen). To assess proliferation we added 2 µM CellTrace Violet reagent (C34557, Life Technologies). To induce T cell activation and proliferation, we stimulated PBMCs 30 minutes after TIGIT/DNAM1 blockade with CD3/CD28 Dynabeads (11131D, ThermoFisher) in a 10:1 PBMC:Dynabead ratio. After 3 days, PBMCs were re-stimulated with 50 ng/ml phorbol myristate acetate (16561-29-8, Sigma-Aldrich) and 1 µg/ml ionomycin (56092-82-1, Simga-Aldrich) for 4 hours, while inhibiting protein transport with 1:1000 BD GolgiStop (10716676, BD Bioscience).

Antibody panels

Four antibody panels were used for flow cytometry analyses. Panel I was used for *ex vivo* quantification of APC CD155 expression (Fig. 1; Supplementary Figs S1A, S3, and S5E–J). We used panel II to quantify CD155 expression and TNF production of LPS-stimulated APCs

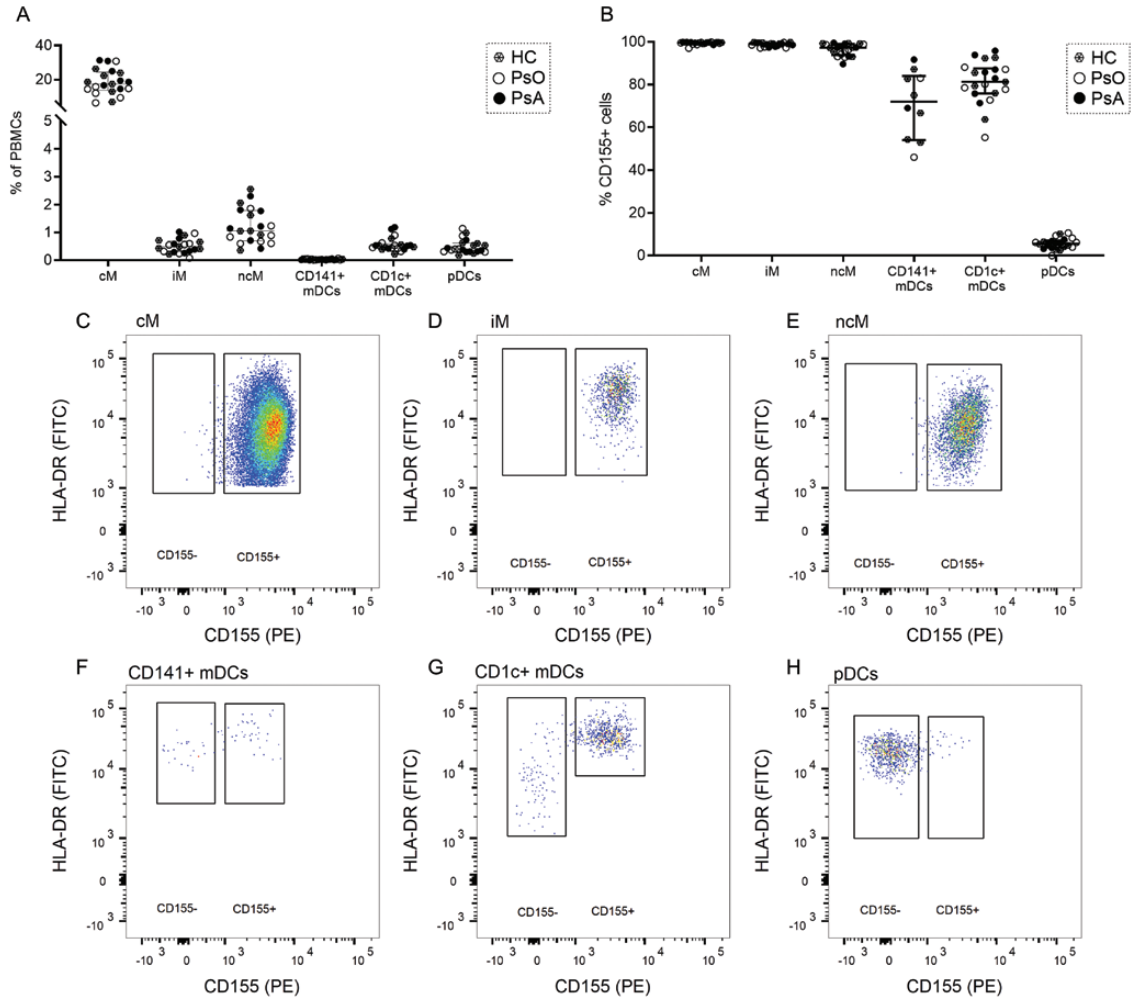

Figure 1. CD155 surface expression by APCs. Pooled flow cytometry analysis of PBMCs *ex vivo*, of healthy controls ($n = 7$, symbol with cross), psoriasis ($n = 7$, open symbol), and psoriatic arthritis ($n = 7$, filled symbol) patients. Shown data are from six APC subsets: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte (ncM), CD141+ myeloid DC (mDC), CD1c+ mDC, and CD303+ plasmacytoid DC (pDC). (A) Proportion of APC subset in PBMCs (gate 'Single Cells' Supplementary Fig. S1A). (B) Proportion of CD155-positive cells in APC subset. (C–H) Gating strategy of the selection of CD155-positive cells in cM (C), iM (D), ncM (E), CD141+ mDC (F), CD1c+ mDC (G), and pDC (H).

(Figs 2 and 3; Supplementary Figs S1B and S4). Panel III was used to assess *ex vivo* TIGIT/DNAM1 expression (Fig. 4; Supplementary Figs S2A and S5A–D), panel IV to quantify the effect of TIGIT/DNAM1 blockade on T cell proliferation (Fig. 5; Supplementary Fig. S2B) and panel V to quantify the effect of TIGIT/DNAM1 blockade on T cell activation (Fig. 5; Supplementary Fig. S2C). Panel I included antibodies against CD1c (APC; 17-0015-42, eBioscience), CD3 (AF700; 300424, Biolegend), CD19 (AF700; 56-0199-42, eBioscience), CD14 (APC-eFluor 780; 47-0149-42, eBioscience), CD16 (BV510; 563829, BD Horizon), CD56 (PE-CF594; 56228, BD Horizon), CD141 (BV711; 563155, BD Horizon), CD155 (PE; 337609, Biolegend), CD303 (PE-Cy7; 354214, Biolegend), HLA-DR (FITC; 347400, BD), and a Fixable Viability Dye (eF450; 65-0863-14, eBioscience). Panel II comprised antibodies targeting CD1c (APC; 17-0015-42, eBioscience), CD3 (AF700; 300424, Biolegend), CD19 (AF700; 56-0199-42, eBioscience), CD56 (AF700; 557919, BD Pharmingen), CD11c (PE-CF594; 562393, BD Horizon), CD14 (PerCP-Cy5.5; 325622, Biolegend), CD16 (BV510; 302048, Biolegend), CD123 (FITC; 11-1239-42, eBioscience), CD141 (BV711; 563155, BD Horizon), CD155 (PE; 337609, Biolegend), HLA-DR (BV-421; 307636, Biolegend), TNF (PE-Cy7; 25-7349-82 eBioscience), and a Fixable Viability Dye (eF780;

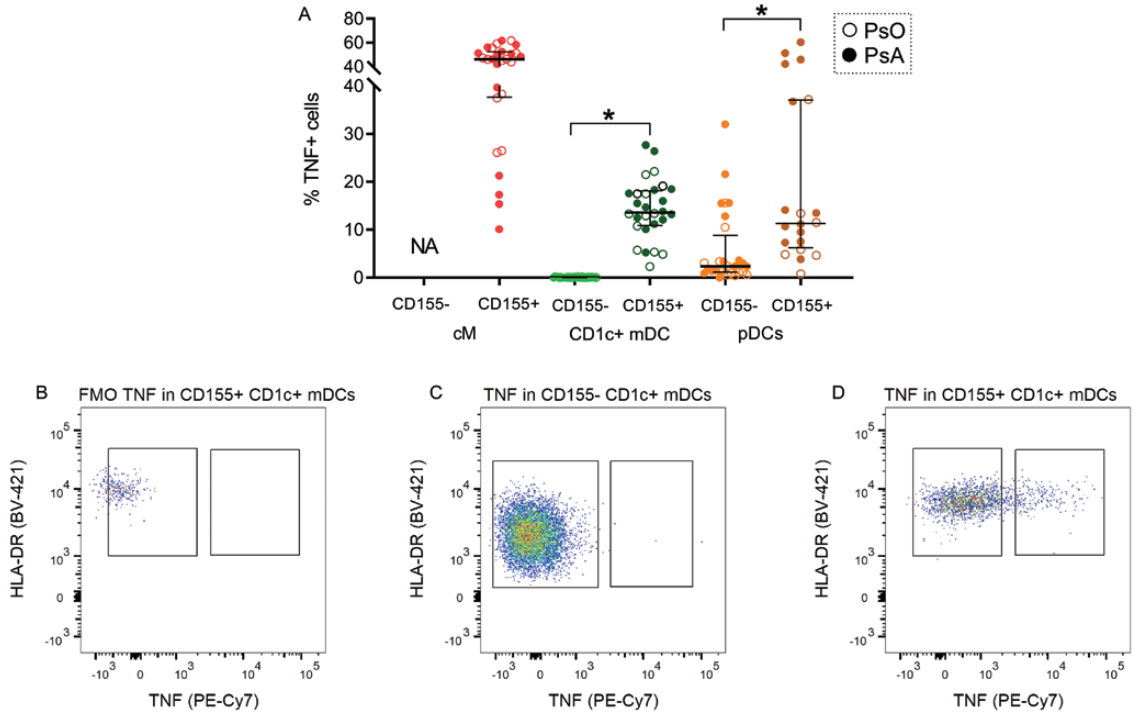

Figure 2. High TNF production by CD155 expressing APCs. Flow cytometry analysis of psoriatic disease patients PBMCs stimulated for 4 hours with LPS (100 ng/ml) in the presence of Brefeldin A (1:1000). (A) Proportion of TNF producing cells within CD155-positive and CD155-negative APC subsets of psoriasis patients (n = 13, open symbol) and psoriatic arthritis patients (n = 15, filled symbol): CD14+CD16- classical monocyte (cM), CD1c+ myeloid dendritic cell (mDC), and CD123+ plasmacytoid dendritic cell (pDC) (detailed gating strategy shown in Supplementary Fig. S1B). (B–D) Representative flow plots of TNF production by CD1c+ mDC: CD155-positive TNF FMO control (B), CD155-negative CD1c+ mDC (C), and CD155-positive CD1c+ mDC (D). *Significant P-value MWU. NA: not applicable.

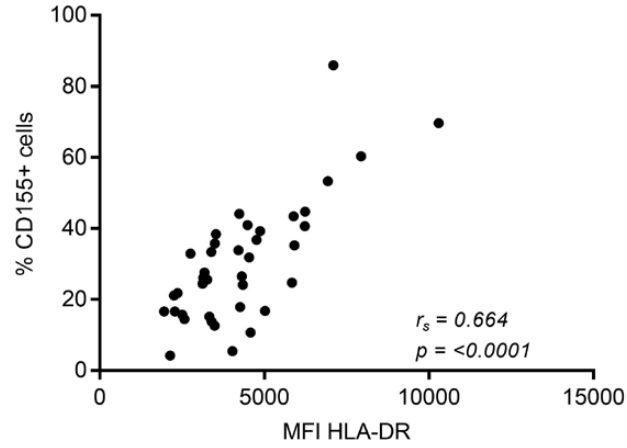

Figure 3. Positive correlation of CD155 and HLA-DR in activated CD1c+ mDC. Flow cytometry analysis of psoriatic disease patients PBMCs stimulated for 4 hours with LPS (100 ng/ml). Shown is the positive correlation of the percentage of CD155-positive CD1c+ mDC and the MFI of HLA-DR expressed by CD1c+ mDC (Spearman's rank correlation coefficient r_s = 0.664 [95% CI 0.433–0.813], P = < 0.0001).

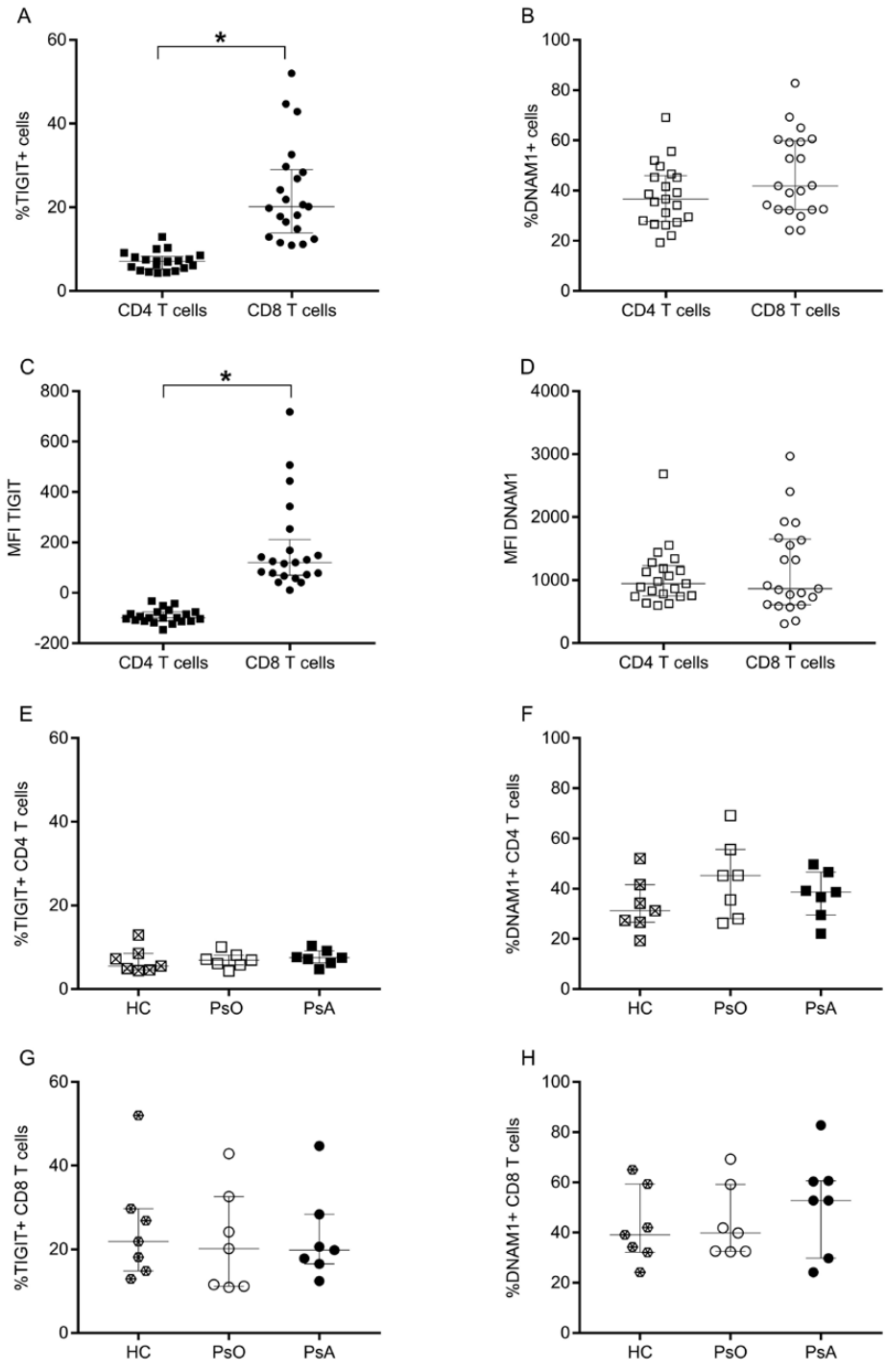

Figure 4. Higher TIGIT and comparable DNAM1 expression by CD8 versus CD4 T cells. Flow cytometry analysis of CD4 (square) and CD8 (circle) T cells *ex vivo* of healthy controls (HC, $n = 7$, symbol with cross), psoriasis (PsO, $n = 7$, blank symbol), and psoriatic arthritis (PsA, $n = 7$, filled symbol) patients. (A–D) Pooled data of all subjects. (E–F) Data of HC, PsO, and PsA patients shown separately. (A) Significantly higher proportion of TIGIT-positive CD8 T cells compared to CD4 T cells. (B) Comparable proportion of DNAM1-positive CD8 and CD4 T cells. (C) Significantly higher TIGIT MFI of CD8 T cell compared to CD4 T cells. (D) Comparable DNAM1 MFI of CD4 and CD8 T cells. (E) Comparable proportion of TIGIT-positive CD4 T cells in HC, PsO, and PsA. (F) Comparable proportion of DNAM1-positive CD4 T cells in HC, PsO and PsA. (G) Comparable proportion of TIGIT-positive CD8 T cells in HC, PsO, and PsA. (G) Comparable proportion of DNAM1-positive CD8 T cells in HC, PsO, and PsA. *Significant *P*-value MWU.

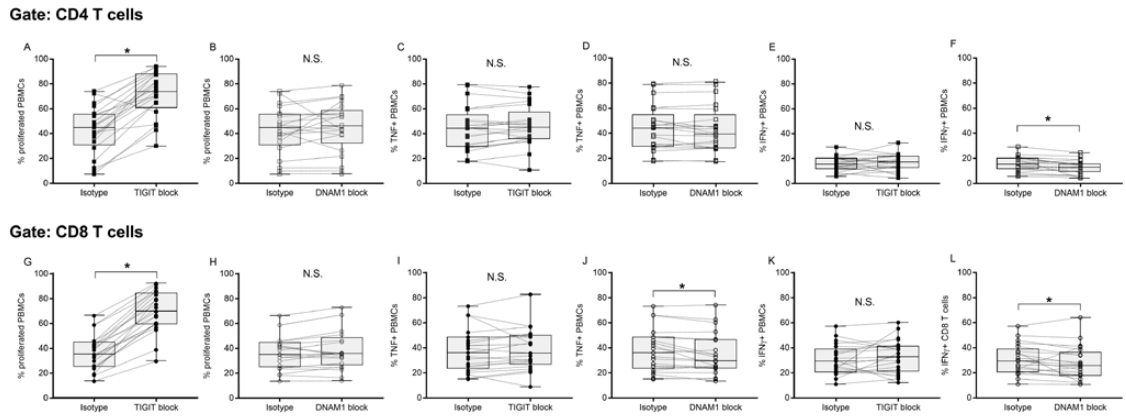

Figure 5. TIGIT blockade increases T cell proliferation, and DNAM1 blockade reduces T cell pro-inflammatory cytokine production. Flow cytometry analysis of PBMCs stimulated for 3 days with CD3/CD28 Dynabeads (PBMC:Dynabead 10:1), after either 10 μg/ml DNAM1 blocking antibody, 10 μg/ml TIGIT blocking antibody or 10 μg/ml DNAM1 and TIGIT blocking antibody isotypes. Pooled data of healthy controls, psoriasis, and psoriatic arthritis patients. Shown are percentages of proliferated T cells, stained with 2 μM CellTrace Violet reagent (A, B, G, and H) and percentages of TNF and IFNγ producing T cells after 4 hours re-stimulation with 50 ng/ml PMA, 1 μg/ml ionomycin in the presence of Brefeldin A (1:1000) (C–F, H–K). (A) TIGIT block significantly increases CD4 T cell proliferation. (B) DNAM1 block has no significant effect on CD4 T cell proliferation. (C) No significant difference in CD4 T cell TNF production after TIGIT blockade (44.5% vs. 45.4%, $P > 0.05$). (D) No significant decrease in TNF production by CD4 T cells after DNAM1 block (44.5% vs. 39.7%, $P > 0.05$). (E) No significant increase in CD4 T cell IFNγ production after TIGIT blockade (15.6% vs. 17.4%, $P > 0.05$). (F) Significantly decreased IFNγ production by CD4 T cells after DNAM1 block (15.6% vs. 13.0%, $P = 0.0015$). (G) TIGIT block significantly increases CD8 T cell proliferation. (H) DNAM1 block has no significant effect on CD8 T cell proliferation. (I) No significant difference in CD8 T cell TNF production after TIGIT blockade (35.8% vs. 36.1%, $P > 0.05$). (J) Significant decrease in TNF production by CD8 T cells after DNAM1 block (36.1% vs. 29.6%, $P = 0.0039$). (K) Trend toward increased CD8 T cell IFNγ production after TIGIT blockade (29.4% vs. 32.0%, $P > 0.05$). (L) Significant decrease in IFNγ production by CD8 T cells after DNAM1 block (29.4% vs. 25.9%, $P = 0.0140$). *Significant *P*-value Wilcoxon-signed rank test.

65-0865-14, eBioscience). Panel III consisted of antibodies toward CD3 (AF700; 557919, BD Pharmingen), CD4 (BV711; 2102790, Sony Biotechnology), CD8 (PE-Cy7; 335822, BD), DNAM1 (APC; 338312, Biolegend), TIGIT (PerCP-Cy5-5; 46-9200-42, eBioscience) and a Fixable Viability Dye (eF780; 65-0865-14, eBioscience). Panel IV included CellTrace Violet reagent (C34557, Life Technologies) and antibodies against CD3 (AF700; 300424, Biolegend), CD4 (Pe-Cy7; 25-0049-42, eBioscience), CD8 (V500; 561617, BD horizon), and a Fixable Viability Dye (eF780; 65-0865-14, eBioscience). Panel V included antibodies against CD3 (AF700; 300424, Biolegend), CD4 (Pe-Cy7; 25-0049-42, eBioscience), CD8 (V500; 561617, BD horizon), TNF (BV421; 562783, BD Horizon), IFN-γ (PerCP-Cy5.5; 15599036, Ebioscience), IL-10 (PE; 554706, BD), and a Fixable Viability Dye (eF780; 65-0865-14, eBioscience).

Flow cytometry

We stained samples by incubation with 25 μl antibody mix diluted in buffer (500 ml phosphate-buffered saline + 5 ml 10% sodium azide + 5 g bovine serum albumin) for 25 min at 4°C. Before intracellular stain of TNF and interferon-gamma (IFNγ), we fixed and permeabilized cells with 100 μl Fixation/Permeabilization Concentrate and Diluent (00-5123-43, 00-5223-56, eBioscience). Phenotypical cell surface markers were used to differentiate between PBMC subsets: T cells (CD3, CD4, and CD8), B cells (CD19), monocytes (CD14 and CD16), natural killer (NK) cells (CD56), plasmacytoid (p)DCs (CD123 or CD303), myeloid (m)DC1 and -2 (CD1c, CD11c, and CD141); detailed gating strategies of panels I-V are shown in Supplementary Figs S1 and S2. Based upon the differential expression of CD14 and CD16, we identified classical (cM), intermediate (iM), and non-classical monocytes (ncM) (Supplementary Fig. S1A). We excluded gated cell populations of ≤ 30 cells. Acquisition was performed on the BD LSRFortessa with four lasers (405, 488, 561, and 635 nm) with DIVA software version 8.0.1. Compensation for spectral overlap and analysis of FCS files was performed using FlowJo version 10.4.

Statistical analysis

We performed contingency analysis of psoriasis and PsA clinical characteristics using χ^2 tests for categorical variables, and independent samples T or Mann–Whitney *U* (MWU) tests for continuous variables. We used MWU tests to compare CD155, DNAM1, and

TIGIT expression, and TNF production between cell subsets and patient groups. The Wilcoxon-signed rank test was used to compare cytokine production between negative controls and DNAM1/TIGIT blocked T cells. Additionally, we used Spearman's rank correlation to correlate clinical parameters and expression of cell surface markers. We considered a *P*-value <0.05 as statistically significant. Statistical analyses were performed using IBM SPSS version 27 and GraphPad Prism 7.00.

Results

High CD155 expression by monocytes and CD1c+ mDC

We used flow cytometry to explore CD155 expression on six PBMC APC subsets: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte (ncM), CD141+ myeloid DC (mDC), CD1c+ mDC, and CD303+ plasmacytoid DC (pDC). Nearly all cM, iM, and ncM expressed CD155 (Fig. 1). In contrast, pDC rarely expressed CD155 and CD141+ mDC showed variable CD155 expression. Overall, CD155 expression among the different APC subsets was comparable in HC, psoriasis, and PsA (Supplementary Fig. S3). To summarize, irrespective of psoriatic disease, monocyte subsets ubiquitously express CD155, while DC shows variable CD155 expression related to their subset.

High TNF production in CD155-positive APCs upon activation

Next, we examined whether activation-induced TNF production by monocyte and DC subsets was related to CD155 expression. For both pDC and mDC, their CD155-positive fraction produced significantly more TNF compared to the CD155-negative fraction (Fig. 2). Considering that all monocyte subsets were ubiquitously positive for CD155, we could not compare TNF production between CD155-negative and CD155-positive fractions. However, the percentage of TNF producing cM correlated positively with the mean fluorescent intensity (MFI) of CD155 on cM (r_s = 0.620 [0.370–0.786], *P* = <0.0001; Supplementary Fig. S4). Again, no differences between psoriasis and PsA were observed (data not shown). In summary, the capacity for TNF production by cM, CD1c+ mDC, and pDC correlates positively with their expression of CD155.

Correlation of CD155 and HLA-DR expression in CD1c+ mDCs

To further investigate a possible role for CD155 in inflammation, we evaluated whether CD155 expression on CD1c+ mDC associates with HLA-DR expression as a marker for matured, activated DC. To this end, we stimulated PBMCs from psoriatic disease patients with LPS (100 ng/ml) for 4 hours and then analyzed by flow cytometry. We found that in CD1c+ mDC, CD155 expression and HLA-DR expression were significantly correlated (r_s = 0.664 (95% confidence interval [CI] 0.433–0.813), *P* < 0.0001; Fig. 3).

High TIGIT expression overall on CD8 T cells and low TIGIT expression on CD4 T cells

As CD155 serves as a ligand for DNAM1 and TIGIT on T cells, we quantified baseline expression of these receptors on CD4 and CD8 T cells. TIGIT expression was significantly higher on CD8 T cells, compared to CD4 T cells (*P* < 0.0001; Fig. 4A and C), but DNAM1 expression was comparable between CD4 T cells and CD8 T cells (Fig. 4B and D). We observed no differences in TIGIT and DNAM1 expression between HC, psoriasis and PsA patients (Fig. 4E–H).

TIGIT blockade increases T cell proliferation and DNAM1 blockade reduces T cell pro-inflammatory cytokine production

Next, we investigated if CD155 ligation can modulate T cell function through selective interaction with DNAM1 or TIGIT. We therefore included anti-TIGIT or anti-DNAM1 blocking antibodies in short-term cultures in which we stimulated PBMCs using anti-CD3/CD28 Dynabeads. Three days blockade of TIGIT caused a significant increase in CD4 and CD8 T cell proliferation compared to the negative control (*P* < 0.001, isotype control antibody) (Fig. 5A and G). Blockade of DNAM1 did not affect CD8 T cell proliferation (Fig. 5B and H). We observed no significant differences between psoriasis and psoriatic arthritis patients (data not shown).

To further explore the effects of TIGIT and DNAM1 on T cell function, we quantified TNF and IFNγ production by CD4 and CD8 T cells after blockade of either TIGIT or DNAM1-receptors. Overall, TIGIT block did not yield a significant increase in cytokine production for IFNγ nor TNF (Fig. 5C,E,I,K). DNAM1 block, on the other hand, caused a significant decrease in production of IFNγ by CD4 T cells (15.6% vs. 13.0%, *P* = 0.0015) and decreased both TNF production (36.1% vs. 29.6%, *P* = 0.0039; Fig. 5J) and IFNγ production by CD8 T cells (29.4% vs. 25.9%, *P* = 0.0140; Fig. 5L). These findings were comparable for T cells from HC, psoriasis and PsA patients (data not shown). Thus, TIGIT blockade caused an increase in CD4 and CD8 T cell proliferation, while DNAM1 blockade resulted in decreased pro-inflammatory cytokine production by T cells.

TIGIT expression on T cells correlates with APR in psoriatic disease

We finally investigated a possible clinical association of the CD155/DNAM1/TIGIT-axis with psoriatic disease by correlating expression with disease severity measures. We found that TIGIT expression on CD4 T cells negatively correlates with the acute phase reactant (APR) ESR (r_s = –0.7918 [95%CI –0.9457 to –0.3476], P = 0.0052) and shows a trend toward correlation with CRP (r_s = –0.579 [95%CI –0.8701 to 0.01175], P = 0.0521) in psoriasis and PsA patients (Fig. 6A and B). Moreover, CD8 T cell TIGIT expression negatively correlates with both ESR (r_s = –0.705 [95%CI –0.920 to –0.162], P = 0.0189) as CRP (r_s = –0.663 [95%CI –0.900 to –0.125], P = 0.022; Fig. 6C and D). There was no correlation of CD4 or CD8 T cell TIGIT or DNAM1 expression with psoriatic disease activity measures (Supplementary Fig. S5A–D). Also, the proportion of CD155-positive APCs did not correlate with the clinical outcomes (Supplementary Fig. S5E–J).

Discussion

To our knowledge, this study is the first to investigate the role of CD155, DNAM1, and TIGIT in driving inflammation in both psoriasis and psoriatic arthritis. We here confirm that CD155 is highly expressed by human APCs and that CD155 associates with an activated and pro-inflammatory DC phenotype. We confirm a role for TIGIT and DNAM1 in balancing the adaptive inflammatory response. Moreover, our results support the association of low TIGIT expression with systemic inflammation in psoriatic disease.

Our finding that CD155 – the ligand for DNAM1 and TIGIT – is highly expressed on monocytes and mDC is in line with previous research [21–24]. Moreover, our data extend a previously suggested association of CD155 with inflammation, by showing a correlation of CD155 with the production of pro-inflammatory cytokine TNF in classical monocytes, mDC and pDC [16, 25]. Additionally, in psoriasis and PsA patients, we found a positive correlation between CD155 expression on CD1c+ mDC and HLA-DR, a DC maturation and activation marker [26, 27].

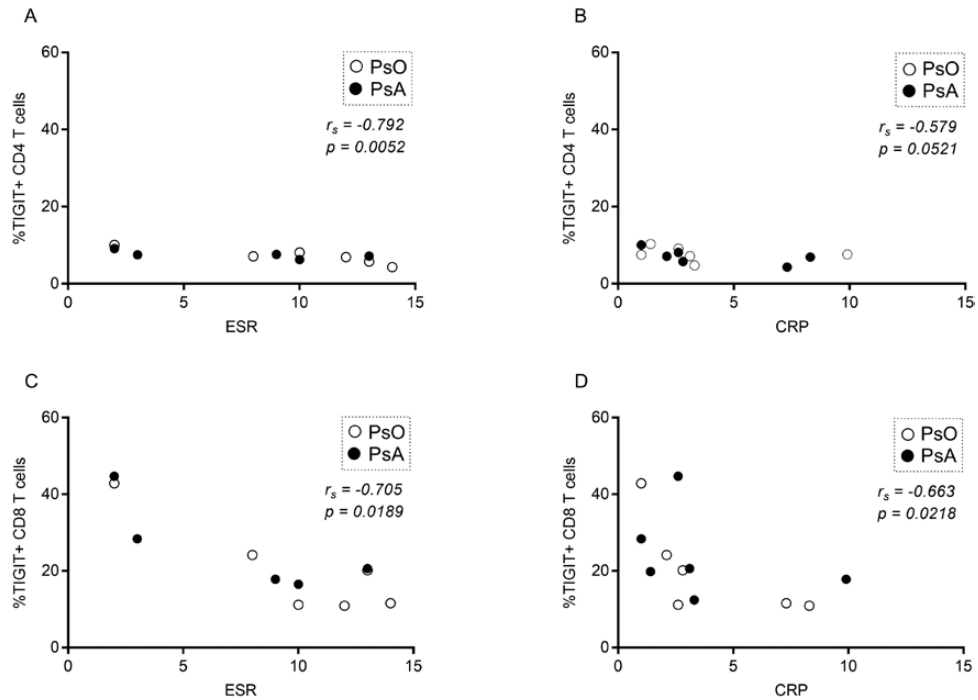

Figure 6. Reduced T cell TIGIT expression associates with increased level of acute phase reactants. Correlation of acute phase reactants in serum and the percentage of TIGIT-expressing T cells analyzed *ex vivo* in psoriasis (n = 7, blank symbol) and psoriatic arthritis (n = 7, filled symbol) patients, using flow cytometry. (A) Significant correlation of percentage TIGIT-positive CD4 T cells and ESR (r_s = –0.7918 [95% CI –0.9457 to –0.3476], P = 0.0052). (B) Trend toward correlation of CD4 TIGIT-positive cells and CRP (r_s = –0.792 [95% CI –0.8701 to 0.01175], P = 0.0521). (C) Significant correlation of percentage TIGIT-positive CD8 T cells and erythrocyte sedimentation rate (ESR) (Spearman's Rank correlation coefficient [r_s] = –0.705 [95% CI –0.920 to –0.162], P = 0.0189). (D) Significant correlation of CD8 TIGIT-positive CD8 T cells and C-reactive protein (CRP) (r_s = –0.663 [95% CI –0.900 to –0.125], P = 0.022).

Altogether, these results propose CD155 as key contributor to inflammation.

Our results demonstrate an important role of TIGIT in the preservation of immune homeostasis, as TIGIT blockade results in increased CD4 and CD8 T cell proliferation and low T cell TIGIT expression associates with systemic inflammation in psoriatic disease. The observed increase in CD8 T cell proliferation after TIGIT blockade may be explained by both a direct effect on the CD8 T cell TIGIT receptor for CD155, as by an indirect through reduced inhibition of conventional CD4 T helper (Th) and regulatory T cells (Treg) [28]. The observed inhibitory effect of TIGIT on T cell proliferation is in line with literature [7, 8]. Previous research in the field of tumor immunology additionally showed that TIGIT blockade enhances T cell-mediated cytokine production, but we did not observe this in our T cell analyses [11, 29]. The absence of increased T cell cytokine production after TIGIT blockade in psoriatic disease possibly relates to the co-existence of CD96 (also known as Tactile), which is a second co-inhibitory receptor for CD155 capable of inhibiting T cell cytokine production *in vitro*, and which might have overruled the effect of our TIGIT block [4, 30]. To the best of our knowledge, the association of TIGIT expression with systemic inflammation in psoriatic disease is not yet published, but is in line with previous research that showed a correlation of CD4 T cell TIGIT expression and skin disease severity (PASI) in psoriasis [11]. Altogether, our results suggest that T cell TIGIT expression is important for immune homeostasis.

Furthermore, our results suggest an important role of DNAM1 in the perpetuation of the adaptive immune response because blockade of DNAM1 resulted in decreased T cell production of pro-inflammatory cytokines. We explain this effect on T cells by both the ceasing of a stimulatory signal, as by more CD155 on APCs available to bind the inhibitory receptors TIGIT and CD96 – both able to reduce effector T cell cytokine production [4, 8]. We did not observe an effect of DNAM1 blockade on T cell proliferation, which is not entirely unexpected. Proliferation is likely controlled by additional T cell co-stimulatory receptors besides DNAM1, at least when stimulated by professional APCs *in vitro* [31]. Moreover, previous research suggests that TIGIT can overrule DNAM1 with regards to T cell proliferation [32]. Explanations for this finding include dose-dependent competition for the ligand CD155 (for which TIGIT has higher affinity), disruption of DNAM1 homo-dimerization by TIGIT, and interference of TIGIT with DNAM1 intracellular signaling cascades [29, 33–35].

We found no direct evidence for disease-specific aberrant expression of the CD155/DNAM1/TIGIT axis relating to T cells in psoriatic disease, although a pathogenic role of TIGIT downregulation on PBMC CD4 T cells had been suggested in psoriasis [11]. Possibly, our relatively small sample size or relatively low disease activity of included patients prevented us from obtaining disease-specific data. However, combining our results from HC and psoriatic patients, we argue that further research is warranted to further elucidate the immunoregulatory role of the CD155/DNAM1/TIGIT axis in psoriatic disease. Indeed, a combination therapy consisting of DNAM1-blocking and TIGIT-stimulating agents might be effective in modulating the adaptive immune response via reduction of T cell proliferation and cytokine production in patient with chronic inflammation.

Our reductionist approach entails an important limitation of our study of the CD155/DNAM1/TIGIT axis. We deem that simplifying this complex network contributed to our step-by-step exploration of its relevance in psoriatic disease. Future studies require additional analyses, such as studying CD155 expression by lymphocytes and non-hematopoietic tissue cells, the effects of TIGIT blockade on NK cell function, the additional CD155 co-inhibitory receptor CD96, and the stimulatory effects of CD112 (an alternative DNAM1 ligand) [4, 5, 7, 36].

In conclusion, we show that CD155 is increased on pro-inflammatory APCs and that the receptors DNAM1 and TIGIT – expressed by T cells – balance the T cell inflammatory response. Moreover, in psoriatic disease, low T cell TIGIT expression is associated with systemic inflammation. Our data supports a contributory role for the CD155/DNAM1/TIGIT axis in a combination therapy, rather than as mono-therapy. Future research exploring how DNAM1 and TIGIT regulate the T cell inflammatory response, could contribute to development of next generation treatments for psoriatic disease.

Supplementary material

Supplementary data are available at *Immunotherapy Advances* online.

Supplementary Figure S1. Gating strategy for CD155 expression and TNF production by APCs. Legend: (A) Gating strategy for CD155 expression by APCs. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-A and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF450. Phenotypical cell surface markers were used to exclude non-APCs: T cells (CD3), B cells (CD19), and natural killer (NK) cells (CD56). We differentiated between six APC subsets using the following gates: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte

(ncM), CD14-CD16-HLA-DR+CD141+ myeloid dendritic cell (mDC), CD14-CD16-HLA-DR+CD1c+ mDC and CD14-CD16-HLA-DR+CD303+ plasmacytoid DC (pDC). Within each APC subset CD155-positive and CD155-negative cells were selected, as shown in primary Fig. 1C–H. (B) Gating strategy for TNF production by activated APCs. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-A and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. Phenotypical cell surface markers were used to exclude non-APCs: T cells (CD3), B cells (CD19), and natural killer cells (CD56). We differentiated between three APC subsets using the following gates: CD14+ classical monocyte (cM), CD14-HLA-DR+CD1c+ mDC and CD14-HLA-DR+CD123+ plasmacytoid DC (pDC). Within each APC subset CD155-positive and CD155-negative cells were selected. Of CD155+ cells, TNF-positive cells were gated (representative plots shown in main Fig. 2C and D). Abbreviations: APC: antigen-presenting cell, cM: classical monocytes, DC: dendritic cell, FSC: forward scatter, HLA-DR: human leukocyte antigen – DR isotype, iM: intermediate monocytes, ncM: non-classical monocytes, pDC: plasmacytoid dendritic cell, SSC: sideward scatter, TNF: tumor necrosis factor.

Supplementary Figure S2. Gating strategy for T cell TIGIT and DNAM1 expression, proliferation and cytokine production. Legend: (A) Gating strategy for *ex vivo* T cell TIGIT and DNAM1 expression. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-A and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. CD3 phenotypical cell surface markers was used to select T cells. Next, CD4 and CD8 T cells were gated. Of both T cell subsets T cells positive and negative for TIGIT and DNAM1 were selected. (B-C) Gating strategies for T cell proliferation and cytokine production. PBMCs were stimulated for 3 days with CD3/CD28 Dynabeads (PBMC:Dynabead 10:1), after either 10 µg/mL DNAM1 blocking antibody, 10 µg/mL TIGIT blocking antibody or 10 µg/mL DNAM1 and TIGIT blocking antibody isotypes. For proliferation, PBMCs were stained with 2 µM CellTrace Violet reagent. For cytokine production, PBMCs were re-stimulated for 4 hours with 50 ng/mL PMA, 1 µg/mL ionomycin in the presence of Brefeldin A (1:1000). (B) Gating strategy for CD4 and CD8 T cell proliferation. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-H and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. CD3 phenotypical cell surface markers was used to select T cells. Next, CD4 and CD8 T cells were gated. Of both T cell subsets the divided population was gated based on the CellTrace Violet stain. (C) Gating strategy for CD4 and CD8 T cell cytokine production. Lymphocytes were determined by the forward and side scatter profile. Cells were gated in a FSC-H and FSC-W dot plot to eliminate doublets. Viable cells were selected based upon negativity for eF780. CD3 phenotypical cell surface markers was used to select T cells. Next, CD4 and CD8 T cells were gated. Of both T cell subsets T cells positive for TNF and IFNγ were selected. Abbreviations: DNAM1: DNAX-accessory molecule-1, IFNγ: interferon gamma, TIGIT: T-cell immunoglobin and ITIM domain, TNF: tumor necrosis factor.

Supplementary Figure S3. CD155 expression by APC subsets comparable in HC, psoriasis and PsA. Legend: Flow cytometry analysis of PBMCs *ex vivo* of healthy controls, psoriasis and psoriatic arthritis patients. Shown are percentages of CD155-positive cells within six APC subsets: CD14+CD16- classical monocyte (cM), CD14+CD16+ intermediate monocyte (iM), CD14-CD16+ non-classical monocyte (ncM), CD141+ myeloid DC (mDC), CD1c+ myeloid mDC and CD303+ plasmacytoid DC (pDC). Overall, CD155 expression within the different APC subsets very similar in HC, psoriasis and PsA ($p > 0.05$), except for a marginally lower iM CD155 expression in HC vs. psoriasis ($p = 0.038$). * Significant difference MWU test. Abbreviations: APC: antigen-presenting cell, cM: classical monocytes, DC: dendritic cell, HC: healthy control, iM: intermediate monocytes, mDC: myeloid dendritic cell, MWU: Mann Whitney U test, nCM: non-classical monocytes, pDC: plasmacytoid dendritic cell, PBMC: peripheral blood mononuclear cell, PsA: psoriatic arthritis.

Supplementary Figure S4. Positive correlation of CD155 and TNF presence in activated classical monocytes. Legend: Flow cytometry analysis of psoriatic disease patients CD14+ classical monocytes (cM) stimulated for 4 hours with 100 ng/mL LPS in the presence of Brefeldin A (1:1000). Shown is the correlation of the percentage TNF producing cM with cM mean fluorescent intensity (MFI) of CD155. The percentage of TNF producing cM is positively correlated with CD155 MFI (Spearman's Rank correlation coefficient $r_s = 0.620$ (0.370 – 0.786), $p = <0.0001$). Abbreviations: cM: classical monocytes, HLA-DR: human leukocyte antigen – DR isotype, LPS: lipopolysaccharide, MFI: median fluorescence intensity, r_s: Spearman's rank correlation coefficient, TNF: tumor necrosis factor.

Supplementary Figure S5. Correlation of psoriatic disease activity measures with both T cell TIGIT and DNAM1 expression, as with APC subsets CD155 expression. Legend: Shown are scatterplots of psoriatic disease severity measures (PASI, CRP, ESR SJC, TJC) and the percentage of TIGIT (A,C) or DNAM1 (B,D) expressing CD8 and CD4 T cells, or the percentage of CD155+ cells within six APC subsets (C-H) in psoriasis and psoriatic arthritis patients, measured by flow cytometry. Spearman's rank correlation coefficients (r_s) were calculated to test correlation. (A) No correlation of PASI, SJC or TJC with the percentage of TIGIT+ CD8 T cells. (B) No correlation of PASI, CRP, ESR, SJC or TJC with the percentage of DNAM1+ CD8 T cells. (C) No correlation of PASI, SJC or TJC with the percentage of TIGIT+ CD4 T cells. (D) No correlation of PASI, CRP, ESR, SJC or TJC with the percentage of DNAM1+ CD4 T cells. (E) No correlation of disease severity measures and the percentage of CD155-positive CD14+CD16- classical monocytes (cM), CD14+CD16+ intermediate monocytes (iM) (F), CD14-CD16+ non-classical monocytes (ncM) (G), CD141+ myeloid dendritic cells (mDC) (H), CD1c+ mDC (I) or CD303+ plasmacytoid DC (pDC) (J). Abbreviations: APC: antigen-presenting cell, cM: classical monocytes, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, iM: intermediate monocytes, mDC: myeloid dendritic cell, ncM: non-classical monocytes, PASI: psoriasis area and severity index, pDC: plasmacytoid dendritic cell, SJC: swollen joint count, TJC: tender joint count.

Supplementary Table S1. Baseline characteristics cohort 1. Categorical data are presented with frequencies (%) and continuous data are shown as mean ± standard deviation (normally distributed variables) or median (interquartile range) (non-normally distributed variables). * Significant difference psoriasis vs PsA (p-value < 0.05). Abbreviations: CRP: C-reactive protein, DMARD: disease modifying anti-rheumatic drug use (past three months), ESR: erythrocyte sedimentation rate, HC: healthy control, NA: not applicable, n.m.: not measured, PASI: psoriasis area and severity index, PsA: psoriatic arthritis, SJC: swollen joint count, TJC: tender joint count.

Supplementary Table S2. Baseline characteristics cohort 2. Categorical data are presented with frequencies (%) and continuous data are shown as mean ± standard deviation (normally distributed variables) or median (interquartile range) (non-normally distributed variables). No significant differences psoriasis vs PsA (p-values ≥ 0.05). Abbreviations: CRP: C-reactive protein, DMARD: disease modifying anti-rheumatic drug use (past three months), ESR: erythrocyte sedimentation rate, HC: healthy control, NA: not applicable, PASI: psoriasis area and severity index (range 0–72), PsA: psoriatic arthritis, SJC: swollen joint count, TJC: tender joint count.

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Author contributions

E.L., T.R., and M.B. were responsible for conceptualization. Funding acquisition was accounted for by T.R. and E.L. E.L. and M.B. designed the methodology. M.O. and E.L. performed the experiments. M.J., J.P., E.L., and M.B. were primarily responsible for data analysis and writing the original draft. All authors contributed substantially to reviewing and editing the manuscript before submission.

Conflict of interest

T.R.D.J. is employed in and has stock with AbbVie. He had no input in study design or interpretation of its results. The other authors have no conflict of interest or disclosures.

Data availability

The data underlying this article were in part generated through funding by Janssen Inc. Data will be shared on reasonable request to the corresponding author with permission of Janssen Inc.

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