Natural killer cells and their function in Takayasu's arteritis

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ABSTRACT

Objective. *Takayasu's arteritis (TAK) is a chronic, large-vessel systemic vasculitis. Immune inflammatory response plays a crucial role in the pathogenesis of TAK. Natural killer (NK) cells are one of the major immunoregulatory cell groups of the immune system, but their role in TAK pathogenesis is unclear. We aimed to investigate the role of peripheral blood NK cells in TAK pathogenesis.*

Methods. *The study consisted of 47 TAK patients and 27 healthy controls. Peripheral blood natural killer (NK) cells and their $CD56^{Dim}$/$CD56^{Bright}$ subsets were phenotyped using CD3 and CD56 surface markers. Functional potential was assessed by production of granzyme B, perforin and interferon (IFN)-γ.*

Results. *TAK patients had decreased numbers of NK cells in the peripheral blood ($p<0.001$) relative to healthy controls. The percentages of $CD56^{Bright}$ ($p<0.05$) and $CD56^{Dim}$ NK cells ($p<0.001$) from TAK patients were also decreased. The expressions of Granzyme B ($p<0.001$), Perforin ($p<0.001$) in NK cells were lower in TAK patients to compared control group, but no differences in the percentage of IFN-γ producing cells was observed between TAK patients and healthy control. There is no difference in the percentage of NK cells or $CD56^{Bright}$ or $CD56^{Dim}$ NK cells between active and inactive TAK. However, granzyme B-expressing NK cell percentage was significantly decreased in active TAK compared to inactive TAK ($p<0.05$).*

Conclusion. *Our findings concluded that NK cell numbers and cytotoxicity are reduced in TAK patients.*

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Introduction

Takayasu's arteritis (TAK) is a chronic, large-vessel systemic vasculitis. It primarily affects the aorta and its branches. However, other large vessels including pulmonary arteries, as well as medium-sized coronary arteries, could also be involved (1). The involved arteries were characterised by stenosis, occlusion, aneurysm or vascular dissection, causing ischaemia and dysfunction of the tissues and organs. TAK patients can occur with stroke, loss of vision, myocardial infarction, even death in severe cases (2, 3). According to a recent review, 50% of TAK patients will relapse and experience a vascular complication within 10 years from diagnosis (4). As a systemic autoimmune disease, immune inflammatory response plays a crucial role in the pathogenesis of the disease (5). Immunohistochemical studies of aortic tissue from TAK patients showed the infiltrating cells were comprised of macrophages, $CD4^+$ T cells, $CD8^+$ T cells, γδ T cells, natural killer (NK) cells and neutrophils (6).

NK cells are an important part of the immune system and play an important role in antiviral infections, tumours, transplant rejection and immune regulation (7). NK cells can be classified as two subsets according to the expression level of CD56: $CD56^{Dim}$ subset (approximately 90% in peripheral blood) and the minority $CD56^{Bright}$ subset (8). The main functional characteristics of $CD56^{Dim}$ NK cells are that they produce less cytokine but increased potential to produce perforin and Granzyme B. In contrast, the important function of the $CD56^{Bright}$ population has been shown to be the secretion of immunoregulatory cytokines, including interferon (IFN)-γ, tumour necrosis factor (TNF)-α and other inflammatory factors (9). In addition, NK cells can also regulate adaptive immunity by cross-reacting with cytokines or chemokines and other immune cells (such as T cells, B cells and dendritic cells) (7).

It has been reported that NK cells are involved in the regulation of various autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), and Behçet's disease (BD), etc. (10). The latest genome-wide association studies (GWAS), enhancer en-

richment analysis and pathway analysis also suggest that NK cells play a key role in TAK (11). Therefore, this study investigated the peripheral blood NK cells and their functional in TAK pathogenesis.

Materials and methods

Patients

The study group consisted of 47 TAK patients (mean age 39.30±11.19 years) who were referred to Beijing Anzhen Hospital, affiliated with Capital Medical University in China and 27 age- and sex-matched healthy controls (mean age 35.7±7.4 years). All the TAK patients fulfilled the criteria for classification of TAK developed by the American College of Rheumatology (ACR) in 1990. Patients who had chronic or current infections, tumours, haematologic diseases, other autoimmune diseases, lymphoproliferative disorders, hepatosplenic diseases or a history of allergic diseases were also excluded. Activity of disease was evaluated by the National Institute of Health (NIH) in 1994 and The Indian Takayasu Activity Clinical Activity Score (ITAS) (12, 13). This study was approved by the Ethics Committee of Beijing Anzhen Hospital.

Cell flow cytometry

Samples were processed using whole blood lysis to analyse NK cell subsets. Surface phenotypes of NK cell subsets were identified using the following antibodies: FITC anti-human CD3 and PerCP-Cy5.5 anti- human CD56 (all from BD Pharmingen). For intracellular cytokine staining, blood samples were stimulated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) and 1 μg/mL ionomycin (Sigma) for 5 hours at 37°C. After fixation and permeabilisation, samples were incubated with antibodies against PE anti-human INF-r, PE-Cy594 anti- human Granzyme B and BV421 anti- human Perforin (all from BD Pharmingen).

Flow cytometry data were acquired using BD LSRFortessa (BD Biosciences) and analysed by analysed with FlowJo v. 7.6.4 software (Tree Star Inc., USA). NK cells were defined as $CD3^-CD56^+$, subdivided into $CD3^-CD56^{Dim}$ and $CD3^-CD56^{Bright}$ subsets (8). The lymphocyte population was identified by assessment of size and granularity of cells using light scatter properties (forward scatter (FSC) *vs*. side scatter (SSC)) and NK percentage expressed as a proportion of total gated lymphocytes.

Statistical analysis

All statistical studies were carried out with the SPSS program V.19.0 (SPSS, Chicago, Illinois, USA). Data in this study was numerical data. According to the normality, variables were described as means ± SE or as medians. We performed univariate analysis by the Student's t-test or Wilcoxon-Mann-Whitney test for numerical data according to normality. p-values <0.05 denoted the statistically significant difference.

Results

Demographic data and clinical features of 47 TAK patients

Among the 47 TAK patients in this study, the male to female ratio of this study was 1:10.75 (4 males, 43 females). The average age was about 39 years and the average disease onset age was about 31 years. Type V was the most common angiographic classification pattern. The median NIH level was <2 points and the median ITAS-A level was <5 points. (Table I). Among these patients, 29 were treated with several medications and 18 patients were untreated with any medication (Table II).

Table I. Demographic data and clinical features of 47 TAK patients.

Gender	
Female	43 (43/47=91.5%)
Male	4 (4/47=8.5%)
Age (years, mean ± SE)	39.30 ± 11.19
Age of onset (years, mean ± SE)	31.64 ± 10.08
Angiographic classification	
Type I	6 (12.8%)
Type IIa	0 (0)
Type IIb	10 (21.3%)
Type III	4 (8.5%)
Type IV	4 (8.5%)
Type V	23 (48.9%)
ESR [mm/h, median (P25, P75)]	11.28 (5.00, 14.00)
CRP [mg/L, median (P25, P75)]	2.99 (0.35, 4.02)
NIH [median (P25, P75)]	1.32 (0.00, 2.00)
ITAS-A [median (P25, P75)]	3.85 (0.00, 8.00)
ITAS-2010 [median (P25, P75)]	3.55 (0.00, 8.00)

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; NIH: National Institute of Health; ITAS: The Indian Takayasu's Clinical Activity Score; ITAS-A: ITAS combined with the acute phase response (either ESR or CRP).

Table II. Pharmacological treatments of 29 TAK patients.

Medications	n (%)
Glucocorticoids (GCs)	24 (82.8%)
Methotrexate (MTX)	15 (51.7%)
Tocilizumab (TCZ)	13 (44.8%)
Mycophenolate mofetil (MMF)	13 (44.8%)
Cyclophosphamide (CTX)	8 (27.6%)
Tacrolimus (FK506)	2 (6.9%)
Antimalarials (Hydroxychloroquine, HCQ)	2 (6.9%)
Tripterygium (Tii)	2 (6.9%)
Leflunomide (LEF)	1 (3.4%)
Azathioprine (AZA)	1 (3.4%)

Reduced numbers of circulating NK cells in TAK patients

In order to determine whether NK cells participate in the development of TAK, we analysed the peripheral blood of patients with TAK collected in Beijing Anzhen Hospital. Accordance with previous literature (8), human NK cells defined in this study as $CD3^-$ $CD56^+$ lymphocytes. NK cells expressed as a percentage of total gated lymphocytes in the peripheral blood of all 47 TAK patients (6.55% (3.13, 8.42)) were found to be significantly reduced compared to healthy controls (11.20% (8.20, 15.3), p<0.001) (Fig. 1).

$CD56^{Bright}$ and $CD56^{Dim}$ NK cells are significantly decreased in the peripheral blood of TA patients

NK cells can be classified as $CD56^{Bright}$ and $CD56^{Dim}$ NK cells according to the

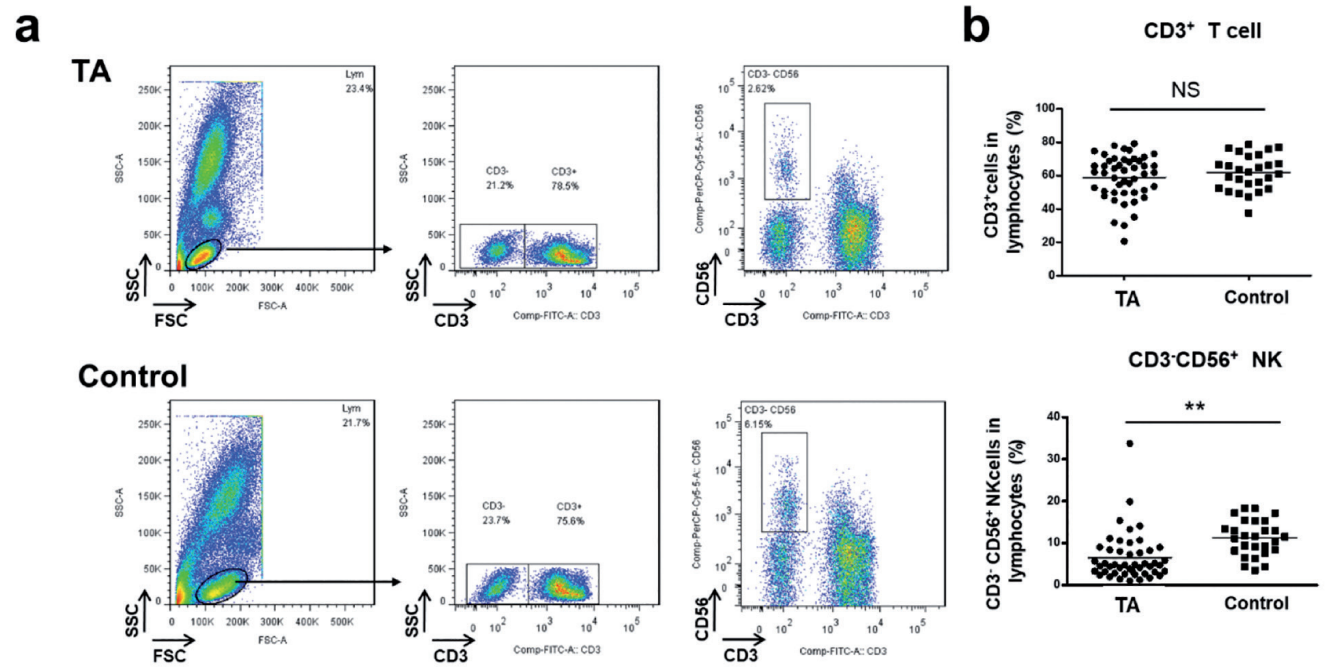

Fig. 1. Reduced numbers of circulating NK cells in TAK patients. (**a**) Flow cytometry analysis of $CD3^{-}CD56^{+}$NK cells in TA patients (n=47) and control groups (n=27). (**b**) Scatter plot shows the percentages $CD3^{+}$ T cells and $CD3^{-}CD56^{+}$ NK cells in total gated lymphocytes.
** $p< 0.001$ compared with control groups. NS indicates not significant.

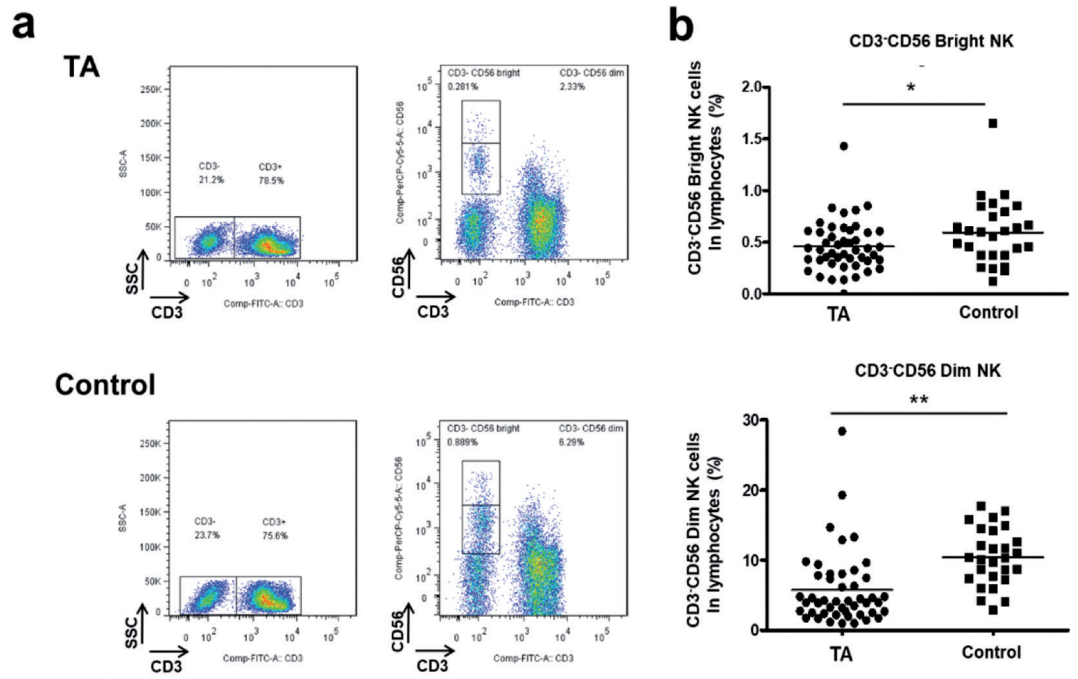

Fig. 2. $CD56^{Bright}$ and $CD56^{Dim}$ NK cells are significantly decreased in the peripheral blood of TAK patients.
(**a**) Flow cytometry analysis of $CD56^{Bright}$ and $CD56^{Dim}$ NK cells subsets in TAK patients (n=47) and control groups (n=27).
(**b**) Scatter plot shows the percentages $CD56^{Bright}$ and $CD56^{Dim}$ NK cells in total gated lymphocytes.
*p<0.05, **p<0.001 compared with control groups.

expression level of CD56. We analysed changes in $CD56^{Bright}$ and $CD56^{Dim}$ NK cells in two subpopulations of NK cells in peripheral blood of TAK patients. TAK patients had a significantly lower proportion of $CD56^{Bright}$ (TAK=0.50 (0.42, 0.61%) *vs*. Control=0.59 (0.37, 0.79)%, (p<0.05)) and $CD56^{Dim}$ (TAK=5.77 (5.18, 7.83)% *vs*. Control=10.42 (7.38, 14.20)%, (p<0.001)) NK cell subsets compared to healthy controls relative to total lymphocytes. (Fig. 2).

Impaired cytotoxicity of NK cells in TAK patients

The main function of NK cells is to produce inflammatory factors and exert cytotoxic effects (9). To explore the function of NK cells, we analysed the frequency of Granzyme B, Perforin and IFN-γ-secreting NK cells using flow cytometry. The percentages of Granzyme B (TAK=84.03 (77.90, 90.60)% *vs*. Control =90.94 (89.60, 94.70)%, (p<0.001)) and Perforin (TAK=74.14 (66.60, 82.90)% *vs*. Control=81.63

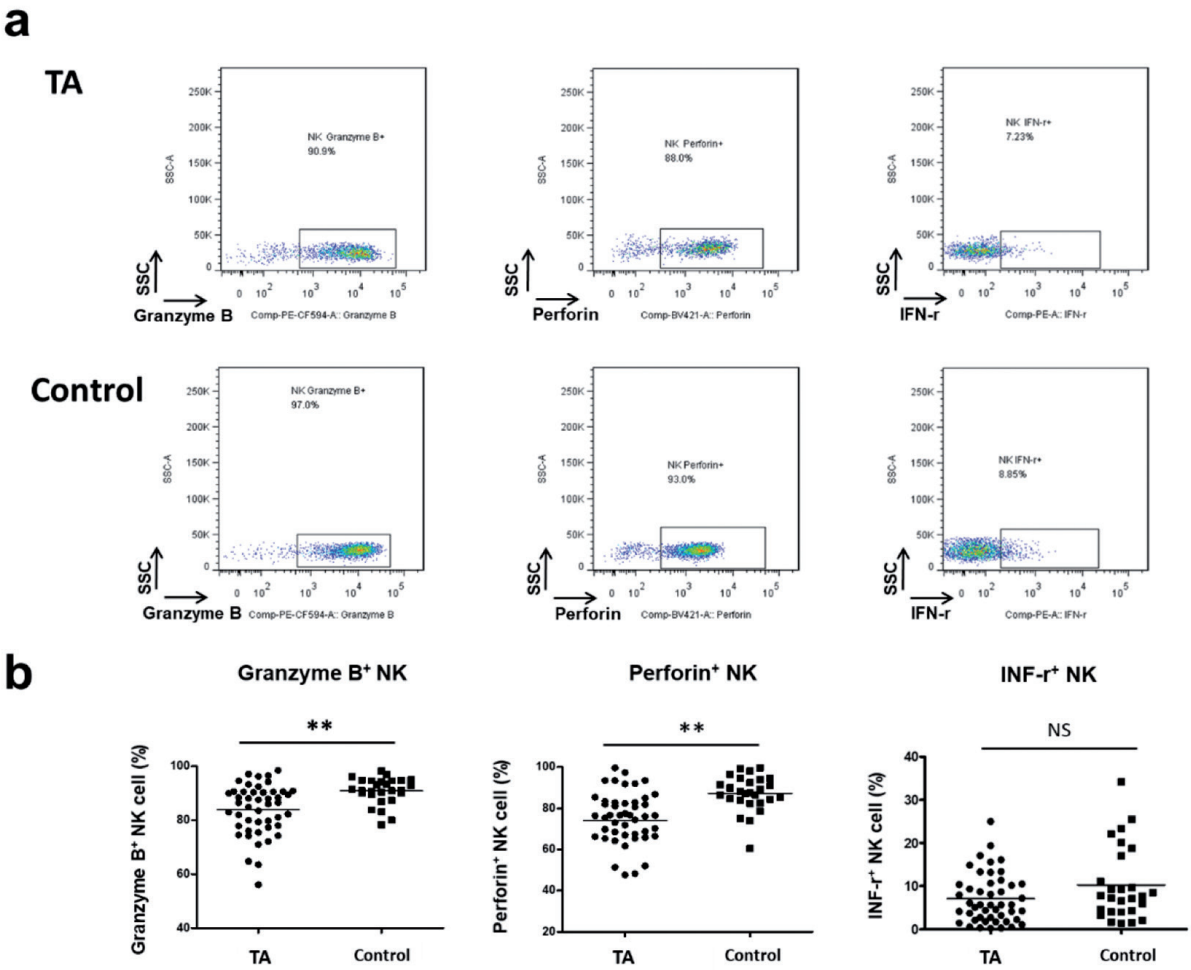

Fig. 3. Impaired cytotoxicity of NK cells in TAK patients.
(**a**) Flow cytometry histogram plots showing intensity of intracellular cytokine staining for Granzyme B, Perforin and IFN-γ following 5-hour PMA/Ionomycin stimulation (Representative plots are shown).
(**b**) Scatter plot shows the percentages of Granzyme B, Perforin and IFN-γ-secreting NK cells in total gated NK cells.
**p<0.001 compared with control groups. NS indicates not significant.

(82.5, 94.10)%, (p<0.001) producing NK cells were lower in TAK patients compared to control group. By contrast, no difference in the percentage of IFN-γ producing cells was observed between TAK patients and healthy control (TA=7.08 (2.26, 10.50) % *vs*. Control =10.29 (4.01, 17.00) %, (p=0.14), Fig. 3).

Granzyme B-expressing NK cell percentage was significantly decreased in active TAK compared to inactive TAK

In terms of the association between NK cells and TAK disease activity, we saw no difference in the percentage of NK cells or NK cell subsets between groups with active (NIH level was≥2 points) and inactive TAK (NIH level was <2 points). In contrast, analysis of granzyme B-expressing NK cell percentage demonstrated a significant decrease in active TAK compared to inactive TAK (active TAK=81.37 (75.08, 88.48) % *vs*. inactive TAK=86.81 (83.00, 92.50)% (p=0.031), Table III). Furthermore, we compared the NK cell subsets and intracellular cytokine staining between treated and untreated patients. The results showed there was no difference in the percentage of NK cells or their subsets between groups with treated and untreated TAK patients (Table IV).

Table III. Comparison of NK cells and their subsets in patients with active and inactive TAK.

median (P25, P75)	Active TAK (n=24)	Inactive TAK (n=23)	p-value
NK cell in lymphocyte (%)	5.84 (2.69, 8.20)	7.29 (3.18, 9.04)	0.389
$CD56^{Bright}$ NK in lymphocyte (%)	0.42 (0.30, 0.59)	0.48 (0.32, 0.61)	0.463
$CD56^{Dim}$ NK in lymphocyte (%)	5.23 (2.28, 7.71)	6.34 (2.55, 8.05)	0.389
Granzyme B^+ NK in NK (%)	**81.37 (75.08, 88.48)**	**86.81 (83.00, 92.50)**	**0.031**
Perforin$^+$ NK in NK (%)	70.20 (65.40, 81.20)	78.26 (68.90, 85.00)	0.142
IFN-γ^+ NK in NK (%)	7.22 (2.03, 10.48)	6.94 (5.17, 10.70)	0.924

Discussion

In order to determine whether NK cells participate in the development of TAK, we analysed the number of NK cells in peripheral blood and their function in TAK patients collected in Beijing An-

Table IV. Comparison of NK cells and their subsets in treated and untreated TAK patients.

median (P25, P75)	Treated TAK (n=29)	Untreated TAK (n=18)	*p*-value
NK cell in lymphocyte (%)	5.71 (2.24, 7.86)	6.54 (2.96, 9.30)	0.547
$CD56^{Bright}$ NK in lymphocyte (%)	0.45 (0.30, 0.60)	0.43 (0.32, 0.62)	0.776
$CD56^{Dim}$ NK in lymphocyte (%)	5.73 (2.48, 7.77)	5.84 (2.59, 8.22)	0.887
Granzyme B^+ NK in NK (%)	85.16 (79.60, 90.80)	82.21 (76.10, 89.73)	0.399
$Perforin^+$ NK in NK (%)	76.20 (67.80, 82.85)	70.84 (63.68, 84.00)	0.592
IFN-γ^+ NK in NK (%)	6.94 (1.94, 11.05)	7.30 (3.46, 10.25)	0.852

zhen Hospital. Our results showed that TAK patients had decreased numbers of NK cells in the peripheral blood compared to healthy controls. The percentages of $CD56^{Bright}$ and $CD56^{Dim}$ NK cells from TAK patients were also decreased. The expressions of granzyme B and perforin in NK cells were lower in TAK patients compared to control groups. Granzyme B-expressing NK cell percentage was significantly decreased in active TA compared to inactive TAK. In summary, our findings concluded that NK cell numbers and cytotoxicity are reduced in TAK patients.

TAK is a chronic, granulomatous, large-vessel systemic vasculitis characterised by stenosis, occlusion, and sometimes aneurysm of the aorta and its main branches (14). Although the pathogenesis of TAK has considerably improved during the last decade, the exact pathogenic sequence remains to be elucidated. It is well known that cell-mediated autoimmunity has been strongly implicated in the pathogenesis of TAK (15). Vascular inflammation of TAK possibly originates in the vasa vasorum, followed by infiltration of inflammatory cells (T cells, NK cells, neutrophils and so on) and production of inflammatory factor and cytokines, such as interleukin (IL)-6, TNF-α, IFN-γ, IL-12, and IL-18, leading to the formation of granulomas (16). NK cells are important members of lymphocytes. They can directly kill infected, transformed or autoreactive cells utilising exocytosis of specialised cytotoxic granules. Besides, NK cells can shape the adaptive immune response by cross-talk with other immune cells such as T cells, B cells and dendritic cells and by producing cytokine or chemokines (17, 18). Through their cytotoxic capacities and cytokine production, NK cells modulate autoimmune diseases. A recent review has summarised the correlation between NK cell number or functional alterations and several autoimmune diseases, including RA, SLE, SS, BD and so on (8). The latest genome-wide association studies (GWAS), enhancer enrichment analysis and pathway analysis suggest that NK cells play a key role in TAK (11).

Previous studies showed the percentages and absolute numbers of NK cells, and NK cytotoxicity were significantly lower in the peripheral blood of RA and SLE patients than in that of healthy controls (19). It has also recently been reported a decline of NK cells in patients with seropositive (SP) arthralgia and in BD patients (20, 21). Consistent with these results, our results found the percentages of total NK cells, $CD56^{Bright}$ and $CD56^{Dim}$ NK cells from TAK patients were decreased compared to healthy controls. The mechanism by which NK cells were decreased in the peripheral blood of patients with autoimmune diseases was complex. Several studies have demonstrated accumulation of NK cells in affected tissues of autoimmune patients, with the decreased numbers of NK cells in the peripheral blood. For example, it has been found the infiltrating NK cells in the pancreatic islet of Type 1 diabetes patients (22), and the synovium of RA patients (23, 24). As mentioned previously, histological findings showed NK cell infiltrations in the arterial vascular wall of TAK (16). NK cells mediated antibody dependent cytotoxicity (ADCC) induced by some autoantibodies (such as anti-annexin-V), which directly induced apoptosis in endothelial cells or target cells (5, 25). Besides, Seko et al. showed that NK cells play an important role in the vascular cell injury by expressing and releasing perforin directly onto the surface of arterial vascular cells (6). Other pro-apoptotic pathways such as MICA/NKG2D (Major Histocompatibility Class I Chain-Related A/NK Group 2D), Fas/FasL and 4-1BB/4-1BBL were also involved in vascular injury in TAK (26). These observations and results support the hypothesis that decreased NK cells in the peripheral blood of TAK patients may due to the trafficking of NK cells to aortic tissues. Shibatomi *et al*. demonstrated that combinations of IL-18 and IL-15 or IL-18 and IL-12 induced NK cell death in patients with systemic autoimmune diseases (27). These results indicate that NK cell apoptosis induced by circulating immune complexes and serum cytokines might contribute to NK cell depletion in some autoimmune diseases. Other possible explanations for depletion of circulating NK cells in SLE are that NK cell depletion occurs secondary to disease progression and NK cells generated from haematopoietic stem cells (HSCs) are defective in SLE patients (19). Another alternative possible reason in BD is that depleted NK cells in peripheral blood are a direct consequence of BD pathology.

Besides, we found that the expressions of granzyme B and perforin in NK cells were lower in TAK patients compared to control groups. These results indicated the cytotoxicity of NK cells were markedly suppressed in TAK patients. The cytolytic function of NK cells has an important role in the initiation and progression of autoimmune diseases (28). Park *et al*. examined the cytotoxic effect of NK cells was using flow cytometry in RA, SLE, ankylosing spondylitis (AS), and BD, although reduced cytotoxic activity was found in RA and SLE, no significant differences were seen in patients with BD or AS (19). In the study of Vastert *et al*. the author found that perforin of NK cells and their lytic function were severely reduced in juvenile idiopathic arthritis (JIA) (29). This finding could thus explain why systemic JIA may be complicated by macrophage activation syndrome (MAS). A common hypothesis as to the pathophysiology of MAS proposes a defect in lymphocyte cytolytic activity (30). The pro-inflammatory cytokine environment, particularly IL-6, has been shown to decrease NK cell cytolytic function (30). It is known that IL-6 plays a vital role in the pathogenesis

of TAK (31). This can partly explain the mechanism of impaired cytotoxicity of NK cells in TAK patients.

IFN-γ is a powerful cytokine, a prototype of an effector cytokine in anti-pathogen immune responses (32). A study of pro-inflammatory cytokine transcripts of peripheral blood mononuclear cells has also shown that patients with TAK had higher mRNA gene expression of IFN-γ (33). In the aorta from TAK patients, an increased expression of IFN-γ has been described (34). IFN-γ levels in active patients at baseline and good correlation of this cytokine with conventional markers of disease activity (ESR an CRP) as well as with IL-6 (35). IFN-γ is a potent immunoregulatory protein mainly secreted by $CD4^+$ and $CD8^+$ T cells and by NK cells (36). D. Saadoun *et al*. demonstrated the presence of IFN-γ-producing T cells in vascular inflammatory infiltrates in patients with TAK. In our study, no differences in the IFN-γ expression of NK cells was observed between TAK patients and healthy control. The expressions of IFN-γ in $CD4^+$ and $CD8^+$ T cells in TA are being further studied by our research group. One study reported IFN-γ expression on $CD56^{bright}$ NK cells was increased in SLE patients (37). The percentage of IFN-γ producing $CD56^{Bright}$ NK cells was also higher in BD patients compared to healthy controls (21). These results suggested that the expressions IFN-γ in different cell subsets and diseases are different. Yu *et al*. hypothesised that the process of human NK differentiation progresses from a $CD56^{Bright}$ to a $CD56^{dim}$ phenotype (38). Therefore, the author suggested that $CD56^{Dim}$ NK cells have a high turnover under SLE conditions and have to be replaced, and consequently, their precursor cells ($CD56^{Bright}$) are released as recent immigrants from the bone marrow and/or the lymph nodes sharing a less differentiated phenotype (37). However, some authors thought there is a shift from the $CD56^{Dim}$ population to the $CD56^{Bright}$ subset in SLE and BD (21, 39). So further study concerning NK phenotype differentiation might provide a better understanding the role of IFN-γ expression of NK cells in the process of TAK pathogenesis.

In terms of the correlation between NK cells and TAK disease activity, we saw no difference in the percentage of NK cells or NK cell subsets between groups with active and inactive TAK. Consistent with our results, there were also no statistically significant differences in the absolute number and frequency of NK cells between the active SLE and inactive SLE groups (37). The NK cell percentage population was not correlated significantly with the DAS28 in RA patients (40). But our results found granzyme B-expressing NK cell percentage was significantly decreased in active TAK compared to inactive TAK. This indicated in granzyme B may play an important role in TAK disease activity. The importance of NK cells in autoimmunity might be associated with different NK cell subsets and different stages of corresponding diseases (8). Our results showed there was no difference in the percentage of NK cells or their subsets between groups with treated and untreated TAK patients. It was reported that NK cells/total lymphocytes (%) was significantly reduced in azathioprine-treated BD patients compared to healthy controls but not for BD patients on other forms of therapy (colchicine, prednisolone and mycophenolate mofetil) (21). In this study, the medication of these BD patients was used as single/monotherapy. But TAK patients in our study were mostly treated with several medications. Therefore, investigations with larger study group and prospective study concerning the influence of drug therapy on NK cells are clearly required.

In summary, this present study suggests that the total NK cells number and$CD56^{Bright}$ /$CD56^{Dim}$ subsets are depleted in the peripheral blood of TAK patients. The cytotoxicity of NK cells was reduced in TAK patients.

To our knowledge, this study is the first to show the NK cells and their function in TAK. The limitation of this study is that the precise mechanism for the number and function change of NK cells was not fully elucidated. Because TAK is a rare disease, collection of arterial tissue from TAK patients was difficult. NK cell phenotypes and their function were only analysed in peripheral blood specimens. Therefore, further studies are clearly required to better understand the mechanism of NK depletion in TAK patients and the relative influence of disease activity.

References

1. KESER G, AKSU K, DIRESKENELI H: Takayasu arteritis: an update. *Turk J Med Sci* 2018; 48: 681-97.
2. TOMBETTI E, MASON JC: Takayasu arteritis: advanced understanding is leading to new horizons. *Rheumatology* (Oxford) 2019; 58: 206-19.
3. COMARMOND C, BIARD L, LAMBERT M *et al*.: Long-term outcomes and prognostic factors of complications in Takayasu arteritis: a multicenter study of 318 patients. *Circulation* 2017; 136: 1114-22.
4. ELEFANTE E, BOND M, MONTI S *et al*.: One year in review 2018: systemic vasculitis. *Clin Exp Rheumatol* 2018; 36 (Suppl. 111): S12-32.
5. ESPINOZA JL, AI S, MATSUMURA I: New Insights on the Pathogenesis of Takayasu Arteritis: Revisiting the Microbial Theory. *Pathogens* 2018; 7 (3).
6. SEKO Y, MINOTA S, KAWASAKI A *et al*.: Perforin-secreting killer cell infiltration and expression of a 65-kD heat-shock protein in aortic tissue of patients with Takayasu's arteritis. *J Clin Invest* 1994; 93: 750-8.
7. GIANCHECCHI E, DELFINO DV, FIERABRACCI A: NK cells in autoimmune diseases: Linking innate and adaptive immune responses. *Autoimmun Rev* 2018; 17: 142-54.
8. ZHANG C, TIAN Z: NK cell subsets in autoimmune diseases. *J Autoimmun* 2017; 83: 22-30.
9. WU Y, TIAN Z, WEI H: Developmental and functional control of natural killer cells by cytokines. *Front Immunol* 2017; 8: 930.
10. FOGEL LA, YOKOYAMA WM, FRENCH AR: Natural killer cells in human autoimmune disorders. *Arthritis Res Ther* 2013; 15: 216.
11. TERAO C, YOSHIFUJI H, MATSUMURA T *et al*.: Genetic determinants and an epistasis of LILRA3 and HLA-B*52 in Takayasu arteritis. *Proc Natl Acad Sci USA* 2018; 115: 13045-50.
12. KERR GS, HALLAHAN CW, GIORDANO J *et al*.: Takayasu arteritis. *Ann Intern Med* 1994; 120: 919-29.
13. MISRA R, DANDA D, RAJAPPA SM *et al*.: Development and initial validation of the Indian Takayasu Clinical Activity Score (ITAS2010). *Rheumatology* (Oxford) 2013; 52: 1795-801.
14. GRAYSON PC, MAKSIMOWICZ-MCKINNON K, CLARK TM *et al*.: Distribution of arterial lesions in Takayasu's arteritis and giant cell arteritis. *Ann Rheum Dis* 2012; 71: 1329-34.
15. ARNAUD L, HAROCHE J, MATHIAN A, GOROCHOV G, AMOURA Z: Pathogenesis of Takayasu's arteritis: a 2011 update. *Autoimmun Rev* 2011; 11: 61-7.
16. KESER G, AKSU K, DIRESKENELI H: Discrepancies between vascular and systemic inflammation in large vessel vasculitis: an important problem revisited. *Rheumatology* (Oxford) 2018; 57: 784-90.

17. VIVIER E, RAULET DH, MORETTA A *et al*.: Innate or adaptive immunity? The example of natural killer cells. *Science* 2011; 331: 44-9.
18. VIVIER E, TOMASELLO E, BARATIN M, WALZER T, UGOLINI S: Functions of natural killer cells. *Nat Immunol* 2008; 9: 503-10.
19. PARK YW, KEE SJ, CHO YN *et al*.: Impaired differentiation and cytotoxicity of natural killer cells in systemic lupus erythematosus. *Arthritis Rheum* 2009; 60: 1753-63.
20. CHALAN P, BIJZET J, KROESEN BJ, BOOTS AM, BROUWER E: Altered natural killer cell subsets in seropositive arthralgia and early rheumatoid arthritis are associated with autoantibody status. *J Rheumatol* 2016; 43: 1008-16.
21. HASAN MS, RYAN PL, BERGMEIER LA, FORTUNE F: Circulating NK cells and their subsets in Behçet's disease. *Clin Exp Immunol* 2017; 188: 311-22.
22. DOTTA F, CENSINI S, VAN HALTEREN AG *et al*.: Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. *Proc Natl Acad Sci USA* 2007; 104: 5115-20.
23. PRIDGEON C, LENNON GP, PAZMANY L, THOMPSON RN, CHRISTMAS SE, MOOTS RJ: Natural killer cells in the synovial fluid of rheumatoid arthritis patients exhibit a CD-56^{bright}, CD94^{bright}, CD158 negative phenotype. *Rheumatology* (Oxford) 2003; 42: 870-8.
24. DALBETH N, CALLAN MF: A subset of natural killer cells is greatly expanded within inflamed joints. *Arthritis Rheum* 2002; 46: 1763-72.
25. TRIPATHY NK, SINHA N, NITYANAND S: Anti-annexin V antibodies in Takayasu's arteritis: prevalence and relationship with disease activity. *Clin Exp Immunol* 2003; 134: 360-4.
26. SEKO Y, SUGISHITA K, SATO O *et al*.: Expression of costimulatory molecules (4-1BBL and Fas) and major histocompatibility class I chain-related A (MICA) in aortic tissue with Takayasu's arteritis. *J Vasc Res* 2004; 41: 84-90.
27. SHIBATOMI K, IDA H, YAMASAKI S *et al*.: A novel role for interleukin-18 in human natural killer cell death: high serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. *Arthritis Rheum* 2001; 44: 884-92.
28. SHI FD, VAN KAER L: Reciprocal regulation between natural killer cells and autoreactive T cells. *Nat Rev Immunol* 2006; 6: 751-60.
29. VASTERT SJ, VAN WIJK R, D'URBANO LE *et al*.: Mutations in the perforin gene can be linked to macrophage activation syndrome in patients with systemic onset juvenile idiopathic arthritis. *Rheumatology* (Oxford) 2010; 49: 441-9.
30. CRAYNE CB, ALBEITUNI S, NICHOLS KE, CRON RQ: The immunology of macrophage activation syndrome. *Front Immunol* 2019; 10: 119.
31. KONG X, SUN Y, MA L *et al*.: The critical role of IL-6 in the pathogenesis of Takayasu arteritis. *Clin Exp Rheumatol* 2016; 34 (Suppl. 97): S21-7.
32. WEYAND CM, GORONZY JJ: Immune mechanisms in medium and large-vessel vasculitis. *Nat Rev Rheumatol* 2013; 9: 731-40.
33. TRIPATHY NK, CHAUHAN SK, NITYANAND S: Cytokine mRNA repertoire of peripheral blood mononuclear cells in Takayasu's arteritis. *Clin Exp Immunol* 2004; 138: 369-74.
34. SAVIOLI B, ABDULAHAD WH, BROUWER E, KALLENBERG CGM, DE SOUZA AWS: Are cytokines and chemokines suitable biomarkers for Takayasu arteritis? *Autoimmun Rev* 2017; 16: 1071-8.
35. GOEL R, KABEERDOSS J, RAM B *et al*.: Serum Cytokine Profile in Asian Indian Patients with Takayasu Arteritis and its Association with Disease Activity. *Open Rheumatol J* 2017; 11: 23-9.
36. ASSENMACHER M, SCHEFFOLD A, SCHMITZ J, SEGURA CHECA JA, MILTENYI S, RADBRUCH A: Specific expression of surface interferon-gamma on interferon-gamma producing T cells from mouse and man. *Eur J Immunol* 1996; 26: 263-7.
37. HENRIQUES A, TEIXEIRA L, INES L *et al*.: NK cells dysfunction in systemic lupus erythematosus: relation to disease activity. *Clin Rheumatol* 2013; 32: 805-13.
38. YU J, MAO HC, WEI M *et al*.: CD94 surface density identifies a functional intermediary between the CD56^{bright} and CD56^{dim} human NK-cell subsets. *Blood* 2010; 115: 274-81.
39. HERVIER B, BEZIAT V, HAROCHE J *et al*.: Phenotype and function of natural killer cells in systemic lupus erythematosus: excess interferon-gamma production in patients with active disease. *Arthritis Rheum* 2011; 63: 1698-706.
40. AGGARWAL A, SHARMA A, BHATNAGAR A: Role of cytolytic impairment of natural killer and natural killer T-cell populations in rheumatoid arthritis. *Clin Rheumatol* 2014; 33: 1067-78.