Concise report

Anti-NKG2A autoantibodies in a patient with systemic lupus erythematosus

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Abstract

Objectives. To characterize a novel anti-NKG2A autoantibody detected in a patient with SLE during a severe flare, and in a cross-sectional study investigate the occurrence of such autoantibodies in patients with SLE and primary SS (pSS).

Methods. Serum or IgG from patients with SLE, pSS and healthy volunteers were assayed for blocking of anti-NKG2A or HLA-E binding to peripheral blood mononuclear cells or CD94/NKG2A- and CD94/NKG2C-transfected Ba/F3 cells. The anti-NKG2A autoantibodies were evaluated for effect on NK cell degranulation in response to HLA-E-transfected K562 cells. IFN-α was determined by an immunoassay and disease activity by the SLEDAI score.

Results. Anti-NKG2A autoantibodies, which blocked binding of HLA-E tetramers to CD94/NKG2A-transfected cells and impaired NKG2A-mediated inhibition of NK cell activation, were observed in a patient with SLE. The presence of anti-NKG2A autoantibodies was associated with high SLE disease activity (SLEDAI score 14 and 16) and increased serum IFN-α. Of 94 SLE, 60 pSS and 30 healthy donor sera, only the index patient serum contained anti-NKG2A autoantibodies.

Conclusion. The presence of autoantibodies targeting NKG2A is a rare event, but when such autoantibodies occur they may promote excessive NK cell function. This can contribute to the pathogenesis by increasing the killing of cells and the release of autoantigens. Our findings highlight the possible importance of NK cells in the SLE disease process.

Key words: systemic lupus erythematosus, autoantibodies, autoimmunity, Sjögren’s syndrome, NKG2A receptor, HLA-E.

Introduction

SLE is an autoimmune disease characterized by immune complex (IC) formation and ongoing IFN-α production. The source of IFN-α is considered to be plasmacytoid dendritic cells (pDCs) activated by ICs containing autoantibodies and nucleic acids [1]. We recently demonstrated that NK cells can strongly promote IFN-α production by pDCs [2, 3], but the molecular mechanisms regulating the crosstalk between NK cells and pDCs in SLE remain poorly characterized.

SLE patients may have a reduced number of NK cells in circulation, but they are functionally competent and display an activated phenotype [4–6]. Moreover, NK cells from patients with active SLE produce more IFN-γ upon stimulation with exogenous cytokines, and the frequency of IFN-γ+ NK cells correlates with serum IFN-α levels [6]. Together, these observations suggest a connection between alterations in NK cells and the excessive IFN-α production in SLE.

In the present study, we identified anti-NKG2A autoantibodies in a patient with SLE during a severe flare. These autoantibodies efficiently blocked the binding of HLA-E tetramers to the heterodimeric CD94/NKG2A receptor complex, resulting in impaired CD94/NKG2A-mediated inhibition of NK cell cytotoxicity against HLA-E-expressing target cells. Two cohorts of patients with SLE or primary SS
(pSS) were screened for anti-NKG2A autoantibodies. The potential role of anti-NKG2A autoantibodies in the disease process is discussed.

Patients and methods

Case report

A 23-year-old man was admitted to Uppsala University hospital with a severe flare of SLE. The patient had a history of alopecia areata at the age of 5 years and was diagnosed with SLE at the age of 16 years. The patient responded to HCQ and prednisolone treatment. At 20 years of age, the patient developed small vessel vasculitis with digital ulcers and a year later alveolitis and deep venous thrombosis followed by lung embolism. APS was diagnosed and the patient received high doses of prednisolone, AZA and warfarin. Despite this treatment and cyclosporine or MTX, the disease worsened.

At admission, the patient presented with vasculitis and deep venous thrombosis in the left leg, inflammatory skin rashes, proteinuria where biopsy showed glomerulonephritis (WHO VB) and progressive alveolitis with a diffusion capacity reduced by 50%. An ANA titre of 1/800 and the presence of anti-SM, anti-RNP and cryoglobulinaemia was noted together with prominent classic complement pathway activation. The patient had a positive Coomb test, but normal haemoglobin levels and leucocyte counts. Low-grade proteinuria (~0.5 g/day) and normal GFR were scored. Dalteparin was started and plasmapheresis x3 was instituted, followed by i.v immunoglobulin treatment (0.4 g/kg body weight ×5) and subsequent CYC therapy (100 mg/day). The patient went into remission and CYC was replaced by MMF (500 mg bid). Ten years later the patient has low-grade proteinuria (0.5 g/day) and remains in remission. The study was approved by the local ethical committee at Uppsala University. Informed consent was obtained according to the Declaration of Helsinki from all patients and controls.

Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy individuals by density gradient centrifugation. The murine Ba/F3 cell lines, transfected with human CD94/NKG2A, CD94/NKG2C/FLAG-DAP-12 or NKG2D/GFP-DAP10 [7, 8], were kindly provided by Dr Lewis Lanier (University of California San Francisco, San Francisco, CA, USA). An HLA-E-transfected K562 cell line was kindly provided by Dr Jakob Michaelsson (Karolinska Institute, Stockholm, Sweden). HLA-E expression on K562 cells was stabilized by incubation with 10 mM of the HLA-G signal peptide (VMAPRTLFL) in OPTI-MEM. K562 cells were transfected with 1 mg/ml IgG at 37°C for 30 min and thereafter stained as specified. To detect IgG binding or blocking of HLA-E tetramer binding, 5 × 10⁴ Ba/F3 transfectants were resuspended in PBS containing 10% FBS and purified IgG or sera for 30 min at 4°C. After washing, bound IgG was detected using PE-labelled donkey-F(ab')₂ anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), whereas blocking of HLA-E tetramer binding was detected using PE-labelled HLA-E tetraramers.

To evaluate NK cell responses, 2 × 10⁶ PBMCs were incubated 30 min with 0.5–1 mg/ml IgG, followed by coincubation with HLA-E-transfected K562 cells, pulsed with or without peptide, for 1 h and analysed as described [10]. Flow cytometry data were acquired on FACS Canto II or LSR Fortessa (BD Biosciences) and analysed using FlowJo (Treestar, Ashland, OR, USA).

Serum IFN-γ

Serum IFN-γ was determined by an immunoassay as previously described [11].

Statistical analysis

Differences in NK cell degranulation following pre-incubation with IgG was assessed with Wilcoxon signed-rank test using Prism (GraphPad Software, San Diego, CA, USA).

Results

Anti-NKG2A autoantibodies in an SLE patient

Serendipitously, we found that pre-incubation of PBMCs from healthy individuals with IgG from an SLE patient (index patient) abolished anti-NKG2A (clone Z199) staining on NK cells and T cells (Fig. 1A and B, data not shown). The CD94/NKG2A heterodimer is an inhibitory receptor expressed on subsets of NK cells and CD8⁺ T cells [9]. CD94/NKG2A serves as a gauge of classical HLA molecule expression on target cells through binding to HLA-E that is stabilized by signal peptides derived from classical HLA molecules. In contrast, CD94/NKG2C is a closely related activating receptor complex that displays considerably lower affinity for HLA-E with HLA-derived signal peptides, but has some specificity for HLA-E with cyto-megalovirus-derived peptides [12]. The loss of anti-NKG2A (Z199) binding was not sensitive to inhibition of endocytosis by the Src-family kinase inhibitor PP2 and occurred within minutes (data not shown), suggesting that the loss of anti-NKG2A binding was the result of
competitive binding by serum proteins rather than receptor down-regulation. Establishing the presence of anti-NKG2A autoantibodies, CD94/NKG2A- but not CD94/NKG2C- or NKG2D-transfected cells detected by flow cytometry using a fluorescently labelled anti-human IgG antibody. (D) Binding of fluorescently labelled HLA-E tetramer to CD94/NKG2A- or CD94/NKG2C-transfected Ba/F3 cells, as determined by flow cytometry. (E) CD94, NKG2A and NKG2C stainings of PBMCs from healthy volunteers incubated with indicated IgG.

Together, the results reveal the presence of anti-NKG2A autoantibodies in the serum of the index patient able to block binding of CD94/NKG2A heterodimers to HLA-E.

Anti-NKG2A autoantibodies antagonize NKG2A-mediated inhibition of NK cell activation

Because CD94/NKG2A inhibits NK cell cytotoxicity, we evaluated the effect of patient IgG on NK cell activation. Degranulation of NK cells against K562 cells, with or without peptide-loaded HLA-E, was quantified as CD107a mobilization on NK cells. As index patient autoantibodies competed with Z199 mAb for binding to NKG2A, we specifically analysed degranulation of CD3 CD56bright NK cells (where most cells express CD94/NKG2A) as well as CD3 CD56dimNKG2C+ NK cells. IgG from the index patient, but not healthy donor IgG or IgG from another SLE patient without anti-NKG2A autoantibodies, abrogated HLA-E/peptide-dependent CD94/NKG2A-mediated inhibition of NK cells. In contrast, NKG2C-mediated activation, as assessed on CD3 CD56dimNKG2C+ NK cells, was not perturbed (Fig. 2A and B). Thus the anti-NKG2A autoantibodies specifically impaired CD94/NKG2A-mediated inhibition of NK cell activation by target cells expressing HLA-E.
Comparison between disease activity and the presence of anti-NKG2A autoantibodies

Next we investigated whether the presence of anti-NKG2A autoantibodies followed disease activity in the index patient. Blocking of anti-NKG2A (Z199) binding to NK cells and IFN-α concentration were determined in sera from four different time points and compared with the SLE disease activity index (SLEDAI) score. Interestingly, the two sera with high anti-NKG2A (Z199)-blocking capacity contained increased IFN-α and were sampled during high disease activity, whereas sera obtained during remission lacked anti-NKG2A-blocking activity and detectable IFN-α (Fig. 2C).

Prevalence of anti-NKG2A autoantibodies

Finally, to evaluate the prevalence of anti-NKG2A autoantibodies among patients with SLE and the aetiologically related disease pSS, we screened 94 SLE, 60 pSS and 30 healthy donor sera for their capacity to block HLA-E tetramer binding to CD94/NKG2A-transfected cells. Only
serum from the index patient interfered with HLA-E binding (Fig. 2D).

Discussion

In this report we describe for the first time a patient with SLE who, during a disease flare, harboured anti-NKG2A autoantibodies. These autoantibodies specifically blocked the interaction of HLA-E with CD94/NKG2A, without affecting binding to CD94/NKG2C. Consequently the anti-NKG2A autoantibodies from the index patient prevented NKG2A-mediated inhibition of NK cell activation by target cells expressing HLA-E, whereas NKG2C-mediated activation was unperturbed.

More than 100 autoantibodies have been described in SLE, some are frequent and correlate with disease activity (e.g. anti-dsDNA), some are associated to disease manifestations (e.g. phospholipid antibodies and thrombosis), whereas others are only described in a few patients [13]. We found anti-NKG2A autoantibodies in just one SLE patient, and only during a disease flare, suggesting that anti-NKG2A autoantibodies are rare. Nonetheless, given the association with active SLE characterized by severe tissue necrosis in this patient, it is tempting to speculate on the role of anti-NKG2A autoantibodies in the disease process. Beside anti-NKG2A autoantibodies, the patient harboured anti-SM and anti-RNP autoantibodies, which together with RNA-containing autoantigens, released from cells during excessive necrosis and apoptosis, can form interferon-inducing ICs [1]. Thus the possible link between the presence of anti-NKG2A autoantibodies, tissue necrosis, high serum IFN-α levels and increased disease activity is intriguing.

Anti-NKG2A autoantibodies may augment the type I IFN system activation in different ways. First, blockade of CD94/NKG2A inhibition may increase the killing of autologous cells, releasing autoantigens that create IFN-inducing ICs. Second, blockade of CD94/NKG2A inhibition may impact NK cell–pDC interactions, promoting IFN-α production by pDCs. However, the addition of anti-NKG2A antibodies to cell cultures with pDCs and NK cells stimulated with interferogenic ICs did not augment IFN-α production (results not shown).

Anti-NKG2A autoantibodies may also promote antibody-dependent cellular cytotoxicity or complement-mediated elimination of CD94/NKG2A-expressing NK cells. Since CD94/NKG2A+ NK cells exert immunoregulatory functions, depletion of these cells may exacerbate autoimmune reactions [14]. Based on findings in mice that activated CD4+ T cells are protected from NK cell lysis by CD94/NKG2A interactions and antibody-mediated blockade of such interactions can augment NK-mediated killing of pro-inflammatory CD4+ T cells, the use of blocking anti-NKG2A antibodies for treatment of autoimmune diseases has been postulated [15, 16]. The exact consequences for the human immune system of autoantibodies targeting NKG2A remain to be established, not least in SLE.

In conclusion, the present report describes a novel autoantibody in SLE that may affect immune cell interactions involved in the disease pathogenesis, possibly contributing to both the disease process and clinical manifestations.

Rheumatology key messages

- We describe a novel autoantibody targeting NKG2A in a patient with SLE.
- The anti-NKG2A autoantibody inhibited HLA-E binding and could contribute to the pathogenesis of SLE.

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