Increased co-expression of the natural killer cell receptor NKG2D and further natural killer cell receptors on CD4+ T cells in granulomatosis with polyangiitis

Sir, Granulomatosis with polyangiitis (GPA) is a rare chronic inflammatory disorder of unknown aetiology, characterised by necrotising granulomatosis of the upper and/or lower respiratory tract and a systemic autoimmune small-vessel vasculitis preferentially affecting pulmonary and renal vessels. Typically, proteinase 3-specific anti-neutrophil cytoplasmic autoantibodies (PR3-ANCA) are detectable in the blood of patients suffering from this disease. T cells play an important role in the pathogenesis of GPA. T cells in GPA are persistently activated and predominantly show an effector memory phenotype (1, 2). Anomalous expression of activating receptors and concurrent reduced expression of inhibitory receptors has been reported on T cells in GPA (3).

NKG2D (natural-killer group 2, member D) is an activating C-type lectin-like receptor, which is constitutively expressed on CD8+ T cells and natural killer (NK)-cells, but not on CD4+ T cells in healthy individuals. NKG2D differs from other NKG2 members as it apparently lacks an antagonist and thus, inhibition of its signalling (4). Anomalous expression of NKG2D on CD4+ T cells has been described in rheumatoid arthritis and GPA suggestive of altered T-cell regulation (5-7).

In this study we report on the co-expression of NKG2D with further NK receptors on T cells in GPA, i.e. the activating NK receptor NKG2C and the inhibitory receptors CD85j (leukocyte IgG-like receptor) and “killer cell immunoglobulin-like receptor” (KIR) KIR2DL2/D3 (CD158b) on CD4+ and CD8+ T cells. The expression of NKG2D, NKG2C, CD85j and CD158b was assessed by flow cytometry on CD4+ and CD8+ T cells from peripheral blood of 30 GPA patients and 20 healthy controls (HC). The phenotype of the CD4+NKG2D+ T-cell population and the CD4+NKG2D+ T-cell population from GPA patients and human control differed strongly in the expression of the analysed surface receptors. GPA patients showed a significant increase in the percentage of CD4+NKG2D+ T cells expressing the activating receptor NKG2C (14.0±2.4% vs. 0.2±0.1%, mean±SEM, p<0.0001, Mann-Whitney U-test) and the inhibitory receptors CD158b (18.6±3.2% vs. 6.6±2.0%, mean±SEM, p=0.0007, Mann-Whitney U-test) and CD85j (35.5±5.3% vs. 2.7±0.9%, mean±SEM, p=0.0001, Mann-Whitney U-test) (Fig. 1A). In contrast, a significant difference in the CD4+NKG2D+ T-cell population of healthy controls was discovered only for the percentages of NKG2C+ cells (7.4±2.3% vs. 0.1±0.0%, mean±SEM, p=0.001, Mann-Whitney U-test) (Fig. 1B). CD4+NKG2D+ T cells revealed a major proportion of CD28- T cells in comparison to CD4+NKG2D- T cells (Fig. 1A-B). The phenotype of the CD8+NKG2D+ T-cell population was also compared with the CD8+NKG2D- population. The percentages of CD158b+ and CD85j+ cells in both T-cell populations were comparable in GPA patients, whereas a decreased frequency of NKG2C+ cells was found in the CD8+NKG2D- compartment (2.0±5.8% vs. 2.3±5.8%, mean±SEM, p=0.007, Mann-Whitney U-test) (Fig. 1C). In healthy controls there was no difference observed in the frequencies of CD28-, NKG2C+, CD158b+, CD85j+ cells within the NKG2D+ and NKG2D- CD8+ T-cell populations (Fig. 1D). Our results indicate that a significant difference in the co-expression of the activating NK-receptors NKG2C as well as the inhibitory receptors CD158b and CD85j is confined to the CD4+NKG2D+ T-cell population in GPA. Anomalous expression

Table I. Clinical and laboratory characteristics of patients with GPA and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Remission (n=8)</th>
<th>Active (n=22)</th>
<th>Healthy controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; median, range)</td>
<td>62 (27-68)</td>
<td>55 (35-78)</td>
<td>54 (22-68)</td>
</tr>
<tr>
<td>Disease duration (months; median, range)</td>
<td>51 (13-112)</td>
<td>68 (1-266)</td>
<td></td>
</tr>
<tr>
<td>BVAS (median, range)</td>
<td>0</td>
<td>8 (1-21)</td>
<td></td>
</tr>
<tr>
<td>PR3-ANCA positive</td>
<td>8/8</td>
<td>20/22</td>
<td></td>
</tr>
<tr>
<td>RTX/VCY/aza/MTX/LEF</td>
<td>0/0/0/2/1</td>
<td>2/11/1/6/2</td>
<td></td>
</tr>
</tbody>
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BVAS: Birmingham Vasculitis Activity Score (9). Remission was defined as absence of clinical disease activity with a BVAS: 0. Disease activity was defined as a clinical manifestation of new-onset or recurrent disease activity with a BVAS ≥1 requiring intensified immunosuppressive therapy; RTX: rituximab; CYC: cyclophosphamide; AZA: azathioprine; MTX: methotrexate; LEF: leflunomide.
of the activating NKG2D receptor could facilitate cytolytic activity of CD4+ T cells interacting with the NKG2D-ligand MIC (MHC class I chain-related polypeptide) independent of T-cell receptor activation (6, 8). Co-expression of further NK-receptors on CD4+NKG2D+ T cells – as shown in our study – could alter signals transmitted by NKG2D through the interaction of NKG2C as well as CD158b and CD85j with their ligands HLA-E, HLA-C, and HLA-G, respectively, in inflammatory lesions in GPA. Depending on the presence or absence of those ligands in inflammatory lesions, NKG2D-mediated signals would be either up- or down-regulated. Further studies are needed to demonstrate ligand expression other than MIC in inflammatory lesions in GPA (6).

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References