Clinical Immunology 202 (2019) 18–28

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

The inhibitory receptors on NK cells and CTLs are upregulated in adult and adolescent patients with secondary hemophagocytic lymphohistiocytosis

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ARTICLE INFO

Keywords:
Hemophagocytic lymphohistiocytosis
Natural killer cells
Cytotoxic T lymphocytes
Surface receptors

ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) includes primary HLH (pHLH) and secondary HLH (sHLH). Mutations that cause abnormal functions in natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) are frequently identified in pHLH. However, why NK cells and CTLs exhibit abnormal functions in sHLH remains unclear. Here, we demonstrated that NK cells in sHLH exhibited a high expression of inhibitory receptor NKG2A and a low expression of activating receptor NKG2D. Besides, the expression of HLA-E on lymphocyte, the adaptor of NKG2A on NK cells, was elevated in sHLH. Moreover, CTLs in sHLH patients expressed a higher level of functional exhaustion markers PD-1, TIM-3 and LAG-3 as well as a lower secretion of IFN-γ and CD107a upon stimulation. In addition, the expression of MHC-I on lymphocytes was decreased. Taken together, our study indicates a potentially pathological mechanism of sHLH and may open up new avenues for the development of immunotherapies against sHLH.

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome [1]. It is characterized by fever, hepatosplenomegaly and cytopenia [2]. The two types of HLH include primary HLH (pHLH) that is triggered by genetic defects, and secondary HLH (sHLH) which is induced by infection, cancer or autoimmune disease. The impaired function of NK cells and cytotoxic T lymphocytes (CTLs), which is shared by all forms of HLH, contributes to the initiation of HLH [1–3]. In pHLH, genetic mutations involving perforin and the degranulation pathway abrogate the function of NK cells and CTLs in eliminating infected cells and cancer cells [4]. Consequently, existing antigenemia stimulates antigen-specific T cells and the expanded CTLs induce the hyperactivation of macrophages and cytokine storm [4].

Despite the advances in the diagnosis of sHLH, the pathophysiological mechanism of sHLH remains unclear. Recently, the impaired function of NK cells and CTLs in the pathogenesis of disease has gained increasing concerns, and several studies have demonstrated that high cytokine levels may weaken the function of NK cells and CTLs [5,6]. The function of NK cells depends heavily on the activation of NK cell surface receptors. NK cell surface receptors can be mainly categorized into inhibitory receptors and activating receptors based on their function. The activating receptors include natural cytotoxic receptors (NKp46, NKp44, and NKp30), NKG2D, KIR2DS, and their activating co-receptors 2B4 and CD16. The inhibitory receptors include KIR2DL, KIR3DL, and CD94/NKG2A [7]. The balance between the inhibitory signaling and activating signaling determines the consequence of NK cells and target cells interactions [8]. Once the balance is disturbed, the function of NK cells may be compromised.

CTLs can provide protections against the sustained insults from intracellular pathogens. Previous studies suggest that the abnormal function of CTLs can play a role in the pathogenesis and progression of inflammatory and neoplastic diseases [9,10]. When CTLs are in contact with target cells, perforin can be released and embedded into the target cell membrane to form a polymer-like tubular structure through which extracellular fluids can enter the target cells, leading to cell lysis. Also, CTLs can secrete granzyme, entering target cells from the pore and inducing apoptosis of target cells [11]. If the function of CTLs is

Abbreviations: CD, cluster of differentiation; CD107a, lysosomal-associated membrane protein-1; CTL, cytotoxic T cell; DNA, deoxyribonucleic acid; EBV, Epstein-Barr virus; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; HCMV, human cytomegalovirus; HLA, human leukocyte antigen; HLH, hemophagocytic lymphohistiocytosis; IFN-γ, interferon-γ; LAG3, lymphocyte activation gene 3; MHC, major histocompatibility complex; NK cell, natural killer cell; PBS, phosphate buffer solution; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death protein 1; TIM3, T cell immunoglobulin domain and mucin domain-containing protein 3

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https://doi.org/10.1016/j.clim.2019.03.006
Received 30 August 2018; Received in revised form 15 February 2019; Accepted 22 March 2019
Available online 23 March 2019

impaired, some diseases may be triggered. It is well-established that the dysfunction and exhaustion of CTLs are chief issues for ineffective virus-elimination in chronic infectious diseases [12]. Several studies found that the functional exhaustion of CTLs may lead to a deficiency in CTLs function [13,14], and low MHC-I expression in the target cells protect them from the cytotoxic effects of CTLs [15]. While these recent studies have suggested the potential regulatory mechanisms by which NK cells and CTLs sustain cytotoxicity, the exact mechanism that leads to the loss of cytotoxicity on NK cells and CTLs is still not well understood in sHLH.

Here, we analyzed the expression of NK cell-activating receptor NKG2D, NK cell-inhibitory receptor NKG2A and NKG2A adaptor HLA-E in sHLH and found that the function of NK cells is impaired in sHLH. The high expression of PD-1, LAG-3, TIM3 as well as the deficiency in IFN-γ and CD107a production confirm the exhaustion of CTLs in sHLH. Besides, the expression of MHC-I on lymphocytes decreased, which hampers the proper function of CTLs. Together, our study indicates a potentially pathological mechanism of sHLH and may open up new avenues for the development of immunotherapies against sHLH.

2. Materials and methods

2.1. Healthy volunteers and patients

Diagnosis of HLH was based on the criteria set in the HLH-2004 protocol [16]. A total of 33 sHLH patients was recruited into this study, who presented at the Department of Hematology at Beijing Friendship Hospital from October 2017 to July 2018. All sHLH patients enrolled did not have a documented molecular mutation (PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, AP3B1, SH2D1A, BIRC4, ITK, CD27, MAGT1). Additionally, 22 healthy subjects were invited to participate as controls. The study was explained to all subjects, who provided signed informed consent. The present study was approved by the Ethics Committee of Beijing Friendship Hospital, which is affiliated with Capital Medical University.

2.2. Sample collection, antibodies, and flow cytometry

Flow cytometry was performed using fresh samples collected in ethylenediaminetetraacetic acid (EDTA) and processed within 4 h of collection. One hundred microliters of whole blood were added to the appropriate tubes (control and sample tubes were the same size).

Antibodies were used according to the product manual: anti-human CD3 Alexa Fluor® 700 (eBioscience, clone OKT3, CAT# 56-0037-42), anti-human CD4 eFluor® 506 and eFluor 450 (eBioscience, clone RPA-T4, CAT# 69-0049-42 and clone OKT-4, CAT# 48-0048-42), anti-human CD8a eFluor® 450 and eFluor 506 (eBioscience, clone RPA-T8, CAT# 48-0088-42 and clone RPA-T8, CAT# 69-0088-42), anti-human CD19 FITC (eBioscience, clone HIB19, CAT# 11-0199-42), anti-human CD56 (NCAM) PerCP-eFluor® 710 (eBioscience, clone CMSSB, CAT# 46-0567-42), anti-human CD159a (NKG2A) FITC (Miltenyi, clone REA110, CAT# 130-098-818), anti-human CD314 (NKG2D) PE (eBioscience, clone1D11, CAT# 12-5878-42), anti-human CD335 (NKp46) APC (eBioscience, clone 9E2, CAT# 17-3359-42), anti-human HLA-E PE (eBioscience, clone 3D12HLA-E, CAT# 12-9953-42), anti-human HLA-ABC APC (eBioscience, clone W6132, CAT# 17-9983-42), anti-human CD279 (PD-1) PerCP-eFluor® 710 (eBioscience, clone eBioJ105, CAT# 46-2799-42), anti-human CD223 (LAG-3) FITC (eBioscience, clone 3DS223H, CAT# 11-2239-42), and anti-human CD366 (TIM3) PE (eBioscience, clone F38-2E2, CAT# 12-3109-42). The cells were incubated with the indicated antibodies for 20 min at room temperature and then with 2 mL of 1× red blood cell lysis (Solarbio, CAT# R1010) for 15 min at room temperature, centrifuged, and washed once with 1× phosphate buffered solution (PBS, pH 7.2–7.4 from Solarbio, CAT# P1020). The samples were tested on CytoFLEX (Beckman Coulter).

2.3. Intracellular cytokine staining

For intracellular cytokine staining, PBMCs were isolated by standard Ficoll-Hypaque gradient centrifugation and then cultured with a 1× Cell Stimulation Cocktail (plus protein transport inhibitors) (500×) (eBioscience, CAT# 00-4975-93) in the presence of RPMI-1640 supplemented with 2% FBS for 4.5 h. The Cell Stimulation Cocktail is a cocktail of phorbol 12-myristate 13-acetate (PMA), ionomycin, brefeldin and monensin. Dead cells were removed by Fixable Viability Dye (FVD) (eBioscience, CAT# 65-0866-18), then cells were incubated with the CD3 Alexa Fluor® 700 and CD8a eFluor® 450 antibodies, then fixed and permeabilized with an IC Fixation Buffer (eBioscience, CAT# 00-8222-49) and permeabilization buffer (eBioscience, CAT# 00-8333), and stained with anti-human IFN gamma APC-eFluor® 780 (eBioscience, clone 4S·B3, CAT# 47-7319-42). FVD is a viability dye that can be used to irreversibly lable dead cells prior to cryopreservation, fixation, and/or permeabilization proce dures.

2.4. CD107a staining

PBMCs were isolated by standard Ficoll-Hypaque gradient centrifugation, cultured with CD3 and P815 cell for 4 h, and then stained with anti-human CD3 FITC (4A Biotech, clone OKT3, CAT# FHF003-100), anti-human CD8a PerCP-Cyanine5.5 (eBioscience, clone RPA-T8, CAT# 45-0088-42), and anti-human CD107a PE (eBioscience, clone eBioH4A3, CAT# 12-1079-42).

2.5. Statistical analysis

SPSS version 19.0 was used for statistical analysis. All the data that conformed to the normal distribution were represented by $x \pm SD$, and the *t*-test was used for comparison, whereas non-normally distributed data were presented using the median and range, and the Wilcoxon's rank-sum test was used. Significant statistical difference was defined as $P < .05$.

3. Results

3.1. Characteristics of the enrolled participants

A total of 33 patients with sHLH were enrolled (21 males, 12 females) in the study, with a median age of 32 years (range: 13–56 years). Eighteen patients presented with EBV infection as the primary disease. Seven patients presented with lymphoma as the primary disease. Two patients were diagnosed with natural killer cell leukemia, and two presented with rheumatological disease as the primary disease. The primary disease of four patients was not clear (Table 1). 22 healthy individuals were included in the control group (10 males, 12 females), with a median age of 33 years (range: 23–65 years).

Table 1
The causes of sHLH.

Primary disease	N (percent)	Male/female
EBV-HLH	18 (54.55%)	13/5
Lym-HLH	7 (21.21%)	5/2
NKL-HLH	2 (6.06%)	2/0
MAS	2 (6.06%)	0/2
Unknown	4 (12.12%)	1/3

EBV-HLH: EBV associated HLH; Lym-HLH: lymphoma associated HLH.
NKL-HLH: natural killer cell leukemia associated HLH.
MAS: rheumatological disease associated HLH
Unknown: The primary disease is not clear.

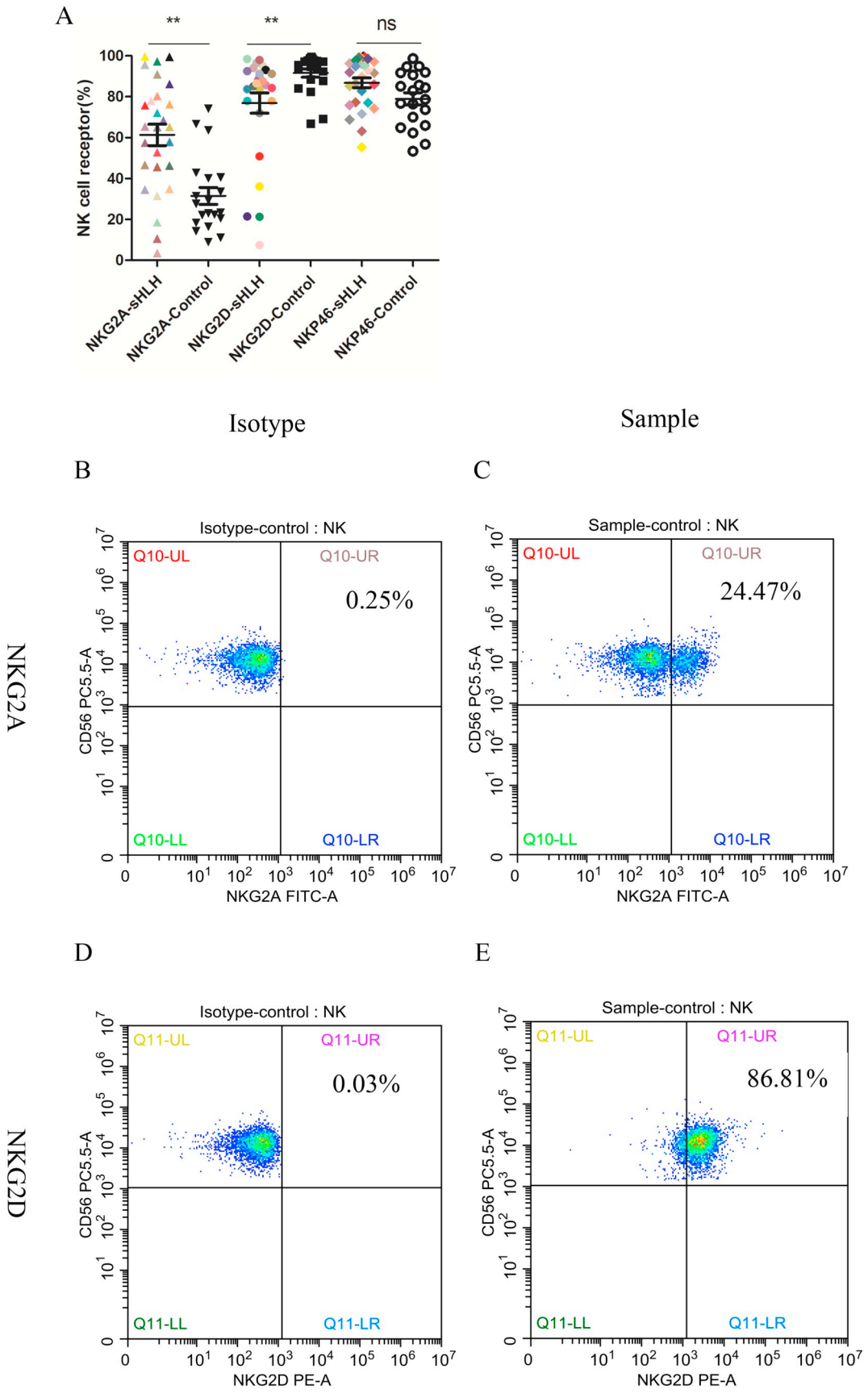


Fig. 1. Upregulation of NKG2A and downregulation of NKG2D in sHLH patients.

A. The different expressions of NK cell surface receptors NKG2A, NKG2D and NKP46 in sHLH and control group (** $P < .01$; ns not significant).

B–M. The representative images for the expressions of NK cell surface receptors NKG2A, NKG2D and NKP46 in sHLH and control group.

(B, D, F) The representative image for the expression of NKG2A, NKG2D and NKP46 in control group (negative control); (C, E, G) The representative image for the expression of NKG2A, NKG2D and NKP46 in control group; (H, J, L) The representative image for the expression of NKG2A, NKG2D and NKP46 in sHLH group (negative control); (I, K, M) The representative image for the expression of NKG2A, NKG2D and NKP46 in sHLH group;

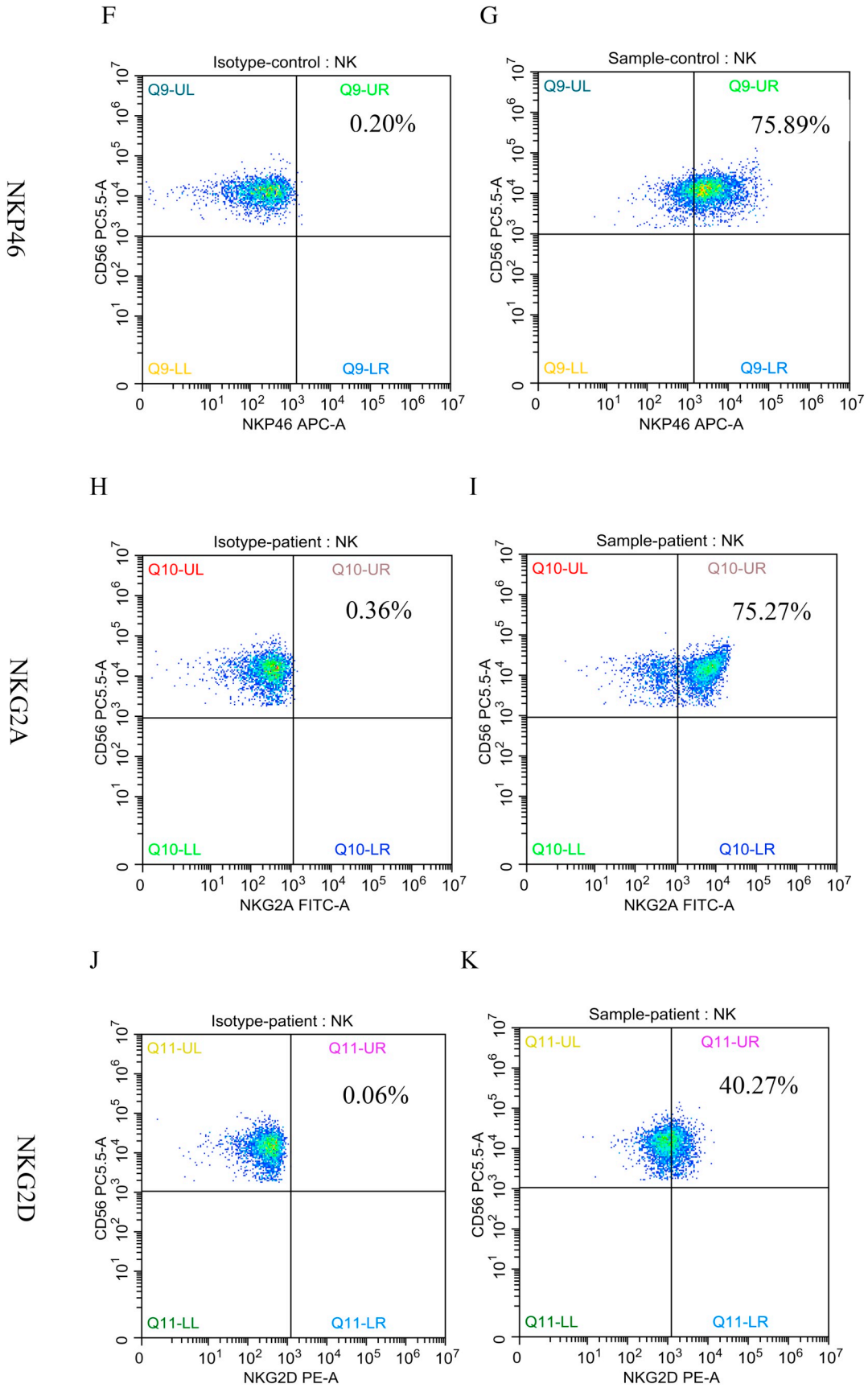


Fig. 1. (*continued*)

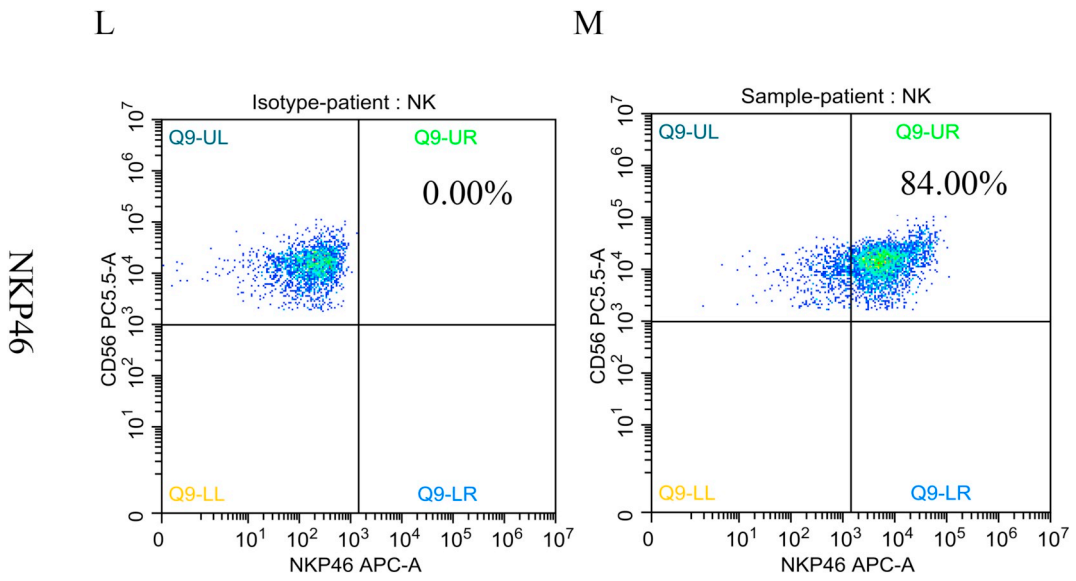


Fig. 1. (*continued*)

3.2. Upregulation of NKG2A and downregulation of NKG2D in sHLH patients

The numbers of NK cells in six sHLH patients were too low and were thus excluded from further analysis. The expression of NKG2A, NKP46 and NKG2D in 27 patients with sHLH was analyzed. As shown in Fig. 1, compared to the control group, the patients with sHLH exhibited significantly higher levels of NKG2A (61.35 ± 27.04 vs. 31.73 ± 17.98, $P = .001$), significantly lower levels of NKG2D (87.42(7.42–98.46) vs. 91.34 ± 9.30, $P = .009$). No significant difference of NKP46 expression were found between sHLH and control group(86.14 ± 13.94 vs.79.89 ± 12.71, $P = .116$).

3.3. Upregulation of HLA-E in sHLH patients

Based on the upregulation of NKG2A and downregulation of NKG2D, we hypothesized that the suppression of NK cell in sHLH patients may be induced by the increasing combination of NKG2A and HLA-E on lymphocytes. To test this hypothesis, the expression of HLA-E on lymphocytes was detected in the 19 subsequently enrolled sHLH patients (CD4 + T, $n = 19$; CD8 + T, $n = 19$; CD19 + B, $n = 17$; CD56 + NK, n = 17) and 11 controls. As shown in Fig. 2, the sHLH patients, compared to the control group, exhibited significantly higher HLA-E expression in CD4+ T cells (1.35 ± 0.54 vs. 0.73 ± 0.40, $P = .002$), CD8+ T cells (1.48 ± 0.63 vs. 0.85 ± 0.50, $P = .009$), CD19+ B cells (1.72 ± 1.09 vs. 0.82 ± 0.37, $P = .005$) and CD56+ NK cells (1.93 ± 0.75 vs. 1.23 ± 0.55, $P = .014$).

3.4. Downregulation of MHC-I in sHLH patients

The expression of HLA-ABC on lymphocytes was detected in 18 enrolled HLH patients (CD4 + T, $n = 18$; CD8 + T, $n = 18$; CD19 + B, $n = 17$; CD56 + NK, $n = 16$) and 12 controls. Fig. 3 shows that sHLH patients exhibited significantly lower HLA-ABC expression levels in CD4+ T cells (18.93 ± 11.01 vs. 35.03 ± 18.35, $P = .005$), CD8+ T cells (17.91 ± 10.37 vs. 38.57 ± 24.68, $P = .016$), CD19+ B cells (45.31 ± 25.94 vs. 79.71 ± 33.17, $P = .035$) and CD56+ NK cells (26.69 ± 21.76 vs. 43.01 ± 22.60, $P = .064$) than control group.

3.5. Functional exhaustion of CTLs in sHLH

To evaluate the functional exhaustion of CTLs, the expression of PD-1, TIM-3 and LAG-3 as well as the production of IFN-γ and CD107a after stimulation in 33 patients with sHLH and 22 controls was analyzed. The expression of PD-1, TIM-3 and LAG-3 on CTLs of the sHLH patients was 40.73 ± 22.64, 15.97 ± 14.45, and 0.73, (0–37.41), respectively. And the expression of PD-1, TIM-3, and LAG-3 on CTLs from the control group was 14.48 ± 7.98, 1.95 ± 1.52, and 0.01(0–2), respectively. As shown in Fig. 4A, it appears that levels of PD-1, TIM-3 and LAG-3 were significantly increased in sHLH patients in comparison with that in control group ($P = 2.53\ E-07$, $3.85\ E-06$; < 0.0001). After stimulation, IFN-γ secretion and CD107a expression in the CTLs were analyzed. In sHLH group, two patients exhibited a CTLs number below the level of detection and thus were excluded from the analysis. Fig. 4B shows that the CD8+ T cells of sHLH patients exhibited impaired IFN-γ secretion compared to the 13 controls (37.30 ± 24.46 vs. 55.17 ± 22.23, $P = .034$). Fig. 4C shows that among the 26 sHLH patients and 21 controls, the sHLH patients exhibited lower CD107a expression levels compared to the controls (4.49 ± 2.71 vs. 6.07 ± 2.14, $P = .035$).

4. Discussion

HLH includes pHLH that is induced by genetic defects, and sHLH that is induced by infection, cancer, or autoimmune disease [2]. Mutations that cause abnormal functions in NK cells and CTLs are frequently identified in patients with pHLH. However, why NK cells and CTLs exhibit abnormal functions in sHLH patients remains unclear. Previous studies have demonstrated that abnormal function of NK cells and CTLs plays a role in the pathogenesis and progression of several diseases, including inflammatory and neoplastic diseases [9,10]. Our study indicated that the impaired function of NK cells and CTLs may contribute to the pathogenesis of sHLH.

In this study, we analyzed the inhibitory receptor NKG2A and the activating receptor NKG2D and NKP46 in sHLH. We found that the sHLH patients exhibited increased expression of NKG2A and decreased expression of NKG2D compared with controls. However, there is no significant difference in the NKP46 expression between pHLH and control group. As we all know, the balance between the inhibitory signaling and activating signaling determines the cytolytic activity of NK cells [8]. In normal tissues, killer cell Ig-like receptors (KIRs) interact with MHC-I molecules, predominantly inhibit the signal conduction, and prevent NK cell activation. Cancer cells and virus-infected cells express low levels of MHC-I molecules that stimulate the activating receptor on NK cells. Consequently, NK cells are activated to eliminate

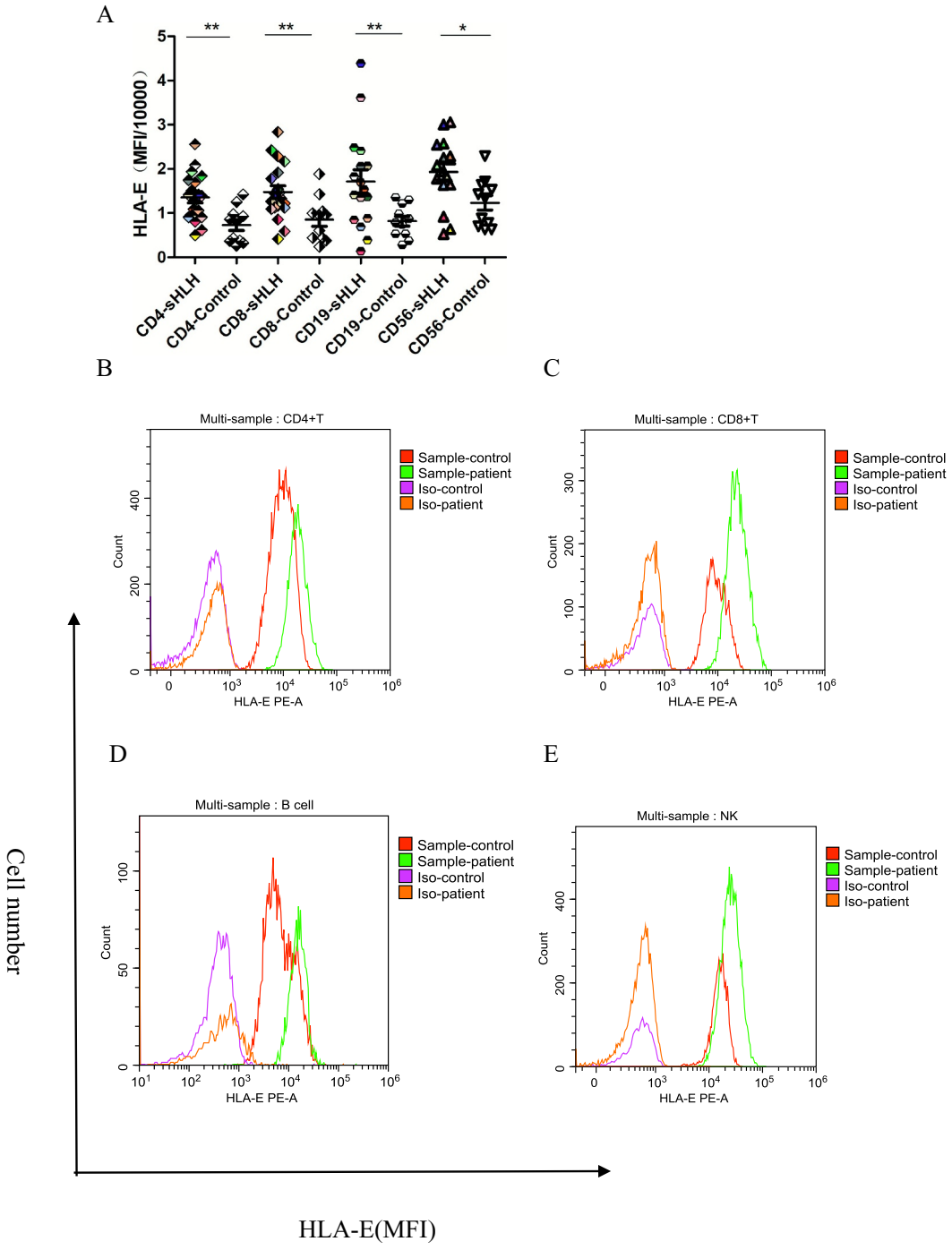


Fig. 2. Upregulation of HLA-E in sHLH patients.
A. The different expressions of HLA-E in CD4 + T cell, CD8 + T cell, CD19 + B cell and CD56 + NK cell in sHLH and control group (**P < .01).
B–E. The representative images for the expressions of HLA-E in CD4 + T cell, CD8 + T cell, CD19 + B cell and CD56 + NK cell in sHLH and control group.

aberrant cells and secrete cytokines [17]. A recent study demonstrated that the interaction of NKG2A and HLA-E enhances the inhibitory signaling in NK cells. CD94/NKG2A inhibiting receptors differ from CD94/NKG2C activating receptors, wherein inhibiting signal transmitting receptors are more sensitive to interactions with most known HLA-E/peptide complexes than those transmitting activating signals [18]. We analyzed NKG2A adaptor HLA-E in the follow-up experiment. In addition, previous studies have shown that high MHC-I expression inhibits NK cell activation. We hypothesized that high MHC-I expression and NGK2A induce NK cell suppression, which occurs in sHLH. Thus, we included the evaluation of HLA-E and MHC-I in the follow-up experiment. As these assays were performed in the follow-up experiment, only a portion of the enrolled patients were tested. The results showed that sHLH patients exhibited higher HLA-E expression levels and lower MHC-I expression levels. A recent study showed that in HCMV-infected patients, the upregulation of HLA-E in the target cells inhibited NK cell activation and protected the target cells from NK cell-induced cell death due to low MHC-I expression [19]. Our results were consistent with this theory and we speculated that the high expression of NKG2A and HLA-E in sHLH may inhibit the function of NK cells.

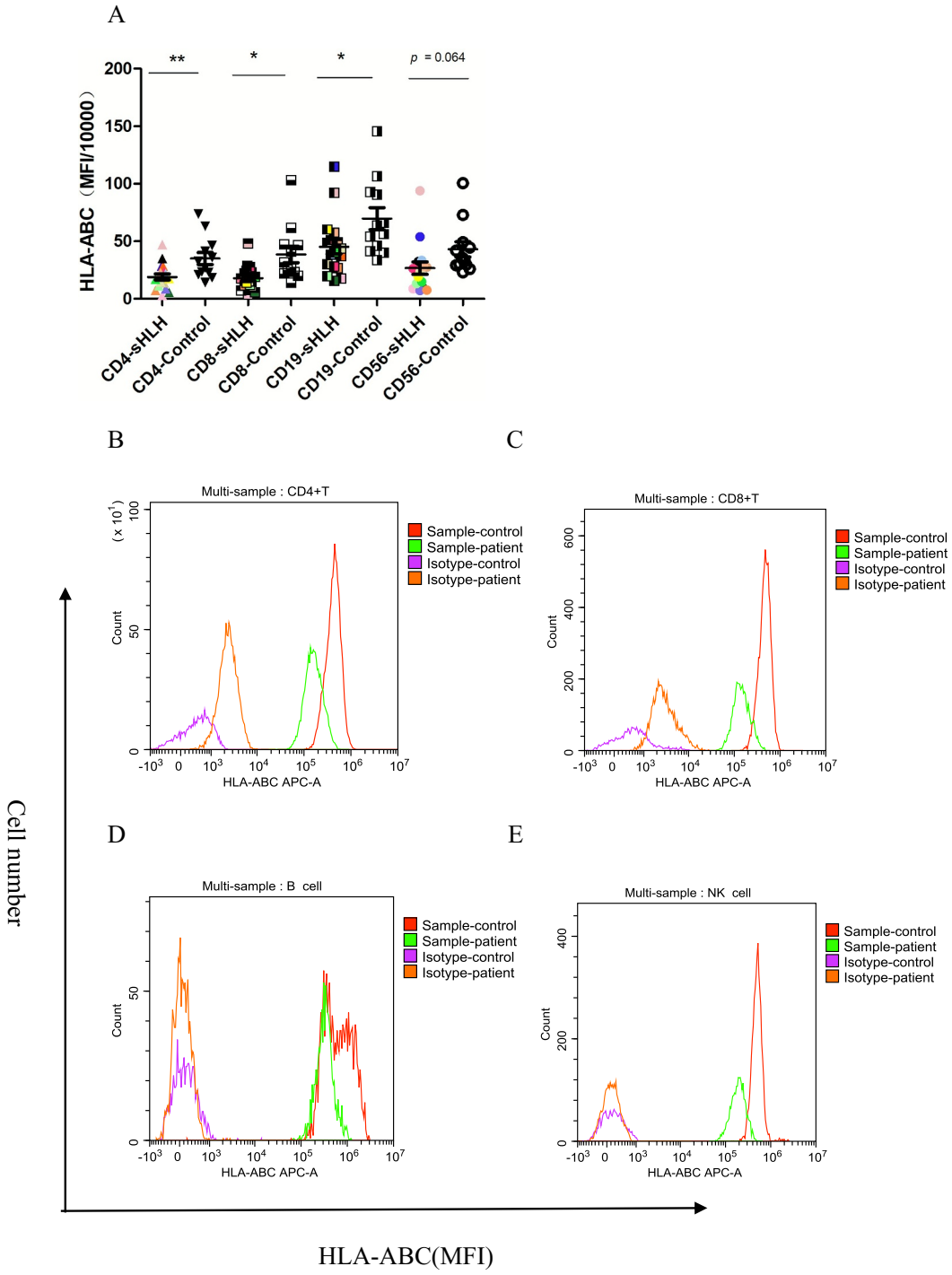


Fig. 3. Downregulation of MHC-I in sHLH patients. A. The different expressions of HLA-ABC in CD4 + T cell, CD8 + T cell, CD19 + B cell and CD56 + NK cell in sHLH and control group (**P < .01; *P < .05).
B–E. The representative images for the expressions of HLA-ABC in CD4 + T cell, CD8 + T cell, CD19 + B cell and CD56 + NK cell in sHLH and control group.

Furthermore, NK cells target pathological T cells in mice and human by activating receptors such as NKG2D [20–23]. Resting T cells are not targeted by NK cells, whereas activated T cells may upregulate the adaptors of NKG2D. The crosslink of NKG2D on NK cells and its adaptor on T cells promotes cytotoxicity of NK cells [24], while the crosslink of NKG2A and its adaptor protects T cells from being targeted by NK cells [25]. In a collagen-induced arthritis mouse model, the block of inhibitory receptors on NK cells by NKG2A antibodies promote the cytotoxicity of NK cells against pathogenic T cells and slows the disease progression [26]. And a large number of studies using inhibitory receptor-KO mouse models or receptor blocking models have demonstrated that the expression of inhibitory receptor adaptors on T cells protects T cells from the cytotoxicity of NK cells. These results suggest that the inhibitory receptors on NK cells may serve as a target for novel therapeutic strategies for autoimmune disease and reducing the cytotoxicity of NK cells on T cells may alleviate symptoms of autoimmune disease. In HLH, however, T cells are hyperactivated and the elimination of the hyperactivated T cells may be conducive to the relief of the disease. In a mouse model with impaired cytotoxicity of NK cells and CTLs, NK-cell cytotoxicity was sufficient to protect mice from the fatal outcome that characterizes HLH-

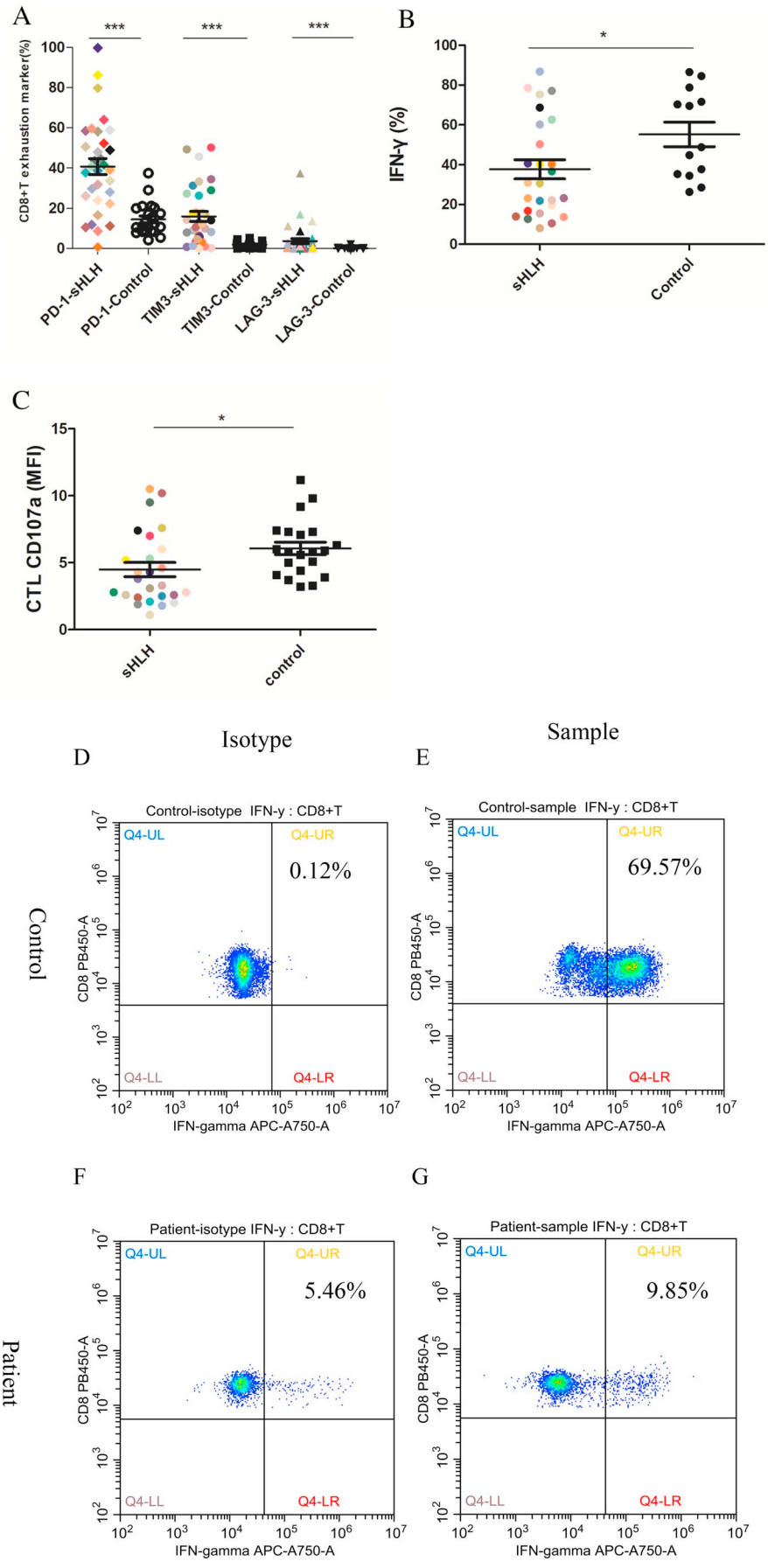
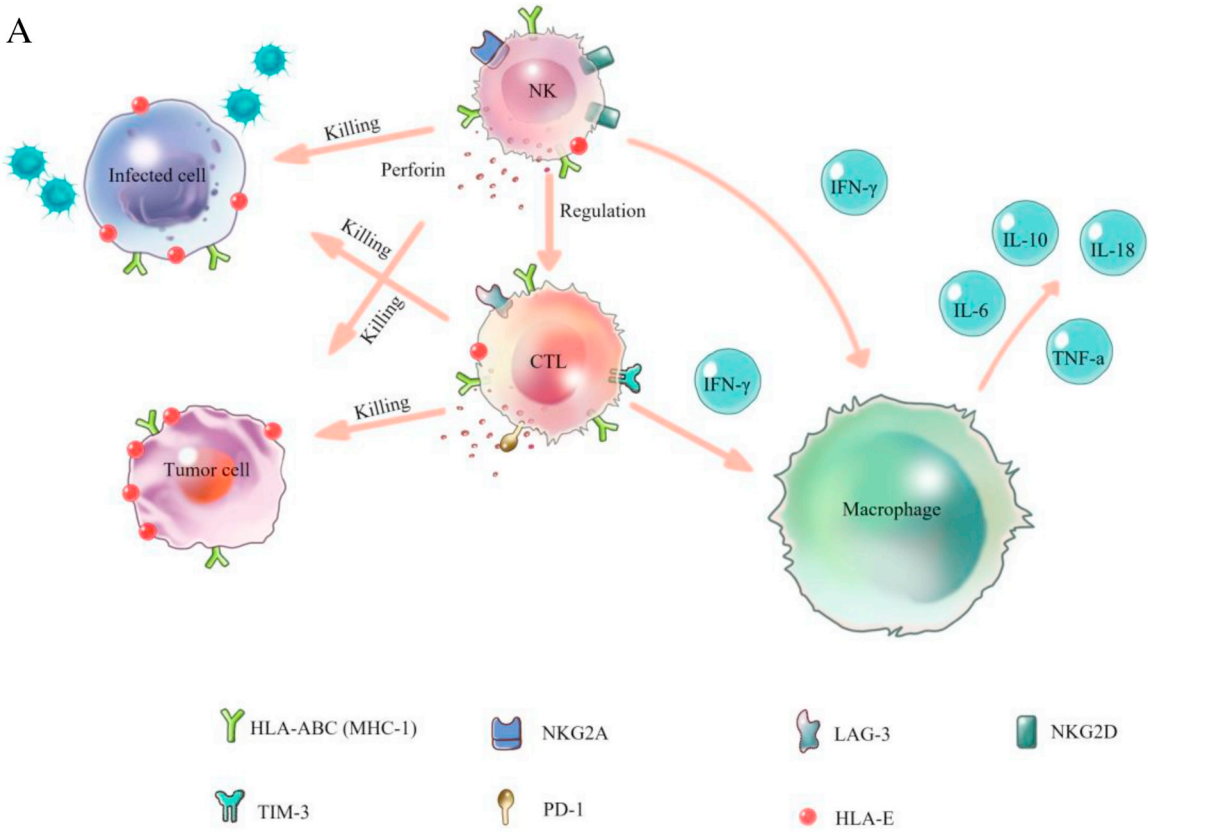


Fig. 4. Functional exhaustion of CTLs in sHLH.
A. The different expressions of CD8 + T cell exhaustion markers PD-1, TIM-3, and LAG-3 in sHLH and control group (*** $P < .001$).
B. The different secretions of IFN-γ in sHLH and control group after stimulation (* $P < .05$).
C. The different secretions of CD107a in sHLH and control group after stimulation (*$P < .05$).
D–G. The representative images for the secretions of IFN-γ in sHLH and control group after stimulation.
(D) The representative image for the secretion of IFN-γ in control group (negative control); (E) The representative image for the secretion of IFN-γ in control group; (F) The representative image for the secretion of IFN-γ in sHLH group (negative control); (G) The representative image for the secretion of IFN-γ in sHLH group;



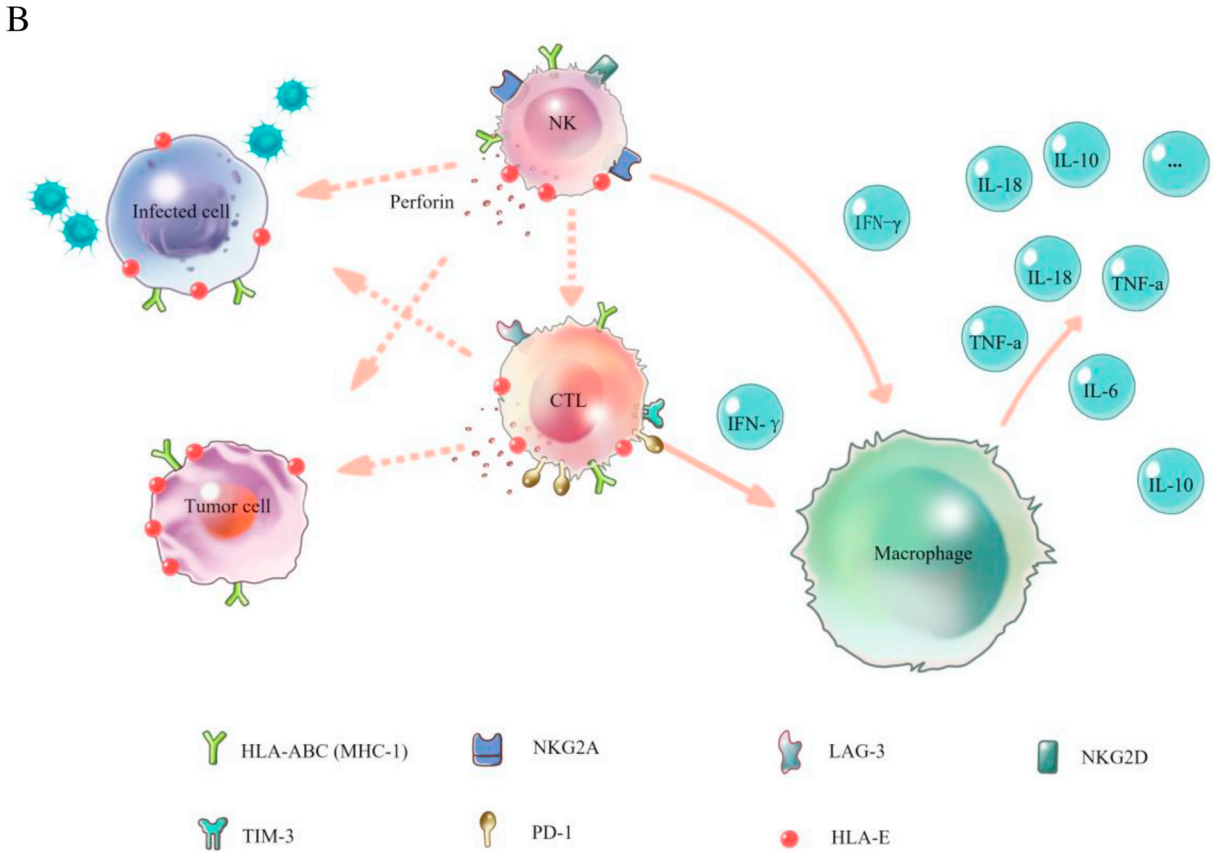


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Fig. 5. The interactions between the NK cells, CTLs and molecules.
A. The interactions between the NK cells, CTLs and molecules in normal setting: (1) CTLs and NK cells eliminate tumour cells and/or infected cells; (2) the NK cells and CTLs can secret IFN-γ to activate the macrophages and induce the secretion of cytokines in macrophages; (3) When the tumour cells and/or infected cells are cleared, the NK cells will eliminate the activated CTLs; (4) The elimination of activated CTLs prevent the secretion of IFN-γ and the hyperactivation of macrophages, and consequently inhibit the generation of cytokine storm.
B. The interactions between the NK cells, CTLs and molecules in sHLH setting: (1) NK cells exhibit a high expression of inhibitory receptor NKG2A and a low expression of activating receptor NKG2D; (2) The expression of HLA-E on lymphocytes elevates; (3) CTLs express a high level of PD-1, TIM-3 and LAG-3 as well as a lower secretion of IFN-γ and CD107a; (4) The expression of MHC-I on lymphocytes decreases; (5)The changes of NKG2A, NKG2D and HLA-E compromise the function of NK cells, and impair the elimination of hyperactivated CTLs; (6) CTLs exhibit both high expression of exhaustion markers and impaired CTLs function; (7) The hyperactivated CTLs induce the hyperactivation of macrophages and cytokine storm.

like disease and was also sufficient to reduce HLH-like manifestations. Mechanistically, NK-cell cytotoxicity reduced tissue infiltration by inflammatory macrophages and downmodulated LCMV-specific T-cell responses by limiting hyperactivation of CTLs [10]. Similarly, our results also indicated that the cytotoxicity of NK cells was inhibited by more interaction of NGK2A and its adaptors, as well as the less interaction of NKG2D and its adaptors. The abnormal cytotoxicity of NK cells consequently impairs the elimination of hyperactivated T cells, and leads to the hyperactivation of macrophages and cytokine storm in sHLH (Fig. 5).

In pHLH, gene mutations involving perforin and the degranulation pathway lead to the loss of NK cells and CTLs function, which induces HLH. In sHLH, without the gene mutations concerning NK cells and CTLs function, the impaired CTLs function may be attributed to the functional exhaustion of CTLs. T cell functional exhaustion is characterized by low cytokine secretion, reduced proliferation, and increased PD-1, LAG-3, and TIM-3 expression [27,28]. In our study, the expression of PD-1, TIM-3 and LAG-3 on CTLs in sHLH were significantly increased and the production of IFN-γ and CD107a after stimulation were significantly decreased.

So, the sHLH patients exhibited both high expression of exhaustion markers on CTLs and impaired CTLs function (Fig. 5). Moreover, our study demonstrated that sHLH patients exhibit low HLA-ABC expression levels. The decrease in HLA-ABC molecules in sHLH protects the target cells from the cytotoxicity of CTLs, which may induce the exhaustion of CTLs [29].

In summary, our study demonstrated that NK cells in sHLH patients exhibited a high expression of inhibitory receptor NKG2A and a low expression of activating receptor NKG2D. Besides, the expression of HLA-E on lymphocyte, the adaptor of NKG2A on NK cells, was elevated in sHLH. Moreover, CTLs in sHLH patients expressed a higher level of functional exhaustion markers PD-1, TIM-3 and LAG-3 as well as a lower secretion of IFN-γ and CD107a upon stimulation. In addition, the expression of MHC-I on lymphocytes decreased. Taken together, our study indicates a potentially pathological mechanism of sHLH and may open up new avenues for the development of immunotherapies against sHLH. Anti-NKG2A humanized blocking antibodies may be combined with anti-PD-1 for the treatment of sHLH. The combined therapy is likely to restore NK cell and CTLs function, effectively eliminate infected cells or cancer cells, and end the persistent inflammatory responses.

Declaration of interest

None.

Funding

This work had obtained Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding (ZYLX201702), Beijing Municipal Administration of Hospitals' Ascent Plan (DFL20180101), Beijing Natural Science Foundation (7181003).

Acknowledgements

All people involved in this project are named as authors, therefore we do not have acknowledgements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.03.006.

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