Notch-1 Signaling Mediated Dendritic Cells - Induced Autologous NK Cell Proliferation and Cytotoxicity Activity to Cancer Cell

Supaporn Suparak[1] **Kanokwan Ngueanchanthong**[1] **Busarawan Sriwanthana**[2] **and Somchai Sangkitporn**[3]

[1]*National Institute of Health, Department of Medical Sciences, Tiwanond Road, Nonthaburi 11000*

[2]*Retired Government Official 23/30 Phaholyothin 60, Khu Khot, Lam Luka, Pathum Thani 12130*

[3]*Pink Park Village, Queen Sirikit Centre for Breast Cancer Foundation 47, Nong Chok, Bangkok 10530, Thailand*

ABSTRACT Natural killer (NK) cells mediated immune responses via cytotoxic activity, or secreting cytokines. The interaction of NK cells with dendritic cells (DCs) contributes to NK cell-mediated anti-tumor or anti-microbial responses. However, the cellular and molecular mechanisms for controlling DC-mediated NK cell cytotoxicity interaction are largely unknown. Here, we showed an involvement of Notch-1 signaling interaction in augmenting NK cell cytotoxicity mediated by DCs. Stimulation of DCs from 6 healthy donors with lipopolysaccharide (LPS) could induce Notch-1 receptor and enhance NK cell proliferation (64.71±1.89%) and their cytotoxicity activity (42.18±1.04%) against K562 cells (human erythroleukemic cell line). DC-mediated NK cell cytotoxicity was suppressed by Notch-1 inhibitor, a γ-secretase inhibitor, (11.91±0.62%), as compared to the LPS treated-DCs ($p < 0.05$, paired t-test). These results suggested a possible mechanism of DCs mediated NK cell expansion and cytotoxicity regarding to Notch-1 receptor, at least in part. These findings provided an insight that the modulation of Notch-1 signaling could be a strategy to eradicate tumors or to suppress NK cell-mediated diseases.

Keywords: Dendritic cells, NK cells, Proliferation, Cytotoxicity, Notch-1

Corresponding author E-mail address: supaporn.su@dmsc.mail.go.th

Received: 6 November 2020 *Revised: 26 April 2021* *Accepted: 13 May 2021*

Introduction

Natural killer (NK) cells and dendritic cells (DCs) are specialized cell of the innate immune system. The cooperative interaction between DCs and NK cells plays a key role not only in the regulation of innate immunity but also in the promotion of appropriate downstream adaptive responses.[1, 2, 3] DC-mediated NK cell activation occurs mainly through both the release of soluble factors and cell-to-cell contacts. DCs release interleukin-12 (IL-12) and interleukin-15 (IL-15) after antigen uptake and enhance NK cell proliferation and cytolytic activity.[4, 5, 6]

The Notch signaling pathway regulates cell proliferation, apoptosis and cell fate decisions during development and adult tissue homeostasis.[7] In mammalian cells there are four Notch receptors (Notch 1-4), that are a group of highly conserved transmembrane receptors. Notch signaling is initiated by the binding of the Notch receptor to Notch ligands on neighboring cells. There are five Notch ligands (Jagged1, Jagged2, Delta-like 1 (DLL1), DLL3, and DLL4).[7] Notch pathway is associated with DC differentiation.[8] The expression of Notch protein in antigen presentation cells and matured DC was demonstrated.[9] The lipopolysaccaraide (LPS) -induced maturation of monocyte-derived DCs is regulated by the Notch signaling through the up-regulation of the chemokine receptor CXCR4 was shown.[10] The triggering Notch receptors can promote dendrite outgrowth and MHC II expression on DCs. Both dendrite outgrowth and MHC II expression are essential for DCs migration, antigen uptake, and/or antigen presentation.[10] Notch signaling blockage with γ-secretase inhibitor, N- [N-(3, 5-difluorophenacetyl)-1-alany1-S-phenyglycine t-butyl ester (DAPT), influenced the differentiation of DCs.[11] DAPT can hydrolyze Notch binding protein to block the Notch signal, and consider having the effect to down regulate the Notch-1 expression at the mRNA level.[12]

The previous studies demonstrated Notch-1 signaling promotes the development and activation of NK cells.[13] The studied in mice model demonstrate Notch-1 signaling controls DCs mediated NK cell activation.[14, 15] The relevance of DCs -NK cell cross-talk in the control of infectious diseases and tumor growth were controversial.[16] In this study, we addressed whether DCs could mediate autologous NK cell proliferation and cytotoxicity.

A human erythroleukemic cell line, K562, target cells, was used for assessing NK cell cytotoxic activity. K562 cells were susceptible for assessing human natural killer activity with reduced expression of HLA class I and heightened expression of ligands for activator NK receptors.[17] The effect of blockade Notch-1 signaling on DCs mediated NK cell proliferation and cytotoxicity was determined. This study may provide the modulation of Notch-1 mediated DCs and NK cells signaling could be a strategy to eradicate tumors or to suppress NK cell- mediated diseases.

Materials and Methods

Cells and culture

Human erythroleukemic cell line (K562) were obtained from the American Type Culture Collection (ATCC CCL-243TM, Manassas, VA, USA) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, CA, USA) at 37°C in a humidified 5% CO_2 incubator. K562 was susceptible sensitive cell line for assessing human natural killer activity.

Isolation of human peripheral blood mononuclear cells and ex vivo generation of DCs

Ex vivo generation of DCs was performed as described in a previous study.[18] Leukocyte-enriched buffy coats, 6 healthy donors, from Thai Red Cross served as sources of human peripheral blood mononuclear cells (PBMCs) with no data of donor for personal identification. PBMCs were isolated using density-gradient centrifugation with Ficoll-Hypaque (IsoPrepTM; Robbins Scientific, Sunny-vale, CA, USA) and washed twice with phosphate-buffered saline (PBS) (Welgene, USA). Positive selection for CD14+ PBMCs was performed using anti-CD14-MicroBeads, MS+/RS+ columns, and MiniMACS separator (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). DCs were generated from CD14+ PBMCs and plated in 6-well plates with RPMI 1640 complete medium (10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 4 mmol/L L-glutamine). The cytokine cocktail of Granulocyte-macrophage colony-stimulating factor (GM-CSF; Miltenyi Biotech, Bergisch Gladbach, Germany) and interleukin-4 (IL-4; Miltenyi Biotech, Bergisch Gladbach, Germany) was added to a final concentration of 1,000 and 500 U/ml, respectively, at day 0, 2, and 4 in 500 μl of fresh medium/well. On day 5 or 6, the immature DCs were transferred to new plates and half of the medium was replaced with fresh medium containing, LPS 1 μg/ml (Escherichia coli, serotype 0127:B8; Sigma-Aldrich, St. Louis, MO, USA) for 2 days to mature. Differentiate of human monocyte to DCs was detected by surface marker staining (anti-CD14-FITC, anti-CD11c-FITC, anti-HLA-DR-PE, anti-Notch-1-PE monoclonal antibody; Thermo Fisher Scientific, Carlsbad, CA, USA) and measured with Flow cytometer FACS Calibur instrument (BD Biosciences, San Jose, CA, USA).

Treatment DCs by γ-secretase inhibitor (GSI)

The DCs Notch signaling pathway was inhibited as described previously.[11] DAPT (N-[(3, 5-difluorophenyl) acetyl]-L-alanyl-2-phenyl] glycine-1,1-dimethylethyl) is a kind of GSI (Sigma-Aldrich, St. Louis, MO, USA) was added at 1, 5, 10 μm on day 6 of DCs culture. DCs viability was checked whether the DAPT did not affect the maturation of the cells. DCs surface molecules was detected by surface marker staining (anti-Notch-1-PE, anti-HLA-DR-PE monoclonal antibody; Thermo Fisher Scientific, Carlsbad, CA, USA) and measured with Flow cytometer FACS Calibur instrument (BD Biosciences, San Jose, CA, USA).

Isolation and culture of NK cells

The isolation and culture of autologous NK cells from 6 healthy donors was done as described in a previous study.(19) The Human NK cell (CD3-CD56+) isolation kit EasySep™ Human CD56 Positive Selection Kit (STEM CELL technologies, Vancouver, Canada) was used according to the manufacturer's instructions using Falcon 5-ml polystyrene round-bottom tubes on the EasySep magnet. PBMC suspensions at a density of 1×10^8 cells/ml were centrifuged at 300 × g for 10 min at 4°C and suspended in 1 ml of recommended medium in a 5-ml polystyrene tube. A total of 50 µl EasySep NK cell isolation cocktail was added, and cells were incubated at room temperature (15–25°C) for 10 min. Subsequently, 100 µl EasySep Streptavidin RapidSpheres was added, and cells were incubated at room temperature (15–25°C) for 5 min. Cell suspensions were brought up to a total volume of 2.5 ml by adding the recommended medium, and placed into the magnet for 5 min at room temperature (15–25°C). The EasySep magnet was removed and the desired fraction was poured off into a 15-ml tube three times following 5 min separations in the magnet. The isolated cells in the 15-ml tube were NK cells. The number of NK cells was evaluated using (anti-CD3-FITC, anti-CD56-PE monoclonal antibody; Thermo Fisher Scientific, Carlsbad, CA, USA) and measured with Flow cytometer FACS Calibur instrument (BD Biosciences, San Jose, CA, USA).

DCs mediated NK cells proliferation assay

The NK proliferation assays was done as described in a previous study.(20) NK cells were stained with 0.5 µM Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) (Thermo Fisher Scientific, Carlsbad, CA, USA) for 10 min at 37°C, washed twice using complete medium. The stained NK cells were then incubated with DCs conditions (non-treated DCs control, LPS-treated DCs, DAPT-treated DCs) in RPMI-1640 plus 5% human serum in 96-well round bottom plates for 4 days at 37°C with 5% CO_2. Before acquisition, 1 µl of 1 mg/ml propidium iodide (PI) (Sigma Aldrich, St. Louis, MO, USA) was added to each well. The culture cells were acquired on a Guava® easyCyte Flow Cytometers and analyzed using Guava software (Millipore Corp, Hayward, CA, USA). Percentages of live proliferating NK cells showing CFSE-negative and PI-negative were calculated. All experiments were from 6 donors of independent experiments performed in triplicate.

DCs mediated NK cell cytotoxicity assay

The NK cytotoxicity assay was done as described in a previous study.(20) NK cells were co-cultured with DCs conditions (non-treated DCs control, LPS-treated DCs, DAPT-treated DCs) in RPMI 1640 complete medium in 96 U-bottom well plates at 37°C in a humidified 5% CO_2 incubator overnight. The cytotoxicity of NK cells against target tumor cells (K562 cells)

were measured by a flow cytometry-assay using CFSE (Thermo Fisher Scientific, Carlsbad, CA, USA) staining of K562 target cells. Briefly, K562 cells were stained with 0.5 μM CFSE for 10 min at 37°C. Cells were then washed twice using complete media. CFSE-stained K562 target cells were placed in a 96-well round bottom plate in triplicate and then mixed with NK/DC co-cultures cells. The plates were centrifuged at 1,500 rpm for 3 min and then incubated for 4 h at 37°C with 5% CO_2. Before acquisition, 1 μl of 1 mg/ml propidium iodide (PI) (SigmaAldrich, St. Louis, MO, USA) was added to each well. The co-culture cells were acquired on a Guava® easyCyte Flow Cytometers and analyzed using Guava software (Millipore Corp, Hayward, CA, USA). Percentages of dead K562 target cells showing CFSE-positive and PI-positive were calculated after subtracting the percentage of spontaneous death of target cells. All experiments were from 6 donors of independent experiments performed in triplicate.

Statistical analysis

Statistical analysis pair t-test was performed using SPSS 26.0 statistical software package (SPSS, Inc., Chicago, IL, USA). The p < value < 0.05 was considered to be statistically significant. Results were from 6 donors of independent experiments and expressed as mean ± S.D.

Results

Notch-1 signaling blockade reduced the DCs mediated NK cell proliferation in vitro

We investigated whether inhibition of Notch-1 signaling could affect the DCs mediated NK cell proliferation in vitro. The DCs-mediated autologous NK cells proliferation was performed from 6 healthy volunteer donors and determined by flow cytometry. The autologous NK cells (CD3-CD56+) were purified from peripheral blood mononuclear cells by positive selection and co-cultured with DCs conditions (non-treated DCs control, LPS treated-DCs, DAPT treated -DCs) as stimulators of NK cells. The mean NK cell proliferation from 6 donors of independent experiments was shown in Table 1. NK cell proliferating was significantly increased after co-cultured with LPS-treated DCs (64.71 ± 1.89%) compared with the non-treated DCs control (25.13 ± 1.85%). NK cells co-cultured with DAPT treated -DCs significantly reduced the DCs mediated-NK cell proliferation (26.57 ± 1.42%) ($p < 0.05$, pair t-test).

Table 1 The percentages of autologous NK cell proliferation and NK cell mediated cytotoxicity on K562 target cells.

NK cell culture conditions	%NK cell proliferation						
	Donor 1	**Donor 2**	**Donor 3**	**Donor 4**	**Donor 5**	**Donor 6**	**Mean**
NK co-cultured with non- treated DCs (control)	27.75 ± 2.56	25.89 ± 3.87	23.87 ± 3.76	24.61 ± 4.21	26.12 ± 3.17	22.51 ± 4.76	25.13 ± 1.85
NK co-cultured with LPS- treated DCs	67.94 ± 3.47	64.97 ± 4.56	62.15 ± 4.54	63.91 ± 2.89	65.04 ± 3.97	64.23 ± 4.50	64.71 ± 1.89
NK co-culture with DAPT-treated DCs	28.62 ± 4.89*	26.41 ± 2.56*	25.74 ± 2.56*	24.84 ± 2.56*	25.87 ± 2.56*	27.91 ± 2.56*	26.57 ± 1.42*
NK cell culture conditions	**%NK cell cytotoxicity**						
	Donor 1	**Donor 2**	**Donor 3**	**Donor 4**	**Donor 5**	**Donor 6**	**Mean**
NK co-cultured with non-activated DCs (control)	15.23 ± 4.21	13.56 ± 3.57	14.25 ± 2.58	13.87 ± 4.59	12.98 ± 3.60	13.74 ± 2.61	13.94 ± 0.75
NK co-cultured with LPS-activated DCs	42.75 ± 3.22	41.98 ± 2.87	42.59 ± 3.14	41.07 ± 2.59	43.69 ± 3.50	40.98 ± 3.47	42.18 ± 1.04
NK co-cultured with DAPT-treated DCs	12.13 ± 2.56*	10.98 ± 4.57*	11.84 ± 3.21*	12.46 ± 3.91*	11.42 ± 2.60*	12.61 ± 3.61*	11.91 ± 0.62*

Remark: All experiments were performed in triplicate and expressed as the mean ± standard deviation. *, $p < 0.005$, pair t-test (NK co-cultured with LPS treated-DCs vs co-cultured with DAPT-treated DCs)

Figure 1 was result of donor 1 from 6 donors of independent experiments. NK cell proliferation was significantly increased after stimulation with LPS-treated DCs (Figure 1B, 67.94%) compared with the DCs control (Figure 1A, 27.75%). DAPT treatment reduced the DCs mediated NK cell proliferation in vitro (Figure 1C, 28.62%). We suggested that NK cells proliferation mediate, at least in part, DCs Notch-1 signaling interaction.

Notch-1 signaling blockade reduced the DCs mediated NK cell cytotoxicity in vitro

To investigate whether inhibition of Notch-1 signaling could affect the DCs mediated NK cell cytotoxicity in vitro. The NK cell cytotoxicity on K562 susceptible target cells was determined after co-culturing with DCs.

The mean NK cell cytotoxicity killed K562 target cells from 6 donors of independent experiments was shown in Table 1. The significantly increased of K562 target cells killed after co-culture with LPS treated-DCs (42.18 ± 1.04%) compare to DCs control (13.94 ± 0.75%). co-culture with DAPT treated -DCs significantly reduced the NK cell mediated cytotoxicity (11.91 ± 0.62%) ($p < 0.005$, pair t-test).

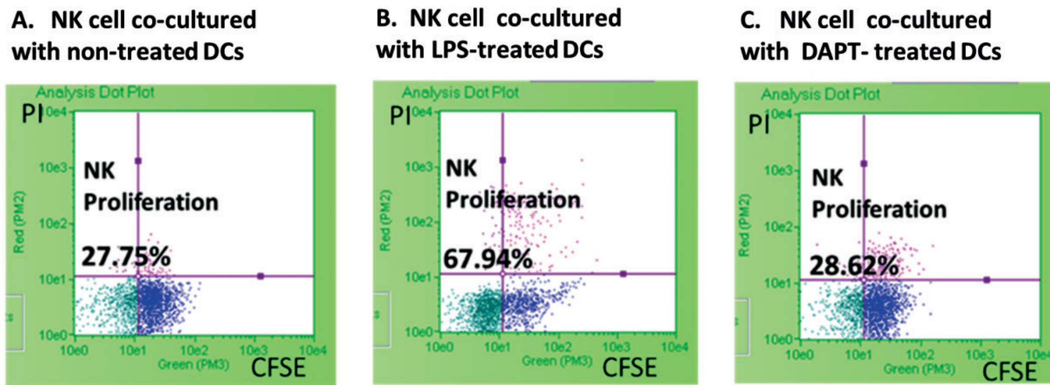

Figure 1 Notch-1 signaling blockade reduced the DCs mediate NK cells proliferation. Autologous NK cells were co-cultured with DCs and determined NK cell proliferation using CFSE-based flow cytometry, the NK live proliferating cells shown the CFSE-negative and PI-negative, lower left. The NK proliferation was significantly increased after co-culture with LPS treated-DCs (B) compared with non-treated DCs control (A). Co-culture with DAPT treated-DCs reduced NK cell proliferation (C).

Figure 2 was NK cytotoxicity result of donor 1 from 6 donors of independent experiments. NK cell cytotoxicity was significantly increased after co-culture with LPS-treated DCs (Figure 2B, 42.75%) compared with the DCs control (Figure 2A, 15.23%). DAPT treatment reduced the DCs mediated NK cell cytotoxicity in vitro (Figure 2C, 12.13%). We suggested that DCs mediated NK cytotoxicity, at least, via Notch-1 signaling dependent.

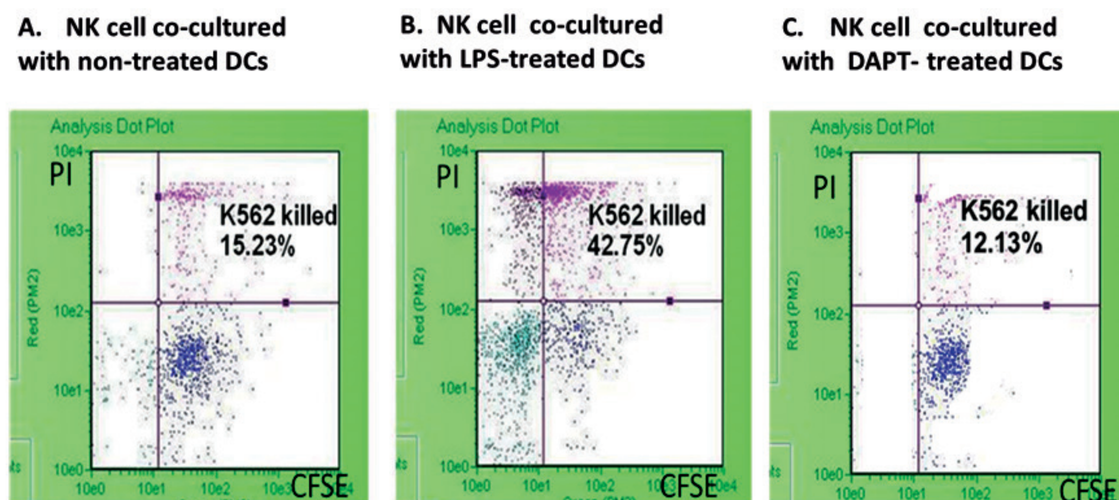

Figure 2 Inhibition of Notch-1 signaling reduced the DCs mediate NK cells cytotoxicity. The cytotoxicity ability of autologous NK cells on K562 cells was measured by CFSE-based flow cytometry assay. The dead K562 target cells showed the

CFSE-positive and PI-positive, upper right. The NK cytotoxicity was increased after co-cultured with LPS-treated DCs (B) compared with control DCs (A). Co-culture with DAPT treated-DCs reduced NK cell cytotoxicity (C). Data shown were donor 1 from 6 donors of independent experiments.

DAPT treatment reduced the expression of DCs cell surface molecules

The surface molecules of DCs and NK cells were determined by flow cytometry after co-culturing for 4 days. The NK cells retained the CD56+CD3- phenotype (data not shown). The DCs Notch-1 receptor and HLA-DR were shown at low level expression in non-treated DCs control (Figure 3A) and increased in LPS-treated DCs (Figure 3B). The decrease of these surface molecules was observed in DAPT treated-DCs (Figure 3C). This suggested the expression of DCs cell surface molecules might be involved to DCs mediated NK cell proliferation and cytotoxicity activity.

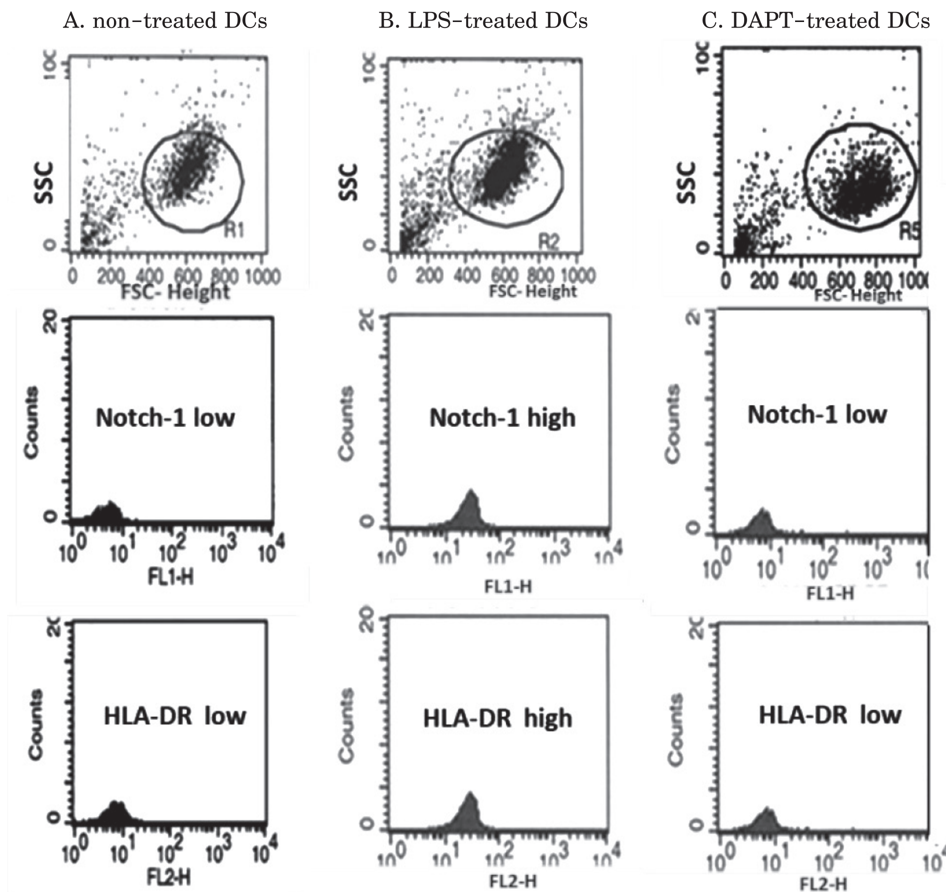

Figure 3 DAPT treatment reduced the expression of DCs cell surface molecules. The DCc surface expression was determined by flow cytometry after 4 days co-culture with autologous NK cells. Notch-1 receptor and HLA-DR surface molecules were expressed at low level in non-treated DCs (A) and increased in LPS-treated DCs (B). These surface molecules were reduced after DAPT-treated DCs (C). Data shown were donor 1 from 6 donors of independent experiments.

Discussion

Notch signaling controlled the development and activation of the innate and adaptive immune system[21] including the development and activation of T-cells.[9] Notch receptors on T-cells play a regulators role of T-cell differentiation[22] and induction of tumor-specific cytotoxic T-cells and memory T-cells.[23] Effector CD8+T-cells cytokine production regulated by the Notch signaling was shown.[24] Notch ligands are expressed on stromal and epithelial cells in the marginal zone of the spleen, thymic epithelium and bone marrow stromal cells, or on stromal cells at inflammatory sites such as rheumatoid arthritis synovium.[25, 26]

This study demonstrated the stimulation of DCs with LPS can enhanced expansion of autologous NK cells and their cytotoxicity activity. Notch-1 signaling blockade reduced the DCs mediated NK cell proliferation and cytotoxicity in vitro. One mechanism by which TLR modulates Notch signaling is by inducing Notch receptor and ligand expression.[27] There is amble evidence that activation of macrophages and DCs with TLR ligands leads to induction of Notch receptors and ligands including Jagged1, DLL1, and DLL4.[27] TLR signaling indirectly promotes Notch pathway activation and expression of Notch target genes. The Notch target genes can be induced by LPS in both Notch-independent and Notch-dependent manners. Notch signaling was activated after LPS stimulation.[28] The expression of Jagged 1, a Notch ligand, induced by LPS occurred in a JNK-dependent manner. In addition, Notch target genes were upregulated by early Notch-independent activation followed by delayed Notch-dependent activation after LPS stimulation. Disruption of Notch signaling by DAPT attenuated the LPS-induced inflammatory responses.[28] Dendritic cell-mediated NK cell activation controlled by Jagged2–Notch interaction was demonstrated. Stimulation of NK cells by Jagged2 enhanced the antitumor cytolytic activity of NK cells in vivo and in vitro.[13] We demonstrated the inhibition of Notch-1 signaling reduced the expression of DCs cell surface molecules. It suggests that Notch-1 signaling can play a role in DC-mediated NK cell activation through interaction with Notch-1 receptor and HLA-DR surface molecules.

DCs and NK cells interaction is a bi-directional activation, once activated, NK cells can eliminate the more immature DCs, allegedly tolerogenic DCs and activate DCs subsequently to adaptive immune response.[29, 30] Most of the studies on DCs subsets are emerging with distinct biologic functions of naturally occurring DCs subsets. In human blood, at least three DCs subsets can be distinguished, plasmacytoid DCs, CD141+ and CD1c+ myeloid/conventional DCs, each with distinct functional characteristics.[31] DC-mediated NK cells activation is based on DCs derived from monocytes, generated from the culture with different cytokines. The interactions between ex vivo isolated human DCs subsets and NK cells have not been largely elucidated. Plasmacytoid and myeloid peripheral blood DCs can activate NK cells and enhance cytolytic activity. However, it is challenging to obtain high enough due to their low frequencies.[31] Different of DC subsets in the human system is leading to new insights of innate cell interactions, particularly for the cross-talk occurring between these DC subsets and NK cells.[32, 33] As DC subsets show a specific

distribution in human tissues, therefore, DCs and NK cells interactions should be considered as a more complex of cell subset networking to optimize the response against microorganisms and cancer cells.

Regulation of Notch in cancer immunity is a very attractive approach in the ongoing revolution in cancer immunotherapy.[34, 35] The rescue of Notch-1 signaling in antigen-specific CD8+ T-cells overcomes tumor-induced T-cells suppression and enhances immunotherapy in cancer was revealed.[36] Our data show the Notch signaling effect on DCs mediated NK cell effector function. Thus, manipulation of Notch ligands could provide to induce NK cells immunity in immunosuppressive conditions including cancer or to inhibit certain NK cell activation on autoimmune diseases.

Conclusion

This study described DCs - mediated autologous NK cell proliferation and cytotoxicity function in vitro requiring Notch-1 signaling. The inhibition of DCs Notch-1 signaling reduced the autologous NK cells proliferation and cytotoxicity activity towards K562 target cells. DAPT treatment reduced the expression of DCs cell surface molecules HLA-DR and Notch-1 receptor. We suggested the expanded autologous NK cells cytotoxicity mediated by DCs Notch-1 interaction, at least in part, Notch-1 receptor involved.

Acknowledgements

This research was supported by grants from Department of Medical Sciences, Ministry of public health, Thailand. We would like to thank Mrs. Siripakorn Sangkitporn, Adivisory expert for thoughtful support and encouragement. Finally, thank to Miss Tawanchai Sangcharoen and Transfusion Transmitted Pathogen Section research team for technical support.

References

1. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. Science 2011; 331(6013): 44-9.
2. Voss M, Bryceson YT. Natural killer cell biology illuminated by primary immunodeficiency syndromes in humans. Clin Immunol 2017; 177: 29-42.
3. Law RH, Lukoyanova N, Voskoboinik I, Caradoc-Davies TT, Baran K, Dunstone MA, et al. The structural basis for membrane binding and pore formation by lymphocyte perforin. Nature 2010; 468(7322): 447-51.
4. Thomas R, Yang X. NK-DC crosstalk in immunity to microbial infection. J Immunol Res 2016; 2016: 6374379. (7 pages).
5. Mahmood S, Upreti D, Sow I, Amari A, Nandagopal S, Kung SK. Bidirectional interactions of NK cells and dendritic cells in immunotherapy: current and future perspective. Immunotherapy 2015; 7(3): 301-8.

6. Marcenaro E, Carlomagno S, Pesce S, Moretta A, Sivori S. NK/DC crosstalk in anti-viral response. Adv Exp Med Biol 2012; 946: 295-308.
7. Radtke F, Fasnacht N, Macdonald HR. Notch signaling in the immune system. Immunity 2010; 32(1): 14-27.
8. Cheng P, Zhou J, Gabrilovich D. Regulation of dendritic cell differentiation and function by Notch and Wnt pathways. Immunol Rev 2010; 234(1): 105-19.
9. Sauma D, Espejo P, Ramirez A, Fierro A, Rosemblatt M, Bono MR. Differential regulation of Notch ligands in dendritic cells upon interaction with T helper cells. Scand J Immunol 2011; 74(1): 62-70.
10. Wang YC, Hu XB, He F, Feng F, Wang L, Li W, et.al. Lipopolysaccharide-induced maturation of bone marrow-derived dendritic cells is regulated by notch signaling through the up-regulation of CXCR4. J Biol Chem 2009; 284(23): 15993-6003.
11. Qian XQ, Chen LL, Cheng Q, Tian Y, Luo XF, Wan XY. Inhibition of Notch 1 receptor influenced the differentiation of Lin-CD45RA-dendritic cell precursors within ovarian carcinoma microenvironment. BMC Immunol 2016; 17(1): 14. (9 pages).
12. Juillerat-Jeanneret L, Flohr A, Schneider M, Walter I, Wyss JC, Kumar R, et al. Targeted γ-secretase inhibition to control the Notch pathway in renal diseases. J Med Chem 2015; 58(20): 8097-109.
13. Kijima M, Yamaguchi T, Ishifune C, Maekawa Y, Koyanagi A, Yagita H, et al. Dendritic cell-mediated NK cell activation is controlled by Jagged2-Notch interaction. Proc Natl Acad Sci USA 2008; 105(19): 7010-5.
14. Felices M, Ankarlo DE, Lenvik TR, Nelson HH, Blazar BR, Verneris MR, et al. Notch signaling at later stages of Natural Killer cell development enhances KIR expression and functional maturation. J Immunol 2014; 193(7): 3344-54.
15. Tchekneva EE, Goruganthu MUL, Uzhachenko RV, Thomas PL, Antonucci A, Chekneva I, et al. Determinant roles of dendritic cell-expressed Notch Delta-like and Jagged ligands on anti-tumor T cell immunity. J Immunother Cancer 2019; 7(1): 95. (17 pages).
16. Zitvogel L, Terme M, Borg C, Trinchieri G. Dendritic cell-NK cell cross-talk: regulation and physiopathology. Curr Top Microbiol Immunol 2006; 298:157-74.
17. West WH, Cannon GB, Kay HD, Bonnard GD, Herberman RB. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. J Immunol 1977; 118(1): 355-61.
18. Li DY, Gu C, Min J, Chu ZH, Ou QJ. Maturation induction of human peripheral blood mononuclear cell-derived dendritic cells. Exp Ther Med 2012; 4(1): 131-4.
19. Sanchez-Martinez D, Allende-Vega N, Orecchioni S, Talarico G, Cornillon A, Vo DN, et al. Expansion of allogeneic NK cells with efficient antibody-dependent cell cytotoxicity against multiple tumors. Theranostics 2018; 8(14): 3856-69.
20. Tario JD Jr, Muirhead KA, Pan D, Munson ME, Wallace PK. Tracking immune cell proliferation and cytotoxic potential using flow cytometry. Methods Mol Biol 2011; 699: 119-64.

21. Janghorban M, Xin L, Rosen JM, Zhang XH. Notch signaling as a regulator of the tumor immune response: to target or not to target? Front Immunol 2018; 9: 1649. (10 pages).
22. Auderset F, Schuster S, Fasnacht N, Coutaz M, Charmoy M, Koch U, et.al. Notch signaling regulates follicular helper T cell differentiation. J Immunol 2013; 191(5): 2344-50.
23. Laky K, Evans S, Perez-Diez A, Fowlkes BJ. Notch signaling regulates antigen sensitivity of naive CD4+ T cells by tuning co-stimulation. Immunity 2015; 42(1): 80-94.
24. Radtke F, MacDonald HR, Tacchini-Cottier F. Regulation of innate and adaptive immunity by Notch. Nat Rev Immunol 2013; 13(6): 427-37.
25. Tanigaki K, Honjo T. Regulation of lymphocyte development by Notch signaling. Nat Immunol 2007; 8(5): 451-6.
26. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. J Exp Med 2007; 204(7): 1653-64.
27. Shang Y, Smith S, Hu X. Role of Notch signaling in regulating innate immunity and inflammation in health and disease. Protein Cell 2016; 7(3): 159-74.
28. Tsao PN, Wei SC, Huang MT, Lee MC, Chou HC, Chen CY, et al. Lipopolysaccharide-induced Notch signaling activation through JNK-dependent pathway regulates inflammatory response. J Biomed Sci 2011; 18(1): 56. (9 pages).
29. Ferlazzo G, Morandi B. Cross-Talks between natural killer cells and distinct subsets of dendritic cells. Front Immunol 2014; 5: 159. (7 pages).
30. Fessenden TB, Duong E, Spranger S. A team effort: natural killer cells on the first leg of the tumor immunity relay race. J Immunother Cancer 2018; 6(1): 67. (3 pages).
31. Van Elssen CH, Oth T, Germeraad WT, Bos GM, Vanderlocht J. Natural killer cells: the secret weapon in dendritic cell vaccination strategies. Clin Cancer Res 2014; 20(5): 1095-103.
32. Bol KF, Schreibelt G, Rabold K, Wculek SK, Schwarze JK, Dzionek A, et al. The clinical application of cancer immunotherapy based on naturally circulating dendritic cells. J Immunother Cancer 2019; 7(1): 109. (13 pages).
33. Nasi A, Bollampalli VP, Sun M, Chen Y, Amu S, Nylén S. Immunogenicity is preferentially induced in sparse dendritic cell cultures. Sci Rep 2017; 7: 43989. (12 pages).
34. Renrick AN, Dunbar ZT, Shanker A. Update on the current revolution in cancer immunotherapy. Immunotherapy 2019; 11(1): 15-20.
35. Amon L, Hatscher L, Heger L, Dudziak D, Lehmann CHK. Harnessing the complete repertoire of conventional dendritic cell functions for cancer immunotherapy. Pharmaceutics 2020; 12(7): 663. (80 pages).
36. Sierra RA, Thevenot P, Raber PL, Cui Y, Parsons C, Ochoa AC, et al. Rescue of Notch-1 signaling in antigen-specific CD8+T cells overcomes tumor-induced T-cell suppression and enhances immunotherapy in cancer. Cancer Immunol Res 2014; 2(8): 800-11.

บทบาทของ Notch-1 signaling ในการเพิ่มประสิทธิภาพ Dendritic Cells เพื่อกระตุ้นการเพิ่มจำนวน และการทำลายเซลล์มะเร็งของ Natural Killer Cells

สุภาพร สุภารักษ์[1] กนกวรรณ เงื่อนจันทร์ทอง[1] บุษราวรรณ ศรีวรรธนะ[2] และสมชาย แสงกิจพร[3]
[1]สถาบันวิจัยวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ ถนนติวานนท์ นนทบุรี 11000
[2]นักวิชาการอิสระ 23/30 พหลโยธิน 60 ลำลูกกา ปทุมธานี 12130
[3]บ้านพิงพัก มูลนิธิศูนย์มะเร็งเต้านมเฉลิมพระเกียรติ 47 หนองจอก กรุงเทพมหานคร 10530

บทคัดย่อ เนเจอรอล คิลเลอร์เซลล์ หรือ เอ็นเคเซลล์ (Natural Killer (NK) cells) กระตุ้นการตอบสนองในระบบภูมิคุ้มกัน โดยการเข้าทำลายเซลล์แปลกปลอมหรือสารหลั่งไซโตไคน์ การส่งสัญญาณระหว่าง NK cells กับเซลล์เดนไดรติก (Dendritic cells, DCs) ก่อให้เกิดการทำลายเซลล์มะเร็งหรือต่อต้านจุลินทรีย์ก่อโรค แต่กลไกระดับเซลล์และระดับโมเลกุลที่ควบคุมปฏิสัมพันธ์ระหว่าง NK cells และ DCs ซึ่งส่งผลเป็นพิษต่อเซลล์มะเร็งนั้นยังไม่ทราบแน่ชัด ในที่นี้เราแสดงถึงบทบาทของ Notch-1 signaling บน DCs มีส่วนร่วมทำให้ NK cytotoxicity เพิ่มขึ้น และเมื่อเพาะเลี้ยง DCs จากอาสาสมัครสุขภาพดี จำนวน 6 คน มาทดสอบเพาะเลี้ยงร่วมกับ autologous NK cells ก็ช่วยให้ NK cells มีการเพิ่มจำนวน (64.71±1.89%) และการทำลายเซลล์มะเร็ง Human erythroleukemic cell line (K562) เพิ่มขึ้นด้วย (42.18±1.04%) แต่การทำลายเซลล์มะเร็งของ NK cells นี้มีปริมาณลดลงโดยการเพาะเลี้ยงร่วมกับ DCs ที่กระตุ้นด้วยสาร Notch-1 inhibitor ชื่อ γ-secretase inhibitor (11.91±0.62%) เมื่อเปรียบเทียบกับ DCs ที่กระตุ้นด้วย lipopolysaccharide (LPS) ($p < 0.05$, paired t-test) การศึกษานี้ แสดงให้เห็นว่าการกระตุ้นหรือยับยั้งการแสดงออกของ Notch-1 receptor มีอิทธิพลต่อประสิทธิภาพของ DC ในการกระตุ้น NK cells เพื่อทำลายเซลล์มะเร็ง ดังนั้นจึงอาจนำผลการศึกษาที่ได้นี้ไปประยุกต์ใช้เพื่อการรักษาผู้ป่วยมะเร็งหรือโรคอื่น ๆ ที่เกี่ยวข้องกับ NK cell ต่อไป

คำสำคัญ : เซลล์เดนไดรติก, เอ็นเคเซลล์, การเพิ่มจำนวนเซลล์, ความเป็นพิษต่อเซลล์, Notch-1