Molecular Immunology 106 (2019) 22–28

Contents lists available at ScienceDirect

Molecular Immunology

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

IL-17 production by NKG2D-expressing CD56+ T cells in type 2 diabetes

Wisitsak Phoksawat[a,b], Amonrat Jumnainsong[b,c], Naruemon Leelayuwat[d,e], Chanvit Leelayuwat[b,c,*]

[a] Graduate School, Khon Kaen University, Khon Kaen, 40002, Thailand
[b] The Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand
[c] Department of Clinical Immunology and Transfusion Sciences, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand
[d] Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand
[e] Exercise and Sport Sciences Development and Research Group, Khon Kaen University, Khon Kaen, 40002, Thailand

ARTICLE INFO

Keywords:
CD56+ T cells
Type 2 diabetes
NKG2D
IL-17
HbA1c

ABSTRACT

T cells expressing CD56 (identified as CD3+CD56+) play a potential role in activation or regulation of other immune cells by secreting various cytokines. We hypothesized that these cells expressing the natural group 2, member D (NKG2D) could produce high levels of interleukin (IL)-17 in type 2 diabetes (T2D). CD56 + T cells expressing NKG2D of T2D patients, particularly in poor glycemic control (PC) predominantly produced higher IL-17 compared to the NKG2D negative population. IL-17 production of CD56 + T cells with NKG2D + was positively correlated with the level of HbA1c (N = 22, R^2 = 0.120 and P = 0.044). Interestingly, CD56+ T cells with NKG2D^{Hi} of T2D patients had significantly higher IL-17 production than those of CD56 + T cells with NKG2D^{Low} (P = 0.027) and showed statistically significant with P-value < 0.001 compared to CD56 + T cells with NKG2D^{Hi} of non-diabetic individuals (ND). In summary, CD56 + T cells expressing NKG2D, especially in the NKG2D^{Hi} population may be involved in pathogenesis and severity of T2D via IL-17.

1. Introduction

T cells expressing the natural killer marker CD56 (identified as CD3+ and CD56+), also defined as the natural killer T (NKT)-like cells or probably NKT cells, are a special subset of T lymphocytes (Van Kaer, 2004; Koreck et al., 2002; Hodge et al., 2012). A hallmark of this cell type is a capability in rapid and effective production of many cytokines, such as interleukin (IL)-4, IL-10, IL-13, IL-17, IL-22, IFN-γ and TNF-α which can sequentially stimulate or regulate the response of other immune cells (Kumar and Delovitch, 2014; Wu and Van Kaer, 2009). Moreover, they also present the competency of cytotoxicity by secreting perforin and granzymes (Hodge et al., 2013; Kuylenstierna et al., 2011; Aggarwal et al., 2014). In the last decade, the study of phenotypes and functions of CD56 + T cells in spontaneity and diseases has been broadly discovered (Liew and Kubes, 2015).

The comprehension of alteration of immune cell phenotypes and functions under the disease may lead to the discovery of new targeted cells for therapeutic propose. In autoimmune diseases, the percentages of CD3+CD56+ NKT cells were significantly reduced in peripheral blood of psoriasis patients compared to controls and were significantly increased after treatment (Koreck et al., 2002). Furthermore, although invariant NKT cells of patients with type 1 diabetes (T1D) had no difference in their frequency from the healthy volunteers, they were significantly increased in IL-17 production indicating the involvement in

Abbreviations: T2D, type 2 diabetes; NKT cells, the natural killer T cells; NK cells, the natural killer cells; IL, interleukin; NKG2D, the natural killer group 2, member D receptor; CD, cluster of differentiation; ND, non-diabetic individuals; WC, well glycemic control; PC, poor glycemic control; N, the number of samples; PBMCs, peripheral blood mononuclear cells; HbA1c, the glycated hemoglobin A1c; NGSP, the national glycohemoglobin standardization program; IFCC, the international federation of clinical chemistry and laboratory medicine; PMA, phorbol-12-myristate-13-acetate; TCR α/β, T cell receptor alpha/beta; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; PBS, phosphate-buffered saline; mAbs, monoclonal antibodies; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; PI3K, the phosphoinositide 3-kinase; DAP10, DNAX-activation protein 10; PKC-θ, the protein kinase C theta; PKC-δ, the protein kinase C delta; JAK, the Janus kinase; STAT3, the signal transducer and activator of transcription 3; RORγt, the retinoic acid receptor-related orphan receptor gamma t; NFAT, the nuclear factor of activated T cells; BATF, the basic leucine zipper ATF-like transcription factor; NKAP, the NF kappa B activating protein; PLZF, the promyelocytic leukemia zinc finger; NA, not available

* Corresponding author at: Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand.
E-mail addresses: wisitsakphok@kkumail.com (W. Phoksawat), amonrat@kku.ac.th (A. Jumnainsong), naruemon@kku.ac.th (N. Leelayuwat), chanvit@kku.ac.th (C. Leelayuwat).

https://doi.org/10.1016/j.molimm.2018.12.008
Received 13 June 2018; Received in revised form 20 November 2018; Accepted 9 December 2018
Available online 18 December 2018

the pathogenesis and development of the disease (Li et al., 2014). In lung transplantation, NKT-like cells producing IL-17 of transplant patients were significantly higher and were negatively correlated with the time post-transplant suggesting a role of these cells in pro-inflammatory cytokine production associated with graft rejection (Hodge et al., 2012).

In our previous study, we have identified the pathogenic CD4 + T subpopulation expressing the natural killer group 2, member D (NKG2D) receptor in patients with type 2 diabetes (T2D). These cells could produce a high level of IL-17 and may be associated with the pathogenesis and severity of the disease (Phoksawat et al., 2016). However, IL-17 productions of CD56 + T cells associated with NKG2D in T2D patients have not been reported. In the current study, we hypothesize that CD56 + T cells expressing NKG2D produce a high amount of IL-17 in T2D associated with pathogenesis of the disease.

2. Materials and methods

2.1. Study subjects

Blood samples of type 2 diabetes (T2D) patients (N = 34) were obtained from Srinagarind and Khon Kaen Hospitals including poor glycemic control (PC; N = 19; HbA1c > 8.5% or > 69 mmol/mol) and well glycemic control (WC; N = 15; HbA1c ≤ 8.5% or ≤ 69 mmol/mol). T2D subjects who had diabetic complications and received anti-inflammatory medication were excluded. Different glycemic control groups were considered according to the previous study representing impaired innate immune cell function in T2D (Chanchamroen et al., 2009). In addition, 21 cases of non-diabetic individuals (ND) were included in this project. The subject characteristics were described as our previous study (Phoksawat et al., 2016). The study project was approved by the Ethical Committee of Khon Kaen University (HE 571,109) and with the 1964 Helsinki declaration and its later amendments.

2.2. Flow cytometry of surface markers

Fresh peripheral blood collected in EDTA or heparin anticoagulant was stained by the mixture of anti-CD3-Fluorescein Isothiocyanate (FITC) (UCHT1, BD Pharmingen™, San Diego, CA, USA), anti-CD56-Peridinin Chlorophyll Protein Complex-Cy5.5 (PerCP-Cy5.5) (HCD56, BioLegend, San Diego, CA, USA) and anti-NKG2D-Allophycocyanin (APC) (149810, R&D systems, Minneapolis, MN, USA) for 15 min at room temperature in the dark. BD FACS™ lysing solution (BD Biosciences, San Jose, CA, USA) was then used for red blood cell lysis for 10–15 min before washing with 1X PBS solution. After that, cells were analyzed by flow cytometric analysis using FACSCanto™ II flow cytometer (BD Biosciences, USA). The service was provided by Research Instrument Center, Khon Kaen University, Thailand. Additionally, CD56+ and NKG2D + were gated based upon minus one PerCP-Cy5.5 and isotype control APC (R&D systems, USA), respectively. The median fluorescent intensity of NKG2D was used as a cut-off of low or high NKG2D expressions (Hanaoka et al., 2010; Weiss-Steider et al., 2011; Gubbels et al., 2010).

2.3. IL-17 production assay using phorbol-12-myristate-13-acetate (PMA) and ionomycin stimulators

Peripheral blood mononuclear cells (PBMCs) from patients with T2D (N = 22) including WC (N = 10) and PC (N = 12), and non-diabetic individual (N = 12) were isolated using Lymphosep Lymphocyte Separation Medium (Biowest, Nuaillé, France). Cells were activated by 100 ng/ml phorbal-12-myristate-13-acetate (PMA) (Sigma Aldrich®, Saint Louis, MO, USA) and 1000 ng/ml ionomycin calcium salt (Sigma Aldrich®, USA), and simultaneously incubated with 3 μg/ml brefeldin A (eBioscience, San Diego, CA, USA) for 2 h at 37 °C (Phoksawat et al., 2016; Zeng et al., 2012). Activated PBMCs were stained with surface antibodies as follows: anti-CD3-Phycoerythrin (PE) (SK7, eBioscience, USA), anti-CD56 PerCP-Cy5.5 (HCD56, BioLegend, USA) and anti-NKG2D APC (149810, R&D systems, USA) for 15 min in the dark before fixation with 4% formaldehyde and permeabilization with permeabilization/wash buffer (R&D systems, USA). After that, cells were intracellularly stained with anti-IL-17 A FITC (eBio64DEC17, eBioscience, USA) for 30 min prior to flow cytometric analysis. The clinical characteristics of ND and T2D patients who were enrolled in analysis of IL-17 production were demonstrated as shown in Table 1.

Table 1
Clinical characteristics of ND and T2D patients enrolled in analysis of IL-17 production.

Parameters	ND Mean ± SD	T2D Mean ± SD	*P*-value
Number of subjects	12	22	NA
Age with range (years)	50.0 (42-60)	56.5 (47-64)	0.002
Gender (male/female, ratio)	2/10, 0.20	4/18, 0.22	0.912
FBS (mg/dL)	83.3 ± 8.3	164.2 ± 44.8	< 0.001
HbA1c (NGSP), %	NA	9.0 ± 2.0	NA
HbA1c (IFCC), mmol/mol	NA	75.0 ± 21.7	NA
Blood pressure			
Systolic (mmHg)	123.8 ± 10.1	127.8 ± 14.9	0.417
Diastolic (mmHg)	74.2 ± 8.8	76.0 ± 10.3	0.676
Lipid profiles			
TC (mg/dL)	215.8 ± 48.1	196.5 ± 44.4	0.099
TG (mg/dL)	146.3 ± 67.1	175.0 ± 95.6	0.428
LDL-C (mg/dL)	137.9 ± 39.2	127.1 ± 47.6	0.449
HDL-C (mg/dL)	56.7 ± 10.8	46.6 ± 8.2	0.005

2.4. IL-17 production assay using various stimulators of specific monoclonal antibodies (mAbs)

Ninety-six well plates were coated with each of 10 μg/mL anti-TCR α/β (IP26, BioLegend, USA) alone, 10 μg/mL anti-NKG2D (1D11, BD Pharmingen™, USA) alone or anti-TCR α/β combine with anti-NKG2D for at least 3 h at 37 °C. Isolated PBMCs (approximately, 5×10^5 cells/well) from 3 patients with T2D were added into the coated plate and incubated with 3 μg/ml brefeldin A for 1 h at 37 °C (Phoksawat et al., 2016) prior to surface and intracellular staining according to the protocol described in above.

2.5. Statistical analyses

Flow cytometric data were analyzed by the BD FACSDivas™ (BD Biosciences, USA). The normal distribution was tested by Shapiro-Wilk test using SPSS (SPSS Inc., Chicago, IL, USA). Statistical analyses for intergroup comparisons were performed using the GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Parametric data were tested by the unpaired t-test, while non-parametric data were tested by the Mann-Whitney *U* test The correlations were assessed by Pearson's correlation or Spearman's correlation. The power of test (1-β) was also examined by Post-hoc analysis using the GPower (ver. 3.1) software (Heinrich Heine University Düsseldorf, Germany). The data were analyzed as median of percentages. Median of fluorescent intensity was used to be a cut-off for separating low and high expression of NKG2D on CD56 + T cells.

3. Results

3.1. CD56 + T cell frequency and NKG2D expression

The frequency of CD56 + T cells of T2D patients did not differ from the non-diabetic individuals (ND; Supplementary Fig. S1A). Neither, was there statistically significant association amongst the groups of WC,

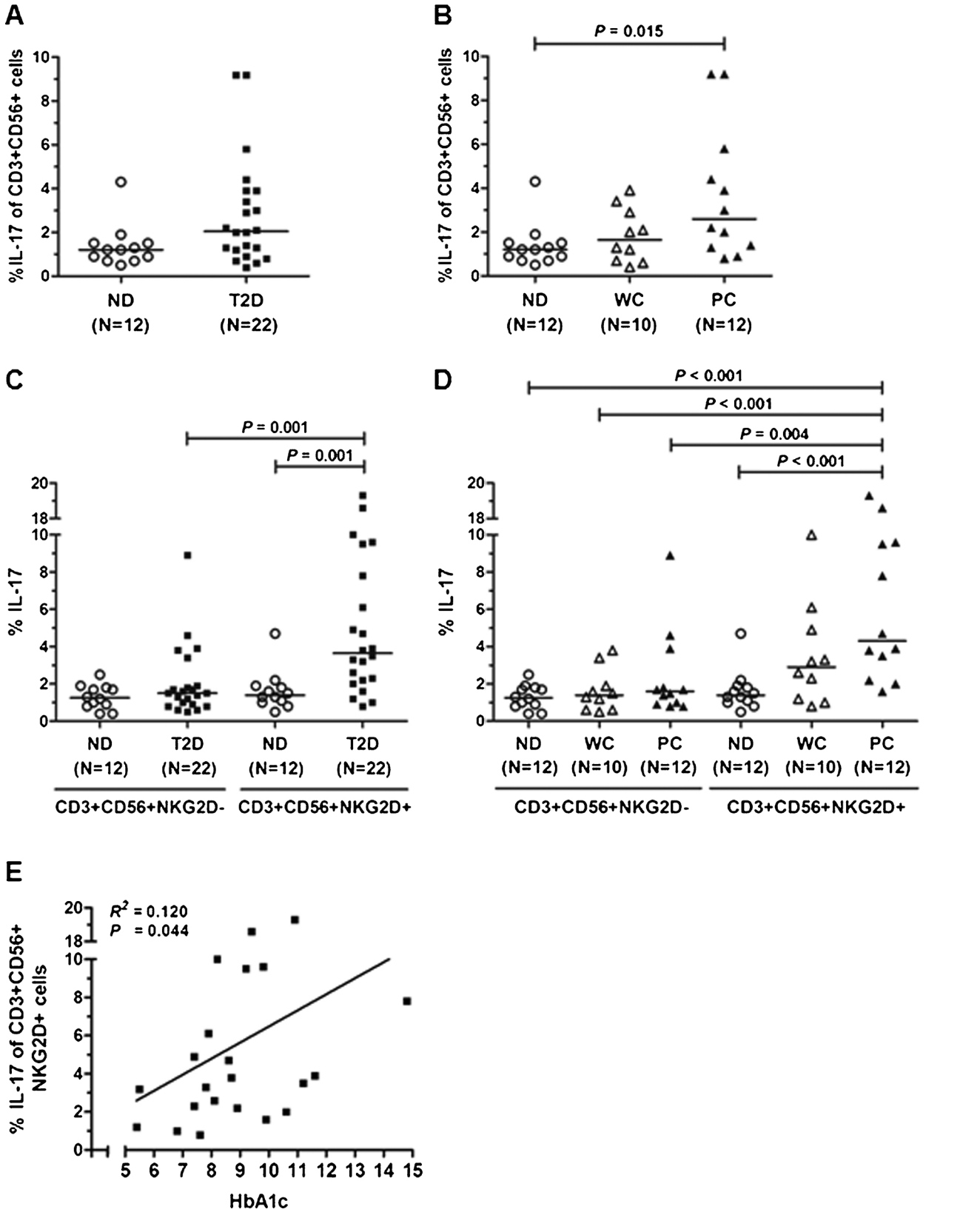

Fig. 1. Interleukin (IL)-17 production by CD56 + T cells expressing the natural group 2, member D (NKG2D) of patients with type 2 diabetes (T2D). (A) CD56 + T cells of T2D patients had higher median of IL-17 than those of non-diabetic individuals (ND) group. (B) Regarding the HbA1c level, Poor glycemic control (PC) group had significantly higher IL-17 production of CD56 + T cells than those of ND group. (C) CD56 + T cells with NKG2D positive of T2D patients produced significantly higher IL-17 than those of CD56 + T cells with NKG2D negative. Moreover, there was a significant difference of IL-17 production amongst CD56 + T cells with NKG2D + of T2D and ND. (D) CD56 + T cells with NKG2D positive from T2D patients with PC predominantly produced the highest IL-17 level that showed statistically significant amongst the groups. (E) IL-17-producing CD56 + T cells with NKG2D positive were positively associated with HbA1c (N = 22, R^2 = 0.120, P = 0.044). white circle: non-diabetic individuals (ND), black square: patients with T2D, white triangle: well glycemic control (WC) group, black triangle: poor glycemic control (PC) group, N = number of patients.

PC and ND (Supplementary Fig. S1B). Moreover, NKG2D expression on CD56 + T cells of T2D patients was not significantly different when compared to those of ND (Supplementary Fig. S1C) and neither amongst the groups (Supplementary Fig. S1D).

3.2. IL-17 production of CD56 + T cells in T2D patients

IL-17 produced by CD56 + T cells of T2D patients had higher median than those of ND, but a statistical difference was not observed (Fig. 1A). Regarding the HbA1c level, the PC group showed the highest median of IL-17 production and was statistically significant with $P = 0.015$ when was compared to the ND group (2.6% vs 1.2%) as shown in Fig. 1B.

We then analyzed IL-17 production of CD56 + T cells compared between NKG2D + and NKG2D-. CD56 + T cells with NKG2D + of T2D patients had significantly higher IL-17 production than those of CD56 + T cells with NKG2D- (3.7% vs 1.5%, $P = 0.001$; Fig. 1C). Evidently, there was a significant difference of IL-17 production of CD56 + T cells with NKG2D + between T2D and ND (3.7% vs 1.4%, $P = 0.001$; Fig. 1C). Additionally, CD56 + T cells with NKG2D + of PC predominantly produced IL-17 when compared to CD56 + T cells with NKG2D- of PC (4.3% vs 1.6%, $P = 0.004$), WC (4.3% vs 1.4%, $P < 0.001$) and ND (4.3% vs 1.3%, $P < 0.001$). Patients with PC had significantly higher IL-17 production of CD56+ T cells with NKG2D+ than those of ND group either (4.3% and 1.4%, $P < 0.001$; Fig. 1D).

3.3. Correlation analyses

There was no correlation between the level of HbA1c and CD56 + T cell frequency, HbA1c and the percentages of NKG2D expression on CD56 + T cells, and HbA1c and the percentages of IL-17-producing CD56 + T cells (data not shown). Interestingly, however, the HbA1c level was positively correlated with IL-17-producing CD56 + T cells with NKG2D+ ($N = 22$, $R^2 = 0.120$, $P = 0.044$; Fig. 1E).

3.4. CD56 + T cells with high intensity of NKG2D expression promoted higher level of IL-17

T cells expressing CD56 with NKG2D + were separated into low and high intensity defined as $NKG2D^{Low}$ and $NKG2D^{Hi}$, respectively. Median of fluorescent intensity was used for the cut-off between the low and high groups. A representative data analysis of IL-17 production between the low and high NKG2D expressions was displayed in Fig. 2. We found that CD56 + T cells with $NKG2D^{Hi}$ of T2D patients produced significantly IL-17 than those of CD56 + T cells with $NKG2D^{Low}$ of both ND and T2D patients (4.7% vs 1.5%, $P < 0.001$ and 4.7% vs 2.3%, $P = 0.027$, respectively). Predominantly, CD56+ T cells with $NKG2D^{Hi}$ of T2D patients showed statistically significant with $P < 0.001$ compared to CD56+ T cells with $NKG2D^{Hi}$ of the ND group (4.7% vs 1.5%). Moreover, CD56 + T cells with $NKG2D^{Low}$ of T2D patients had significantly different of IL-17 production from the ND group (2.3% vs 1.5%, $P = 0.043$; Fig. 3A).

Regarding the HbA1c level, CD56 + T cells with $NKG2D^{Hi}$ of PC outstandingly produced IL-17 when compared to CD56 + T cells with $NKG2D^{Low}$ of PC (6.2% vs 2.9%, $P = 0.028$), WC (6.2% vs 2.0%, $P = 0.004$) and ND (6.2% vs 1.5%, $P < 0.001$). A statistical significance of IL-17 production of CD56 + T cells with $NKG2D^{Hi}$ between PC and WC (6.2% vs 3.5%, $P = 0.047$), between PC and ND (6.2% vs 1.5%, $P < 0.001$), and between WC and ND group (3.5% vs 1.5%, $P = 0.034$) were observed. Furthermore, T2D patients with PC had also significantly higher IL-17 production of CD56+ T cells with $NKG2D^{Low}$ than those of the ND group (2.9% and 1.5%, $P = 0.020$; Fig. 3B).

3.5. CD56 + T cells stimulated via specific anti-NKG2D could produce IL-17

PBMCs obtained from 3 cases of T2D patients were stimulated by specific monoclonal antibodies (mAbs) including anti-TCRα/β alone, anti-NKG2D alone or anti-TCR α/β plus anti-NKG2D. CD56 + T cells with NKG2D + could produce a high level of IL-17 via activation of anti-NKG2D alone, while CD56 + T cells with NKG2D negative slightly produced IL-17 (Fig. 4).

4. Discussion

Type 2 diabetes (T2D) is a chronic metabolic disease characterized by hyperglycemia and insulin resistance of tissues and associated with low-grade systemic inflammation (American Diabetes Association, 2014; Duncan et al., 2003; Donath and Shoelson, 2011). Under T2D condition, there are many alterations of the immune cell responses (Shu et al., 2012). We have previously demonstrated the aberration of NKG2D expression on $CD4+CD28^{null}$ T subset producing highly pro-inflammatory cytokine, IL-17, in T2D (Phoksawat et al., 2016). In this study, we established a phenotypic and pro-inflammatory characteristic of CD56 + T cells involving pathogenesis of T2D. This is the first evidence of IL-17 producing CD56 + T cells expressing NKG2D in T2D patients.

Regarding the sample size, the power of test (1-β) was also examined by Post-hoc analysis using the GPower (ver. 3.1) software. The power was calculated based on the different means of the percentages of IL-17 production by CD3 + CD56 + NKG2D + cells between the two independent groups. Apparently, the power (1-β) was 0.8006271 or 80%. The characteristic features of T2D patients and ND were described as our previous study showing variously statistical significance (Phoksawat et al., 2016). We expected that only T2D condition may affect the phenotype of CD56 + T cells. Comparing with the ND subjects who had the age of < 45, 45–50 and > 50 years, we confirmed that the age had no effect on the frequency and NKG2D expression of CD56 + T cells (data not shown). Almeida-Oliveira A et al. (Almeida-Oliveira et al., 2011) showed that both NKT cell and NKT NKG2D + cell frequency of the elderly adults (> 60 years) had no statistically significant from the adult group (19–59 years). In addition, dyslipidemia, especially the aberration of a high-density lipoprotein-cholesterol (HDL-C) level, had no effect on the frequency and NKG2D expression of CD56 + T cells.

We found that the CD56 + T cell frequency of T2D patients did not differ from ND. Similarly, Guo et al. (2012) and Dworacka et al. (2014) showed that NKT cell count of T2D patients had no significant difference when compared with the control group. We demonstrated that NKG2D expression on CD56 + T cells of T2D patients was not significantly different compared with ND. However, Guo et al. (2012) previously showed a statistical significance between the two groups. We also analyzed NKG2D expression on CD56 + T cells from 23 patients with T2D who had been obviously recorded for the duration of the disease. However, the statistical significance was not observed amongst the groups of patients who were diagnosed of T2D for < 5 compared with 5–10 and > 10 years.

The characteristic of CD56 + T cells to produce IL-17 by stimulation with PMA and ionomycin stimulation was explored. Our results showed that the pro-inflammatory cytokine, IL-17, was prominently produced by CD56 + T cells under T2D, especially in patients who had poorly controlled glycemia. Evidently, CD56 + T cells with NKG2D + of T2D patients, especially in PC patients, could significantly produce higher IL-17 than those of CD56 + T cells with NKG2D-, whilst this phenomenon did not occur in the ND group. Therefore, NKG2D may be associated with the low-grade inflammation of this disease through the production of IL-17. A previous study reported that NKT-like cells of stable lung transplant patients might play a vital role associated with graft rejection. The IL-17-producing NKT-like cells were increased with

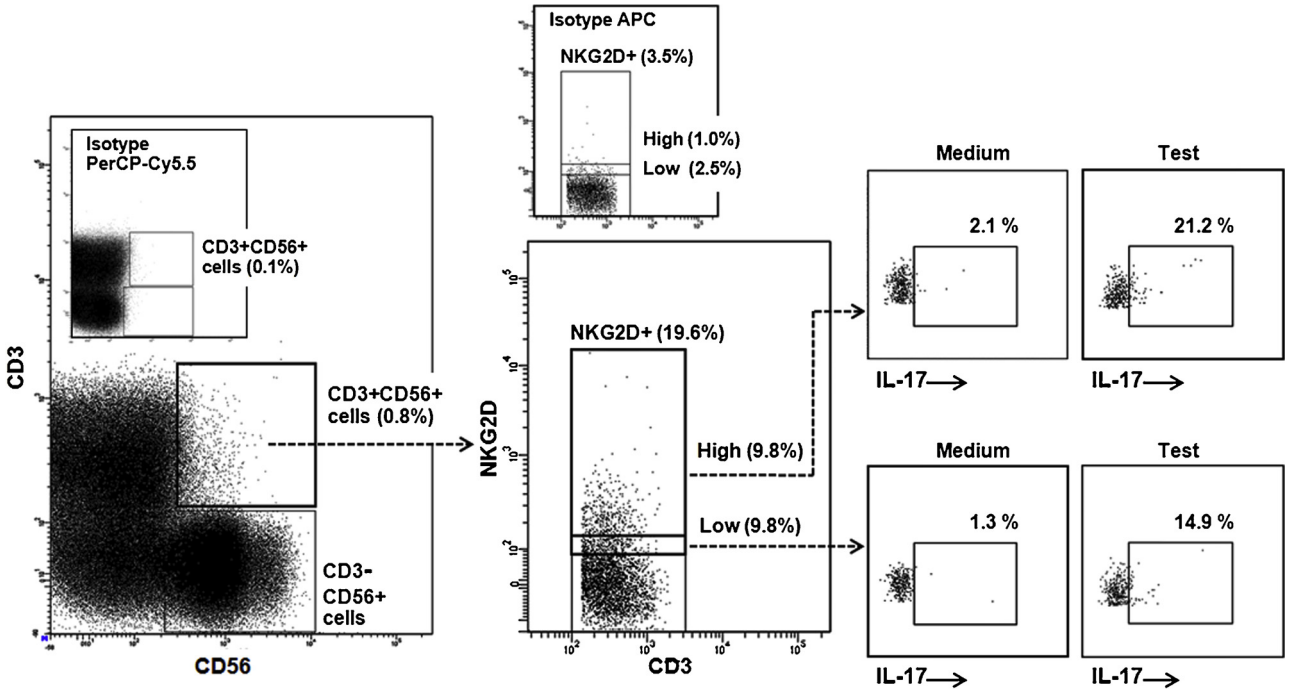

Fig. 2. A representative data analysis of interleukin (IL)-17 production compared between low and high intensity of the natural group 2, member D (NKG2D) expression on CD56 + T cells. Isolated peripheral blood mononuclear cells (PBMCs) were activated by phorbal-12-myristate-13-acetate (PMA) and ionomycin calcium salt. CD56 + T cells were gated on CD3 + CD56 + prior to analysis of NKG2D expression on their surface. On gated NKG2D positive population, median of fluorescent intensity was used to be a cut-off for separating low expression ($NKG2D^{Low}$) and high expression of NKG2D ($NKG2D^{Hi}$). Finally, the percentage of IL-17 production was demonstrated in each population. Medium: non-stimulation, Test: stimulation.

negative correlation with time post-transplant (Hodge et al., 2012). Moreover, invariant NKT cells of T1D patients could produce significantly higher IL-17 than those of the healthy volunteers (Li et al., 2014). The appropriate blood glucose was necessary for the regulation of immune cell responses. Expectedly, this study found that the HbA1c level was positively correlated with IL-17 production of CD56 + T cells with NKG2D + indicating the degree of inflammation depended on the glycemic control status. CD56 + T and CD4 + with NKG2D + may be prognosis markers of chronic inflammation via IL-17 production.

CD56 + T cells with $NKG2D^{Hi}$ of T2D patients, especially in the PC group, showed a statistical significance of IL-17 production amongst the groups. Interestingly, they also showed significant difference of IL-17 level from the ND group. We suggested that this cell population may be importantly involved in the pathogenesis of the disease.

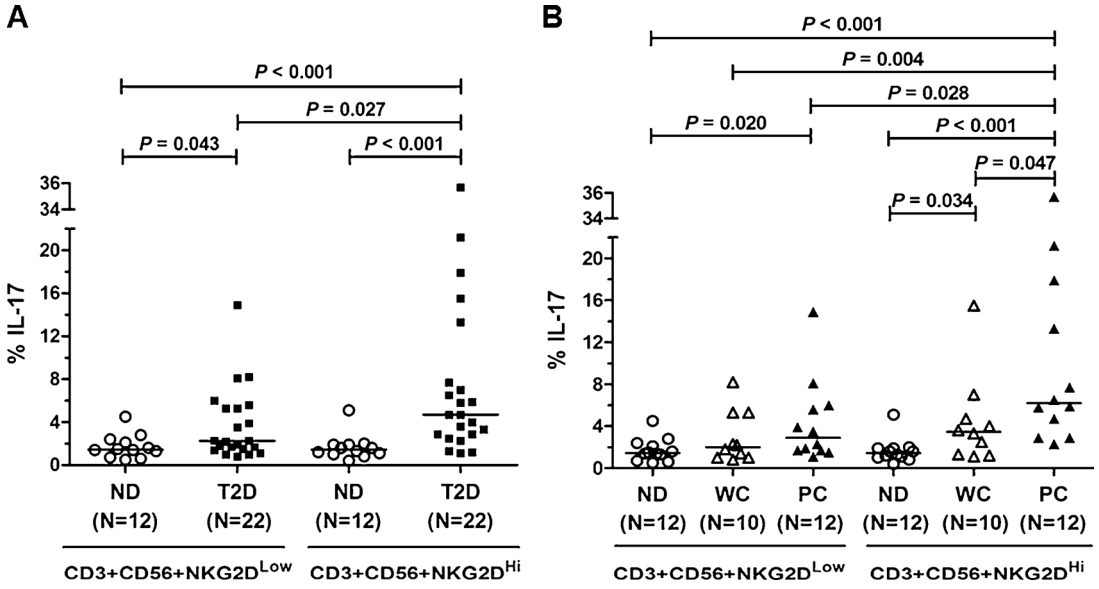

Fig. 3. CD56 + T cells with high intensity of the natural group 2, member D expression ($NKG2D^{Hi}$) promoted high level of interleukin (IL)-17. (A) CD56 + T cells with $NKG2D^{Hi}$ of type 2 diabetes (T2D) patients had significant higher level of IL-17 production than those of CD56 + T cells with $NKG2D^{Low}$ of both T2D and non-diabetic individuals (ND). Moreover, T2D patients had significantly higher IL-17 production of CD56 + T cells with $NKG2D^{Hi}$ than those of the ND group. (B) CD56 + T cells with $NKG2D^{Hi}$ of patients with poor glycemic control (PC) showed the highest median of IL-17 and were significantly different amongst the groups. white circle: non-diabetic individuals (ND), black square: patients with T2D, white triangle: well glycemic control (WC) group, black triangle: poor glycemic control (PC) group, N = number of patients.

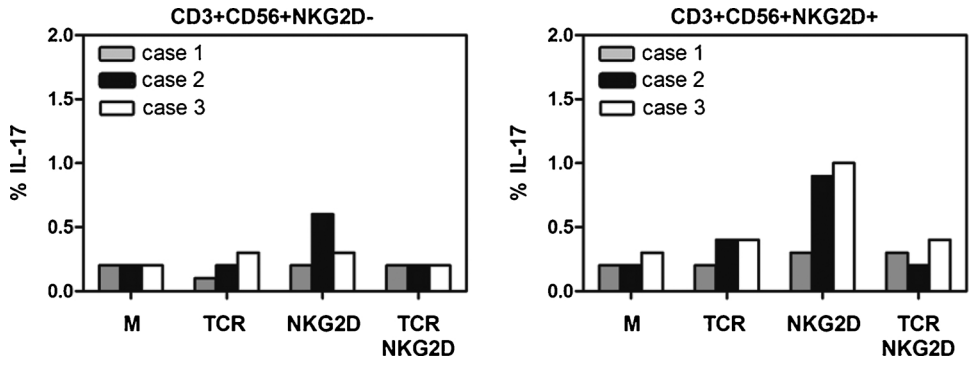

Fig. 4. High interleukin (IL)-17 production of CD56 + T cells expressing the natural group 2, member D (NKG2D) stimulated by specific anti-NKG2D. Isolated peripheral blood mononuclear cells (PBMCs) from 3 cases of patients with type 2 diabetes (T2D) were stimulated by different specific monoclonal antibodies (mAbs). Predominantly, CD56 + T cells with NKG2D positive stimulated by anti-NKG2D alone could produce higher IL-17 than those of CD56 + T cells with NKG2D negative.

Apparently, IL-17 is associated with the expression of NKG2D, we, thus, investigated whether stimulation of the NKG2D receptor was associated with the IL-17 production in CD56 + T cells. PBMCs from selected 3 patients with T2D were stimulated by specific mAbs such as anti-TCR α/β alone (IP26), anti-NKG2D (1D11) alone or anti-TCR α/β combined with anti-NKG2D. IP26 was used for stimulation instead of OKT3 because OKT3 reacted against the same epsilon (ε) chain as did SK7 which was used for surface staining. This was to avoid the cross-block binding effect. IP26 was used successfully to co-stimulate NK-like T cells along with anti-NKG2D, 1D11 (Vallejo et al., 2011). CD56 + T cells in the presence of NKG2D produced higher level of IL-17 than those of CD56 + T cells with NKG2D negative, particularly when they were stimulated by anti-NKG2D alone, indicating that NKG2D stimulation might be associated with the IL-17 production signaling pathway and presumably played as TCR-independent receptor. Contrariwise, the activation of anti-TCR α/β represented the inhibitory effect of IL-17 production. We are in the process of investigating the incidence. As shown by Kuylenstierna et al. (2011) that NKG2D expressed on invariant NKT cells could play both a direct TCR-independent activation and co-stimulation with CD1d to trigger the cytolytic function. Besides activation of the phosphorinositide 3-kinase (PI3K), NKG2D associated with the DNAX-activation protein 10 (DAP10) may also induce the downstream signaling through the protein kinase C theta (PKC-θ). PKC-delta (δ) and the Janus kinase (JAK) would transmit the signal into the signal transducer and activator of transcription 3 (STAT3) and the retinoic acid receptor-related orphan receptor gamma t (RORγt), which are the important transcription factors for IL-17 productions (Fiocco et al., 2014; Jain et al., 1999; Lopez-Larrea et al., 2008; Tan et al., 2006; Vivier et al., 2013; Upshaw and Leibson, 2006; Yang et al., 2011). Likewise, PKC-θ signaling may sequentially stimulate the nuclear factor of activated T cells (NFAT) before inducing of the basic leucine zipper ATF-like transcription factor (BATF) and RORγt, respectively (Jordan-Williams et al., 2013; Wang et al., 2012; Baitsch et al., 2012). Furthermore, the NF kappa B activating protein (NKAP) associated with the promyelocytic leukemia zinc finger (PLZF) can induce IL-17 production via RORγt in invariant NKT cells (Thapa et al., 2016). However, the association between NKAP and NKG2D signaling has not been well-defined.

5. Conclusions

Our study is the first report to demonstrate the functional characteristic of IL-17 producing CD56 + T cells expressing NKG2D in patients with T2D. These cells may be potentially pathogenic triggering low-grade inflammation and driving a severity of T2D along with $CD4+CD28^{null}$NKG2D + cells, especially in the PC group.

Conflict of interest

The authors have declared no conflict of interests. All authors have approved the manuscript.

Accessibility of data

All data can be accessible upon request.

Acknowledgements

WP is holding a scholarship under the Post-doctoral Program from Research Affairs and Graduate School, Khon Kaen University (58336). This work is supported by Khon Kaen University Research Grant 2014-2016 and The Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Exercise and Sport Sciences Development and Research Group, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molimm.2018.12.008.

References

Aggarwal, A., Sharma, A., Bhatnagar, A., 2014. Role of cytolytic impairment of natural killer and natural killer T-cell populations in rheumatoid arthritis. Clin. Rheumatol. 33, 1067–1078.
Almeida-Oliveira, A., Smith-Carvalho, M., Porto, L.C., Cardoso-Oliveira, J., Ribeiro Ados, S., Falcao, R.R., Abdelhay, E., Bouzas, L.F., Thuler, L.C., Ornellas, M.H., Diamond, H.R., 2011. Age-related changes in natural killer cell receptors from childhood through old age. Hum. Immunol. 72, 319–329.
American Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care 37 (1), S81–90.
Baitsch, L., Fuertes-Marraco, S.A., Legat, A., Meyer, C., Speiser, D.E., 2012. The three main stumbling blocks for anticancer T cells. Trends Immunol. 33, 364–372.
Chanchamroen, S., Kewcharoenwong, C., Susaengrat, W., Ato, M., Lertmemongkolchai, G., 2009. Human polymorphonuclear neutrophil responses to Burkholderia pseudomallei in healthy and diabetic subjects. Infect. Immun. 77, 456–463.
Donath, M.Y., Shoelson, S.E., 2011. Type 2 diabetes as an inflammatory disease. Nat. Rev. Immunol. 11, 98–107.
Duncan, B.B., Schmidt, M.I., Pankow, J.S., Ballantyne, C.M., Couper, D., Vigo, A., Hoogeveen, R., Folsom, A.R., Heiss, G., 2003. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 52, 1799–1805.
Dworacka, M., Wesolowska, A., Wysocka, E., Winiarska, H., Iskakova, S., Dworacki, G., 2014. Circulating $CD3^{+}56^{+}$ cell subset in pre-diabetes. Exp. Clin. Endocrinol. Diabetes 122, 65–70.
Fiocco, U., Accordi, B., Martini, V., Oliviero, F., Facco, M., Cabrelle, A., Piva, L., Molena, B., Caso, F., Costa, L., Scanu, A., Pagnin, E., Atteno, M., Scarpa, R., Basso, G., Semenzato, G., Punzi, L., Doria, A., Dayer, J.M., 2014. JAK/STAT/PKCdelta molecular pathways in synovial fluid T lymphocytes reflect the in vivo T helper-17 expansion in psoriatic arthritis. Immunol. Res. 58, 61–69.
Gubbels, J.A., Felder, M., Horibata, S., Belisle, J.A., Kapur, A., Holden, H., Petrie, S., Migneault, M., Rancourt, C., Connor, J.P., Patankar, M.S., 2010. MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. Mol. Cancer 9. https://doi.org/10.1186/1476-4598-9-11.
Guo, H., Xu, B., Gao, L., Sun, X., Qu, X., Li, X., Liu, S., Feng, J., Wang, J., Tang, Y., Yan, G., Gao, X., Jiang, Y., 2012. High frequency of activated natural killer and natural killer T-cells in patients with new onset of type 2 diabetes mellitus. Exp. Biol. Med.? (Maywood) 237, 556–562.
Hanaoka, N., Jabri, B., Dai, Z., Ciszewski, C., Stevens, A.M., Yee, C., Nakakuma, H., Spies, T., Groh, V., 2010. NKG2D initiates caspase-mediated CD3zeta degradation and

lymphocyte receptor impairments associated with human cancer and autoimmune disease. J. Immunol. 185, 5732–5742.
Hodge, G., Hodge, S., Li-Liew, C., Reynolds, P.N., Holmes, M., 2012. Increased natural killer T-like cells are a major source of pro-inflammatory cytokines and granzymes in lung transplant recipients. Respirology 17, 155–163.
Hodge, G., Holmes, M., Jersmann, H., Reynolds, P.N., Hodge, S., 2013. The drug efflux pump Pgp1 in pro-inflammatory lymphocytes is a target for novel treatment strategies in COPD. Respir. Res. 14, 63.
Jain, N., Zhang, T., Kee, W.H., Li, W., Cao, X., 1999. Protein kinase C delta associates with and phosphorylates Stat3 in an interleukin-6-dependent manner. J. Biol. Chem. 274, 24392–24400.
Jordan-Williams, K.L., Poston, S., Taparowsky, E.J., 2013. BATF regulates the development and function of IL-17 producing iNKT cells. BMC Immunol. 14. https://doi.org/10.1186/1471-2172-14-16.
Koreck, A., Suranyi, A., Szony, B.J., Farkas, A., Bata-Csorgo, Z., Kemeny, L., Dobozy, A., 2002. $CD3^+CD56^+$ NKT cells are significantly decreased in the peripheral blood of patients with psoriasis. Clin. Exp. Immunol. 127, 176–182.
Kumar, V., Delovitch, T.L., 2014. Different subsets of natural killer T cells may vary in their roles in health and disease. Immunology 142, 321–336.
Kuylenstierna, C., Bjorkstrom, N.K., Andersson, S.K., Sahlstrom, P., Bosnjak, L., Paquin-Proulx, D., Malmberg, K.J., Ljunggren, H.G., Moll, M., Sandberg, J.K., 2011. NKG2D performs two functions in invariant NKT cells: direct TCR-independent activation of NK-like cytolysis and co-stimulation of activation by CD1d. Eur. J. Immunol. 41, 1913–1923.
Li, S., Joseph, C., Becourt, C., Klibi, J., Luce, S., Dubois-Laforgue, D., Larger, E., Boitard, C., Benlagha, K., 2014. Potential role of IL-17-producing iNKT cells in type 1 diabetes. PLoS One 9. https://doi.org/10.1371/journal.pone.0096151.
Liew, P.X., Kubes, P., 2015. Intravital imaging – dynamic insights into natural killer T cell biology. Front. Immunol. 6. https://doi.org/10.3389/fimmu.2015.00240.
Lopez-Larrea, C., Suarez-Alvarez, B., Lopez-Soto, A., Lopez-Vazquez, A., Gonzalez, S., 2008. The NKG2D receptor: sensing stressed cells. Trends Mol. Med. 14, 179–189.
Phoksawat, W., Jumnainsong, A., Leelayuwat, N., Leelayuwat, C., 2016. Aberrant NKG2D expression with IL-17 production of $CD4^+$ T subsets in patients with type 2 diabetes. Immunobiology 222, 944–951.
Shu, C.J., Benoist, C., Mathis, D., 2012. The immune system's involvement in obesity-driven type 2 diabetes. Semin. Immunol. 24, 436–442.
Tan, S.L., Zhao, J., Bi, C., Chen, X.C., Hepburn, D.L., Wang, J., Sedgwick, J.D., Chintalacharuvu, S.R., Na, S., 2006. Resistance to experimental autoimmune encephalomyelitis and impaired IL-17 production in protein kinase C theta-deficient mice. J. Immunol. 176, 2872–2879.
Thapa, P., Chen, M.W., McWilliams, D.C., Belmonte, P., Constans, M., Sant'Angelo, D.B., Shapiro, V.S., 2016. NKAP regulates invariant NKT cell proliferation and differentiation into ROR-gammat-expressing NKT17 cells. J. Immunol. 196, 4987–4998.
Upshaw, J.L., Leibson, P.J., 2006. NKG2D-mediated activation of cytotoxic lymphocytes: unique signaling pathways and distinct functional outcomes. Semin. Immunol. 18, 167–175.
Vallejo, A.N., Hamel Jr., D.L., Mueller, R.G., Ives, D.G., Michel, J.J., Boudreau, R.M., Newman, A.B., 2011. NK-like T cells and plasma cytokines, but not anti-viral serology, define immune fingerprints of resilience and mild disability in exceptional aging. PLoS One 6, 13. https://doi.org/10.1371/journal.pone.0026558.
Van Kaer, L., 2004. Regulation of immune responses by CD1d-restricted natural killer T cells. Immunol. Res. 30, 139–153.
Vivier, E., Ugolini, S., Nunes, J.A., 2013. ADAPted secretion of cytokines in NK cells. Nat. Immunol. 14, 1108–1110.
Wang, X., Chuang, H.C., Li, J.P., Tan, T.H., 2012. Regulation of PKC-theta function by phosphorylation in T cell receptor signaling. Front. Immunol. 3, 8. https://doi.org/10.3389/fimmu.2012.00197.
Weiss-Steider, B., Soto-Cruz, I., Martinez-Campos, C.A., Mendoza-Rincon, J.F., 2011. Expression of MICA, MICB and NKG2D in human leukemic myelomonocytic and cervical cancer cells. J. Exp. Clin. Cancer Res. 30, 8. https://doi.org/10.1186/1756-9966-30-37.
Wu, L., Van Kaer, L., 2009. Natural killer T cells and autoimmune disease. Curr. Mol. Med. 9, 4–14.
Yang, X.P., Ghoreschi, K., Steward-Tharp, S.M., Rodriguez-Canales, J., Zhu, J., Grainger, J.R., Hirahara, K., Sun, H.W., Wei, L., Vahedi, G., Kanno, Y., O'Shea, J.J., Laurence, A., 2011. Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. Nat. Immunol. 12, 247–254.
Zeng, C., Shi, X., Zhang, B., Liu, H., Zhang, L., Ding, W., Zhao, Y., 2012. The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications. J. Mol. Med. (Berl.) 90, 175–186.