Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY ORIGINAL ARTICLE

Increased proportion of CD56^{bright} natural killer cells in active and inactive systemic lupus erythematosus

Danika Schepis,[1] Iva Gunnarsson,[2] Maija-Leena Eloranta,[3] Jon Lampa,[2] Stefan H. Jacobson,[4] Klas Kärre[1] and Louise Berg[1]

[1]*Department of Microbiology, Tumour and Cell Biology, Strategic Research Center IRIS, Karolinska Institutet, Stockholm,* [2]*Department of Medicine, Unit of Rheumatology, Karolinska Institutet, Stockholm,* [3]*Department of Medical Sciences, Systemic Autoimmunity Group, Uppsala University, Uppsala, and* [4]*Department of Nephrology, Danderyd University Hospital, Stockholm, Sweden*

doi:10.1111/j.1365-2567.2008.02887.x
Received 31 January 2008; revised 11 May 2008; accepted 15 May 2008.
Correspondence: Dr D. Schepis, Department of Microbiology, Tumour and Cell Biology, Box 280, Karolinska Institutet, 17177 Stockholm, Sweden.
Email: danika.schepis@ki.se
Senior author: Louise Berg, email: louise.berg@ki.se

Summary

Natural killer (NK) cells belong to the innate immune system but can also affect adaptive immune reactions. This immune regulatory function is often ascribed to the CD56^{bright} subpopulation of NK cells that is prevalent in secondary lymphoid tissues and has potent cytokine-producing ability. The NK cells have been described as affecting autoimmune disease and stimulating B-cell production of antibodies, but their role in systemic lupus erythematosus (SLE) pathology has not been extensively studied. We have studied NK cells in SLE, a B-cell-driven systemic autoimmune disease, and phenotypically characterized peripheral blood NK cells in comparison to NK cells from patients with immunoglobulin A nephritis, rheumatoid arthritis and healthy individuals. We have found an increased proportion of CD56^{bright} NK cells in SLE, regardless of disease activity. We detected a somewhat increased expression of the activating receptor NKp46/CD335 on NK cells from SLE patients, although neither the percentage of NK cells of all lymphocytes nor the expression of other NK receptors analysed (LIR-1/CD85j, CD94, NKG2C/CD159c, NKG2D/CD314, NKp30/CD337, NKp44/CD336, CD69) differed between patient groups. We show that type I interferon, a proinflammatory cytokine known to be abundant in SLE, can cause increases of CD56^{bright} NK cells *in vitro*. We confirmed that serum levels of interferon-α were increased in active, but not in inactive, disease in the SLE patient group. In conclusion, we found an increased proportion of CD56^{bright} NK cells in the blood of SLE patients, although it remains to be examined whether and how this relates to the disease process.

Keywords: CD56^{bright} natural killer cell; natural killer cells; systemic lupus erythematosus; type I interferon

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease affecting many organs, including kidneys and skin. SLE is characterized by many immunological abnormalities such as hyperactive B cells and overproduction of type I interferons (IFN, reviewed by Rönnblom *et al.*).[1] Autoantibodies detectable in SLE are primarily specific for nuclear antigens that are possibly exposed to the immune system as the result of disease-associated defects in the clearance of apoptotic cells. The resulting antigen–antibody immune complexes accumulate in the skin and kidneys and cause inflammatory reactions including IFN responses,[2] possibly through activation via Toll-like receptors.[3] Interferon is considered central to innate immune activation and causes, among many other things, the activation of dendritic cells increasing their ability to promote subsequent adaptive immune responses.

Natural killer (NK) cells are lymphocytes belonging to the innate immune system. They mediate early protection against infections and lyse transformed cells and cells coated with antibody. They also secrete cytokines, predominantly IFN-γ.[4] In humans, NK cells are defined by expression of CD56 and lack of CD3 and make up approximately 10% of peripheral blood lymphocytes. There are two major subpopulations of human NK cells, the CD56^{dim} NK cells, which express killer immunoglobulin-

like receptors (KIR) and CD16 (FcγRIIIa), and the CD56^{bright} NK cells which lack expression of KIR and CD16 and which express the high-affinity interleukin-2 (IL-2) receptor α subunit CD25. These two sets of human NK cells differ in their function and distribution. The CD56^{dim} NK cells make up approximately 90% of blood NK cells and have a high cytolytic capacity, while the CD56^{bright} NK cell is the main NK cell type found in secondary lymphoid tissue and at sites of inflammation. It is conceivable that CD56^{bright} NK cells may affect autoimmune diseases by promoting T-cell activation in lymphoid tissues[5] and subsequently B-cell responses. It is also possible that NK cells may play a more direct role in promoting B-cell responses during SLE, in part through CD40/CD154 interactions between these cell types.[6,7]

We set out to analyse phenotypic abnormalities in peripheral blood NK cells from patients with SLE. As control groups, healthy donors and two other groups of patients affected by inflammatory disorders, IgA nephritis (IgAN) and rheumatoid arthritis (RA), were included. We found an increase in the proportion of CD56^{bright} NK cells in patients with SLE regardless of disease activity. Furthermore, type I IFN could induce increased proportions of CD56^{bright} NK cells *in vitro*. Levels of IFN-α were elevated in the patients with SLE in our study, although only in those with active disease.

Materials and methods

Patients

Twenty-three female patients (mean age 44 years, range 19–80) with SLE from the Department of Rheumatology at the Karolinska University Hospital were included in the study; their informed consent was obtained. All patients fulfilled at least four of the American College of Rheumatology (ACR) criteria for SLE.[8] Disease manifestations, ongoing immunosuppressive therapy and routine laboratory analyses, including analysis of anti-double-stranded DNA antibodies (fluorescent enzyme immunoassay, normal < 10 U/ml), were recorded.

At the inclusion visit, disease activity was estimated using the SLE Activity Measurement (SLAM) and a score of 7 or more was defined as active disease. Of the 23 patients with SLE, three were sampled twice, once during active and again during inactive phases of disease. For patient characteristics see Table 1.

As control populations, 10 patients with biopsy-proven IgAN (seven men/three women, mean age 42 years, range 19–66), 13 patients with RA (four male/nine female, mean age 54, range 29–74, all fulfilling ACR criteria for RA;[9] none treated with methotrexate, one with myocrisine, two patients had no disease-modifying drugs; seven patients had prednisolone doses below 10 mg/day; no patient was being treated with or had earlier been given biologicals) and 20 healthy controls (10 men/10 women, mean age 44 years, range 25–65) were included.

All patients gave their informed consent to participate and the study was approved by the local ethical committee.

Fluorescence-activated cell sorting (FACS) staining of patient samples

Peripheral blood mononuclear cells (PBMC) were separated by density centrifugation and stained for cell surface markers within 4 hr of sampling. Cells were multi-colour stained using monoclonal antibodies specific for: CD3 (cone SK7), CD56 (clone B159 or NCAM16.2), CD16 (clone NKP15) and CD94 (clone HP-3D9, all directly conjugated and from BD Biosciences, San José, CA), NKp30/CD337 (clone Z25, Beckman Coulter, Marseille, France), NKp44/CD336 (clone Z231, Beckman Coulter), CD69 (clone FN50, Dako, Glostrup, Denmark), NKG2D/CD314 (clone 149810, R&D Systems, Minneapolis, MN), NKG2C/CD159c (clone 134591, R&D Systems), NKp46/CD335 (kindly provided by Marco Colonna, Washington University, St Louis, MO) and LIR1/CD85j (clone M405, kindly provided by Amgen Inc., Thousand Oaks, CA). The unconjugated antibodies were detected by phycoerythrin-conjugated polyclonal goat anti-mouse IgG (Dako). As negative control, mouse IgG1 was used (Dako). The NK cells were defined as CD3$^-$ CD56$^+$ lymphocytes and CD56^{bright} NK cells were defined as CD3$^-$ CD56$^+$ CD16$^-$ lymphocytes.

In vitro *stimulation with cytokines*

PBMC from healthy blood donors were grown in Iscove's modified Dulbeccos' medium (IMDM; Sigma-Aldrich, St Louis, MO) supplemented with antibiotics, L-glutamine, non-essential amino acids, β-mercaptoethanol, sodium pyruvate (all from Sigma-Aldrich), 5% inactivated human serum, 5% inactivated fetal calf serum (Sigma-Aldrich, IMDM complete), in the presence or absence of the following recombinant human cytokines: IL-2 (200 U/ml, PeproTech, London, UK), IL-15 (20 ng/ml, PeproTech) and universal type I IFN (1000 U/ml, PBL, Piscataway, NJ). After 3 days of culture the cells were stained for CD3, CD56 and CD16. As a negative control, phycoerythrin-conjugated IgG1 (BD Biosciences) was used. To determine the percentage of dead cells, cultured cells were stained using 7-amino-actinomycin D (7AAD; BD Biosciences) in combination with antibodies specific for CD3, CD56 and CD16, followed by analysis by flow cytometry. To delineate the degree of proliferation of CD56^{dim} and CD56^{bright} NK cells in response to cytokines, bromodeoxyuridine (BrDU) was added on day 2 of culture and PBMC were stained with monoclonal antibodies specific for BrDU in combination with CD3, CD56 and CD16, and analysed by flow cytometry. The NK cell function was analysed by adding 0·5 million

Table 1. Patient characteristics

Age (years)	Present disease manifestations	DMARD	Pred. (mg/day)	Anti-dsDNA (U/ml)	SLAM score
Active SLE patients					
39[1]	Nephritis, arthritis	None	5	68	8
26	Skin, haematological	None	6·25	251	14
56	Arthritis	AZA	10	ND	13
34	Nephritis, arthritis	Chlorambucil	15	139	9
43	Nephritis, skin	None	5	> 400	26
52	Nephritis	None	10	314	8
64	Haematological	None	5	ND	12
19	Nephritis	AZA	20	ND	16
29	Nephritis, lymphadenopathy	None	15	> 400	25
23[1]	Nephritis, APS	None	0	65	9
47	Skin, haematological, fever, nephritis		30[2]	> 400	16
36	Arthritis, mental depression	AZA	ND	56	12
37[1]	Nephritis	MMF	8·75	52	9
45	Nephritis	None	7·5	15	10
44	Haematological, hair loss, musculoskeletal	AZA, AM	5	Neg	15
39			**8·1**	**104**	**12**
Inactive SLE patients					
38	Inactive	AZA, AM	2·5	Neg	1
59	Inactive	None	6	Neg	2
69	Inactive	AZA	4	Neg	4
39	Inactive	AM[2]	0	Neg	4
35	Inactive	None	0	110	6
80	Inactive	None	0	Neg	4
58	Inactive	None	0	ND	4
44	Inactive	None	0	Neg	1
39[1]	Inactive	MMF	25	24	6
23[1]	Inactive	None	20	28	6
37[1]	Inactive	MMF	10	ND	5
47			**6**	**Neg**	**4**

Medians shown below columns in bold types.

AM, antimalarials; APS, antiphospholipid syndrome; AZA, azathioprine; DMARD, disease modifying anti-rheumatic drug; MMF, mycophenolate mofetil; ND, not determined; Pred., prednisolone; SLAM, systemic lupus erythematosus activity measurement; SLE, systemic lupus erythematosus.

[1]Patients analysed during active and inactive SLE.

[2]Recent onset.

K562 cells to PBMC cultures grown with medium or in the presence of IL-2, IFN or a combination of IFN and IL-2 at day 2 of culture. After 20 hr, GolgiStop (BD Biosciences) was added, and cells were stained intracellularly for IFN-γ after 4 hr, in combination with antibodies for CD3, CD56 and CD16.

Measurements of IFN-α in serum

To detect IFN-α levels in serum, a dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) with anti-IFN antibodies LT27:273 and LT27:293 was used as described elsewhere.[10]

Statistical analysis

Differences were calculated using analysis of variance (ANOVA) combined with a Tukey post hoc test. For comparisons between two groups of patients, the Student's *t*-test was used. Data in the text are given as median (range).

Results

Patients with SLE have an increased proportion of CD56^{bright} NK cells in blood

To determine whether NK cells play a role in the pathogenesis of SLE, we decided to analyse the phenotype of peripheral blood NK cells in this patient group, and compare it to that of healthy controls. As additional control groups with chronic inflammatory conditions, patients with IgAN and RA were included. These patients have an organ-specific, B-cell-driven autoimmune disease and a systemic T-cell-driven inflammatory disease, respectively. There were no statistically significant differences in the

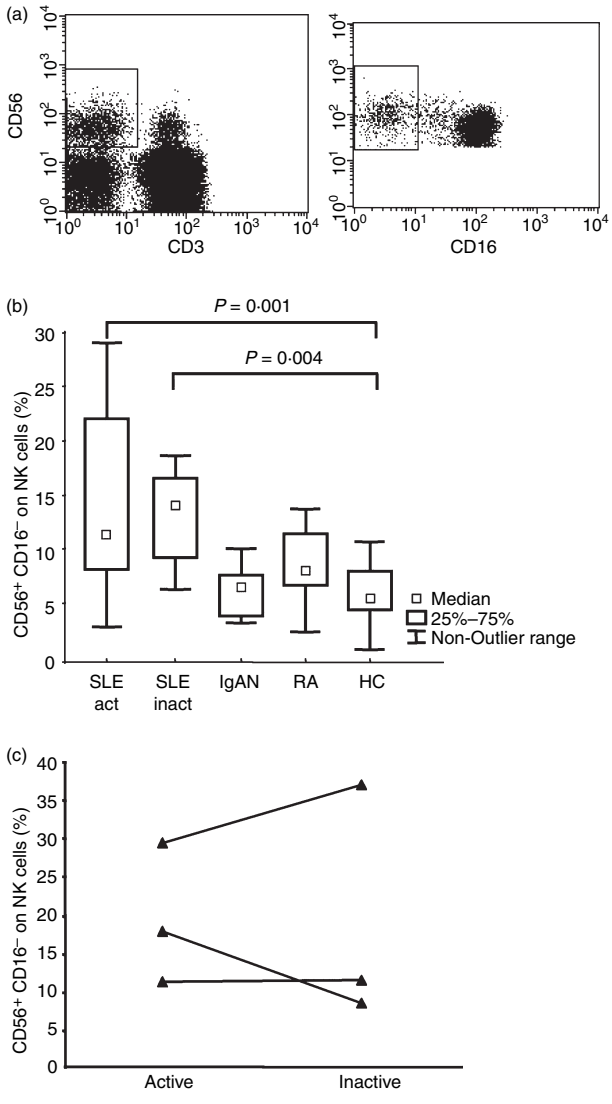

Figure 1. The percentage of CD56^{bright} natural killer (NK) is increased in patients with systemic lupus erythematosus (SLE). (a) On the left, an example of gating of CD3$^-$ CD56$^+$ NK cells on gated blood lymphocytes from a healthy control is shown. On the right, CD56^{bright} NK cells defined as CD16$^-$ NK cells are shown in the square. (b) The percentage of CD56^{bright} NK cells of total blood CD3$^-$ CD56$^+$ NK cells is plotted for patients with SLE during active disease ($n = 15$), patients during inactive disease ($n = 8$), patients with immunoglobulin A nephritis (IgAN) ($n = 10$), patients with rheumatoid arthritis (RA) ($n = 13$) and healthy blood donors ($n = 20$). Analysis of variance test $P = 0{\cdot}0009$, followed by Tukey's post hoc test: active SLE compared to healthy control $P = 0{\cdot}01$, and inactive SLE compared to healthy control $P = 0{\cdot}004$. (c) The percentage of CD56^{bright} NK cells of total blood CD3$^-$ CD56$^+$ NK cells is plotted for three patients with SLE sampled during active and again during inactive diseases. Results from the active sample are included in (b).

proportion of NK cells of all lymphocytes among the patient groups [SLE 11% (0·3–34), IgAN 14% (3–27), RA 13% (1·6–28), healthy controls 15% (5–42), $P = 0{\cdot}7$ by ANOVA]. We also compared the proportions of all NK cells that were CD16$^-$ CD3$^-$ CD56$^+$ (CD56^{bright}) NK cells (Fig. 1a) among the patient groups. A statistically significant difference was found between SLE patients ($n = 23$), which had increased proportions of CD56^{bright} NK cells, and the healthy donors ($n = 20$) (SLE 13% (3–58), healthy controls 5% (1–11), $P < 0{\cdot}001$, *t*-test). Patients with IgAN and RA had normal levels of CD56^{bright} NK cells (Fig. 1b). There was no difference in CD56^{bright} NK cell proportions between healthy males and females (data not shown). To better understand the reason for this increased proportion of CD56^{bright} NK cells, we divided the patients with SLE according to disease activity, defining patients with SLAM score of ≥ 7 as having 'active disease'. In addition, samples were taken from three patients with SLE during active disease and again during inactive disease (Fig. 1c). There was no difference in the proportion of CD56^{bright} NK cells in SLE in relation to severity of disease. No association between the proportion of CD56^{bright} NK cells and presence of nephritis, medical treatment or anti-double-stranded DNA antibodies could be distinguished within the SLE patient group (data not shown).

We also analysed the expression of different NK cell inhibitory and activating receptors (LIR-1/CD85j, CD94, NKG2C/CD159c, NKG2D/CD314, NKp30/CD337, NKp44/CD336, CD69) but found no differences in the expression of these markers on NK cells among the patient groups (data not shown). The expression of NKp46/CD335 was slightly increased on NK cells from patients with SLE, but only when analysed on CD56^{dim} NK cells separately [median fluorescence intensity (MFI) on CD56^{dim} cells was 159 (100–242) for active SLE, 118 (47–273) for inactive SLE, 91 (44–136) for IgAN, 89 (45–171) for healthy controls; $P = 0{\cdot}002$ active SLE compared to healthy controls, ANOVA followed by Tukey]. Expression of NKp46/CD335 on CD56^{bright} NK cells was somewhat higher than that for CD56^{dim} NK cells [MFI 199 (62–379) and 101 (47–242) respectively, for all individuals].

Type I IFN increased the proportion of CD56^{bright} cells *in vitro*

We hypothesized that cytokines with known stimulatory effects on NK cells may cause the increase in the proportion of CD56^{bright} cells shown. We cultured PBMC from healthy donors for 3 days in the presence of IL-15, IL-2 or type I IFN, or in the presence of the combination of IL-2 and type I IFN. IL-15 and IL-2 decreased the proportion of CD56^{bright} NK cells in culture after 3 days, possibly as a result of NK cell maturation. In contrast, type I IFN increased the proportion or CD56^{bright} NK cells in culture significantly (Fig. 2), while a combination of IL-2 and type I IFN did not affect the proportion of CD56^{bright} cells in the cultures compared with the

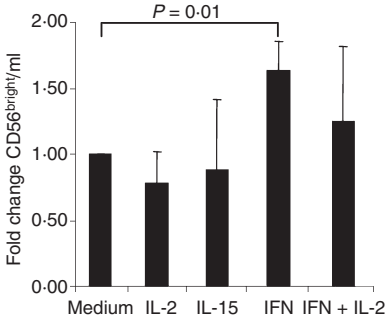

Figure 2. Culture of peripheral blood mononuclear cells (PBMC) in the presence of type I interferon (IFN) increases the number of $CD56^{bright}$ NK cells. The PBMC from healthy donors ($n = 4$) were cultured for 3 days in the presence of the indicated cytokines. The number of $CD56^{bright}$ NK cells per ml of cell culture was analysed and compared to the number found in the medium control. The fold increase in $CD56^{bright}$ NK cell is plotted. Paired *t*-test: $P = 0{\cdot}01$ unstimulated compared to IFN-stimulated.

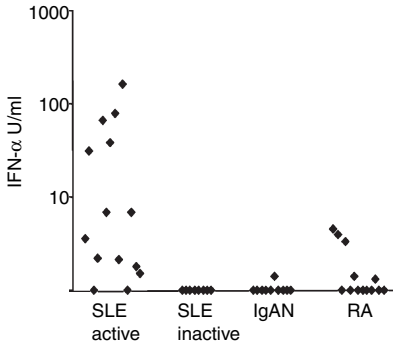

Figure 3. Serum levels of interferon-α (IFN-α) are elevated in patients with systemic lupus erythematosus (SLE) with active disease. Serum from patients with active SLE ($n = 14$), inactive SLE ($n = 8$), immunoglobulin A nephritis (IgAN, $n = 10$) and rheumatoid arthritis (RA, $n = 13$) was analysed for levels of IFN-α. Levels are expressed as U/ml. $P = 0{\cdot}02$ on analysis of variance.

medium control. As values differed considerably between blood donors, the figure shows normalized values, where the number of $CD56^{bright}$ NK cells per ml culture medium is expressed as the fold change of that found in the medium control. An increased proportion of $CD56^{bright}$ NK cells in presence of IFN could be the result of at least three different mechanisms: (1) increased proliferation of $CD56^{bright}$ NK cells, (2) increased death of $CD56^{dim}$ NK cells or (3) alterations in phenotype induced by IFN treatment. To study this, we analysed the proliferation of the respective NK cell subtype using BrDU incorporation and subsequent FACS staining. There was no proliferation in IFN-treated cells from healthy blood donors, while IL-2 and IL-15 induced proliferation predominantly of the $CD56^{bright}$ NK cells [56% (39–73) of $CD56^{bright}$ NK cells; 7·5% (2–17) of $CD56^{dim}$ NK cells, $n = 4$]. Stainings using 7AAD to analyse the deaths of the two NK cell subtypes indicated a slightly higher proportion of dead cells among the $CD56^{bright}$ compared to $CD56^{dim}$ NK cells (data not shown). Investigation of IFN-γ production in gated subsets of NK cells stimulated with the classical NK target cell line K562 determined no increased function on a per cell basis of NK cells from patients with SLE (data not shown). In general, $CD56^{bright}$ NK cells responded to a higher degree (data not shown), confirming earlier reports.[11]

We hypothesized that the *in vitro* effect of type I IFN on proportions of NK subpopulations could also be operative *in vivo* and we therefore set out to search for a possible correlation between IFN-α serum levels and the proportion of $CD56^{bright}$ NK cells. Levels of IFN-α in serum were analysed for all patient groups and found to be highly elevated only in the SLE group with active disease (Fig. 3). There was no correlation between the IFN-α levels and the proportion of $CD56^{bright}$ NK cells in this group, nor in the SLE patient population as a whole (data not shown).

Discussion

We have described an increase in the proportions of peripheral blood $CD56^{bright}$ NK cells in SLE, evident during inactive as well as during active disease. This argues against a therapy-associated effect because most patients with inactive disease did not receive any treatment. In addition, *in vitro* studies showed that IFN, a cytokine previously implicated in SLE, can increase the proportion of this NK cell subtype.

To our knowledge, this is the first description of a treatment-independent increase in the proportion of blood $CD56^{bright}$ NK cells in non-infectious diseases. $CD56^{bright}$ NK cells have previously been described as increasing during treatment with IL-2 receptor blockade in uveitis[12] and in multiple sclerosis,[13] as well as during IFN-β treatment in multiple sclerosis,[14] and there is an increased proportion of $CD56^{bright}$ NK cells during chronic human immunodeficiency virus infection.[15] There are several possible mechanisms that may explain our finding, including differential homing of NK cell populations in disease,[16] specific proliferation of $CD56^{bright}$ NK cells and an increased output of immature $CD56^{bright}$ NK cells[17,18] from bone marrow or from lymphoid tissue.[19] $CD56^{bright}$ NK cells develop in lymphoid tissue and can be found in close proximity to T cells where they can affect adaptive immune responses,[5] with possible implications for autoimmune adaptive responses. Another possibility is a described difference in resistance to apoptosis, where $CD56^{bright}$ NK cells survive better in oxidant-rich environments.[20,21] It is noteworthy that the increased proportion of $CD56^{bright}$ NK cells is not just a consequence of any autoimmune disease affecting the kidneys or the joints, because patients with IgAN and RA showed a different pattern.

Whether NK cells are disease promoting or provide protection from autoimmune disease is not clear, and this

seems to depend on the particular disease studied. It is also conceivable that NK cells exert different functions depending on disease stage, i.e. in the initiation of disease processes compared with the perpetuation of inflammation (reviewed by Johansson *et al.*).[22] Patients with SLE[23] as well as the *lpr* mouse model for SLE,[24] exhibit low NK cell activity which may be partly explained by a low expression of the adaptor protein DAP12 in SLE patients.[25] Furthermore, an association in time between disease development and low NK cell activity has been described in the *lpr* mouse.[26] In a mouse model of another B-cell-dependent autoimmune disease, experimental autoimmune myasthenia gravis (EAMG), Shi *et al.* showed that depletion of NK cells during the priming phase protected from disease development by reducing IFN-γ production by CD4$^+$ T cells and increasing production of the anti-inflammatory cytokine transforming growth factor-β, while levels of pathogenic antibodies decreased.[27]

The connection between the accumulation of CD56^{bright} NK cells and IFN stimulation *in vitro* is of particular interest because IFN has been implicated in SLE (reviewed by Rönnblom *et al.*).[1] Serum levels of IFN are increased in patients with SLE[28] and overproduction of IFN induces lupus-like manifestations.[29] Furthermore, 129Sv mice deficient for interferon alpha receptor (IFNAR) are protected from experimental lupus[30] and IFN-α therapy for unrelated disorders is associated with autoimmune manifestations, including lupus-like symptoms.[31] We hypothesized that the SLE-associated increase in CD56^{bright} NK cell proportion described here could be induced by high IFN-α levels. Interestingly, we found that IFN could increase the proportion of CD56^{bright} NK cells *in vitro*, but that this was the result of neither an increased proliferation of CD56^{bright} NK cells nor the death of CD56^{dim} NK cells in cell cultures. We conclude that the IFN-induced increase of the number of CD56^{bright} NK cells is the result of phenotypic changes. This could either be a result of a decreased rate of maturation from CD56^{bright} to CD56^{dim} NK cells, or of an increased rate of change from the CD56^{dim} to the CD56^{bright} NK cell phenotype. Considering the described ability of CD56^{bright} NK cells to develop into CD56^{dim} cells,[17] we favour the first mentioned possibility.

We analysed IFN-α serum levels in our patient groups and found the levels to be increased in the SLE patients with active disease, while the group of patients with inactive SLE had undetectable levels. Hence, there is no straightforward correlation between increased serum levels of IFN-α and the accumulation or CD56^{bright} NK cells even if some of the patients with heightened CD56^{bright} counts were patients with active disease. PBMC from SLE patients with undetectable IFN serum levels display increased levels of the IFN-α-inducible protein MxA, which is indicative of *in vivo* IFN-α production.[32] It is therefore possible that low levels of IFN-α, undectectable in serum by our method, induce the increase in proportion of CD56^{bright} NK cells in SLE described here. The abundance of CD56^{bright} NK cells in SLE, a cell type with high cytokine-secreting ability, may play a role in the pathology of SLE. It is intriguing to speculate on a genetic rather than a disease-associated cause of this accumulation of potentially proinflammatory NK cells, which may contribute to the risk of developing SLE.

Acknowledgements

Mathula Thangarajh is acknowledged for her enthusiasm. The project was supported by funds from the Swedish Medical Research Council, the King Gustav the V's 80th birthday fund, Karolinska Institutet, the Swedish Association against Rheumatism and the Swedish Foundation for Strategic Research.

References

1 Rönnblom L, Eloranta ML, Alm GV. The type I interferon system in systemic lupus erythematosus. *Arthritis Rheum* 2006; **54**:408–20.

2 Vallin H, Perers A, Alm GV, Rönnblom L. Anti-double-stranded DNA antibodies and immunostimulatory plasmid DNA in combination mimic the endogenous IFN-alpha inducer in systemic lupus erythematosus. *J Immunol* 1999; **163**: 6306–13.

3 Barrat FJ, Meeker T, Gregorio J *et al.* Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. *J Exp Med* 2005; **202**:1131–9.

4 Yokoyama WM, Kim S, French AR. The dynamic life of natural killer cells. *Annu Rev Immunol* 2004; **22**:405–29.

5 Martin-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, Sallusto F. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nat Immunol* 2004; **5**:1260–5.

6 Blanca IR, Bere EW, Young HA, Ortaldo JR. Human B cell activation by autologous NK cells is regulated by CD40–CD40 ligand interaction: role of memory B cells and CD5$^+$ B cells. *J Immunol* 2001; **167**:6132–9.

7 Gao N, Dang T, Yuan D. IFN-gamma-dependent and -independent initiation of switch recombination by NK cells. *J Immunol* 2001; **167**:2011–8.

8 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**:1271–7.

9 Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**:315–24.

10 Vallin H, Blomberg S, Alm GV, Cederblad B, Rönnblom L. Patients with systemic lupus erythematosus (SLE) have a circulating inducer of interferon-alpha (IFN-alpha) production acting on leucocytes resembling immature dendritic cells. *Clin Exp Immunol* 1999; **115**:196–202.

11 Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA. Human natural killer cells:

a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 2001; **97**:3146–51.

12 Li Z, Lim WK, Mahesh SP, Liu B, Nussenblatt RB. Cutting edge: *in vivo* blockade of human IL-2 receptor induces expansion of CD56(bright) regulatory NK cells in patients with active uveitis. *J Immunol* 2005; **174**:5187–91.

13 Bielekova B, Catalfamo M, Reichert-Scrivner S *et al.* Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci U S A* 2006; **103**:5941–6.

14 Saraste M, Irjala H, Airas L. Expansion of $CD56^{bright}$ natural killer cells in the peripheral blood of multiple sclerosis patients treated with interferon-beta. *Neurol Sci* 2007; **28**:121–6.

15 Titanji K, Sammicheli S, De Milito A *et al.* Altered distribution of natural killer cell subsets identified by CD56, CD27 and CD70 in primary and chronic human immunodeficiency virus-1 infection. *Immunology* 2008; **123**:164–70.

16 Campbell JJ, Qin S, Unutmaz D, Soler D, Murphy KE, Hodge MR, Wu L, Butcher EC. Unique subpopulations of $CD56^{+}$ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J Immunol* 2001; **166**:6477–82.

17 Chan A, Hong DL, Atzberger A *et al.* $CD56^{bright}$ human NK cells differentiate into $CD56^{dim}$ cells: role of contact with peripheral fibroblasts. *J Immunol* 2007; **179**:89–94.

18 Romagnani C, Juelke K, Falco M *et al.* $CD56^{bright}$ $D16^{-}$ killer Ig-like receptor NK cells display longer telomeres and acquire features of $CD56^{dim}$ NK cells upon activation. *J Immunol* 2007; **178**:4947–55.

19 Freud AG, Becknell B, Roychowdhury S *et al.* A human CD34(+) subset resides in lymph nodes and differentiates into $CD56^{bright}$ natural killer cells. *Immunity* 2005; **22**:295–304.

20 Thoren FB, Romero AI, Hermodsson S, Hellstrand K. The $CD16^{-}$/$CD56^{bright}$ subset of NK cells is resistant to oxidant-induced cell death. *J Immunol* 2007; **179**:781–5.

21 Harlin H, Hanson M, Johansson CC, Sakurai D, Poschke I, Norell H, Malmberg KJ, Kiessling R. The $CD16^{-}$ CD56(bright) NK cell subset is resistant to reactive oxygen species produced by activated granulocytes and has higher antioxidative capacity than the $CD16^{+}$ CD56(dim) subset. *J Immunol* 2007; **179**: 4513–9.

22 Johansson S, Berg L, Hall H, Hoglund P. NK cells: elusive players in autoimmunity. *Trends Immunol* 2005; **26**:613–8.

23 Erkeller-Yusel F, Hulstaart F, Hannet I, Isenberg D, Lydyard P. Lymphocyte subsets in a large cohort of patients with systemic lupus erythematosus. *Lupus* 1993; **2**:227–31.

24 Pan LZ, Dauphinee MJ, Ansar Ahmed S, Talal N. Altered natural killer and natural cytotoxic cellular activities in lpr mice. *Scand J Immunol* 1986; **23**:415–23.

25 Toyabe S, Kaneko U, Uchiyama M. Decreased DAP12 expression in natural killer lymphocytes from patients with systemic lupus erythematosus is associated with increased transcript mutations. *J Autoimmun* 2004; **23**:371–8.

26 Takeda K, Dennert G. The development of autoimmunity in C57BL/6 lpr mice correlates with the disappearance of natural killer type 1-positive cells: evidence for their suppressive action on bone marrow stem cell proliferation, B cell immunoglobulin secretion, and autoimmune symptoms. *J Exp Med* 1993; **177**:155–64.

27 Shi FD, Wang HB, Li H, Hong S, Taniguchi M, Link H, Van Kaer L, Ljunggren HG. Natural killer cells determine the outcome of B cell-mediated autoimmunity. *Nat Immunol* 2000; **1**:245–51.

28 Preble OT, Black RJ, Friedman RM, Klippel JH, Vilcek J. Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. *Science (New York, NY)* 1982; **216**:429–31.

29 Zhuang H, Kosboth M, Lee P *et al.* Lupus-like disease and high interferon levels corresponding to trisomy of the type I interferon cluster on chromosome 9p. *Arthritis Rheum* 2006; **54**:1573–9.

30 Santiago-Raber ML, Baccala R, Haraldsson KM, Choubey D, Stewart TA, Kono DH, Theofilopoulos AN. Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. *J Exp Med* 2003; **197**:777–88.

31 Rönnblom LE, Alm GV, Oberg K. Autoimmune phenomena in patients with malignant carcinoid tumors during interferon-alpha treatment. *Acta Oncol (Stockholm, Sweden)* 1991; **30**:537–40.

32 von Wussow P, Jakschies D, Hochkeppel H, Horisberger M, Hartung K, Deicher H. MX homologous protein in mononuclear cells from patients with systemic lupus erythematosus. *Arthritis Rheum* 1989; **32**:914–8.

