

Expansion of CD161 expressing CD8⁺ single-positive and CD4⁺CD8⁺ double-positive PR3-specific T-cells in granulomatosis with polyangiitis

Sirs,
Granulomatosis with polyangiitis (GPA) is a rare chronic inflammatory disorder of unknown aetiology characterised by chronic granulomatous inflammation and systemic autoimmune vasculitis associated with antineutrophilic cytoplasmic autoantibodies specific for proteinase 3 (PR3-ANCA). Alterations of the peripheral T-cell compartment have been reported in GPA such as the expansion of circulating CD8⁺ single-positive and CD4⁺CD8⁺ double-positive effector memory T-cells within the total CD3⁺ T-cell population (1, 2). Expression of the C-type lectin receptor CD161 identifies a subset of activated T-cells with innate features in inflammatory disorders. CD161 has been suggested to mark tissue-migrating cells (3). In line with this notion, a larger

proportion of intrahepatic CD8⁺ hepatitis C virus (HCV)-specific T-cells expresses CD161 compared to circulating T-cells in chronic hepatitis C (4). Circulating and tissue-resident gluten-specific T-cells also express CD161 suggestive of chronic activation and migratory capacity of such cells in celiac disease (5). So far, CD161 expression has not been investigated on circulating PR3-specific T-cells in GPA.

To address migratory potential and activation of PR3-specific T-cells, we analysed CD161 expression on circulating CD8⁺ single-positive and CD4⁺CD8⁺ double-positive PR3-specific T-cells in HLA-A2+ GPA-patients (n=21) compared to HLA-A2+ healthy controls (n = 21). The GPA patient cohort has been described before (1). CD161 expression on PR3-specific T-cells was compared to that on cytomegalovirus (CMV)- and Epstein Barr virus (EBV)-specific T-cells. Antigen-specific T-cells were detected using peptide / major histocompatibility class (MHC) class I dextramers containing nonapeptide epitopes of PR3₁₆₉₋₁₇₇ (VLQELNVTV), EBV immediate

early BamHI-M leftward reading frame 1 protein BMLF1₂₈₀₋₂₈₈ (GLCTLVAML) and CMV phosphoprotein pp65₄₉₅₋₅₀₄ (NLVPM-VATV). PR3-, EBV- and CMV-specific T-cells were detected following peptide stimulation with the same peptide as contained in the peptide / MHC class I dextramer (1). Statistical analysis was performed using GraphPad Prism (GraphPad Software 8). Values are given as mean ± SD. Comparisons between two groups were performed using Mann-Whitney U-test for categorical variables. Results were considered significant at *p*<0.05.

In the present study, CD161 expressing PR3-specific T-cells were detected both within the CD8⁺ single-positive and CD4⁺CD8⁺ double-positive cell subsets of the total CD3⁺ T-cell population (Fig. 1A). Notably, PR3-specific T-cells within the CD8⁺ single-positive and CD4⁺CD8⁺ double-positive T-cell subsets were more frequently detected in GPA-patients compared to healthy controls (Fig. 1B). CD161 expression was found on a significantly larger fraction of CD8⁺ single-positive and

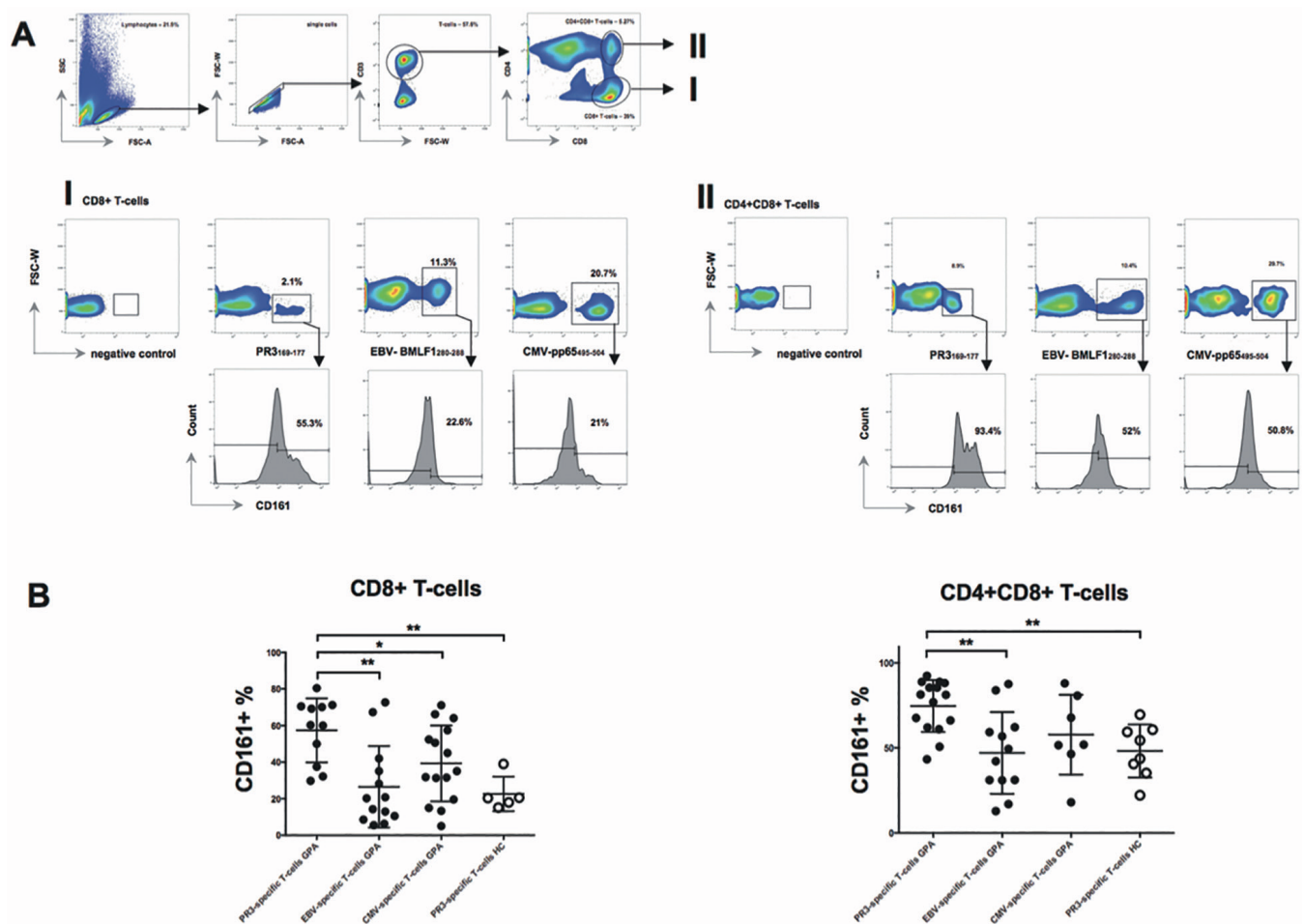


Fig. 1. Expression of CD161 on antigen-specific T-cells. **A:** Gating strategy showing detection of PR3-specific T-cells within CD8⁺ single-positive and CD4⁺CD8⁺ double-positive CD3⁺ T-cells and CD161 expressing cells within CD8⁺ single-positive and CD4⁺CD8⁺ double-positive antigen-specific T-cell subsets. **B:** Percentage of CD161⁺ cells within the CD8⁺ single-positive and CD4⁺CD8⁺ double-positive antigen-specific T-cell subsets.

p*<0.05. *p*<0.01. CMV: cytomegalovirus; EBV: Epstein Barr virus; GPA: granulomatosis with polyangiitis; HC: healthy control; PR3: proteinase 3.

CD4⁺CD8⁺ double-positive PR3-specific T-cells as compared with CMV- and EBV-specific T-cells in GPA (Fig. 1B). There were no differences with regard to the mean fluorescence intensity of CD161 expression between different antigen-specific T-cell subsets (data not shown).

The present study shows an increased percentage of CD161 expressing CD8⁺ single-positive and CD4⁺CD8⁺ double-positive PR3-specific T-cells in GPA. CD161 expression has been shown to identify a subset of activated T-cells with tissue migratory capacity in chronic HCV infection and in celiac disease (3-5). Moreover, CD161 has been implicated as a co-stimulatory molecule in interleukin (IL)-12 and IL-18-driven TCR-independent activation (3). Increased levels of circulating IL-18 and monocytic IL-12 production have also been reported in GPA (6, 7). Alterations of the total peripheral T-cell compartment including the expansion of CD8⁺ single-positive and CD4⁺CD8⁺ double-positive T-cells is driven by antigen and inflammation in GPA (1, 2). Under experimental inflammatory conditions, CD4⁺CD8⁺ double-positive T-cells originate from CD4⁺ and CD8⁺ single-positive cells re-expressing the other co-receptor, *i.e.* CD8 and CD4, respectively (2). Thus, CD161 expression may equip PR3-specific T-cells with additional features for TCR-independent activation and tissue-migration. This may enable PR3-specific T-

cells to eradicate stressed cells even in the presence of low or undetectable amounts of the autoantigen PR3, thereby resembling innate receptor expressing gluten-specific T-cells in coeliac disease (3, 5, 8-10).

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