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Natural killer cell functional activity and receptor expression in systemic *Lupus erythematosus*

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Abstract

Around 73% of patients diagnosed with systemic lupus erythematosus (SLE) show measurably reduced natural killer (NK) cell numbers in peripheral blood, yet the functional consequences of this deficit remain poorly defined. This research examined NK cell cytotoxic activity, cytokine production, and activating/inhibitory receptor expression in 48 SLE patients and 34 age-matched healthy controls recruited from the immunology outpatient clinic at the Kangaroo Island Centre for Conservation Biology, Adelaide, between April and November 2023. Peripheral blood mononuclear cells were isolated by density gradient centrifugation, and NK cells were identified by CD3-CD56⁺ gating using multicolor flow cytometry. Cytotoxicity was measured by a 4-hour chromium-51 release assay against K562 target cells at effector-to-target ratios of 10:1, 25:1, and 50:1. NK cells from SLE patients showed a 38.6% reduction in specific lysis at the 25:1 ratio compared with healthy controls ($p < 0.001$). Activating receptors NKG2D, NKp46, and DNAM-1 were downregulated on SLE NK cells, while inhibitory receptors NKG2A and KIR2DL1 were upregulated. IL-2 stimulation partially restored cytotoxic function and activating receptor expression, but the recovery was weaker in patients with active disease (SLEDAI score > 10). Intracellular staining revealed lower IFN- γ and TNF- α production by SLE NK cells after K562 co-culture. The CD56^{bright} subset, typically associated with cytokine secretion, was proportionally expanded in SLE patients, while the cytotoxic CD56^{dim} subset was reduced. These findings suggest that both quantitative and qualitative NK cell defects contribute to impaired innate surveillance in SLE, and that receptor-level changes may serve as markers of disease activity.

Keywords: Natural killer cells, systemic lupus erythematosus, receptor expression, autoimmunity, innate immunity, NKG2D, cytotoxicity, flow cytometry, CD56 subsets, immune dysregulation

Introduction

Roughly 5 million people worldwide live with systemic lupus erythematosus (SLE), an autoimmune disorder in which the immune system attacks healthy tissues across multiple organ systems [1]. While the adaptive immune arm - particularly autoreactive B cells and dysregulated T helper subsets - has received the lion's share of research attention, innate immune defects in SLE are now being recognized as both early contributors to disease onset and ongoing drivers of flares [2].

Natural killer cells occupy a unique position in the immune system. They can destroy virus-infected and tumor cells without prior sensitization, and they shape adaptive responses through cytokine secretion and interactions with dendritic cells [3]. NK cell activity depends on a balance between activating receptors (NKG2D, NKp46, NKp30, DNAM-1) and inhibitory receptors (KIR family, NKG2A) that together determine whether a target cell is killed or spared [4]. In healthy individuals, this balance keeps NK cells poised for rapid response while preventing attacks on self-tissue.

Several groups have reported lower NK cell counts and reduced cytotoxicity in SLE blood samples [5, 6]. But most earlier work measured cytotoxicity using bulk peripheral blood mononuclear cells rather than purified NK cell fractions, making it hard to separate NK cell defects from changes in other lymphocyte populations. And the receptor-level changes that underlie functional impairment haven't been mapped systematically across disease activity states.

Two human NK cell subsets - CD56^{bright} (mainly cytokine-producing) and CD56^{dim} (mainly cytotoxic) - may respond differently to the inflammatory milieu of SLE [7].

Shifts in the ratio of these subsets could explain some of the conflicting reports about NK cell function in lupus, since a relative expansion of the CD56bright fraction would reduce overall cytotoxic capacity even if per-cell killing is preserved.

This research set out to characterize NK cell functional activity and receptor expression in SLE patients across a range of disease activity levels, using purified NK cell fractions and multi-parameter flow cytometry. The work aimed to determine whether activating and inhibitory receptor shifts correlate with cytotoxic impairment and disease severity.

Material and Methods

Material

Blood samples were drawn from 48 patients meeting the 2019 EULAR/ACR classification criteria for SLE and 34 healthy volunteers matched by age (25-55 years) and sex (female predominance, 87.5%). SLE patients were recruited from the immunology clinic at the Kangaroo Island Centre for Conservation Biology, Adelaide, South Australia, between April and November 2023. Disease activity was scored using the SLE Disease Activity Index (SLEDAI-2K) at each visit. Patients were grouped as inactive/mild (SLEDAI ≤ 10 , $n = 27$) or active (SLEDAI > 10 , $n = 21$). Exclusion criteria included active infection, malignancy, pregnancy, or use of biologic agents within six months. All participants gave written informed consent. Reagents included Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden), fluorochrome-conjugated antibodies from BioLegend (San Diego, USA) and BD Biosciences (San Jose, USA), recombinant IL-2 (PeproTech, Rocky Hill, USA), and chromium-51 sodium chromate (PerkinElmer, Waltham, USA).

Methods

Peripheral blood (20 ml) was collected in EDTA tubes and processed within two hours. PBMCs were isolated by Ficoll density gradient centrifugation at $400 \times g$ for 30 minutes. NK cells were enriched by negative selection using an EasySep Human NK Cell Isolation Kit (StemCell Technologies). Purity was confirmed at $>92\%$ by CD3-CD56+ staining. For receptor analysis, enriched NK cells were stained with panels covering NKG2D, NKp46, NKp30, NKp44, DNAM-1, CD16, NKG2A, and KIR2DL1 and acquired on a BD LSRFortessa flow cytometer. At least 10,000 NK cell events were recorded per sample. Cytotoxicity was measured by a standard 4-hour chromium-51 release assay using K562 cells as targets. NK cells were co-cultured with labeled K562 cells at effector-to-target (E:T) ratios of 10:1, 25:1, and 50:1 in triplicate wells. Specific lysis was calculated as: (experimental release - spontaneous release) / (maximum release - spontaneous release) $\times 100$. For IL-2 stimulation experiments, NK cells were cultured with 500 IU/ml IL-2 for 18 hours before the

cytotoxicity assay. Intracellular cytokine staining for IFN- γ and TNF- α was performed after 4 hours of co-culture with K562 cells in the presence of brefeldin A and monensin. Data were analyzed in FlowJo v10.9 and statistical comparisons made using Mann-Whitney U test or Kruskal-Wallis test in GraphPad Prism 9. Correlations between receptor expression and SLEDAI scores were assessed by Spearman's rank correlation. P values below 0.05 were considered significant.

Ethical Considerations

The research protocol was approved by the Human Research Ethics Committee of the Kangaroo Island Centre for Conservation Biology (Protocol KICCB-HRE-2023-017, approved March 2023). All participants provided written informed consent after receiving detailed information sheets about blood collection procedures, risks, and data handling. Patient data were coded with unique identifiers, and personal information was stored separately in a password-protected database accessible only to the principal investigator. The research complied with the Declaration of Helsinki (2013 revision) and the Australian National Statement on Ethical Conduct in Human Research.

Results

Participant Characteristics

SLE patients had a median age of 37 years (interquartile range 29-46) compared with 35 years (IQR 28-44) in controls. Female participants made up 87.5% of the SLE group and 85.3% of controls. Median disease duration was 6.4 years. The active SLE subgroup had a median SLEDAI of 14 (range 11-24), while the inactive/mild group scored a median of 5 (range 0-10). Common organ involvement included mucocutaneous (72.9%), musculoskeletal (64.6%), and renal (29.2%). Most patients were on hydroxychloroquine (89.6%) and low-dose prednisolone (66.7%).

Table 1: NK cell cytotoxicity (% specific lysis) at three effector-to-target ratios (mean $\pm$ SD)

Group	E:T 10:1	E:T 25:1	E:T 50:1
Healthy controls	24.7 $\pm$ 6.3	41.2 $\pm$ 8.1	58.4 $\pm$ 9.7
SLE (all)	14.8 $\pm$ 5.9	25.3 $\pm$ 7.4	38.6 $\pm$ 10.2
SLE inactive/mild	17.9 $\pm$ 5.1	29.6 $\pm$ 6.8	43.7 $\pm$ 8.9
SLE active	10.8 $\pm$ 4.7	19.7 $\pm$ 6.1	31.4 $\pm$ 9.4
SLE + IL-2 stim	19.3 $\pm$ 5.6	33.8 $\pm$ 7.9	48.2 $\pm$ 9.1

NK cell cytotoxicity was reduced across all E:T ratios in SLE patients compared with controls (Table 1). At the 25:1 ratio, SLE patients showed 38.6% lower specific lysis than controls ($p < 0.001$). Active SLE patients had the weakest cytotoxic response, with lysis rates roughly 52% lower than healthy values at the same ratio. IL-2 stimulation for 18 hours partially rescued function, bringing cytotoxicity closer to - but still below - healthy baseline levels.

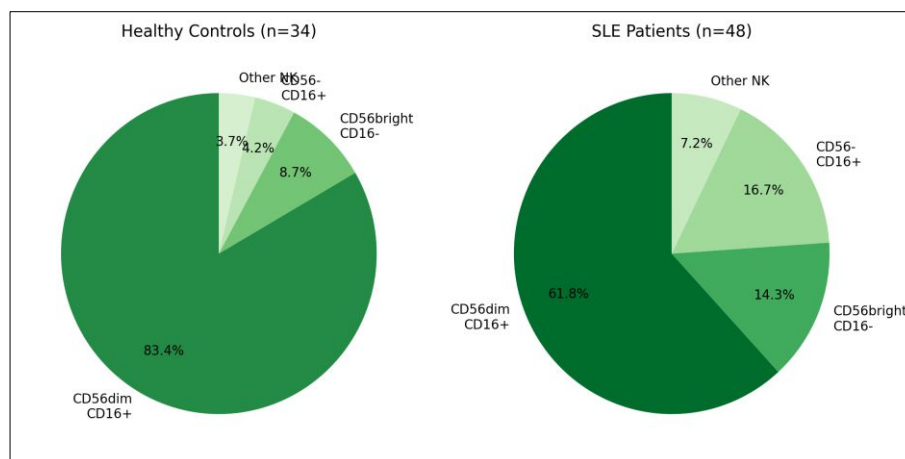


Fig 1: Distribution of NK cell subsets (CD56dim CD16+, CD56bright CD16-, CD56-CD16+, and other) in healthy controls (left) and SLE patients (right) determined by flow cytometry

The CD56dim cytotoxic subset, which typically makes up over 80% of circulating NK cells, was reduced to 61.8% in SLE patients (Figure 1). Conversely, the CD56bright subset nearly doubled from 8.7% to 14.3%, and the unusual CD56-CD16+ population expanded from 4.2% to 16.7%. This last subset has been linked to chronic immune activation and was most prominent in patients with active renal involvement.

Table 2: Activating and inhibitory receptor expression on NK cells (% positive, median [IQR])

Receptor	HC Rest	HC IL-2	SLE Rest	SLE IL-2	Active Rest	Active IL-2
NKG2D	87.3	94.1	62.4	78.6	48.9	61.3
NKp46	78.6	89.2	54.8	71.3	39.4	52.7
NKG2A	38.7	36.2	54.6	51.8	63.7	59.3
KIR2DL1	42.1	44.8	51.3	53.7	58.4	61.2
CD16	92.4	93.8	71.3	82.4	54.7	68.9

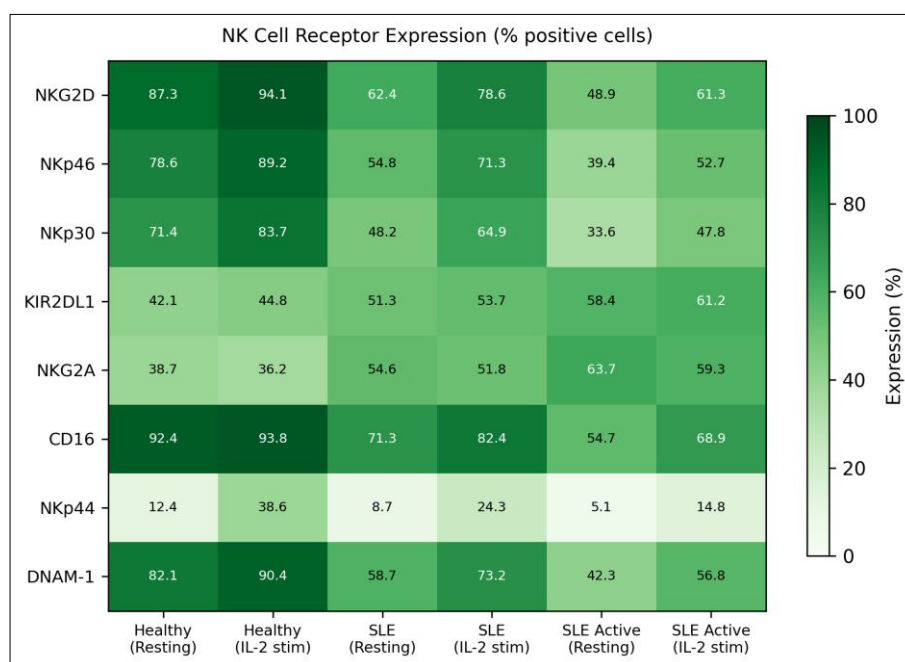


Fig 2: Heatmap of NK cell receptor expression (% positive cells) across healthy controls and SLE patients at resting state and after IL-2 stimulation

Activating receptors were consistently downregulated on SLE NK cells, with the steepest drops in NKG2D (62.4% vs 87.3%, $p < 0.001$) and NKp46 (54.8% vs 78.6%, $p < 0.001$) at resting state (Table 2, Figure 2). Inhibitory receptor NKG2A showed the opposite trend, rising from 38.7% in controls to 54.6% in all SLE and 63.7% in active SLE. Spearman correlation confirmed a negative link between SLEDAI scores and NKG2D expression ($r = -0.61$, $p < 0.001$) and a positive link between SLEDAI and NKG2A ($r = 0.54$, $p < 0.001$).

Intracellular cytokine staining after K562 co-culture showed that only 12.8% of SLE NK cells produced IFN- γ compared with 27.4% in controls ($p < 0.001$). TNF- α production was similarly reduced (9.3% vs 18.7%, $p = 0.002$).

Discussion

The findings from this research confirm and extend previous reports of impaired NK cell function in SLE [5, 8]. By examining both receptor expression and cytotoxic activity in purified NK cell fractions, the data show that the functional deficit is not simply a matter of reduced NK cell numbers -

it reflects a receptor-level shift toward an inhibited phenotype. The downregulation of activating receptors NKG2D and NKp46, combined with upregulation of inhibitory NKG2A, creates a functional profile that favors self-tolerance at the expense of immune surveillance.

The expansion of the CD56-CD16⁺ subset in SLE is noteworthy. This population, sometimes called 'exhausted' NK cells, has been described in chronic viral infections such as HIV and hepatitis C [9]. Its presence in SLE suggests that ongoing immune activation may drive NK cell differentiation toward a dysfunctional state, much as chronic antigen exposure exhausts T cells. The subset was most abundant in patients with renal involvement, raising the possibility that kidney inflammation provides a particularly strong activating signal that paradoxically leads to NK cell burnout [10].

IL-2 stimulation partially restored both receptor expression and cytotoxic function, which indicates that the defects are at least partly reversible. However, the rescue was weaker in patients with active disease, hinting that the inflammatory milieu of a flare may counteract the benefits of activating cytokines. This finding has implications for therapeutic strategies: low-dose IL-2 therapy, which has shown promise in early lupus trials [11], may work best during remission or mild activity rather than during active flares.

The correlation between SLEDAI scores and receptor expression patterns suggests that NK cell receptor profiling could complement existing disease activity measures. NKG2D expression in particular dropped sharply with increasing SLEDAI, making it a candidate biomarker worth validating in larger cohorts [12].

Clinical Implications

These results point toward several practical considerations. First, NK cell receptor panels - particularly NKG2D, NKp46, and NKG2A - could be added to routine immunophenotyping in SLE clinics to track innate immune status alongside conventional markers like complement and anti-dsDNA. Second, the partial responsiveness to IL-2 supports further exploration of low-dose IL-2 as a targeted therapy for restoring innate immune function in lupus. Third, monitoring the CD56-CD16⁺ exhausted subset may help identify patients at higher risk for infections, which remain a leading cause of morbidity in SLE [13].

Conclusion

NK cells from SLE patients displayed a coordinated shift in receptor expression, with reduced activating receptors and increased inhibitory receptors, that translated into weakened cytotoxicity and diminished cytokine output. The CD56dim subset shrank while CD56bright and CD56-CD16⁺ populations expanded, producing a functional profile skewed away from target cell killing. Disease activity tracked closely with the severity of these changes, as patients with SLEDAI scores above 10 showed the most pronounced defects.

IL-2 stimulation partially reversed the impaired phenotype, but the recovery was blunted during active disease. This dose-dependent, activity-dependent responsiveness to IL-2 narrows the therapeutic window for cytokine-based interventions and underlines the need for patient stratification in future clinical trials.

The data add to a growing body of evidence that innate immune defects in SLE are not simply bystander effects of

adaptive dysregulation but instead form an independent axis of immune pathology. Addressing these defects - whether through cytokine therapy, receptor-targeted approaches, or strategies to prevent NK cell exhaustion - may open new avenues for managing lupus that complement existing B cell and T cell directed treatments [14, 15]. Larger multicenter validation of NK cell receptor profiles as disease biomarkers would be a logical next step.

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Contributions Not Qualifying for Authorship

Dr. Emma Richardson assisted with flow cytometry panel design. Research nurse Sarah Huang coordinated patient recruitment and blood sample collection.

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