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CD56^{bright}CD16^{-} natural killer cells are shifted toward an IFN-γ-promoting phenotype with reduced regulatory capacity in osteoarthritis

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ABSTRACT

A subset of natural killer (NK) cells with CD56$^{+/bright}$CD16$^{dim/-}$ expression is recently shown to present critical regulatory functions. Functional characteristics of CD56$^{+/bright}$ NK cells in osteoarthritis (OA) patients remains unknown. Here, we remedied this problem by comparing the NK cells from healthy controls and OA patients. Data showed that the CD56^{bright}CD16^{-} NK subset was significantly enriched in OA patients. These CD56^{bright}CD16^{-} NK cells from OA patients presented significantly higher IFNG transcription and IFN-γ protein secretion than those from healthy controls, both directly ex vivo and after activation via various stimulating reagents, including IL-2/IL-15, K562, and PMA/ionomycin. On the other hand, the transcription and secretion of granzyme A (Gzm-A), Gzm-B, and perforin were significantly lower in CD56^{bright}CD16^{-} NK cells from OA patients than in CD56^{bright}CD16^{-} NK cells from healthy controls. Also, the CD56^{bright}CD16^{-} NK cells from OA patients were less capable of suppressing the proliferation of autologous CD4^{+} T cells, in a manner that was dependent on the expression of Gzm-B and perforin. Interestingly, CD4^{+} T cells co-incubated with CD56^{bright}CD16^{-} NK cells were prone to express a higher level of IFNG, and the CD56^{bright}CD16^{-} NK cells from OA patients were more potent at stimulating IFNG than the CD56^{bright}CD16^{-} NK cells from healthy controls. Overall, our investigation demonstrated that CD56^{bright}CD16^{-} NK cells from osteoarthritis patients were shifted toward an IFN-γ-promoting phenotype and with reduced regulatory functions.

1. Introduction

Despite the fact that self-reactivity exists in almost all human individuals, only 5%–10% of subjects develop autoimmune diseases [1]. This is due to the existence of specialized regulatory immune cells, such as regulatory T (Treg) cells and regulatory B (Breg) cells, which suppress auto-reactive immunity. Dysfunction of the regulatory immune cells not only promotes the development of autoimmune diseases, but is also implicated in many chronic conditions characterized by low-grade but sustained proinflammatory responses, such as obesity, diabetes, cardiovascular diseases, and osteoarthritis [2–5].

In recent years, it is increasingly recognized that a CD56^{bright} subset of the natural killer (NK) cells presents critical regulatory functions in autoimmune diseases [6,7]. Unlike the canonical CD56^{dim}CD16^{bright} NK subset, which makes up for greater than 90% of total circulating NK cells in healthy individuals, the CD56^{bright} subset is CD16$^{-/dim}$ and rare in circulation, but can be found in lymph nodes, inflamed tissues, and solid tumors [8–11]. These CD56^{bright} NK cells express the inhibitory molecule NKG2A, do not express the MHC class I-recognition molecules KIRs, and have higher cytokine secretion but lower cytotoxicity compared to the CD56^{dim} NK cells [12–14]. Upon receiving signals from IL-12, IL-15, and/or IL-18, CD56^{bright} NK cells can express a variety of cytokines, such as IFN-γ, IL-10, IL-13, TNF-β, and GM-CSF, some of which present essential regulatory functions [12,14–16]. Activated NK cells can also eliminate immature dendritic cells via the expression of cytolytic molecules [17,18]. NK cells can also exert regulatory effects on T cells. CD56^{bright} NK cells can utilize and compete with T cells for IL-2 availability by expressing both the high-affinity and the intermediate-affinity forms of the IL-2 receptors [19,20]. Activated CD56^{bright} NK cells can also inhibit the proliferation of autologous T

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cells in a manner associated with the release of granzyme (Gzm) and perforin [21–23]. Additionally, $CD56^{bright}$ NK cells were found to be capable of mediating direct killing of activated T cells [24,25].

Osteoarthritis (OA) is the most common degenerative joint disease with no cure available. Aseptic inflammatory responses are known to contribute to the loosening of joints and prosthetic fixtures and progression of the disease [26]. A number of studies have identified that the synovial fluid of osteoarthritis patients was enriched with $CD56^{+/bright}$ NK cells [27–29]. The role of $CD56^{bright}$ NK cells in osteoarthritis is still unclear. Hence, in this study, we investigated the proportion and function of $CD56^{bright}$ NK cells in OA patients.

2. Materials and methods

2.1. Patient and control recruitment

This study included 30 patients with knee OA, and 30 healthy controls. The OA patient group included 18 female subjects and 12 male subjects, in which the mean age was 66.3 ± 7.1 years, and the mean body mass index (BMI) was 24.6 ± 2.5 kg/m^2. According to the Kellgren-Lawrence system [30], 8 patients were classified as grade 2 OA, 15 patients as grade 3 OA, and 7 patients as grade 4 OA. No treatment was given to the OA patients at the time of recruitment and sample collection. The healthy control group included 20 female subjects and 10 male subjects, in which the mean age was 64.5 ± 5.5 years, and the mean BMI was 24.1 ± 3.1 kg/m^2. All healthy controls and OA patients provided written informed consent for participation in the study and donation of peripheral blood. The ethics committee of Chengdu Second People's Hospital approved this study.

2.2. Sample collection, processing, and storage

Peripheral blood was harvested into citrate containing tubes and layered on top of sterile Ficoll solution (Sigma). Following centrifugation at 300*g* for 30 min, the mononuclear cell layer was collected and washed twice in sterile phosphate-buffered saline (PBS) supplemented with 2% heat-inactivated fetal bovine serum (FBS; Gibco). The cells were then stored in 90% FBS and 10% DMSO (Sigma) at −150 °C. Before use, cells were thawed in complete culture medium (composed of RPMI 1640, 10% FBS, 1× L-glutamine, and 1× penicillin-streptomycin; Gibco) supplemented with 1% DNase (Sigma), washed twice, and rested in complete culture medium overnight at 37 °C and 5% CO_2.

2.3. Flow cytometry

Commercially available antibodies, including anti-human CD3, CD16, CD19, and CD56, were purchased from Biolegend. Fixable Aqua Dead Cell stain was purchased from Invitrogen. Surface staining was performed by incubating the antibodies and the dead cell stain with the total peripheral blood monuclear cells (PBMCs) for 30 min at 4 °C in PBS supplemented with 2% FBS. The PBMCs were then washed twice to remove excess antibodies. Phenotyping was performed using a FACSCanto analyzer on formalin-fixed cells, and $CD56^{bright}CD16^-$ NK cell sorting was performed using a FACSAria sorter on live cells.

2.4. Stimulation and Co-incubation of $CD56^{bright}CD16^-$ NK cells and $CD4^+$ T cells

The following commercially available reagents were used for the stimulation of $CD56^{bright}CD16^-$ NK cells, including recombinant human (rh) IL-2 at 100 UI/mL plus rh IL-15 at 20 ng/mL (R&D), K562 cells at 1/1 ratio with NK cells (ATCC), and PMA at 10 ng/mL plus ionomycin (Sigma) at 100 ng/mL. The K562-stimulated NK cells were further isolated using Human NK Enrichment Kit (Stemcell) to analyze NK-specific effects.

For $CD56^{bright}CD16^-$ NK cell-$CD4^+$ T cell co-incubation, $CD4^+$ T cells were negatively isolated using Human CD4 T Enrichment Kit (Stemcell), and then plated in a 96-well round-bottom plate at 5×10^4 cells each well. The flow cytometry-sorted $CD56^{bright}CD16^-$ NK cells, if present, were then added at 1×10^4–5×10^4 cells each well, as specified in the experiments. The Human T Activator (antiCD3/CD28) beads (Thermo Fisher) were added at 1 bead per T cell. All cell cultures were topped up to 200 μL complete culture medium per well, and incubated under 37 °C and 5% CO_2. For NK- and T cell-specific effects, $CD56^{bright}CD16^-$ NK cells and $CD4^+$ T cells were negatively isolated using the corresponding enrichment kits. For the secretion of soluble molecules, the plates were centrifuged at 300*g* for 5 min, and the supernatant was collected. For the proliferation of $CD4^+$ T cells, 0.1 μCi per well [^{3}H]-Thymidine (Amersham) was added for 8 h to isolated $CD4^+$ T cells. The cells were then harvested and the counts per minute (cpm) were counted in a direct β counter.

2.5. Elisa

The following commercial ELISA kits were used according to the manufacturer's instructions, including Human IFN-γ Quantikine ELISA kit, Human Granzyme A DuoSet ELISA kit, Human Granzyme B DuoSet ELISA kit (R&D), and Human Perforin ELISA kit (Abcam).

2.6. Gene transcription

Total RNA was harvested from isolated NK cells or $CD4^+$ T cells using RNAeasy Mini Kit (Qiagen). cDNA was synthesized using TaqMan Reverse Transcription Reagents (Thermo Fisher). Real-time PCR was performed using pre-designed TaqMan Gene Expression assays (Thermo Fisher), including Hs00989291_m1 for IFNG, Hs00989184_m1 for GZMA, Hs00188051_m1 for GZMB, and Hs00169473_m1 for PRF1. The expression level of each gene was shown as ratio to ACTB.

2.7. Statistics

All data analyses and graphic representations were made using Prism (GraphPad). Comparisons between two groups were made using *t*-test with Welch's correction. Comparisons between multiple groups were made using one-way ANOVA followed by Tukey's post-tests. Comparisons between two groups in multiple parameters were made using two-way ANOVA followed by Sidak's post-tests. Mean ± standard deviation was given for all datasets. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Frequency of $CD56^{bright}CD16^-$ NK cells was slightly enriched in the peripheral blood of OA patients

First, we investigated the composition of circulating NK cells in OA patients and healthy controls. The NK cells were gated as $CD3^-CD19^-CD56^+$ cells in live singlet lymphocytes (Fig. 1a). We found that, based on CD56 vs. CD16 expression, the total NK cells can be separated into two major populations, including the $CD56^{bright}CD16^-$ NK cells and the $CD56^{dim}CD16^+$ NK cells (Fig. 1a). The frequency of $CD56^{bright}CD16^-$ NK cells in total NK cells was slightly higher in OA patients than in healthy controls (Fig. 1b). The OA patients were then separated into stage 2, stage 3, and stage 4 patients based on the Kellgren-Lawrence staging system [30], with higher stages representing more severe symptoms. On average, the frequencies of $CD56^{bright}CD16^-$ NK cells were higher in patients with higher stage of OA, but the differences were not statistically significant (Fig. 1B). The frequencies of $CD56^{bright}CD16^-$ NK cells as a percentage of total live singlet lymphocytes were also slightly higher in OA patients than in healthy controls (Fig. 1c), with no statistically significant difference between patients at different stages.

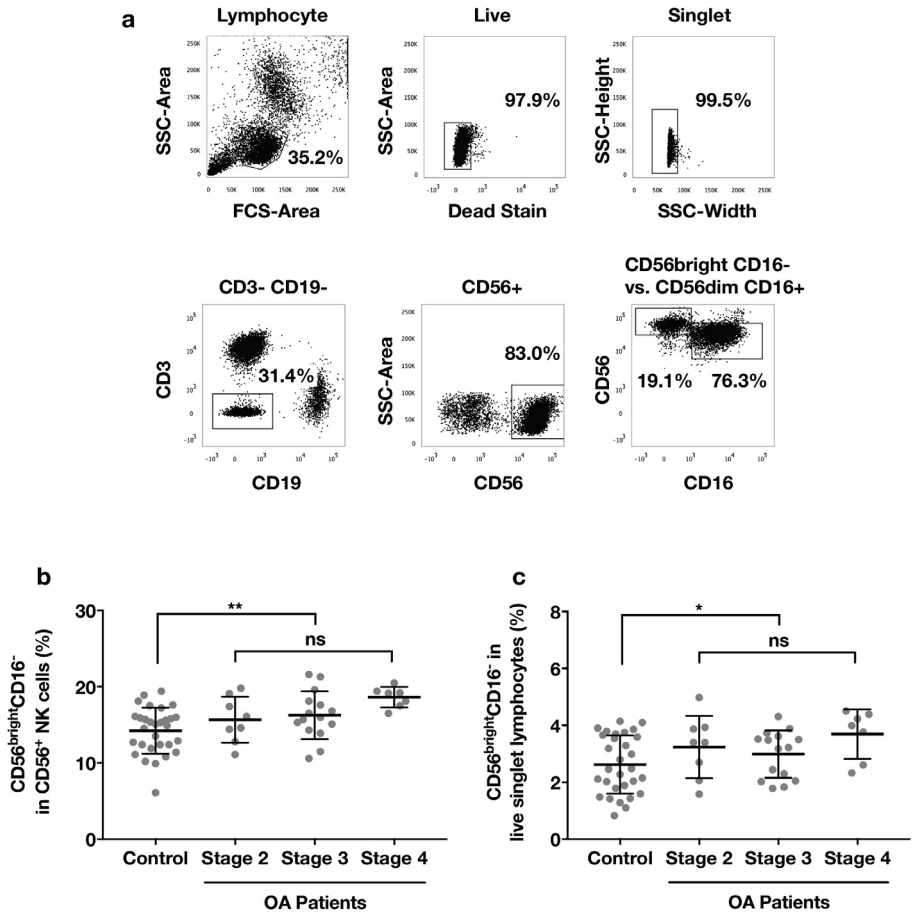

Fig. 1. The frequency of CD56^{bright}CD16^{-} NK cells in OA patients. (a) CD56^{bright}CD16^{-} NK cells were gated stepwise as shown in one representative OA patient. (b) The frequency of CD56^{bright}CD16^{-} NK cells in total NK cells in 30 healthy controls and 30 OA patients. **P < 0.01. ns, not significant. (c) The frequency of CD56^{bright}CD16^{-} NK cells in total live singlet lymphocytes in 30 healthy controls and 30 OA patients. *P < 0.05. ns, not significant.

3.2. CD56^{bright}CD16^{-} NK cells from OA patients displayed higher IFN-γ but lower Gzm-A, Gzm-B, and perforin expression

Next, we investigated whether the CD56^{bright}CD16^{-} NK cells in OA patients were functionally altered compared to those in healthy controls. CD56^{bright} NK cells are early sources of IFN-γ, which stimulates T cells in lymph nodes [11]. In unstimulated CD56^{bright}CD16^{-} NK cells, we examined the IFNG transcription level, which was higher in OA patients than in healthy controls (Fig. 2a). Subsequently, the CD56^{bright}CD16^{-} NK cells were stimulated with various combinations of stimulating agents, including IL-2 and IL-15, MHC-deficient K562 cells, and PMA and ionomycin. CD56^{bright}CD16^{-} NK cells from OA patients presented significantly higher IFNG transcription levels than CD56^{bright}CD16^{-} NK cells from healthy controls following IL-2/IL-15 stimulation and PMA/ionomycin stimulation (Fig. 2a). K562 stimulation, however, did not produce a significant difference (Fig. 2a). The IFN-γ secretion was then examined. Without external stimulation, little IFN-γ secretion was found in the supernatant from either healthy controls or OA patients (Fig. 2b). However, the IFN-γ secretion was readily detectable in IL-2/IL-15-stimulated, K562-stimulated, and PMA/ionomycin-stimulated CD56^{bright}CD16^{-} NK cells, and was significantly higher in OA patients than in healthy controls (Fig. 2b). We also examined the production of IFN-γ by OA stage. No significant difference between various stages of OA patients was observed in in the absence of stimulation (Fig. 2a and b, left panels). With IL-2/IL-15, K562, and PMA/ionomycin, however, CD56^{bright}CD16^{-} NK cells from more advanced OA stages tended to produce more IFN-γ than CD56^{bright}CD16^{-} NK cells from less advanced OA stages.

Subsequently, we examined the Gzm expression and perforin expression by CD56^{bright}CD16^{-} NK cells. In IL-2/IL-15-stimulated CD56^{bright}CD16^{-} NK cells, the expression of GZMA mRNA was not significantly between OA patients and healthy controls (Fig. 3a). The expression of GZMB and PRF1, on the other hand, was significantly lower in OA patients than in controls (Fig. 3a). The secretion of Gzm-A, Gzm-B, and perforin was examined in the supernatant (Fig. 3b), and the results were consistent with the mRNA results. No significant differences between OA patients at different stages were observed. In K562-stimulated CD56^{bright}CD16^{-} NK cells, the transcription of GZMA, GZMB, and PRF1 was significantly lower in OA patients than in healthy controls (Fig. 3c). The Gzm-A, Gzm-B, and perforin concentration was also significantly lower in OA patients than in controls (Fig. 3d). Again, no significant difference between different stages of OA patients was seen.

3.3. CD56^{bright}CD16^{-} NK cells from OA patients presented capacity to suppress autologous CD4^{+} T cell proliferation

It has been shown that CD56^{bright} NK cells regulated immune responses via perforin-dependent manner, possibly by mediating the elimination of proinflammatory autologous CD4^{+} T cells [23]. Gzm-B expression was also shown to promote CD56^{bright} NK cell-mediated regulation by suppressing the proliferation of autologous CD4^{+} T cells [21]. Hence, we investigated the suppressive effects of CD56^{bright}CD16^{-} NK cells in OA patients and healthy controls. The

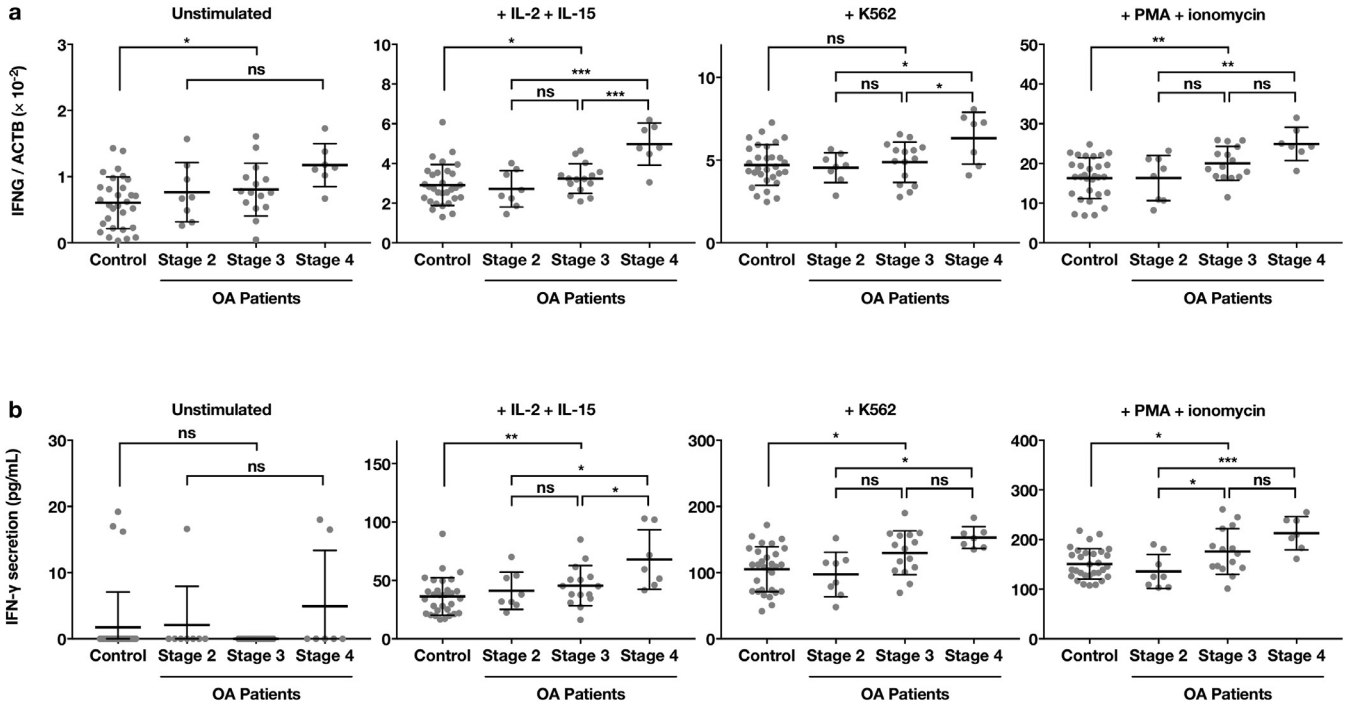

Fig. 2. IFN-γ expression by CD56^{bright}CD16^{-} NK cells in OA patients. (a) CD56^{bright}CD16^{-} NK cells were unstimulated, or stimulated with IL-2 and IL-15, K562 cells, or PMA and ionomycin for 6 h. The IFNG transcription was then examined in NK cells. ns, not significant. *P < 0.05. **P < 0.01. ***P < 0.001. (b) CD56^{bright}CD16^{-} NK cells were unstimulated, or stimulated with IL-2 and IL-15, K562 cells, or PMA and ionomycin for 6 h. The IFN-γ secretion was examined in the supernatant. ns, not significant. *P < 0.05. **P < 0.01. ***P < 0.001.

effect of CD56^{bright}CD16^{-} NK cells on the proliferation of autologous CD4^{+} T cells was examined. CD56^{bright}CD16^{-} NK cells were pre-stimulated with IL-2 and IL-15. The NK cells were then added at increasing ratios to autologous CD4^{+} T cells, which were stimulated with anti-CD3/CD28. Overall, in both healthy controls and OA patients the proliferation capacity of CD4^{+} T cells presented a downward trend with increasing levels of CD56^{bright}CD16^{-} NK cells (Fig. 4a). Between healthy controls and OA patients, the CD56^{bright}CD16^{-} NK cells from OA patients were significantly less effective at suppressing CD4^{+} T cell proliferation than the CD56^{bright}CD16^{-} NK cells from healthy controls (Fig. 4a). We previously observed that Gzm-B and perforin expression levels were significantly lower in IL-2/IL-15-stimulated CD56^{bright}CD16^{-} NK cells from OA patients (Fig. 3a and b). Here, we analyzed the transcription of GZMA, GZMB, and PRF1 in CD56^{bright}CD16^{-} NK cells after pre-stimulation with IL-2/IL-15 and co-incubation with CD4^{+} T cells. The GZMB and PRF1 transcription levels were significantly lower in OA patients than in healthy individuals (Fig. 4b). To confirm whether GZMB and PRF1 were involved in CD56^{bright}CD16^{-} NK cell-mediated suppression of CD4^{+} T cell proliferation, the Gzm-B inhibitor Z-AAD-CMK and the exocytosis inhibitor EGTA were added to the coculture of NK cells and CD4^{+} T cells. The proliferation of CD4^{+} T cells was then investigated (Fig. 4c). We found that both Z-AAD-CMK and EGTA could significantly reduce CD56^{bright}CD16^{-} NK-mediated suppression of CD4^{+} T cells. Furthermore, in the absence of Z-AAD-CMK and EGTA, the CD56^{bright}CD16^{-} NK-mediated suppression was significantly weaker in OA patients than in healthy controls. In the presence of Z-AAD-CMK and EGTA, however, no significant difference between OA patients and healthy controls was found (Fig. 4c).

3.4. CD56^{bright}CD16^{-} NK cells from OA patients promoted IFN-γ response in autologous CD4^{+} T cells

Since CD56^{bright}CD16^{-} NK cells from OA patients presented elevated IFN-γ expression, in the next step, we examined the IFN-γ response by CD4^{+} T cells following incubation with CD56^{bright}CD16^{-} NK cells. CD4^{+} T cells from healthy controls or OA patients were incubated alone, or with autologous CD56^{bright}CD16^{-} NK cells. The IFNG transcription was then examined in isolated CD4^{+} T cells. In most healthy controls and OA patients, the IFNG transcription in CD4^{+} T cells was higher in the presence than in the absence of autologous CD56^{bright}CD16^{-} NK cells (Fig. 5). In addition, the increase in IFNG transcription was more pronounced in OA patients than in healthy controls (Fig. 5).

4. Discussion

Immune dysregulations are increasingly recognized as an instigating factor in OA. Several previous studies demonstrated that CD56$^{+/bright}$ NK cells were enriched in the synovial fluid of OA patients [27–29]; however, no functional investigation was performed. In this study, we remedied this problem by comparing the function of CD56^{bright}CD16^{-} NK cells in healthy controls and OA patients. In circulating NK cells, the following discoveries were made. First, the CD56^{bright}CD16^{-} NK cells were significantly enriched in OA patients than in healthy controls. Second, the CD56^{bright}CD16^{-} NK cells from OA patients presented significantly higher IFNG transcription and IFN-γ protein secretion than the CD56^{bright}CD16^{-} NK cells from healthy controls, directly ex vivo as well as after activation with various stimulating reagents. However, this tendency could not be extended to other genes, as the transcription and secretion of Gzm-A, Gzm-B, and perforin was significantly lower in CD56^{bright}CD16^{-} NK cells from OA patients than in CD56^{bright}CD16^{-} NK cells from healthy controls. Third, the CD56^{bright}CD16^{-} NK cells from OA patients were less capable of suppressing the proliferation of autologous CD4^{+} T cells, in a Gzm-B-dependent and perforin-dependent manner. And fourth, the CD56^{bright}CD16^{-} NK cells from OA patients could promote the IFN-γ expression from autologous CD4^{+} T cells more potently than the CD56^{bright}CD16^{-} NK cells from healthy controls. Overall, these results suggested that compared to the CD56^{bright}CD16^{-} NK cells from healthy controls, the CD56^{bright}CD16^{-}

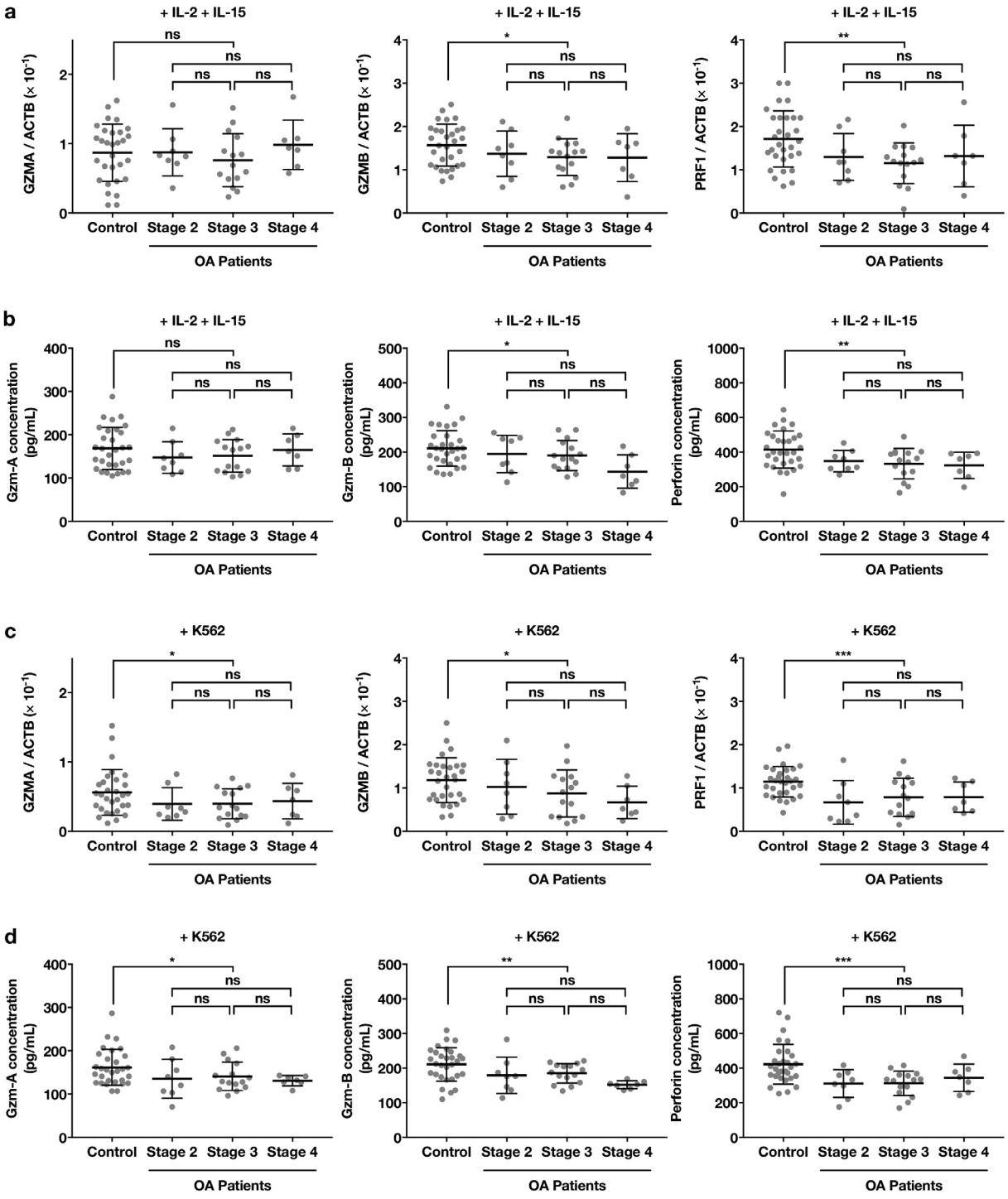

Fig. 3. Gzm and perforin expression by $CD56^{bright}CD16^{-}$ NK cells in OA patients. (a) and (b) $CD56^{bright}CD16^{-}$ NK cells were stimulated with IL-2 and IL-15 cells for 6 h. (c) The GZMA, GZMB, and PRF1 transcription levels were then examined in NK cells, and (d) the Gzm-A, Gzm-B, and perforin concentrations were examined in the supernatant. ns, not significant. *$P < 0.05$. **$P < 0.01$. (c) and (d) $CD56^{bright}CD16^{-}$ NK cells were stimulated with K562 cells for 6 h. (a) The GZMA, GZMB, and PRF1 transcription levels were then examined in NK cells, and (b) the Gzm-A, Gzm-B, and perforin concentrations were examined in the supernatant. ns, not significant. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

NK cells from OA patients presented altered functional characteristics, with higher capacity to induce IFN-γ response from $CD4^{+}$ T cells, and lower capacity to suppress $CD4^{+}$ T cell proliferation.

Within the OA group, the patients were further distinguished into stage 2, stage 3, and stage 4 patients according to the Kellgren-Lawrence system. Overall, no significant differences in the frequencies of $CD56^{bright}CD16^{-}$ NK cells were observed between stage 2, stage 3, and stage 4 OA patients. The OA patients with more advanced disease tended to present higher capacity to express IFN-γ. No significant differences in Gzm and perforin expression were observed between OA patients of different severities.

It remains to be determined whether dysregulations in NK cell-mediated responses could directly contribute the severity of the disease. A main caveat of this study is that the sources of samples were from the circulating fraction of NK cells. However, it is known that NK cells can infiltrate the synovial tissue [29]. Whether synovial NK cells in OA

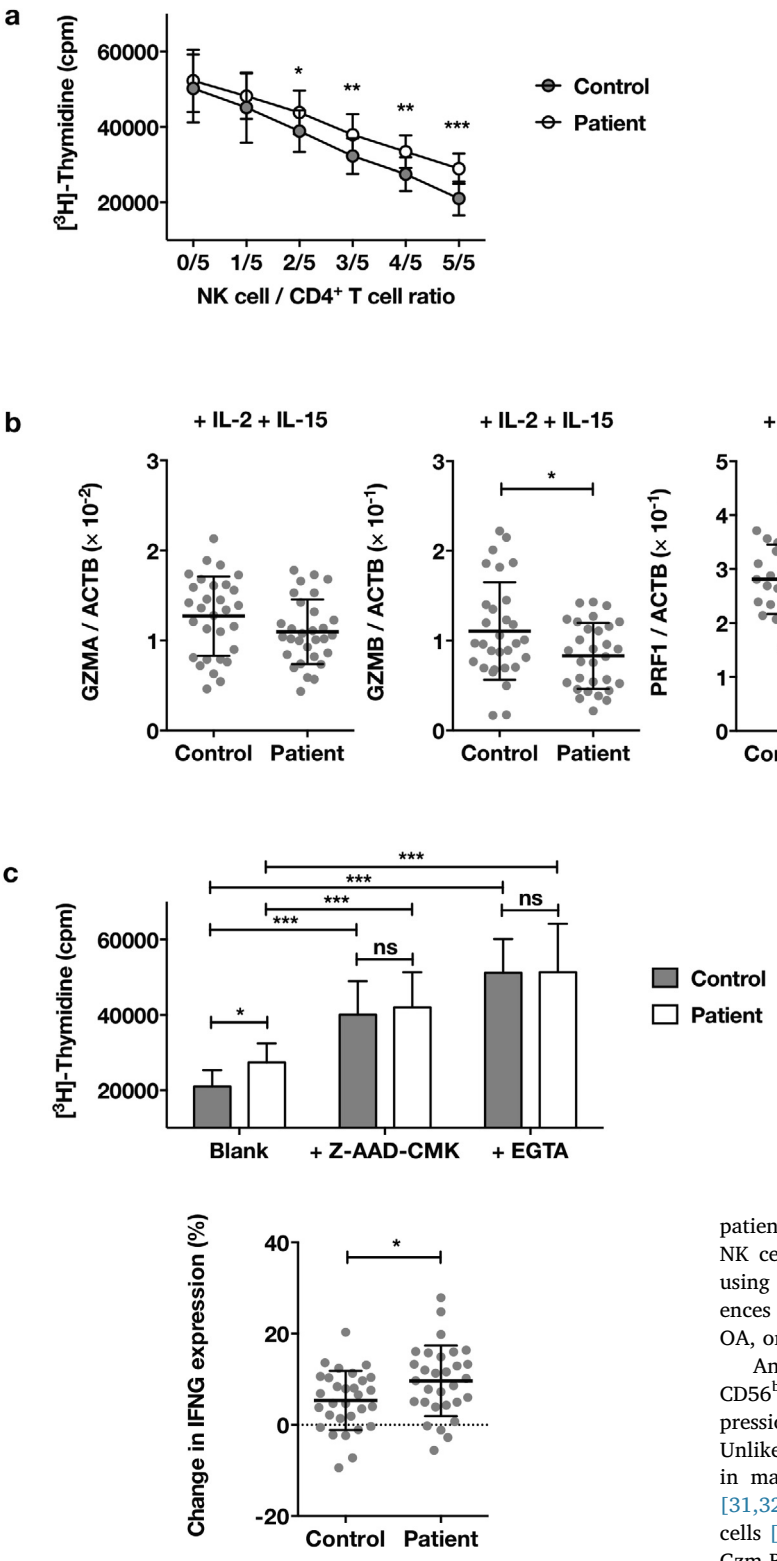

Fig. 4. Suppression of autologous $CD4^+$ T cells by $CD56^{bright}CD16^-$ NK cells in OA patients. (a) $CD56^{bright}CD16^-$ NK cells were pre-stimulated with IL-2 and IL-15 overnight, and then co-incubated with anti-CD3/CD28-stimulated autologous $CD4^+$ T cells at increasing NK/T cell ratios. The proliferation of $CD4^+$ T cells was examined using $[^3H]$-Thymidine incorporation. *P < 0.05. **P < 0.01. ***P < 0.001. (b) $CD56^{bright}CD16^-$ NK cells were pre-stimulated with IL-2 and Il-15 overnight, and then co-incubated with anti-CD3/CD28-stimulated autologous $CD4^+$ T cells at 1/1 NK/T cell ratio. The NK cells were then isolated by negative selection, and the GZMA, GZMB, and PRF1 transcription levels were then examined. *P < 0.05. (c) $CD56^{bright}CD16^-$ NK cells were pre-stimulated with IL-2 and Il-15 overnight, and then co-incubated with anti-CD3/CD28-stimulated autologous $CD4^+$ T cells at 1/1 NK/T cell ratio, in the absence of inhibitors or in the presence of Z-AAD-CMK or EGTA. ns, not significant. *P < 0.05. ***P < 0.001.

Fig. 5. $CD56^{bright}CD16^-$ NK cell-mediated effects on IFNG transcription by $CD4^+$ T cells. Anti-CD3/CD28-stimulated autologous $CD4^+$ T cells were incubated alone or with autologous IL-2/IL-15-pre-stimulated $CD56^{bright}CD16^-$ NK cells at 1/1 ratio. The $CD4^+$ T cells were then isolated and the IFNG transcription was examined. The change in IFNG expression with or without autologous NK cells was calculated as change (%) = (IFNG expression with NK cells − IFNG expression without NK cells)/(IFNG expression without NK cells) × 100. *P < 0.05.

patients also exhibited the dysregulations presented by the circulating NK cells should be investigated in future studies. Also, experiments using animal models should be conducted to establish whether differences in NK cells alone could initiate and promote the development of OA, or vice versa.

Another caveat of this study is that, we do not yet know why the $CD56^{bright}CD16^-$ NK cells in OA patients presented higher IFN-γ expression capacity and lower Gzm and perforin expression capacity. Unlike the situation in T cells, the IFNG gene is epigenetically accessible in mature NK cells, which produce IFNG transcripts constitutively [31,32]. Low levels of GZMB transcripts were also found in resting NK cells [33]. However, NK cells do not constitutively express IFN-γ and Gzm-B protein. Activation of NK cells is required to upregulate IFNG and GZMB transcription and translation. The cytokines IL-2, IL-12, and IL-15 were shown to enhance the expression of transcription factors that are involved in optimal IFNG transcription [31]. IL-12 signaling, in addition, was shown to increase IFNG mRNA stability [34]. Activation of cytotoxic molecules, on the other hand, depended on the interactions between various activating and inhibiting receptors, such as NKG2D, Ly49, KIR, and others [35]. Dysregulation of these signals might contribute to disruptions in NK function. For example, the infiltrating macrophages and synovial lining cells in OA patients could express IL-

12 [36], which could promote IFN-γ without directly inducing cytotoxicity. Further research is needed to investigate the underlying causes of NK dysregulations.

Based on our discoveries, it might be possible to predict the likelihood of an individual to develop OA based on the characteristics of the NK cells. However, it should be noted that many differences seen between OA patients and healthy controls, and between OA patients at various stages, were only marginally significant. Many OA and healthy datasets presented large overlapping regions in range, and no clear-cut differences were observed. To an extent, we thought that this was expected, given that OA was not regarded as a highly inflammatory type of arthritis, and the role of the immune system was considered contributory but not determinant. In addition, the OA patients were a heterogeneous group with patients at different stages of severity. Hence, the clinical significance of these discoveries remains unclear. Whether OA development could be better suppressed via manipulation of NK cells should be investigated in animal models.

Declaration of Competing Interest

None.

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