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EXTENDED REPORT

NKG₂D stimulated T-cell autoreactivity in giant cell arteritis and polymyalgia rheumatica

Christian Dejaco¹,² Christina Dufner,¹,³ Juman Al-Massad,⁴ Annette D Wagner,⁴ Joon-Keun Park,⁴ Johannes Fessler,² Ariane Aigelsreiter,⁵ Franz Hafner,⁶ Sandra Vega,⁷ William Sterlacci,⁸,⁹ Beatrix Grubeck-Loebenstein,⁷ Alexander Tzankov,⁸,¹⁰ Thomas Ness,¹¹ Luigi Boiardi,¹² Carlo Salvareni,¹² Michael Schirmer¹

ABSTRACT

Objective To investigate functional expression of NKG₂D on CD4 and CD8 T-cells in patients with giant cell arteritis (GCA) and polymyalgia rheumatica (PMR).

Methods Peripheral blood was drawn from patients with GCA (n=16), PMR (n=78) and healthy controls (HC, n=64). Tissue samples were obtained from GCA patients and controls. Proliferation and cytokine production assays were performed using CFSE and intracellular IFN-γ or TNF-α staining, respectively, and flow cytometry analysis. Immunofluorescence and immunohistology were applied to analyse the presence of NKG₂D-expressing T-cells and NKG₂D-ligands in temporal arteries, respectively. mRNA levels of NKG₂D-ligands were determined by RT-PCR.

Results In both GCA and PMR patients, NKG₂D was preferentially expressed on senescent CD4⁴CD₂⁸⁻ and CD8⁴CD₂⁸⁻, as well as on CD8⁴CD₂⁸⁻ T-cells. Frequencies of senescent T-cells were increased in GCA and PMR patients compared to HC. In GCA tissue samples, infiltrating T-cells were predominately CD₂⁸⁻. NKG₂D expressing T-cells concentrated around the vasa vasorum of the adventitia. Antigenic stimulation induced rapid up-regulation of NKG₂D on CD4⁴CD₂⁸⁻ and CD4⁴CD₂⁸⁻ T-cells, whereas TNF-α and interleukin-15 enhanced NKG₂D expression on senescent CD4 and CD8 T-cells only. NKG₂D cross-linkage augmented anti-CD3 triggered proliferation, IFN-γ and TNF-α production of CD8 T-cells. In CD4⁴CD₂⁸⁻ T-cells, NKG₂D ligation resulted in increased IFN-γ production only. NKG₂D ligands were expressed in temporal arteries from GCA patients, particularly in the adventitial and medial layers of affected vessels.

Conclusions NKG₂D is functionally expressed on CD4⁴CD₂⁸⁻ and CD8⁴CD₂⁸⁻ T-cells in GCA and PMR. NKG₂D-ligands are present in temporal arteries and may co-stimulate NKG₂D expressing T-cells.

INTRODUCTION

Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) are closely related clinical conditions that are currently understood as different states of the same disease.¹–¹³ Both GCA and PMR are a result of an inadequate immune reaction against an unknown auto-antigen present in the adventitia of large vessels.¹ In GCA, T-cells enter the adventitial layer via the vasa vasorum and produce large amounts of pro-inflammatory cytokines, including interferon-γ (IFN-γ).¹ ³ IFN-γ activates macrophages to release metalloproteinases (MMPs), disrupting the media and the internal elastic lamina.¹ ³ In PMR, arterial inflammation is restricted to the adventitia accompanied by an intense systemic immune reaction.⁴ The specific T-cell subsets contributing to inflammation in GCA and PMR as well as the micro-milieu regulating T-cell activity are still unexplored.

We and others described a subset of senescent CD4 T-cells accumulating in patients with chronic inflammatory diseases. These T-cells are characterised by the loss of the co-stimulatory molecule CD28, are autoreactive and apoptosis resistant, and reveal up-regulated alternative (co)-stimulatory receptors.⁵–¹⁰ One of these alternative receptors is NKG₂D, a C-type lectin-like activating natural killer (NK)-cell receptor usually expressed on NK-cells and NK/T-cells. NKG₂D interacts with major histocompatibility class I related chain (MICA), MICB and cyto-megalovirus UL-16 binding proteins (ULBPs) 1–3 that are usually up-regulated on malignant transformation, oxidative stress, and viral or bacterial infections.¹¹–¹⁷

In the present study we investigated functional expression of NKG₂D on CD4 and CD8 T-cell subsets in GCA/PMR. The following observations suggest a possible relevance of this pathway for the pathogenesis of the disease: (1) The prevalence of circulating CD4⁴CD₂⁸⁻ T-cells is increased in GCA/PMR, and in rheumatoid arthritis (RA), NKG₂D was functionally expressed on CD8⁻ T-cells.¹⁵ (2) NKG₂D ligands are up-regulated on viral or bacterial infections and chronic infections have been suspected to trigger GCA/PMR for decades. In some studies, microbial antigens were detected in temporal arteries from GCA patients.¹⁸ (3) MHC class I chain-related gene A (MICA) polymorphisms were associated with the occurrence of GCA and Takayasu’s arteritis in previous studies.¹⁹

METHODS

For a detailed description of the materials and methods, see online supplementary file 1.

Patients, cell preparation and short-term cell lines

After written informed consent was obtained, heparinised blood samples were drawn from
Peripheral veins of consecutive GCA (n=16) and PMR (n=78) patients, diagnosed according to the American College of Rheumatology or Bird’s classification criteria, respectively, as well as healthy controls (HC, n=64).20 21 Patients and controls were comparable regarding age and sex. Clinical data were retrieved by chart review and were available in 11 GCA and 67 PMR patients (see table 1 for clinical characteristics). Analysis of NKG2D expression and correlations with clinical data were performed in this subgroup only. Remission or active disease of GCA and PMR patients was retrospectively determined by a rheumatologist (MS, blinded to experimental data) based on documented history and clinical testing as well as acute phase reactants. The study protocol was approved by the institutional review board of Innsbruck Medical University.

Peripheral blood mononuclear cells (PBMCs) were isolated using magnetic bead labelled anti-CD4 and anti-CD8 monoclonal antibodies (mAbs), and short-term cell lines were established as previously described.5 6

**NKG2D regulation, cytokine production and proliferation assays**

CD4 and CD8 T-cells were stimulated with anti-CD3 (3 μg/ml), interleukin (IL)-15 (20 ng/ml), tumour necrosis factor-α (TNF-α; 20 ng/ml), IL-18 (100 ng/ml) or IFN-γ (10 ng/ml). NKG2D expression (quantified by the mean fluorescence intensity (MFI) using flow cytometry) was tested before and at various time points after anti-CD3 (6, 12, 24 h) or cytokine exposure (24, 48, 72 h) exposure.

For proliferation and cytokine production assays, CD4 and CD8 T-cells were stimulated with OKT3±anti-NKG2D or control immunoglobulin. Stimulation with anti-NKG2D mAb was shown to have comparable effects to MICA tetramers in functional assays.22

For proliferation assays, T-cells were labelled with the fluorescent dye 5-(and -6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) and the percentage of proliferating cells was determined by flow cytometry.23 For cytokine assays, cells were permeabilised and intracellularly stained with anti-IFN-γ, anti-TNF-α, anti-IL-4, anti-IL-10 mAbs or control immunoglobulin. Surface staining was done with CD4, CD8 or CD28 mAbs. The MFI was used to quantify the stimulatory effects of OKT3±anti-NKG2D on cytokine production, because the MFI reflecting the proportion of additional cytokine producing cells as well as enhanced expression in pre-stimulated cells is more sensitive to change than measuring the proportion of new cytokine positive cells only.

**Immunohistochemistry, RT-PCR and ELISA for NKG2D ligands**

For immunohistochemistry, we retrospectively identified nine paraffin-embedded cases of histologically confirmed temporal arteritis, four cases with GCA (physician’s final diagnosis, no formal criteria applied) and negative temporal artery histology as well as seven cases without GCA.

To compare mRNA expression of different NKG2D ligands (MICA, MICB and ULBP1–3) in patients with temporal arteritis, we performed real time (RT)-PCR using fresh-frozen temporal artery specimens from five patients with histologically confirmed GCA.

Soluble levels of MICA were tested in blinded sera in duplicates with an ELISA kit (R&D Systems) according to the manufacturer’s instructions.

**Statistical analysis**

Statistical analysis was performed using SPSS V.15.0. The χ² test, the Student t test, the Mann–Whitney U test or the Wilcoxon test were performed as appropriate.

**RESULTS**

**Circulating CD4CD28− and CD8 T-cells express NKG2D in GCA and PMR patients**

To explore the expression of NKG2D on the cell surface of circulating T-cell subsets in GCA and PMR, flow cytometry studies were performed. In the CD4 T-cell subset, NKG2D was more frequently expressed on CD28− T-cells compared to CD28 T-cells (GCA: median 5.7% (range 0–59.1) vs 0.8% (0.1–4.2), p=0.021; PMR: 15.9% (0–81.7) vs 1.5% (0.3–13.1), p<0.001) whereas almost all CD8 T-cells expressed NKG2D (GCA: CD8CD28− 98.3% (91.3–99.4) vs CD8CD28 97.5% (92.3–99.1), NS; PMR: 97.2% (78.5–99.1) vs 95.8% (78.0–100), NS (figure 1A).

NKG2D was more commonly found on CD8CD28 T-cells from GCA and PMR patients than from HC (91.0% (84.7–97.9), p<0.05), whereas on CD8CD28− and CD4 T-cell subsets similar expression levels were observed in patients and controls.

NKG2D expression was increased on CD4CD28− T-cells from GCA/PMR patients (pooled analysis) with active versus inactive disease, whereas no difference was found regarding CD4CD28− T-cells and CD8 T-cell subsets. NKG2D expression was independent from disease duration; however, patients on high-dose corticosteroid treatment showed higher NKG2D expression on CD8CD28− T-cells than did those on low-dose steroids (see online supplementary figure S1).

The frequencies of CD3CD4CD4CD28− and CD3CD4CD8CD28− T-cells were increased in patients with GCA (4.5% out of CD4 T-cells (range 0.2–30.6), p<0.01 and 40.6% out of CD8 T-cells (5.6–75.3), p<0.01, respectively) and PMR (2.1% (0–28.4),

### Table 1 Patients’ characteristics

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<th>GCA (n=11)</th>
<th>PMR (n=67)</th>
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<tr>
<td>Age* (years)</td>
<td>74.5±12.1</td>
<td>70.1±7.7</td>
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<tr>
<td>Female, n (%)</td>
<td>8 (72.7)</td>
<td>57 (85.1)</td>
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<td>Disease duration† (months)</td>
<td>24.0 (6–43)</td>
<td>6.0 (0–78)</td>
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<td>New diagnosis, n (%)</td>
<td>2 (18.2)</td>
<td>15 (23.8)</td>
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<tr>
<td>ESR (mm/1st hour)†</td>
<td>30 (4–92)</td>
<td>32 (2–84)</td>
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<tr>
<td>CRP (mg/l)†</td>
<td>3.6 (0.1–101.0)</td>
<td>6.2 (0–62.0)</td>
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<tr>
<td>Remission, n (%)</td>
<td>3 (27.3)</td>
<td>18 (27.3)</td>
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<tr>
<td>Prednisone dose* (mg/day)</td>
<td>5.6±5.0</td>
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<td>Patients without corticosteroids, n (%)</td>
<td>2 (18.2)</td>
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<td>DMARDs, n (%)</td>
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<td>Methotrexate</td>
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<tr>
<td>Leflunomide</td>
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Differences were tested by the χ² test (proportions), Student t test (data with normal distribution) or the Mann–Whitney U test (data with non-normal distribution) as appropriate.

*Mean (SD, data with normal distribution).
†Median (range, data with non-normal distribution).

CD3, C-reactive protein (normal values 0–6 mg/l); DMARDs, disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate (normal values 0–10 mm/1st hour); GCA, giant cell arteritis; PMR, polymyalgia rheumatica.
Figure 1  Expression of NKG2D on circulating CD4CD28− and CD8 T-cells from giant cell arteritis (GCA)/polymyalgia rheumatica (PMR) patients and presence of NKG2D+ T-cells in temporal artery biopsies. (A) Surface expression of NKG2D (% positive cells out of CD28 or CD28− subsets as indicated) on T-cell populations in PMR and GCA patients. (B) Prevalence of circulating CD3CD4CD28− and CD3CD8CD28− T-cells in healthy controls, PMR and GCA patients (percentage out of all CD3CD4 T-cells). Data were analysed by flow cytometry and are shown as box-plots. The Mann–Whitney U and Wilcoxon tests were used as appropriate; **p<0.01; ***p<0.001. (C) Representative two-colour immunofluorescence staining of a paraffin-embedded temporal artery biopsy from a patient with histologically confirmed GCA. Cells expressing NKG2D (green), CD3 (red) or both (yellow) are shown. Original magnification ×40.

IL-15 led to an up-regulation of NKG2D on CD4CD28 T-cells and CD3CD8CD28 T-cells did not differ between GCA and PMR patients. No association was found between disease activity, disease duration, prednisone dose and prevalence of CD28 T-cells in GCA and PMR (data not shown).

NKG2D expressing T-cells concentrate around adventitial vasculitis in GCA patients

Immunofluorescence analysis indicated the presence of NKG2D expressing CD3 T-cells in the adventitia and media of temporal arteries from GCA patients as shown in figure 1C. NKG2D expressing T-cells particularly concentrated around the adventitial vasculitis in the adventitium (see online supplementary table S1 for quantification of CD3NKG2D T cells). Most T-cells infiltrating temporal arteries from GCA patients were CD4; ≥99% of CD4 and CD8 T-cells were CD28. As shown in online supplementary figures S2 and S3. In comparison, temporal artery specimens from controls revealed no T-cells within the vessel wall.

NKG2D expression is up-regulated by antigenic stimulation and pro-inflammatory cytokines

To test whether T-cell antigen receptor complex stimulation leads to up-regulation of NKG2D, isolated circulating CD4 and CD8 T-cells were stimulated with cross-linked anti-CD3. Up-regulation of NKG2D on the cell surface of CD4CD28 T-cells was detected already after 6 h of stimulation and the highest NKG2D levels were found after 12 h (figure 2A). CD4CD28 T-cells showed a slight, short-lasting up-regulation of NKG2D after 6 h. Expression of NKG2D on CD8CD28 and CD8CD28 T-cells was not influenced by anti-CD3 stimulation (data not shown).

Cytokine stimulation assays were performed using TNF-α (figure 2B), IL-15 (data not shown), IL-18 (data not shown) and IFN-γ (data not shown). Stimulation of T-cells with TNF-α or IL-15 led to an up-regulation of NKG2D on CD4CD28 but not CD4CD28 T-cells. Besides, TNF-α or IL-15 induced up-regulation of NKG2D on CD8CD28 T-cells whereas only stimulation with TNF-α promoted NKG2D expression of CD8CD28 T-cells. The kinetics of NKG2D up-regulation was much slower by cytokine compared to anti-CD3 stimulation: NKG2D expression reached the highest levels after 24–48 h of cytokine exposure. IL-18 and IFN-γ did not influence NKG2D expression levels on CD4 or CD8 T-cells.

NKG2D co-stimulates pro-inflammatory activity of CD4CD28 and CD8 T-cells

To test whether NKG2D expressed on CD4 and CD8 T-cells is functional, proliferation and cytokine production assays were performed.

Whereas NKG2D stimulation alone did not induce cell division of CD8CD28 T-cells as indicated by the CFSE assay (median percentage of cells with at least one cell division: 3.7 (range 0.8–7.0) and 1.5 (1.0–9.5), respectively), NKG2D cross-linkage augmented anti-CD3 mediated proliferation of CD8CD28 T-cells (50.0% (36.7–86.5) vs 37.0% (15.6–58.9) with anti-CD3 only; p<0.05) and CD8CD28 T-cells (69.9 (51.2–83.1) vs 40.6 (27.3–75.3); p<0.05) (figure 3). In the CD4 T-cell subset, anti-CD3 induced proliferation was not further increased by NKG2D (not shown).

In cytokine production assays intracellular accumulation of Th1-type (IFN-γ and TNF-α) and Th2-type cytokines (IL-4 and IL-10) was tested by flow cytometry. NKG2D cross-linkage augmented anti-CD3 induced IFN-γ production of CD8CD28 T-cells (median MFI 135.4 (range 19.0–190.6) vs 42.3 (11.7–170.6); p<0.05) (figure 4). TNF-α production was increased by anti-CD3 plus anti-NKG2D compared to anti-CD3 alone in CD8CD28 T-cells (median MFI 11.9 (range 7.6–57.9) vs 9.6 (5.8–31.3); p<0.05) and CD8CD28 T-cells (9.8 (5.9–11.6) vs 7.7 (5.2–11.0); p<0.05) whereas the co-stimulatory effect was absent in the CD4 T-cell subset (data not shown). Cross-linking NKG2D did not influence IL-4 and IL-10 production in any CD4 or CD8 T-cell subset (data not shown).

Figure 2. Up-regulation of NKG2D by T-cell antigen receptor complex or cytokine stimulation in giant cell arteritis/polymyalgia rheumatica. Purified CD4 or CD8 T-cells were stimulated with plate-bound anti-CD3 or tumour necrosis factor-α (TNF-α) for different periods. (A) shows the stimulatory effect of anti-CD3 on CD4CD28+ and CD4CD28 T-cells and (B) indicates up-regulation of NKG2D on CD4 and CD8 T-cell subsets after exposure to TNF-α. Box-plots indicate mean fluorescence intensity of NKG2D expression of each T-cell subset. Differences were tested for statistical significance with the Wilcoxon test.

*p<0.05; AU, arbitrary units; h, hours; ns, not significant.

Figure 3. NKG2D co-stimulates pro-inflammatory activity of CD4CD28+ and CD8 T-cells. To test whether NKG2D expressed on CD4 and CD8 T-cells is functional, proliferation and cytokine production assays were performed.

Whereas NKG2D stimulation alone did not induce cell division of CD8CD28 or CD8CD28 T-cells as indicated by the CFSE assay (median percentage of cells with at least one cell division: 3.7 (range 0.8–7.0) and 1.5 (1.0–9.5), respectively), NKG2D cross-linkage augmented anti-CD3 mediated proliferation of CD8CD28 T-cells (50.0% (36.7–86.5) vs 37.0% (15.6–58.9) with anti-CD3 only; p<0.05) and CD8CD28 T-cells (69.9 (51.2–83.1) vs 40.6 (27.3–75.3); p<0.05) (figure 3). In the CD4 T-cell subset, anti-CD3 induced proliferation was not further increased by NKG2D (not shown).

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Expression of NKG2D ligands in temporal arteritis

Immunohistochemistry studies indicated a higher MICA expression in temporal artery samples from patients with histologically confirmed temporal arteritis compared to patients with clinical GCA but negative histology in patients without GCA. Representative images are depicted in figure 5. The most notable finding was a higher MICA staining in the adventitial and medial layers of cases with positive versus negative histology. Moderate MICA expression of endothelial cells of intima and vasa vasorum was observed in most tissue specimens regardless of inflammation (see online supplementary table S2 for details). Inflammatory cells (lymphocytes and giant cells) of patients with temporal arteritis also stained positively for MICA.

RT-PCR was performed to compare the expression of NKG2D ligands MICA, MICB and ULBP1–3 in temporal arteritis. As depicted in online supplementary figure S4, median MICA mRNA concentrations were 5.0–20.2-fold higher compared to the other ligands tested. In addition, ULBP1–3 mRNAs were inconstantly expressed (no detectable mRNA in 2/5 (ULBP1) and 1/5 (ULBP2 and ULBP3) temporal artery samples).

Serum levels of soluble MICA in GCA, PMR and HC

Levels of soluble MICA were tested in blinded serum samples from GCA (n=10) and PMR patients (n=70) as well as HC (n=38). The serum concentrations of soluble MICA were similar in GCA (36.3 pg/ml; range 0–341), PMR (77.3;
We were unable to perform CD3^+ NKG2D+ T-cells in peripheral blood of patients with GCA and PMR. NKG2D on T-cells was functional, promoting the production of cytokines, the (3H)thymidine proliferation assay and the utilisation of isolated CD4CD28^− T-cells in GCA/PMR versus RA patients, however, cannot be explained by technical reasons only. Given that NKG2D^+ CD4CD28^− T-cells (and the corresponding NKG2D ligands) are present in the adventitia of GCA lesions, these cells may preferentially secrete IFN-γ to promote vessel wall destruction in temporal arteritis. In contrast, the predominant

0–3293.3) and HC (93.2; 0–3214.4). Soluble MICA concentrations were negatively correlated with the prednisone dose (corrcoeff =−0.47, p=0.002), but were not associated with disease activity or disease duration.

**DISCUSSION**

Our results indicate that NKG2D receptor-expressing CD4CD28^− and CD8 T-cells contribute to the pathogenesis of GCA and PMR. We found NKG2D^+ T-cells and their corresponding ligands in the vessel wall of GCA patients and detected circulating NKG2D^+ T-cells in peripheral blood of patients with GCA and PMR. NKG2D on T-cells was functional, promoting the production of pro-inflammatory cytokines known to be involved in the pathogenesis of these diseases.

NKG2D^+ T-cells concentrated around the vasa vasorum of the adventitial layer in GCA patients, suggesting that these cells enter from peripheral blood to the site of inflammation. Alternatively, they mature locally, up-regulating NKG2D. The site of primary T-cell stimulation is located around the vasa vasora and we showed in our in vitro experiments that antigenic stimulation promotes NKG2D expression of CD4 T-cells. We were unable to perform CD3−CD4/8−NKG2D^+CD28^− multiple stains of tissue sections allowing a detailed characterisation of NKG2D expressing T-cells; however, our sequential and double stainings (see online supplementary figure S3) reveal a predominance of CD4CD28^− T-cells within the inflammatory aggregates around the vasa vasora, whereas CD8CD28^− T-cells occur less commonly. CD28 T-cells are rarely present in GCA lesions. Extrapolating the data from peripheral blood analysis, we hypothesise that most NKG2D^+ T-cells present in temporal arteries of GCA patients are CD4CD28^−. In contrast, most peripheral NKG2D^+ T-cells are CD8, because almost all CD8 but only senescent CD4 T-cells express NKG2D.

The data from our stimulation assays indicate different functional roles of NKG2D for CD4 and CD8 T-cell subsets in GCA/PMR. NKG2D cross-linkage promoted proliferation of CD28CD8 and CD28^−CD8 T-cells, whereas this effect was not observed in CD4CD28^− T-cells. Besides, NKG2D augmented TNF-α production of CD8 T-cell subsets only, whereas IFN-γ production was stimulated in CD8CD28^−, CD8CD28 and CD4CD28^− T-cells. Our in vitro data partially contrast previous observations in RA, in which cell division as well as IFN-γ and TNF-α production of CD4CD28^− T-cells was enhanced by NKG2D ligation. Methodical issues may account for the divergences: ELISA detection of cytokines, the (3H)thymidine proliferation assay and the utilisation of isolated CD4CD28^−NKG2D^+ T-cells in the previous study might have been more sensitive than our approaches. The different effects of NKG2D on IFN-γ and TNF-α production by CD4CD28^− T-cells in GCA/PMR versus RA patients, however, cannot be explained by technical reasons only. Given that NKG2D^+ CD4CD28^− T-cells (and the corresponding NKG2D ligands) are present in the adventitia of GCA lesions, these cells may preferentially secrete IFN-γ to promote vessel wall destruction in temporal arteritis. In contrast, the predominant
cytokine in the RA synovium is TNF-α released by CD4CD28− T-cells after NKG2D stimulation.15,27

NKG2D expression of circulating CD4CD28− T-cells was higher in GCA/PMR patients with active versus inactive disease but was not influenced by disease duration or therapy. The clinical relevance of NKG2D up-regulation on CD8CD28 T-cells by corticosteroids is probably small given the high expression of this receptor on CD8CD28 T-cells in general.

Our in vitro data indicate a preferential up-regulation of NKG2D on CD4CD28+ T-cells by T-cell receptor stimulation as well as TNF-α and IL-15, supporting the observed clinical association between disease activity and NKG2D expression on this subset. IL-18 and IFN-γ had no effect in vitro, which is consistent with previous reports on NK-cells.28 Cross-linkage of CD3 resulted in a more rapid, but shorter lasting up-regulation of NKG2D compared to stimulation with cytokines. The different kinetics were explained by rapid NKG2D surface expression due to redistribution of intracellular proteins by anti-CD3 compared to delayed receptor appearance by induction of NKG2D mRNA by cytokines.15 CD8 T-cells may contribute to the regulation of NKG2D surface expression because they produce large amounts of TNF-α once they are activated by T-cell receptor plus NKG2D stimulation.

The presence and role of NKG2D ligands in vascular lesions of GCA patients has not been investigated so far: we found higher expression of MICA in temporal arteritis specimens compared to probes with normal histology, suggesting that NKG2D ligands may perpetuate the autoimmune response in temporal arteritis. MICA appears to be the most relevant NKG2D ligand as indicated by RT-PCR results. Using immunohistochemistry, MICA was predominately detected in the adventitia and media of inflamed vessels, whereas in Takayasu’s arteritis MICA was preferentially expressed on vascular smooth muscle cells.29 Interestingly, lymphocytes and giant cells also positively stained for MICA, suggesting that inflammatory cells might co-stimulate NKG2D expressing T-cells in a cell to cell contact dependent manner. Previous studies have already demonstrated an up-regulation of MICA on peripheral lymphocytes on T-cell receptor activation.30–31 Another notable observation is the expression of MICA on endothelial and smooth muscle cells in the majority of samples (even in those without inflammation). Apart from stress-induced up-regulation of MICA due to the surgical procedure, our results could explain the high susceptibility of temporal arteries to inflammation. Future studies (eg, using animal models of GCA) should be performed to clarify these issues.

Serum levels of soluble MICA did not differ between GCA, PMR and HC and were much lower compared to levels previously reported in RA.15 This finding is surprising because matrix MMPs are known to induce shedding of NKG2D ligands32–33 and MMPs are also involved in the pathogenesis of GCA, leading to the fragmentation of the interstitial elastic lamina.3 25 This observation could be explained by the fact that most patients were treated with corticosteroids. We found a negative correlation between the prednisone dose and MICA concentrations, and corticosteroids are known to reduce MMP activity.34–35 In addition, genetic variants of MICA associated with GCA, PMR, RA, other autoimmune diseases and carcinoma might have different shedding susceptibilities to MMPs.36–42

In summary, our data indicate that the activating NK-cell receptor NKG2D is functionally expressed on circulating CD4CD28− and CD8 T-cells in patients with GCA and PMR. NKG2D ligands in temporal arteries may co-stimulate NKG2D expressing T-cells in GCA and perpetuate the chronic inflammatory process.

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