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ORIGINAL ARTICLE

Role of IL-24 in NK cell activation and its clinical implication in systemic lupus erythematosus

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Abstract

Objectives Interleukin (IL)-24 has been considered as an inflammatory cytokine in autoimmune diseases. However, conflicting data exist and its biological function remains controversial. Additionally, little is known about its functional impact on natural killer (NK) cells. The aim of this study was to investigate the role of IL-24 in NK cell activation and its clinical implication in systemic lupus erythematosus (SLE).

Methods Serum cohort consisting of 299 SLE patients, 214 RA patients, and 159 healthy controls (HCs) and plasma cohort consisting of 70 SLE patients, 82 RA patients, and 123 HCs were included in evaluating IL-24 concentrations. Impact of IL-24 on NK cell activation was assessed in two NK cell subsets, i.e., $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cells. Human NK-92 cell line was applied to evaluate functional potential of IL-24 on NK cell migration and invasion.

Results Serum and plasma levels of IL-24 were comparable between patients with SLE or RA and HCs. While recombinant human (rh) IL-2 consistently induced an increased expression of CD69 on both $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ cells derived from both healthy subjects and patients with SLE, IL-24 alone was insufficient to activate the $CD56^{dim}$ and $CD56^{bright}$ NK cells. Similarly, while the migratory NK-92 cell numbers were significantly increased with rhIL-2 stimulation, IL-24 alone was unable to enhance NK-92 cell migratory and invasive capacity.

Conclusion Our data indicate that there were no significant differences in serum and plasma concentrations of IL-24 between SLE patients and healthy controls. Recombinant IL-24 has no effect on NK cell activation and migration.

Key points
- *This is the first study to investigate functional potential of IL-24 on NK cell activation.*
- *Recombinant IL-24 lacks functional capacity on NK cell activation in either $CD56^{dim}CD16^{+}$ or $CD56^{bright}CD16^{-}$ NK cell subsets derived from both healthy subjects and patients with SLE.*
- *No significant differences in serum and plasma levels of IL-24 between SLE patients and healthy controls.*

Keywords IL-24 · NK cells subsets · Cell activation · Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with a broad range of clinical manifestations. SLE is characterized by various immunological abnormalities, including self-reactive T and B cells activation leading to an excessive autoantibody production and immune complex formation. Initially, SLE was considered as a classic adaptive

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immune disorder. However, recent advances in the understanding of SLE pathogenesis has emerged the components of innate immune cells also play an important role in the development of SLE, such as natural kill (NK) cells [1, 2]. Human NK cells are typically defined as $CD3^{-}CD56^{+}$ cells and can be divided into two major subpopulations, the $CD56^{dim}$ NK cells which express CD16 ($CD3^{-}CD56^{dim}CD16^{+}$), and the $CD56^{bright}$ NK cells which lack expression of CD16 ($CD3^{-}CD56^{bright}CD16^{-}$) [3, 4]. These two NK cell subsets differ in their distribution and function. The $CD56^{dim}CD16^{+}$ NK cells make up approximately 90% of peripheral blood NK cells and possess a high cytotoxic capacity, while the $CD56^{bright}CD16^{-}$ NK cells are mainly resided in secondary lymphoid tissue or at sites of inflammation with less cytotoxic but potent cytokine-secreting capacity [3, 5]. Several groups have reported a significant lower proportions and total numbers of NK cells in peripheral blood of SLE patients compared with healthy individuals [2, 6, 7], but investigators also showed an increased proportion of $CD56^{bright}$ NK cell subset in peripheral blood of SLE patients with active disease [8].

Interleukin (IL)-24 belongs to the IL-10 family of cytokines and was initially designated as melanoma differentiation antigen 7 (MDA-7) due to its expression in human cancer–derived cell lines and in normal melanocytes [9, 10]. Apart from tumor cells and melanocytes, IL-24 is also produced by activated immune cells such as monocytes, T cells and B cells, suggesting that IL-24 may play a role in immune response [11, 12]. IL-24 shares 20–30% amino acid homology with IL-10, IL-20, and IL-22 and interact with two heterodimeric receptor complexes, IL-20R1/IL-20R2 and IL-22R1/IL-20R2 [9, 13]. It has been reported that the serum levels of IL-24 were significantly elevated in patients with SLE and rheumatoid arthritis (RA) compared with healthy controls (HCs) [14, 15]. However, another group showed that the serum levels of IL-24 in SLE patients did not significantly differ from the healthy individuals [16]. In terms of its biological function, initially several research groups reported that IL-24 could function as a tumor suppressor by inducing apoptosis and inhibiting migration, invasion, and angiogenesis in tumor cells both in vivo and in vitro [17–21]. However, conflicting data exist and the anti-tumor properties of IL-24 remain controversial. For example, while reports showed that IL-24 produced from either transfected Hek cells (Hek-IL-24) or bacterially expressed GST-IL-24 fusion protein (GST-IL-24) could efficiently promote apoptosis and suppress cancer cells [22–25], others were unable to detect such apoptosis-inducing properties in tumor cells with Hek-IL-24 or GST-IL-24 [26–28]. Apart from its tumor suppressor function, IL-24 was also known to be involved in the regulation of immune responses and autoimmune diseases. For example, treatment of the peripheral blood mononuclear cells (PBMCs) with IL-24, purified from the HEK293 cell line, resulted in the induction of IL-6, IFN-γ, TNF-α, IL-1β, IL-12, and GM-CSF [29]. Recombinant human IL-24 (rhIL-24) protein could efficiently induce human monocyte and neutrophil migration [30]. These results indicate that IL-24 may function as a pro-inflammatory cytokine. However, an anti-inflammatory property of IL-24 has also been reported. For example, IL-24 could inhibit the plasma cell differentiation in human germinal center B cells [31]. Furthermore, although the functional properties of IL-24 have been characterized in multiple cell types, its effect on NK cells remains undefined. To this end, we first investigated the expression pattern of IL-24 in SLE patients with active disease in both serum and plasma with a larger sample size. We further assessed the functional effect of IL-24 on NK cell activation in both healthy individuals and SLE patients. Our data indicate that there were no significant differences in serum and plasma concentrations of IL-24 between SLE patients and healthy controls. Recombinant IL-24 has no effect on NK cell activation and migration.

Methods

Study subjects

Two independent cohorts, including serum samples from 299 SLE patients, 214 RA patients, and 159 healthy controls (serum cohort) and plasma samples from 70 SLE patients, 82 RA patients, and 123 healthy controls (plasma cohort), were enrolled in the study. In serum cohort, the SLE or RA patients were recruited from the Department of Rheumatology and Immunology, and the healthy controls were recruited from Health Care Centers at Peking University People's Hospital, respectively. In plasma cohort, the SLE or RA patients were recruited from the Department of Rheumatology, and the healthy controls were recruited from Health Care Centers at Second Affiliated Hospital of Dalian Medical University, respectively. The patients with SLE fulfilled 1982 revised American College of Rheumatology (ACR) classification criteria for SLE [32]. The patients with RA satisfied 1987 revised ACR classification criteria for a diagnosis of RA [33]. The SLE Disease Activity Index (SLEDAI) and the Disease Activity Score (DAS)28 were used to assess the disease activities of SLE and RA, respectively. SLE patients had active disease at the time of blood sampling [SLEDAI: 10.00 (interquartile range, IQR 5.00 to 18.25)]. RA cases had active disease at the time of blood sampling [DAS28 5.55 (IQR 4.07 to 8.26)]. The healthy controls were selected without any disease records. The baseline characteristics of patients and controls are shown in Table 1. For in vitro recombinant IL-24 stimulation experiments, the peripheral blood mononuclear cells (PBMCs) were collected from 14 SLE patients with active diseases and 14 healthy subjects, respectively.

Table 1 Characteristic of the study cohorts

Characteristics	Controls		RA cases		SLE cases	
	Serum (n = 159)	Plasma (n = 123)	Serum (n = 214)	Plasma (n = 82)	Serum (n = 299)	Plasma (n = 70)
Sex. no. female/male	122/37	88/35	162/52	69/13	262/37	65/5
Age (mean ± SD years)	36.3 ± 12.6	31.1 ± 10.1	50.9 ± 14.2	61.0 ± 12.9	36.3 ± 13.8	39.2 ± 14.9
Age of onset (mean ± SD years)	-	-	42.0 ± 14.6	51.1 ± 14.2	30.3 ± 13.3	31.5 ± 13.5
Disease duration (mean ± SD years)	-	-	8.7 ± 8.0	10.4 ± 11.0	5.8 ± 6.5	7.5 ± 7.5
Clinical manifestations, n (%)						
Arthritis	-	-	-	-	282 (65.6)	65 (58.5)
Thrombopenia	-	-	-	-	262 (35.9)	64 (31.3)
Complement depressed	-	-	-	-	295 (93.9)	62 (91.9)
Proteinuria	-	-	-	-	262 (67.2)	64 (73.4)
Anti-dsDNA (positivity)	-	-	-	-	269 (70.6)	65 (81.5)

RA, rheumatoid arthritis; *SLE*, systemic lupus erythematosus; *anti-dsDNA*, anti-double stranded DNA antibodies; *SD*, standard deviation

*"*n*" indicating the number of cases when the data were available and "%" indicating the proportion of positivity of the manifestations

The study was approved by the Research Ethics Committee at Peking University People's Hospital.

Reagents, cells, and antibodies

Recombinant human IL-24 was purchased from R&D Systems (Minneapolis, MN). Recombinant human IL-2 (rhIL-2) was purchased from Beijing SL Pharmaceutical Company (SL PHARM, Beijing, China). PBMCs were separated from 8-mL peripheral blood by the density gradient centrifugation using 6% hydroxyethyl starch plus 9% sodium diatrizoate (Tian Jin Hao Yang Biological Manufacture CO., Ltd, Tianjin, China). The NK-92 cell line was purchased from Shanghai Yubo Biotechnology Co., Ltd (Yubo, Shanghai, China). The NK-92 cells were cultured in α-MEM (Gibco, Waltham, MA), and supplemented with 12.5% heated horse serum (Biological Industries, Kibbutz Beit-Haemek, Israel), 12.5% heated fetal bovine serum (Gibco), 50 units/mL penicillin (Gibco), 50 μg/mL streptomycin (Gibco), and 100 IU/mL rhIL-2 (SL PHARM). Cells were propagated at 37 °C with 5% CO_2 and the experiments were performed under sterile conditions.

The following antibodies were used for fluorescence-activated cell sorting (FACS) staining: Alexa Fluor700-anti-CD3 (BD Biosciences, San Jose, CA), PE-anti-CD56, FITC-anti-CD16, APC-anti-CD69, the isotype-matched anti-IgG1 control (APC), and 7-aminoactinomycin D (7AAD). All antibodies were purchased from (Biolegend, San Diego, CA).

Quantification of human IL-24 protein

Human IL-24 DuoSet enzyme-linked immunosorbent assay (ELISA) Development Kit was used to quantify secreted IL-24 in serum and plasma according to the manufacturer's instruction (R&D Systems, Minneapolis, MN). In brief, serum and plasma samples, which were stored at − 80 °C until ELISA experiments, were run in duplicates, and absorbance was measured at 450 nm. Samples were analyzed individually.

PBMC cultures and flow cytometric analysis

PBMCs were cultured at a concentration of 1×10^6 cells/ml in 12-well culture plates in presence or absence of IL-24 (200 ng/ml) alone or in combination with IL-2 (200 IU/mL). Cells were harvested after 72 h and stained with following antibodies including 7-AAD, Alexa Fluor700 anti-CD3, PE-anti-CD56, FITC-anti-CD16, APC-anti-CD69, and the isotype-matched anti-IgG control (APC). The 7-AAD was used to label the non-viable cells for cell viability assessment. The surface markers of CD3, CD56, and CD16 were used to define two NK cell subsets, i.e., $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cells. The surface marker CD69, an early activation marker of NK cells, was used to assess the NK cell activity. An irrelevant isotype-matched antibody was used as a negative control for CD69. Cells were examined with flow cytometry using a FACS Arial II flow cytometer (Becton Dickinson, Franklin Lakes, NJ), and the obtained flow cytometric data was analyzed using the Flowjo software VX10 (FlowJo, LLC, Ashland, OR). Results were expressed as percentage of positive cells for each marker.

Transwell migration and Matrigel invasion assays

The migratory ability of the NK-92 cells was measured using the Transwell chambers with 8.0 μm pore polycarbonate

membrane (Corning, Corning, NY). The invasive ability of the NK-92 cells was quantified using the Matrigel invasion chambers with 8.0 μm polyester (PET) membrane (Corning). In brief, a total of 1×10^6 NK-92 cells were resuspended into 200 μL serum-free α-MEM and were added to the top wells of the Transwell chambers or the Matrigel invasion chambers. The lower chambers were filled with 500 μL α-MEM supplemented with 10% horse serum and 10% fetal bovine serum with or without IL-24 (either 100 or 200 ng/ml) alone or in combination with IL-2 (200 IU/mL). After incubation at 37 °C for 48 h, the numbers of transmigrated NK cells were counted.

Statistical analysis

Serum and plasma concentrations of IL-24 were compared between cases and controls and the significance of differences between two groups was evaluated using the non-parametric two-sided Mann-Whitney *U* test. The two-tailed independent Student's *t*-test was applied to assess the influence of recombinant IL-24 stimulation on NK cell expression and NK-92 migration or invasion. The two-tailed paired Student's *t*-test with Bonferroni correction was applied to assess the influence of recombinant IL-24 stimulation on NK cell activation. All statistical analyses were conducted using SPSS 22.0 software (IBM SPSS, Armonk, NY). A *p*-value less than 0.05 were considered statistically significant (*$p < 0.05$; **$p < 0.01$; NS, not significant).

Results

Serum and plasma concentrations of IL-24 in SLE patients and healthy controls

Previous study has reported that the serum concentration of IL-24 was significantly elevated in SLE patients compared with healthy individuals [15]. However, another group showed no difference [16]. Additionally, it has been reported that the cytokine expression in plasma was more stable compared with serum; thus, plasma may be a better source than serum for quantifying the cytokine expression [34]. In present study, we measured the concentration of IL-24 in both serum and plasma from patients with SLE or RA and healthy individuals. As shown in Fig. 1, we found majority of serum and plasma samples were under detectable for IL-24, in either patients with SLE and RA or healthy individuals. The positivity rates of IL-24 were similar between SLE, RA, and HC groups in both serum (24.75%, 19.63%, and 27.04%, respectively) and plasma samples (20.00%, 23.17%, and 17.89%, respectively) (Table 2). Similarly, serum and plasma levels of IL-24 were comparable between three groups and no significant differences were observed compared to HC group (all $P > 0.05$, Fig. 1A and 1B). Next, we investigated the possible association between IL-24 concentration and SLE clinical/serological parameters. As shown in Fig. 1C and 1D, there were no any significant differences concerning IL-24 expression in SLE clinical/serological subsets.

Table 2 Serum and plasma IL-24 positivity in cases and HCs

IL-24 positivity	Controls	RA cases	SLE cases
Serum, *n* (%)	43 (27.04)	42 (19.63)	74 (24.75)
Plasma, *n* (%)	22 (17.89)	19 (23.17)	14 (20.00)

RA, rheumatoid arthritis; *SLE*, systemic lupus erythematosus

*"*n*" indicating the number of cases and "%" indicating the proportion of positivity of IL-24

Lack of activation effect in NK cells following stimulation with IL-24

Given that IL-24 exhibited an immunostimulatory effect in activation of myeloid cells, such as monocytes and neutrophils [30] but displayed an inhibitory effect on plasma cell differentiation in human germinal center B cells [31]. NK cells from patients with active SLE displayed an unusual phenotype and impaired functions [2, 7]. Next, we investigated whether in vitro IL-24 had a capacity to induce NK cell activation. Furthermore, it is known that IL-2 exerts potent stimulatory effect on NK cell activation [35]. Thus, in present study the rhIL-2 was applied as a positive control. PBMCs from both healthy controls and SLE patients with active disease were cultured with rhIL-2, rhIL-24 alone, or both in combinations. As shown in Fig. 2, we observed a decreased proportion of total NK cell population ($CD3^-CD56^+$ NK cells) in SLE patient group compared to HCs (Fig. 2A and 2B). This decreased proportion of total NK cells was mainly attributed to reduced frequencies of $CD56^{dim}CD16^+$ NK cell subpopulation in SLE patients (Fig. 2C and 2D). In contrast, the proportion of $CD56^{bright}CD16^-$ NK cells among total NK cells was increased in patient group compared with healthy controls (Fig. 2D). These findings are consistent with previous reports [2, 6–8]. However, in general stimulation with either IL-2 or IL-24 alone or IL-2/IL-24 combination had no effects on expansion of total or subpopulations of NK cells in vitro.

We next examined the ability of IL-24 on NK cell activation by measuring surface expression of CD69, an early activation marker, in $CD56^{dim}CD16^+$ and $CD56^{bright}CD16^-$ NK cell subsets, respectively. As shown in Fig. 3, while the recombinant human IL-2 consistently induced an increased expression of CD69 on both $CD56^{dim}CD16^+$ and $CD56^{bright}CD16^-$ cells, IL-24 alone was insufficient to activate the $CD56^{dim}$ and $CD56^{bright}$ NK cells. IL-24 in combination with IL-2 showed no synergistic effect on activation of

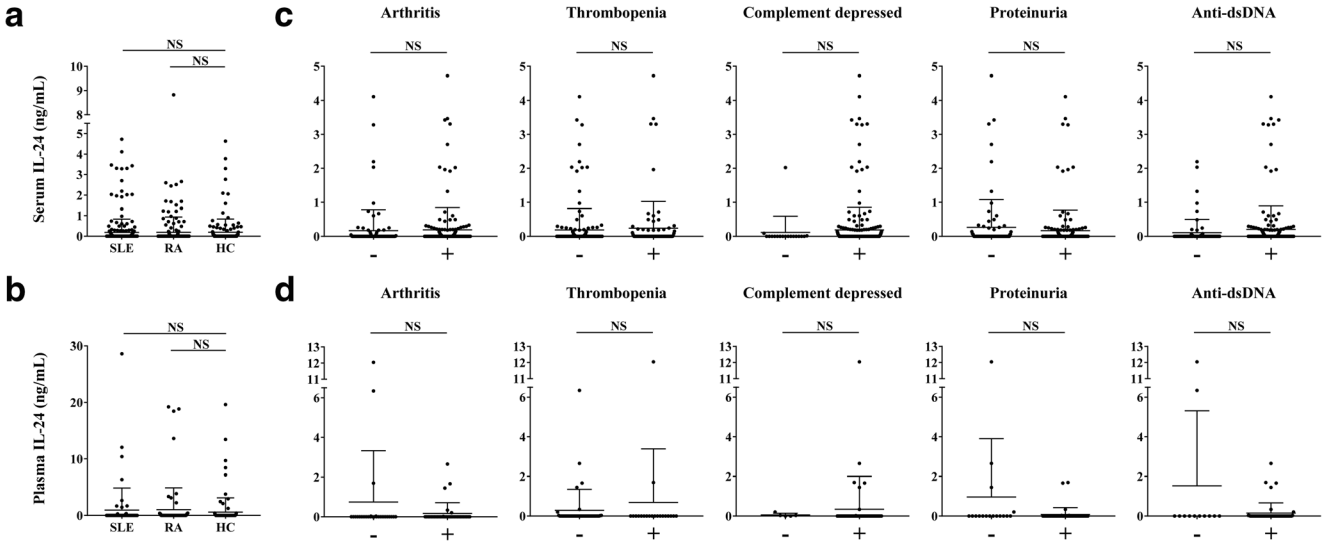

Fig. 1 Serum and plasma concentrations of interleukin (IL)-24 in patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and healthy controls (HCs). **A** and **B** Serum and plasma levels of IL-24 were comparable between SLE patients (serum: $n = 299$, plasma: $n = 70$), RA patients (serum: $n = 214$, plasma: $n = 82$), and HCs (serum: $n = 159$, plasma: $n = 123$). **C** and **D** Serum and plasma levels of IL-24 showed no differences within clinical/serological subphenotypes, including arthritis (serum: $n = 282$, plasma: $n = 65$), thrombopenia (serum: $n = 262$, plasma: $n = 64$), complement depressed (serum: $n = 295$, plasma: $n = 62$), proteinuria (serum: $n = 262$, plasma: $n = 64$), and anti-dsDNA antibodies (serum: $n = 269$, plasma: $n = 65$). All data represent as mean ± standard deviation (SD)

both $CD56^{dim}$ and $CD56^{bright}$ NK cells in comparison with IL-2 alone (Fig. 3A–D). We also evaluated whether the capacity of IL-24 on NK cell activation differs between NK cell subpopulations derived from healthy individuals and patients with SLE. As shown in Fig. 3B, although the administration of recombinant IL-24 induced a decreased expression of CD69 on $CD56^{bright}CD16^{-}$ cells in patient group, in general there were no differences between healthy individuals and patients with SLE concerning the functional capacity of IL-24 on NK cell activation.

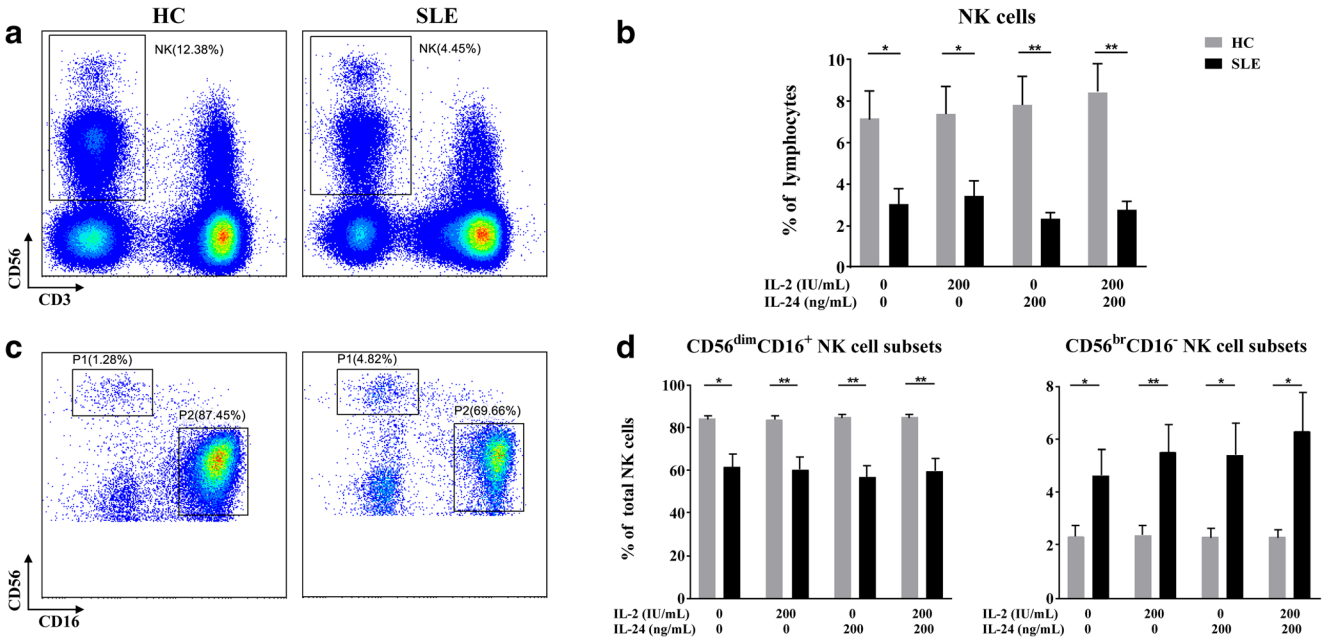

Fig. 2 Influence of recombinant IL-24 stimulation on whole NK cell population ($CD3^{-}CD56^{+}$ NK cells) and $CD56^{dim}CD16^{+}$/$CD56^{bright}CD16^{-}$ NK cell subpopulations. Peripheral blood mononuclear cells (PBMCs) from either healthy individuals (HCs, $n = 14$) or systemic lupus erythematosus (SLE) patients with active disease ($n = 14$) were cultured in presence or absence of IL-24 (200 ng/ml) alone or in combination with IL-2 (200 IU/mL). **A** Defining NK cells by gating $CD3^{-}CD56^{+}$ cells. **B** Gating strategy to define $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cell subsets. **C** A decreased proportion of total NK cell population ($CD3^{-}CD56^{+}$ NK cells) in SLE patient group compared to HCs. **D** Reduced proportions of $CD56^{dim}CD16^{+}$ NK cells and increased frequencies of $CD56^{bright}CD16^{-}$ NK cells from SLE patients compared with HCs. Data represent as mean ± standard error of mean (SEM). $^{*}P < 0.05$, $^{**}P < 0.01$

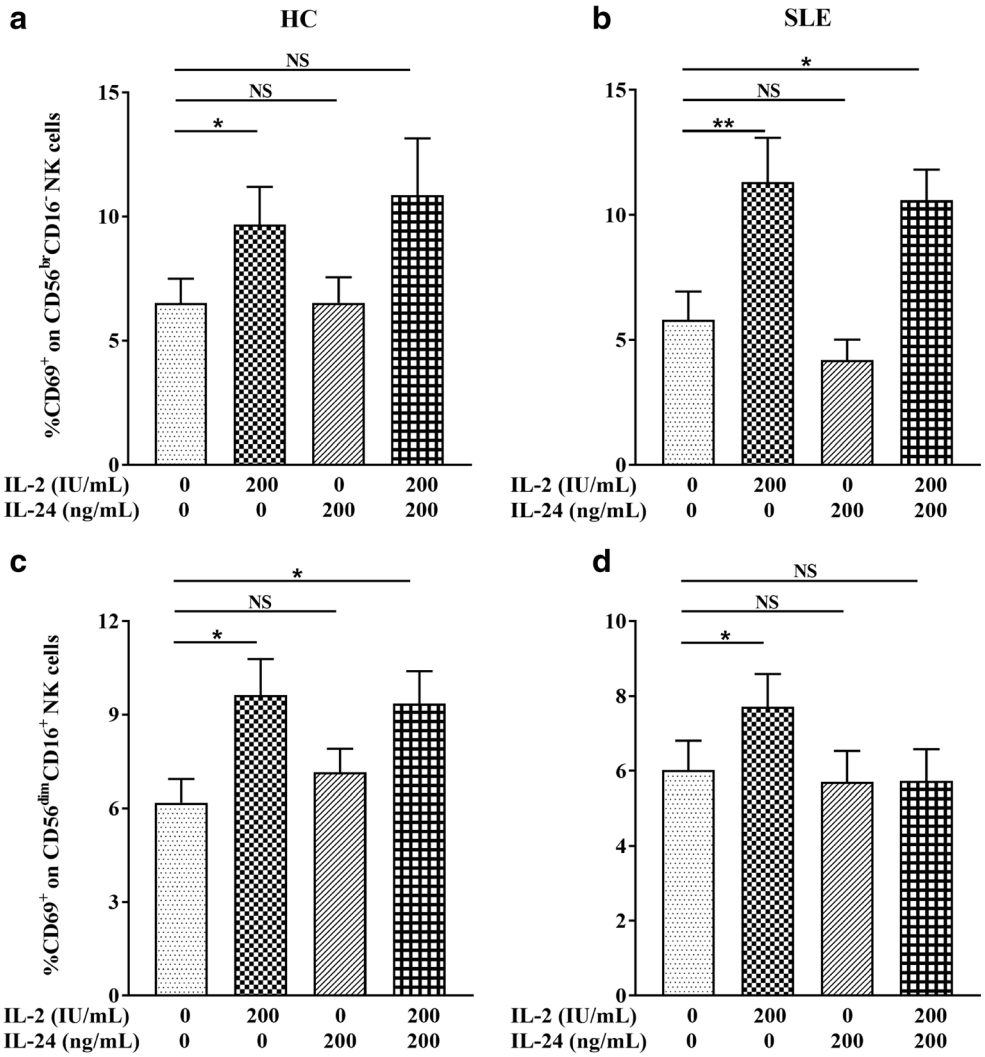

Fig. 3 Influence of recombinant IL-24 stimulation on NK cell activation. Peripheral blood mononuclear cells (PBMCs) from either healthy controls (HCs, n = 14) or systemic lupus erythematosus (SLE) patients with active disease (n = 14) were cultured in presence or absence of IL-24 (200 ng/ml) alone or in combination with IL-2 (200 IU/mL). Activated NK cells were defined by CD69 positive NK cells. **A** and **B** Proportions of $CD69^{+}CD56^{bright}CD16^{-}$ NK cells from either HCs or SLE patients. **C** and **D** Proportions of $CD69^{+}CD56^{dim}CD16^{+-}$NK cells from either HCs or SLE patients. Data represent as mean ± standard error of mean (SEM). $^{*}P < 0.05$, $^{**}P < 0.01$

Recombinant human IL-24 showed no effect on NK cell migration and invasion

Given that NK cell migration is a critical process for its effector function [36], the previous study has reported the recombinant IL-24 capable of inducing human monocyte and neutrophil migration [30]. We next examined the ability of recombinant IL-24 on NK cell migration and invasion in human NK cell lines NK-92 which possesses a high migratory capacity [37]. The migratory and invasive ability of NK-92 cells was quantitatively evaluated using the Transwell migration and Matrigel invasion assays, respectively. Furthermore, NK-92 cell line was dependent on exogenous IL-2 for its viability and proliferation [38] and in vitro IL-2 stimulation induced a strong NK-92 cell migration [35, 39]. Recombinant IL-2 was therefore chosen as an appropriate positive control in migration and invasion assays. As shown in Fig. 4A, while the migratory NK-92 cell numbers were significantly increased with 200 U rhIL-2 stimulation, IL-24 alone was unable to enhance the NK-92 cell migratory capacity. IL-24 in combination with IL-2 showed no synergistic effect on NK-92 cell migration compared with IL-2 alone. Similarly, while the number of Matrigel invading NK-92 cells increased in the presence of rhIL-2, IL-24 alone or in combination with IL-2 showed no such effect on NK-92 cell invasion (Fig. 4B).

Discussion

Previously, two research groups have investigated the expression pattern of IL-24 in SLE patients with modest sample sizes. One group reported that serum levels of IL-24 were significantly higher in SLE patients (n = 75) compared with control subjects (n = 58) [15]. However, another group showed that there was no significant difference in serum concentration of IL-24 between SLE patients (n = 65) and healthy controls (n = 65) [16]. By measuring the concentration of IL-24 with a larger sample size in both serum (SLE patients n = 299 vs. HCs n = 159) and plasma (SLE patients n = 70 vs. HCs n = 123), here we show that IL-24 was undetectable in

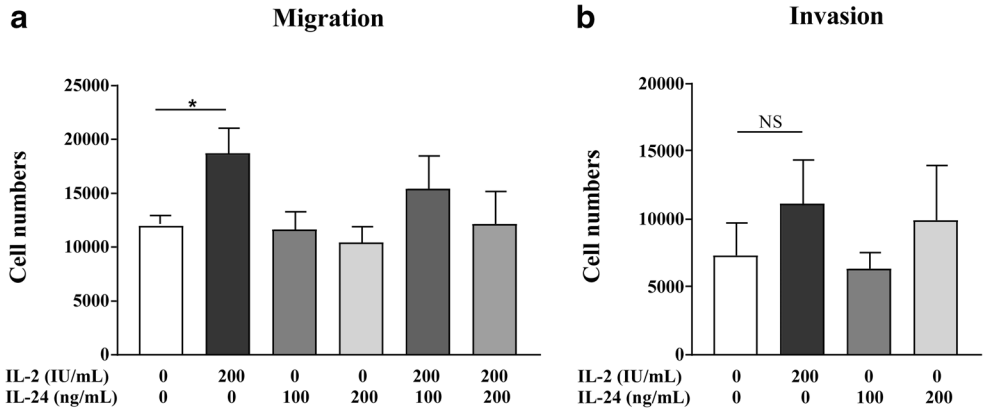

Fig. 4 Influence of recombinant IL-24 stimulation on NK-92 cell migration and invasion. The migratory and invasive abilities of NK-92 cells were investigated by placing 1.0×10^6 NK-92 cells on the top wells of Transwell or Matrigel invasion chambers, respectively. The lower chambers were filled with 500 μL α-MEM supplemented with **A** IL-24 (either 100 or 200 ng/ml) alone or in combination with IL-2 (200 IU/mL) in Transwell migration assay and **B** IL-24 (200 ng/ml) alone or in combination with IL-2 (200 IU/mL) in Matrigel invasion assay. Each bar represents mean ± standard error of mean (SEM) from five separate experiments. *$P < 0.05$

majority of serum and plasma samples from patients with SLE and healthy subjects. There were no differences in positivity of IL-24 between two groups. There were also no differences concerning serum and plasma levels of IL-24 in SLE clinical/serological subsets. Previously, IL-24 has also been shown to be expressed at increased levels in serum or plasma from RA patients [14, 40]. In present study, we found that IL-24 was not detected in majority of RA patients and there were no differences in expression levels of IL-24 either between RA patients and HCs or between the two patient groups in both serum and plasma.

It has been previously reported that IL-24 possesses anti-tumor properties by introducing cancer-specific cell death when overexpressed above the physiologic levels, but conflicting findings also exist and the anti-tumor properties of IL-24 remain controversial [22–28]. IL-24 seems to play a pro-inflammatory role in immune-pathological or autoimmune diseases, such as psoriasis, RA, spondyloarthritis, and inflammatory bowel disease [14, 40, 41]. In vitro treatment of PBMC with the HEK 293 cell line–derived IL-24 induced high levels of pro-inflammatory cytokine secretion, such as IL-6, TNF-α, and IFN-γ [29]. Recombinant IL-24 protein could induce human monocyte and neutrophil migration [30]. Nevertheless, there is also evidence that IL-24 possesses an anti-inflammatory effect in immune response. For example, IL-24 displayed an inhibitory effect on differentiation of human GC B cells into mature plasma cells [31]. This has prompted us to investigate a potential role of IL-24 in NK cell activation and function. We evaluated the impact of rhIL-24 on NK cell activation in both $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cell subsets and found that recombinant IL-24 lacks functional capacity on NK cell activation, neither $CD56^{dim}CD16^{+}$ nor $CD56^{bright}CD16^{-}$ cell subpopulations. Given that the NK cells from patients with active SLE displayed phenotypic and functional abnormalities compared to healthy individuals [2, 7, 8], we further assessed the functional capacity of IL-24 on NK cell activation in primary NK cell subsets derived from both healthy subjects and SLE patients. No difference was observed between healthy individuals and patients with SLE in functional capacity of IL-24 on NK cell activation. Furthermore, we showed that recombinant IL-24 has no effect on NK cell migration and invasion in human NK cell line NK-92.

The possible explanation for our findings could be that IL-24 is a ligand for two heterodimeric receptors, IL-20R1/IL-20R2 and IL-22R1/IL-20R2. Although the common subunit IL-20R2 is expressed in many immune cells, the IL-20R1 and IL-22R1 subunits appear to be not expressed on monocytes, T, B, and NK cells [10, 40]. It may indicate that the immune cells are unlikely to be the main functional targets for IL-24, despite IL-24 can be produced by active immune cells. In view of this, it is not surprising that rhIL-24 could not trigger the NK cell activation and function. Furthermore, it has been shown that the IL-20R1/IL-20R2 and IL-22R1/IL-20R2 receptors function through JAK/STAT signaling pathway [9, 42]. Although it was reported that the IL-24-induced apoptosis of tumor cells could be independent of JAK/STAT pathway and thus presumably independent of the heterodimeric receptors IL-20R1/IL-20R2 and IL-22R1/IL-20R2 signaling [43, 44], so far no direct evidence supports that other types of IL-24 receptors are expressed on myeloid and lymphoid cells.

In summary, our results indicate that the concentrations of IL-24 in both serum and plasma are comparable between SLE patients and HCs. Recombinant IL-24 lacks the functional capacity to activate the $CD56^{dim}$ and $CD56^{bright}$ NK cells in both healthy individuals and SLE patients and has no effect on NK-92 cell migration and invasion.

Authors' contributions Jianping Guo: conceptualization, supervision, formal analysis, funding acquisition, writing—original draft, writing—review and editing. Xia Li: conceptualization, investigation. Yundi Tang:

investigation, methodology, data curation, formal analysis, validation, writing—original draft. Xiaotong Sun: investigation, methodology, resources, data curation, validation. Yuxuan Wang and Huijie Luan: investigation, methodology. Ruijun Zhang: methodology, resources. Fanlei Hu and Xiaoling Sun: supervision.

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Data availability The datasets used during the study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethical approval and consent to participate The study obtained ethical approval from the Ethics committee of Peking University People's Hospital. Informed consent was obtained from all individual participants included in the study.

Disclosures None.

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