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

Sirs,

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 und 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/DL3 (CD158b) on $CD4^+$ and $CD8^+$ T cells. The expression of NKG2D, NKG2C, CD85j and CD158b was analysed 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.* 0.6±0.2%, mean±SEM, p=0.0007, Mann-Whitney U-test) and CD85j (35.3±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, $p=0.01$, 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 $CD158b^+$ und $CD85j^+$ 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±2.9% *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 in (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

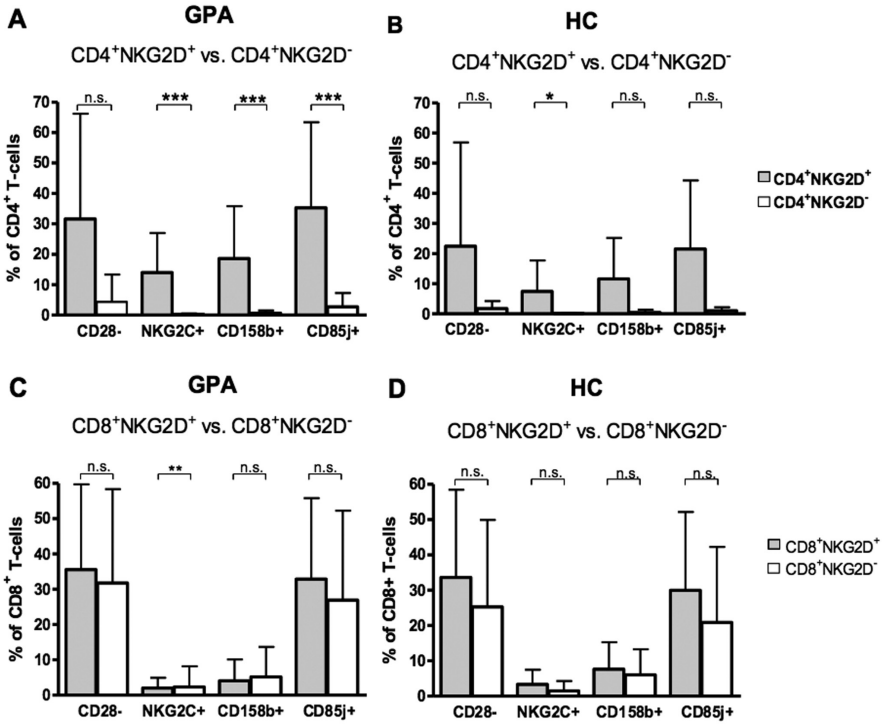

Fig. 1. Increased co-expression of NKG2D and further NK receptors (NKG2C, CD158b, CD85j) within the total $CD4^+$ T-cell population in GPA.

A. Percentages of $CD28^-$, $NKG2C^+$, $CD158b^+$ and $CD85j^+$ T cells in GPA patients (GPA, n=20) within the $CD4^+NKG2D^+$ and $CD4^+NKG2D^-$ T-cell population, respectively, as assessed by flow cytometry. Higher frequencies of NKD2C+ cells were found in GPA.

B. Percentages of $CD28^-$, $NKG2C^+$, $CD158b^+$ and $CD85j^+$ T cells in healthy controls (HC, n=20) within the $CD4^+NKG2D^+$ and $CD4^+NKG2D^-$ T-cell population.

C. Percentages of $CD28^-$, $NKG2C^+$, $CD158b^+$ and $CD85j^+$ T cells in GPA patients (GPA, n=20) within the $CD4^+NKG2D^+$ and $CD4^+NKG2D^-$ T-cell population.

D. Percentages of $CD28^-$, $NKG2C^+$, $CD158b^+$ and $CD85j^+$ T cells in healthy controls (HC, n=20) within the $CD4^+NKG2D^+$ and $CD4^+NKG2D^-$ T-cell population. Mean ± SD, $^*p<0.05$; $^{***}p<0.001$; n.s. = no significance, Mann-Whitney U-test.

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

	Remission (n=8)	Active (n=22)	Healthy controls (n=20)
Sex (male/female)	7/1	13/9	12/8
Age (years: median, range)	62 (27-68)	55 (35-78)	54 (22-68)
Disease duration (months: median, range)	51 (13-112)	68 (1-266)	
BVAS (median, range)	0	8 (1-21)	
PR3-ANCA positive	8/8	20/22	
Steroids yes/no	7/1	20/2	
RTX/CYC/AZA/MTX/LEF	0/0/2/5/1	2/11/1/6/2	

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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