Cellular & Molecular Immunology (2017) 14, 976–985

www.nature.com/cmi

RESEARCH ARTICLE

IL-1β limits the extent of human 6-sulfo LacNAc dendritic cell (slanDC)-mediated NK cell activation and regulates CD95-induced apoptosis

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To function optimally, human blood natural killer (NK) cells need to communicate with other immune cells. Previously, it has been shown that NK cells communicate with 6-sulfo LacNAc dendritic cells (slanDCs), which are able to stimulate NK cells *in vitro*. In this study, we investigated how slanDCs regulate the level of NK cell activation. The secretion of interleukin (IL)-1β by slanDCs during coculture with NK cells increased as a result of signaling via intercellular adhesion molecule-1 on slanDCs following its interaction with lymphocyte function-associated antigen-1 on NK cells. IL-1β induced the expression of Fas receptor (CD95) on NK cells. The binding of Fas ligand (CD178) to CD95 induced the apoptosis of activated NK cells. Moreover, IL-1β also induced increased cyclooxygenase-2 expression in slanDCs, which in turn enabled the cells to secrete prostaglandin (PG)-E2. Consequently, PGE2 acted as a suppressing agent, tuning down the activation level of NK cells. In summary, IL-1β limits the level of NK cell activation by inducing apoptosis and suppression as a homeostatic regulatory function.
Cellular & Molecular Immunology (2017) **14,** 976–985; doi:10.1038/cmi.2016.17; published online 18 April 2016

Keywords: COX-2; Fas-induced apoptosis; immunosuppression; NK cells; slanDCs

INTRODUCTION

Efficient immune responses require coordinated reciprocity between different immune cells. It has been shown that natural killer (NK) cells and dendritic cells (DCs) are two types of innate immune cells that communicate to establish an optimal immune response during infection or tumor infiltration.[1–4] NK cells are a group of innate lymphocytes defined by the surface expression of CD56 and lack of CD3.[5–7] They exert strong cytotoxic effects against tumor cells or virus-infected cells without a need for prior antigen sensitization[8,9] and produce high amounts of cytokines and chemokines to modulate immune responses.[6,8,9] However, the nature of NK cell functions depends on their interactions with other immune cells, such as slanDCs.[8,10]

SlanDCs represent a major subpopulation of proinflammatory human blood DCs and differ from other DC subsets due to their selective expression of 6-sulfo LacNAc1 (a carbohydrate modification of P-selectin glycoprotein ligand-1).[10,11] Functionally, it has been reported that slanDCs efficiently improve the immunomodulatory and cytotoxic potential of NK cells.[3,12,13] A previous study has demonstrated that slanDCs utilize membrane-bound tumor necrosis factor (mTNF)-α to activate NK cells.[13] The role of interleukin (IL)-12 released by slanDCs for NK cell activation has also been reported.[3,13] In addition to IL-12 and TNF-α, slanDCs also produce proinflammatory cytokines such as IL-1β upon stimulation with Toll-like receptor (TLR) 4, TLR7/8 or CD40 ligand.[14,15]

A study has reported significantly increased production of IL-1β by slanDCs after coculture with NK cells, for which direct cell–cell contact was required.[13] However, the detailed mechanism by which NK cells modulate IL-1β production by slanDCs and the typical role of this cytokine during NK-slanDC crosstalk remained unexplored. Therefore, we investigated the potential role of intercellular adhesion molecule (ICAM)-1 signaling in the increased IL-1β production by slanDCs following its interaction with lymphocyte function-associated antigen (LFA)-1 (CD11a and CD18) on NK cells. ICAM-1 is expressed in different cell types and is readily

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Received: 2 September 2015; Revised: 26 February 2016; Accepted: 2 March 2016

upregulated by different inflammatory stimulants.[16,17] It binds to its principal receptor, LFA-1, which is expressed by different immune cells such as lymphocytes, macrophages and neutrophils.[18,19] Although ICAM-1 is well known as an adhesion molecule, its signaling in lymphocytes can also result in cytokine release.[17,20]

IL-1β is a member of the IL-1 proinflammatory cytokine family that is synthesized in its mature, bioactive form from proIL-1β with the help of IL-1 converting enzyme.[21,22] IL-1β is released by different innate immunoregulatory cell types such as monocytes, DCs and macrophages.[21–23] Through binding to its receptor (IL-1R1), IL-1β has an important role in host defense against infection.[22–24] Therefore, our study aimed to investigate whether the increased production of IL-1β during coculture controls the apoptosis of activated NK cells. Moreover, we also studied how IL-1β tunes down the level of activation of NK cells by inducing the expression of cyclooxygenase (COX)-2 in slanDCs. COX-2 is well known as the rate-limiting enzyme for the production of prostaglandin (PG)-E2.[25] PGE2 has been reported as a suppressive factor for immune cells.[26–28]

MATERIALS AND METHODS

Samples

Following the provision of written informed consent, blood samples were obtained from healthy individuals visiting the Institute of Transfusion Medicine at Hannover Medical School. The study was approved by the local ethical committee of Hannover Medical School (MHH Ethics Committee, No. 830).

Cells and culture conditions

Peripheral blood mononuclear cells (PBMCs) were separated using Ficoll density gradient centrifugation. Isolation of NK cells and slanDCs was performed by FACS as previously described.[13] Freshly sorted slanDCs and NK cells were cultured separately (at 1×10^5 cells/100 μl) using RPMI 1640 media (Biochrom; Berlin, Germany) supplemented with 10% FCS, 1 mM L-glutamine, 50 U/ml penicillin, 0.5 mM sodium pyruvate and 50 μg/ml streptomycin. Moreover, freshly isolated slanDCs were cocultured with sorted autologous NK cells at a ratio of 1:5. PBMCs were cultured at a concentration of 3×10^6 cells/ml. The cells were cultured in 24-well suspension culture plates (Greiner; Frickenhausen, Germany) for 16 h and 25 ng/ml lipopolysaccharide (LPS; Sigma; Seelze, Germany) was used to stimulate the slanDCs.

Flow cytometric analysis

Analyses of surface and intracellular molecules of NK cells and slanDCs were performed using the following monoclonal antibodies: anti-CD3-BD Horizon V500, anti-CD45-PerCp, anti-CD107a-FITC, anti-bcl2-FITC, anti-caspase-3-PE, phosphorylated c-Jun N-terminal kinase (JNK)-PE, anti-phosphorylated NF-kB-p65-PE (all from BD Biosciences, Heidelberg, Germany), anti-CD3-APC, anti-CD19-APC-Cy7/BV510, anti-CD54-FITC, anti-CD56-BV510/PE-Cy7, anti-CD95-FITC, anti-CD178-PE, anti-HLA-DR-BV421, anti-IFN-γ-PE (all from Biolegend; San Diego, CA, USA), anti-CD69-PE (Invitrogen, Darmstadt, Germany), anti-slan (M-DC8)-APC/PE (both from Miltenyi Biotec, Bergisch Gladbach, Germany), anti-COX-2-FITC (Cayman Chemical; Ann Arbor, MI, USA) and anti-IL-1β-FITC/PerCp (R&D Systems, Wiesbaden, Germany). The negative controls were included as fluorochrome-conjugated isotype-matched antibodies from the respective companies. The samples were measured using a FACSCanto II flow cytometer.

Detection of cell surface molecules

To evaluate the influence of slanDCs on the expression of CD11a, CD18, CD69, CD95 and CD178 on NK cells, we cocultured NK cells with slanDCs in the presence of LPS. Furthermore, coculture was performed in the presence of 10 μg/ml anti-CD11a (Biolegend) or anti-IL-1β (eBioscience; San Diego, CA, USA) blocking antibodies. NK cells were also incubated without slanDCs in the presence or absence of LPS or 2 ng/ml rhIL-1β. To evaluate ICAM-1 (CD54) and CD178 surface expression on slanDCs, sorted slanDCs or PBMCs were cultured in the presence or absence of LPS. The expression of surface molecules on NK cells and slanDCs was analyzed by flow cytometry.

Detection of intracellular proteins

NK cells were cocultured with slanDCs and LPS in the presence or absence of COX inhibitors (20 μM indomethacin or 5 μM NS398 (both from Cayman Chemical)), 10 μg/ml anti-IL-1β or 0.1 μg/ml anti-CD95 (ZB4) blocking antibody (Biolegend). NK cells and slanDCs were also incubated separately in the presence or absence of LPS, anti-IL-1β, anti-CD54 or anti-CD95 blocking antibodies. The NK cells were negatively separated from slanDCs in MS columns using anti-slan (M-DC8) microbeads (Miltenyi Biotec) according to the manufacturer's protocol. The NK cells (purity >98%) were incubated with 2 μg/ml brefeldin A (Sigma) for 3 h and were then analyzed for their intracellular expression of bcl2, caspase-3 and IFN-γ by flow cytometry as previously described.[7] To measure phosphorylated JNK and phosphorylated NF-κB-p65 levels in slanDCs during coculture, slanDCs were first stimulated with LPS for 16 h to induce the upregulation of CD54 expression. Then, the slanDCs were coincubated with NK cells for 20 min followed by intracellular staining as previously described[13] to analyze phosphorylated JNK and phosphorylated NF-κB-p65. The intracellular IL-1β and COX-2 levels in slanDCs were also analyzed as previously described.[13,27] To assess late apoptosis (Annexin-V^+/$7AAD^+$) and early apoptosis (Annexin-V^+/$7AAD^-$) in NK cells and slanDCs, an Annexin-V/7AAD kit (Beckman Coulter, Krefeld, Germany) was used as previously described.[13] To measure IL-1β and GM-CSF in culture supernatants, a cytometric bead array flex set (BD Biosciences) was used according to the company's instructions; FCAP array software V.3 was used for data analysis. PGE2 in the culture supernatants was measured using a commercially available PGE2 ELISA kit as previously described.[29]

NK cell degranulation assay

NK cells were cocultured with slanDCs and LPS in the presence or absence of COX inhibitors (20 μM Indomethacin or 5 μM NS398). Subsequently, the NK cells were negatively separated from slanDCs as previously described[13] with an NK cell purity of >98%, and NK cell degranulation stimulated by K562 target cells was analyzed using a CD107a assay. The NK cells and K562 cells were cocultured at an E:T ratio of 10:1 in the presence of anti-CD107a-FITC, and Monensin (BD Biosciences) was added 1 h later at a final concentration of 6 μg/ml. Following the 4 h total coincubation, the cells were stained for surface markers, washed and analyzed for the surface expression of CD107a by flow cytometry.

Statistical analysis

Paired two-tailed *t*-tests (to compare two groups) or one-way analysis of variance (ANOVA) followed by Bonferroni posttests (to compare three or more groups) were applied to evaluate the statistical significance of the observed differences. GraphPad Prism software V5 (GraphPad, Inc., La Jolla, CA, USA) was used for statistical analysis.

RESULTS

NK cells enhance IL-1β secretion by slanDCs

Upon stimulation, slanDCs produce several proinflammatory cytokines such as IL-1β, IL-12 and TNF-α.[11,13,15] In a previous study, direct cell–cell contact with NK cells enhanced IL-1β production by slanDCs.[13] However, the mechanism by which NK cells enhance IL-1β production by slanDCs remained unknown. In this study, our blocking experiment using an anti-CD11a antibody revealed that the binding of LFA-1 (CD11a and CD18) on NK cells to ICAM-1 (CD54) on slanDCs induced signaling that activated NF-κB in slanDCs (Figures 1a and b). JNK was also activated in slanDCs by ICAM-1 signaling, as shown in the LFA-1 blocking experiment (Figures 1c and d). It is well known that the activation of the NF-κB and JNK pathways increases the release of various proinflammatory cytokines such as IL-1β.[30,31] It was therefore not unexpected that blocking ICAM-1 signaling in the coculture by using an anti-CD11a blocking antibody abrogated the enhanced secretion of IL-1β by slanDCs (Figure 1e). Alternatively, intracellular staining revealed that the use of an anti-CD54 blocking antibody also abrogated the enhanced secretion of IL-1β by slanDCs in the coculture (Figure 1f). Intracellular staining detected no significant amounts of IL-1β in NK cells cocultured with slanDCs (data not shown). Addition of an anti-CD11a blocking antibody to the slanDC culture did not affect the baseline production of IL-1β by slanDCs (data not shown). In contrast, the presence of the anti-CD11a blocking antibody in the coculture resulted in an increase in apoptotic NK cells and apoptotic slanDCs (Figures 1g and h). To induce ICAM-1 signaling, NK cells upregulated LFA-1 components after coculture with slanDCs in the presence of LPS (Figures 2a–d). Similarly, slanDCs upregulated CD54 in the coculture in the presence of LPS (Figure 2e). Stimulation of PBMCs with LPS also resulted in the significant upregulation of CD54 on slanDCs (Figure 2f).

IL-1β controls the apoptosis of NK cells by inducing the expression of CD95

A previous study reported that slanDCs activate NK cells *in vitro*.[13] However, it was not known whether those activated cells also undergo apoptosis as a homeostatic response. Here we measured a significantly higher proportion of early and late apoptotic NK cells after coculture with slanDCs in the presence of LPS (Figures 3a and b). NK cells expressed more intracellular active caspase-3 (Figures 3c and d), but less of the antiapoptotic molecule bcl-2, after coculture with slanDCs (Figures 3e and f). Our blocking experiments indicated that binding of NK cell CD95 to its ligand CD178 has a role in inducing NK cell apoptosis (Figures 4a and b). Both Fas (CD95) expression and FasL (CD178) expression were upregulated on NK cells upon coculture with slanDCs in the presence of LPS (Figures 4c–e). LPS stimulation was sufficient for slanDCs to upregulate their surface CD178 expression (Figure 4f). Although we do not yet know which molecule is responsible for the upregulation of CD178, IL-1β released during coculture increased the surface expression of CD95 on NK cells (Figures 4g and h). Therefore, blocking of IL-1β also significantly abrogated the occurrence of apoptosis in NK cells (Figures 4i and j). Although the addition of rhIL-1β to the NK cell culture medium upregulated CD95 in NK cells (as shown above in Figure 4g), it did not significantly enhance the level of apoptotic cells (data not shown).

IL-1β limits the level of NK cell stimulation by inducing the release of PGE-2

The function of other molecules, such as mTNF-α and IL-12, has been reported during slanDC-mediated stimulation of NK cell functions such as cytotoxicity and cytokine production.[3,13] However, the regulatory role of IL-1β during the stimulation of NK cells by slanDCs in a coculture has not been addressed. Here we report significantly higher intracellular COX-2 expression in slanDCs cocultured with NK cells (Figure 5a). However, COX-2 expression in slanDCs was significantly reduced in the presence of an anti-IL-1β blocking antibody in coculture (Figure 5b). COX-2 is a rate-limiting enzyme that is required for PGE2 production.[25] Our ELISA results showed remarkable PGE2 production by LPS-stimulated slanDCs. However, there was no significant difference between the concentration of PGE2 in LPS-stimulated slanDC cultures and LPS-stimulated slanDC-NK cell cocultures (Figure 5c). NK cells express the PGE2 degrading enzyme hydroxyprostaglandin dehydrogenase (HPGD; data not shown), and PGE2 has been reported as a suppressive factor for immune cells.[26,27] Consistent with this, the inhibition of PGE2 by COX inhibitors added to the coculture resulted in a further significant increase in NK cell activation (Figure 5d). Furthermore, PGE2 inhibition in the coculture also resulted in significantly increased GM-CSF release (Figure 5e), intracellular IFN-γ (Figure 5f) and CD107a degranulation (Figures 5g and h) in NK cells.

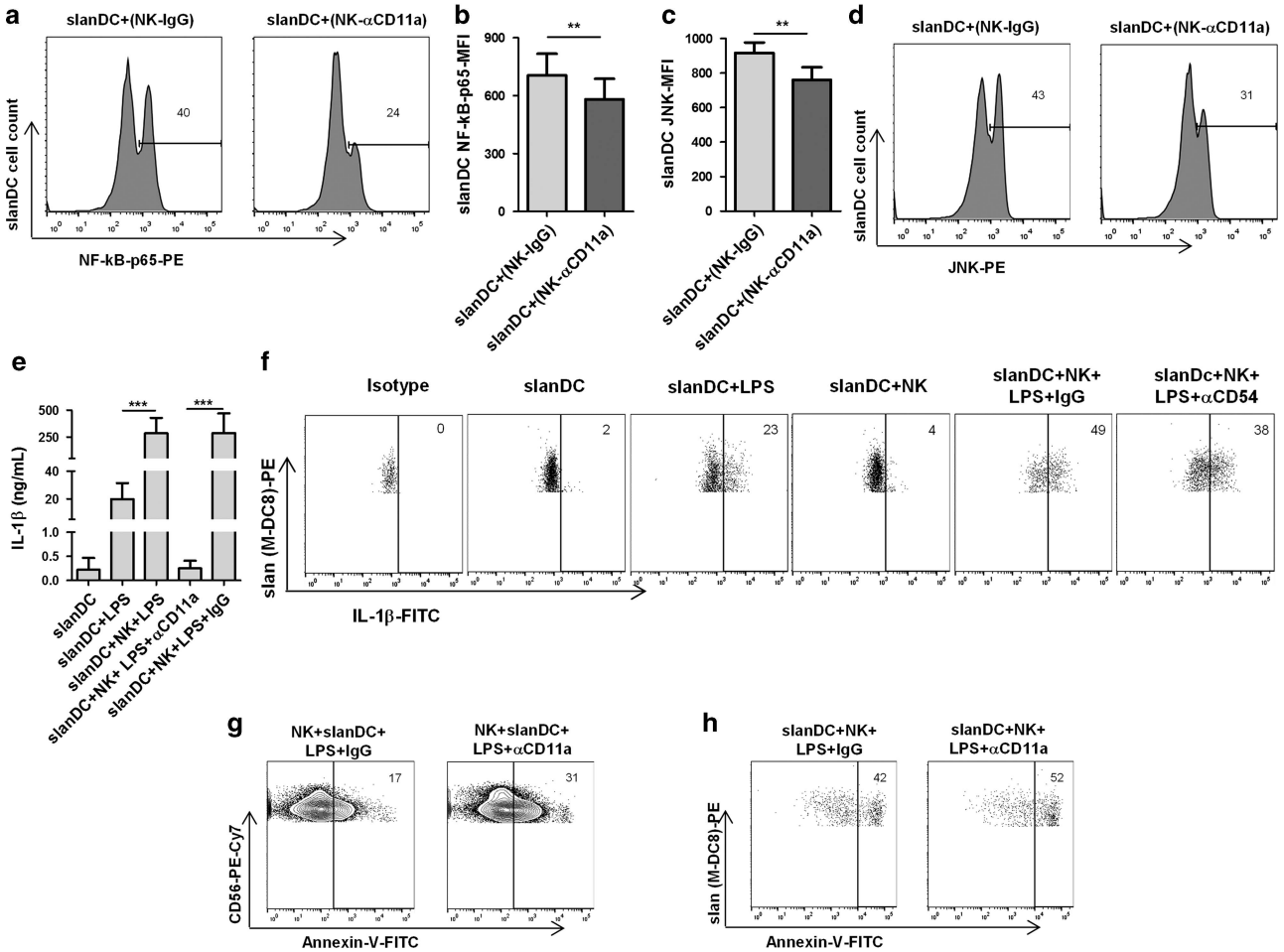

Figure 1 NK cells enhance IL-1β secretion by slanDCs via LFA-1. Freshly isolated slanDCs and NK cells were coincubated for up to 16 h in the presence of LPS, anti-CD11a blocking antibody, anti-CD54 blocking antibody or isotype IgG. NK cells and slanDCs were also cultured separately in the presence or absence of LPS. (**a–d**) LPS-stimulated slanDCs were coincubated with untreated or CD11a-blocked NK cells for 20 min. (**a**) slanDCs were stained for intracellular NF-κB-p65 expression, and a representative histogram is shown. The values represent the percentage of NF-κB-p65$^+$ cells. (**b**) slanDCs were stained for intracellular NF-κB-p65 expression, and the mean fluorescence intensity (MFI) of NF-κB-p65$^+$ cells is shown ($n=5$/group). (**c**) slanDCs were stained for intracellular JNK expression, and the MFI of JNK$^+$ cells is shown ($n=4$/group). (**d**) slanDCs were stained for intracellular JNK expression, and a representative histogram is shown. The values represent the percentage of JNK$^+$ cells. (**e**) Supernatants ($n=5$/group) were analyzed for IL-1β, and the concentration (ng/ml) was measured using a CBA. (**f**) slanDCs were stained for intracellular IL-1β expression, and representative dot plots are shown. The values represent the percentage of IL-1β$^+$ cells. (**g**) NK cells were stained with Annexin-V, and dot plots are shown. The values represent the percentage of Annexin-V$^+$ cells. (**h**) slanDCs were stained with Annexin-V, and dot plots are shown. The values represent the percentage of Annexin-V$^+$ cells. (**b**, **c** and **e**) The data are shown as the mean ± s.d. and are representative of four to five independent experiments. $^{**}P<0.01$ and $^{***}P<0.001$, paired two-tailed *t*-tests (**b**, **c**) or one-way ANOVA followed by Bonferroni posttests (**e**).

A previous study reported that slanDCs do not produce GM-CSF and IFN-γ but that these are produced by NK cells.[13]

DISCUSSION

Human DCs are known to stimulate immunocytes such as NK cells.[1–4,13,32–34] Inflamed peripheral tissue, lymph nodes and the microenvironment of solid tumors have been elucidated as the *in vivo* sites of interaction for NK cells and DCs.[1,2,35] Different subsets of blood DCs use distinct molecular mechanisms during crosstalk with NK cells.[3,13,34,36,37] Previously, slanDCs were reported to stimulate NK cells through mTNF-α and IL-12.[3,12,13] However, there are other cytokines, such as IL-1β, that are released by slanDCs in the coculture. NK cells enhance the release of IL-1β by slanDCs via a mechanism that requires cell–cell contact.[13] Therefore, we were interested in studying the role of increased IL-1β secretion in the coculture. IL-1β is a member of the IL-1 proinflammatory cytokine family that is released primarily by activated monocytes in response to a wide variety of infectious stimuli. Through binding to its receptor (IL-1R1), IL-1β has an important role in host defense by inducing inflammation during infections.[21,23]

In our study, blocking experiments revealed that NK cells used LFA-1 to bind to ICAM-1 on slanDCs, thereby enhancing the release of IL-1β by slanDCs. Addition of an anti-CD11a blocking antibody to the coculture completely abrogated the release of IL-1β to the culture supernatant. CD54 blocking in

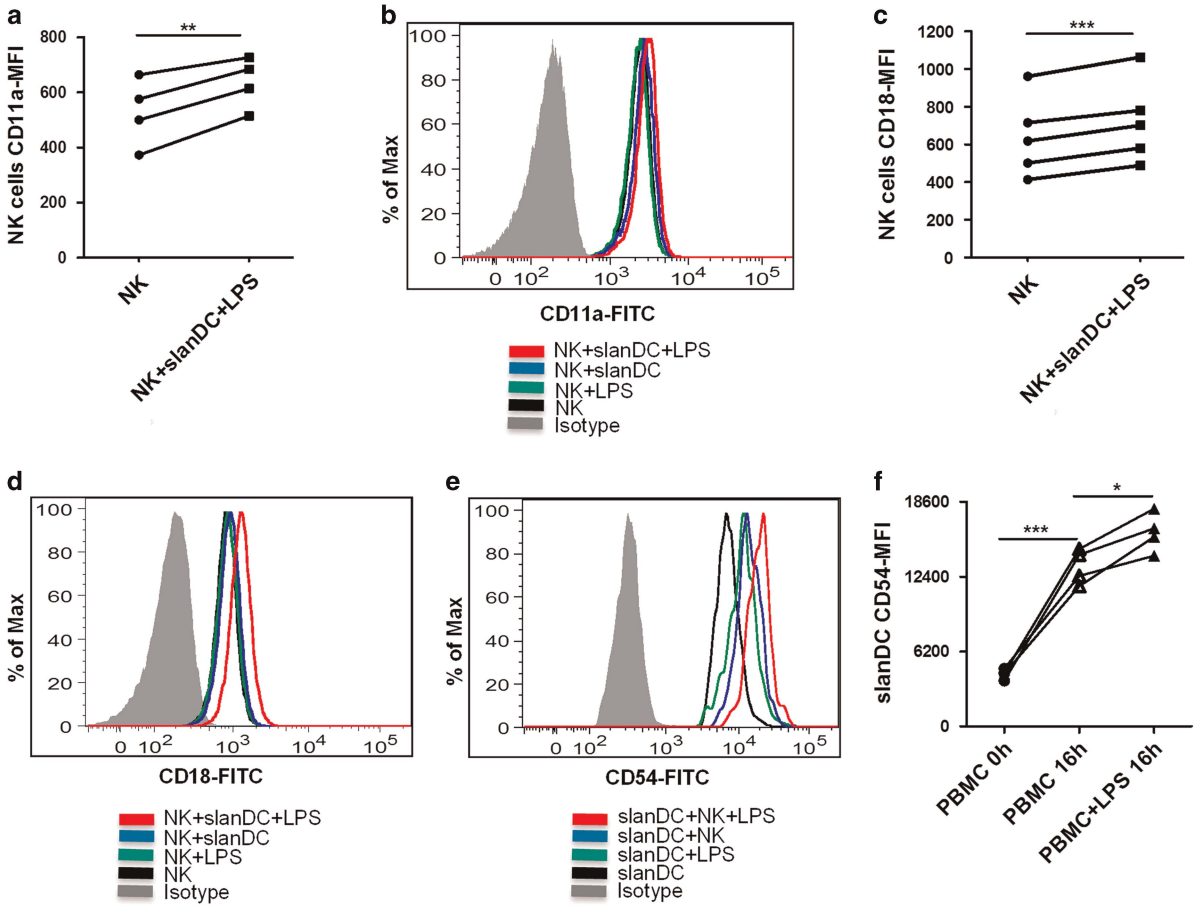

Figure 2 LFA-1 and ICAM-1 are upregulated during NK-slanDC coculture. Freshly isolated slanDCs and NK cells were coincubated for 16 h in the presence or absence of LPS. PBMCs, NK cells and slanDCs were also cultured separately in the presence or absence of LPS. (**a**) NK cells were stained for CD11a expression, and the MFI of $CD11a^+$ cells is shown ($n=4$/group). (**b**) NK cells were stained for CD11a expression, and a representative histogram is shown. (**c**) NK cells were stained for CD18 expression, and the MFI of $CD18^+$ cells is shown ($n=5$/group). (**d**) NK cells were stained for CD18 expression, and a representative histogram is shown. (**e**) slanDCs were stained for ICAM-1 (CD54) expression, and a representative histogram is shown. (**f**) slanDCs were stained for ICAM-1 (CD54) expression, and the MFI of $CD54^+$ slanDCs is shown ($n=4$/group). (**a**, **c** and **f**) The data are shown as the mean ± s.d. and are representative of four to five independent experiments. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$, paired two-tailed *t*-tests (**a**, **c**) or one-way ANOVA followed by Bonferroni posttests (**f**).

the coculture also significantly reduced the proportion of slanDCs expressing IL-1β. Apart from its blocking activity, the anti-CD11a blocking antibody also initiated apoptosis in both NK cells and slanDCs during coculture. Therefore, the complete inhibition of IL-1β release in the presence of anti-CD11a blocking antibody observed in the coculture might be due to the combined effect of blocking and apoptosis. Blocking CD11a in the slanDC culture did not affect the proportion of slanDCs producing IL-1β, indicating that ICAM-1 trans-signaling via LFA-1 on NK cells is essential. LFA-1 and ICAM-1 were upregulated in NK cells and slanDCs, respectively, during coculture. ICAM-1 (CD54) is expressed in various cell types, is readily upregulated by different inflammatory stimuli[16,17] and is well known as an adhesion molecule.[18,20] In lymphocytes, ICAM-1 stimulation can also lead to signaling that results in transcription factor phosphorylation and cytokine release.[19,20] The activation of NF-κB and JNK in slanDCs through ICAM-1 signaling shown in our study is additional evidence of the role of ICAM-1 in downstream slanDC functions. Although they enhanced the release of IL-1β from slanDCs, NK cells themselves were not able to produce significant amounts of IL-1β in the coculture.

As the main objective of this investigation, we studied the role of elevated IL-1β under *in vitro* coculture conditions. First, IL-1β induced the expression of Fas receptor (CD95) in NK cells during coculture with slanDCs. Thus far, unknown factors increased the expression of Fas ligand (CD178) on both NK cells and slanDCs in the coculture. CD95 is a member of the TNF receptor family and is a type I membrane protein expressed by different cell types. The binding of CD178 to CD95 activates the caspase cascade, resulting in apoptosis and cell death.[38] Therefore, we observed a higher proportion of NK cells undergoing apoptosis in the presence of elevated IL-1β during *in vitro* coculture. During 16 h of coculture, the overall proportion of NK cells undergoing apoptosis varied between donors. However, for all donors, the proportion of apoptotic

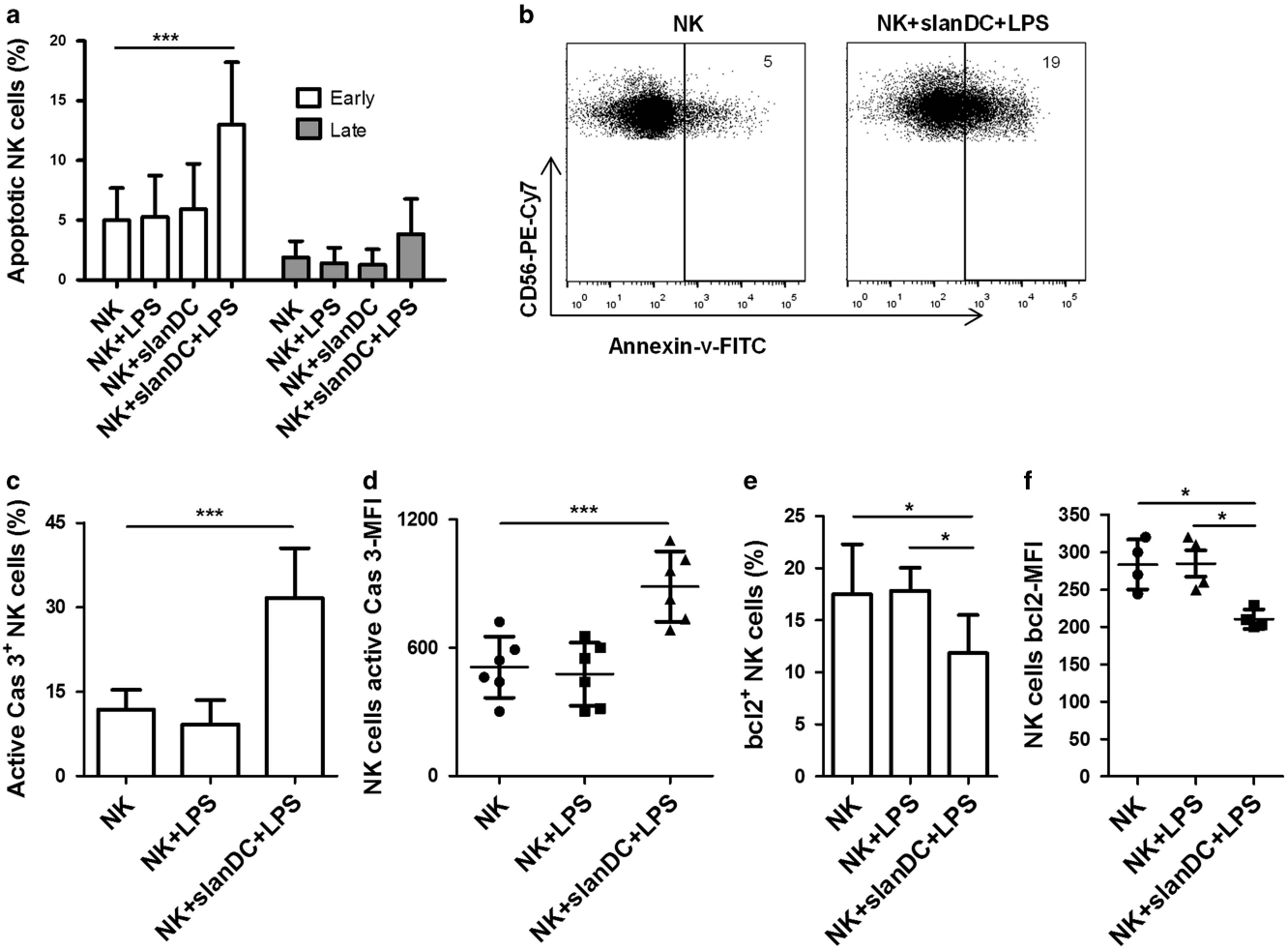

Figure 3 Increased proportion of apoptotic NK cells after coculture with slanDCs. Freshly isolated NK cells and slanDCs were coincubated for 16 h in the presence or absence of LPS. NK cells were also cultured with or without LPS. (**a**) NK cells were stained for Annexin-V and 7AAD and evaluated for apoptosis using flow cytometry. The percentage of early (Annexin-V$^+$7AAD$^-$) and late (Annexin-V$^+$7AAD$^+$) apoptotic cells are shown ($n=9$/group). (**b**) NK cells were stained for Annexin-V, and dot plots are shown. The data are representative of nine independent experiments. The values represent the percentage of Annexin-V$^+$ cells. (**c**) NK cells were stained for intracellular active caspase-3 expression, and the percentage of active caspase-3$^+$ cells is shown ($n=6$/group). (**d**) NK cells were stained for intracellular active caspase-3 expression, and the MFI of active caspase-3$^+$ cells is shown ($n=6$/group). (**e**) NK cells were stained for intracellular bcl2 expression, and the percentage of bcl2$^+$ cells is shown ($n=6$/group). (**f**) NK cells were stained for intracellular bcl2 expression, and the MFI of bcl2$^+$ cells is shown ($n=4$/group). (**a**, **c**–**f**) The data are shown as the mean ± s.d. and are representative of four to nine independent experiments. $^{**}P<0.01$ and $^{***}P<0.001$, one-way ANOVA followed by Bonferroni posttests (**a**, **c**–**f**).

NK cells doubled in coculture with slanDCs in comparison to NK cells cultured alone. In the absence of LPS, slanDCs did not release significant amounts of IL-1β into the coculture supernatant. Concomitantly, CD95 was not upregulated on NK cells, meaning that no significant apoptosis was induced. However, the upregulation of CD95 in NK cells by rhIL-1β was not sufficient to enhance apoptosis. This might indicate the requirement for FasL on slanDCs to induce apoptosis in activated NK cells during coculture. The observation that IL-1β regulates the apoptosis of NK cells by dictating the expression of CD95 on NK cells is a new finding.

Moreover, the increased IL-1β in our *in vitro* coculture served as a regulator of NK cell activation. IL-1β tuned down NK cell activation by modulating COX-2 expression in slanDCs. In the presence of elevated IL-1β, slanDCs expressed higher levels of COX-2. COX-2 has been described as the rate-limiting enzyme for PGE2 production.[25] LPS-stimulated slanDCs produced high levels of PGE2. Although the expression of COX-2 was significantly upregulated in slanDCs cocultured with NK cells compared with slanDCs cultured alone, there was no significant difference between the PGE2 levels in slanDC cultures and slanDC-NK cell cocultures. This might be due to the degradation of PGE2 by NK cell HPGD, a PGE2 degrading enzyme. PGE2 is well known for its suppressive activity on immune cells such as NK cells.[26,27] Previous reports have shown that slanDCs are capable of increasing the activation and function of NK cells

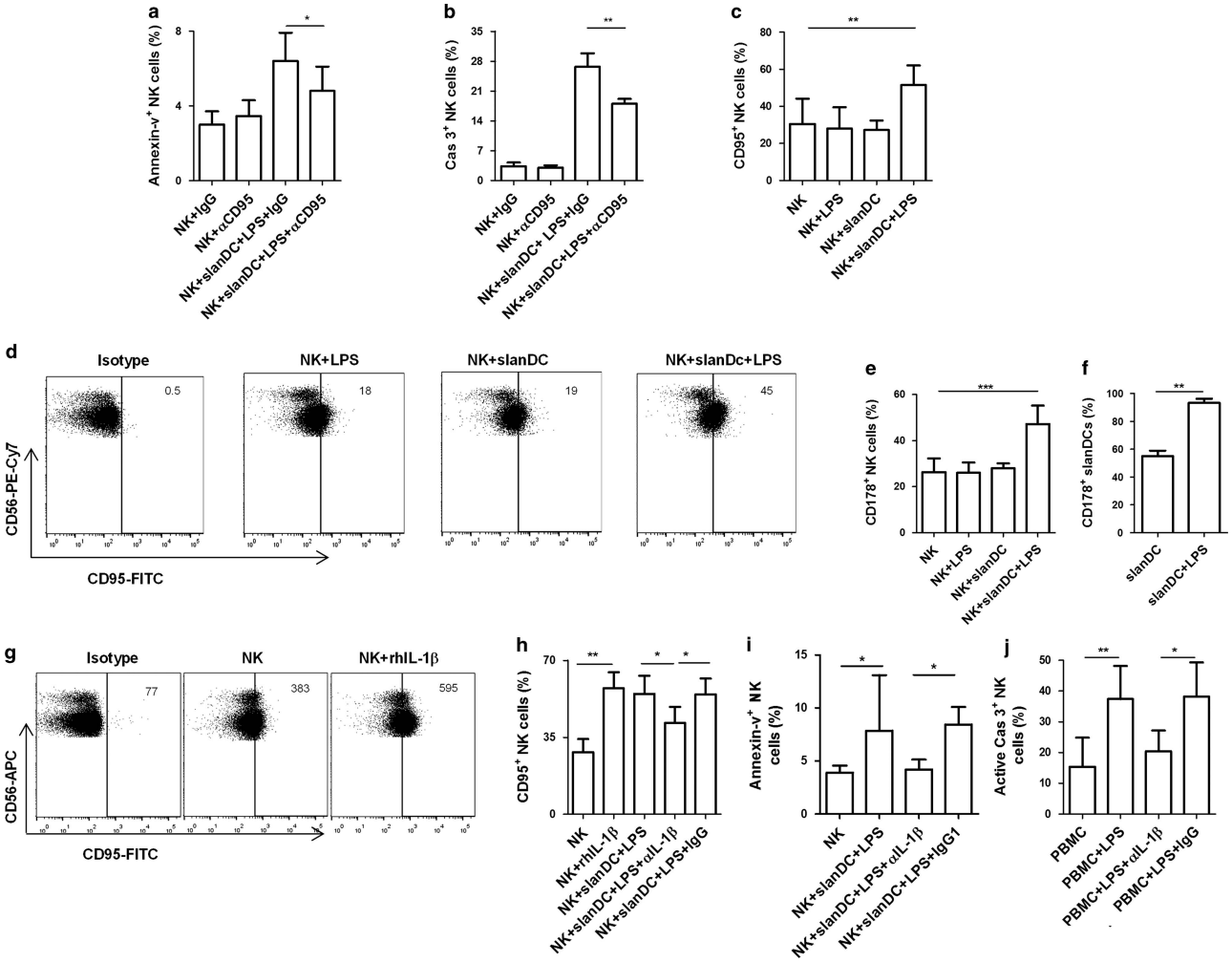

Figure 4 IL-1β induces the expression of CD95 in NK cells to induce apoptosis. Freshly isolated NK cells and slanDCs were coincubated for 16 h in the presence or absence of LPS. The coculture was also performed in the presence of antagonistic anti-CD95 (ZB4), anti-IL-1β blocking antibody or isotype IgG. NK cells or slanDCs were also cultured with or without LPS or rhIL-1β. Finally, NK cells (in PBMCs) were cultured for 16 h with or without LPS, anti-IL-1β blocking antibody or isotype IgG. (**a** and **i**) NK cells were evaluated for apoptosis using flow cytometry, and the percentage of early (Annexin-V$^+$7AAD$^-$) apoptotic cells are shown ($n=5$ and 9/group, respectively). (**b**, **j**) NK cells were stained for intracellular active caspase-3 expression, and the percentage of active caspase-3$^+$ cells is shown ($n=4$ and 6/group, respectively). (**c**, **h**) NK cells were stained for CD95 expression, and the percentage of CD95$^+$ cells is shown ($n=7$ and 5/group, respectively). (**d**, **g**) NK cells were stained for CD95 expression, and dot plots are shown. The data are representative of seven and five independent experiments, respectively. The values represent the percentage (**d**) or MFI (**g**) of CD95$^+$ NK cells. (**e**, **f**) NK cells (**e**) or slanDCs (**f**) were stained for CD178 expression, and the percentage of CD178$^+$ cells is shown ($n=6$/group). (**a**–**c**, **e**, **f**, **h**–**j**) The data are shown as the mean ± s.d. and are representative of four to nine independent experiments. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$, paired two-tailed *t*-tests (**f**) or one-way ANOVA followed by Bonferroni posttests (**a**–**c**, **e**, **h**–**j**).

in cocultures.[3,12,13] In the current study, the production of PGE2 was reduced by the application of COX inhibitors. For PGE2 inhibition, the nonspecific COX inhibitor indomethacin or the COX-2-specific inhibitor NS398 was used. NS398 treatment was used to study the specific effect of COX-2 in the coculture. COX inhibition further enhanced the activation, cytotoxicity and cytokine production of NK cells, and indeed, specific blocking of COX-2 was sufficient to increase NK cell functions. However, the proportion of apoptotic NK cells also increased with stimulation. This suggests that in the absence of PGE2 secretion, IL-1β can still control the level of activation via apoptosis induction in activated NK cells.

In summary, IL-1β acts as a regulatory molecule during the activation of NK cells by slanDCs. The release of IL-1β by slanDCs is enhanced by the induction of ICAM-1 signaling via binding of LFA-1 of NK cells. IL-1β tunes down NK cell activation by inducing COX-2 (a rate-limiting enzyme for the immune cell suppressing PGE2) in slanDCs. Moreover, IL-1β also upregulates the expression of CD95 on NK cells, inducing the apoptosis of activated cells, presumably as a homeostatic measure. This indicates a regulatory role for slanDC-derived IL-1β for an optimal stimulation level of NK cells. This regulatory function of IL-1β might also hold true *in vivo* during infections and inflammatory disorders that induce the

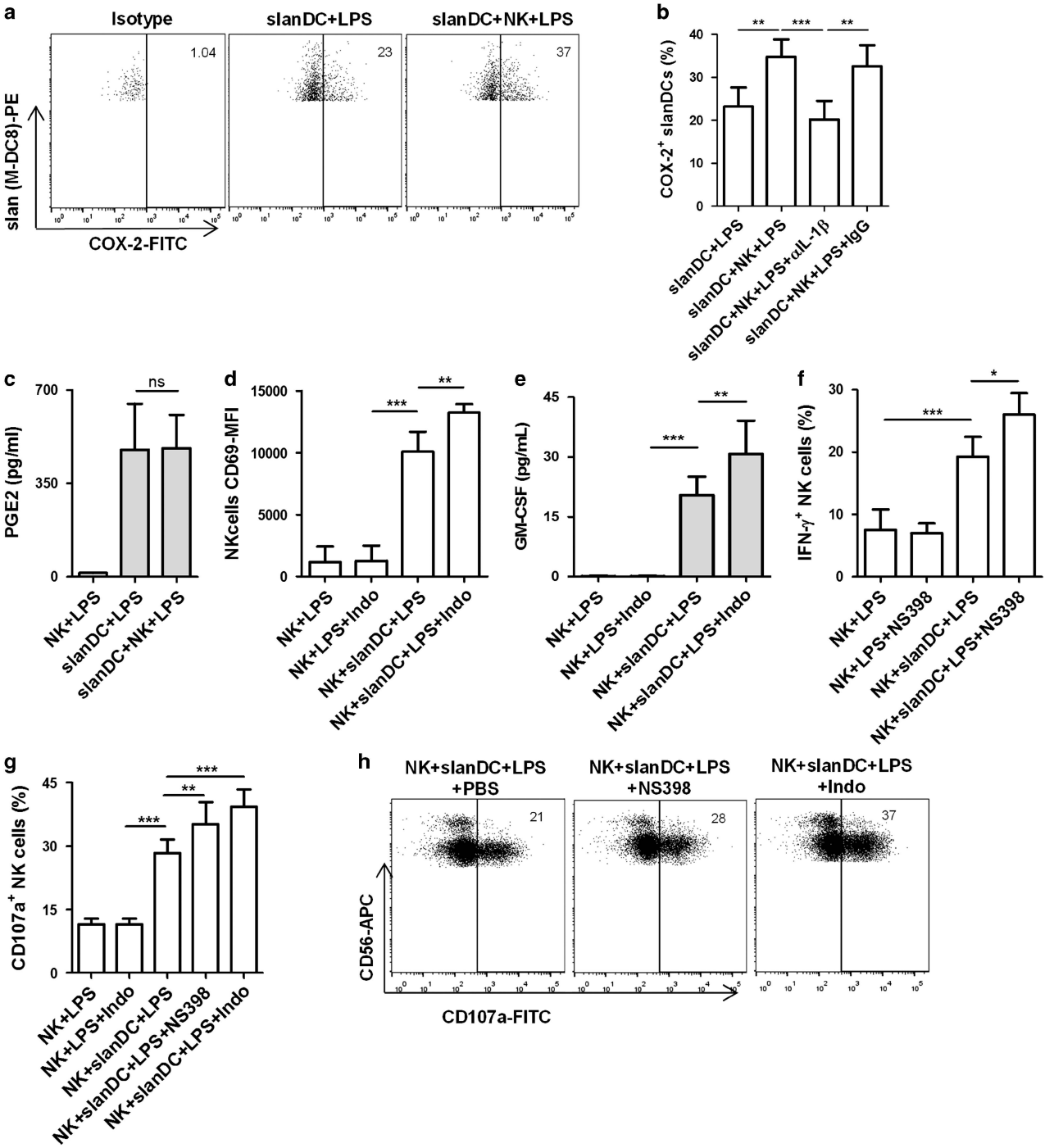

Figure 5 IL-1β induces COX-2 expression in slanDCs to restrain the activity of NK cells. Freshly isolated NK cells and slanDCs were coincubated for 16 h in the presence of LPS. The coculture was also performed in the presence of COX inhibitors [Indomethacin (Indo) or NS398], anti-IL-1β blocking antibody or isotype IgG. NK cells or slanDCs were also cultured with LPS or Indomethacin. (**a**) slanDCs were stained for COX-2 expression, and dot plots are shown. The data are representative of five independent experiments. The values represent the percentage of COX-2$^+$ slanDCs. (**b**) slanDCs were stained for intracellular COX-2 expression, and the percentage of COX-2$^+$ slanDCs is depicted ($n=5$/group). (**c**) Supernatants were analyzed for PGE2 using ELISA, and the concentration (pg/ml) is shown ($n=6$/group). (**d**) NK cells were stained for CD69 expression, and the percentage of CD69$^+$ NK cells is shown ($n=6$/group). (**e**) Supernatants were analyzed for GM-CSF using CBA, and the concentration (pg/ml) is shown ($n=5$/group). (**f**) NK cells were stained for intracellular IFN-γ expression, and the percentage of IFN-γ$^+$ NK cells is shown ($n=4$/group). (**g**) NK cells were separated from slanDCs, and a CD107a degranulation assay was performed using K562 target cells. The percentages of CD107a$^+$ cells are shown ($n=6$/group). (**h**) A CD107a degranulation assay was performed using K562 target cells, and dot plots are shown. The data are representative of six independent experiments. The values represent the percentage of CD107a$^+$ cells. (**b**–**g**) The data are shown as the mean ± s.d. and are representative of four to six independent experiments. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$, one-way ANOVA followed by Bonferroni posttests (**b**–**g**).

release of this cytokine in the body. Therefore, this study suggests the importance of IL-1β during the *in vitro* stimulation of NK cells, which might also be relevant *in vivo*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the cell sorting core facility at Hannover Medical School for their support. The Institute of Transfusion Medicine at Hannover Medical School has provided blood samples from healthy donors. We thank Penelope Charlotte Kay-Fedorov for thoroughly reading and improving the manuscript. This study was supported by grants from the Deutsche Forschungsgemeinschaft (DFG): SFB738/A5, Deutsche Zentrum für Infektionsforschung (DZIF): TTU 04.802, Hannover Biomedical Research School (HBRS), REBIRTH Cluster of Excellence and Niedersächsische Krebsgesellschaft e.V.

AUTHOR CONTRIBUTIONS

DMT, FA, DC, GA and RJ performed experiments and discussed the data; DMT, FA and RJ analyzed the results and made the figures; DMT, RJ and RES designed the research; DMT wrote the manuscript.

1 Cooper M. NK cell and DC interactions. *Trends Immunol* 2004; **25**: 47–52.
2 Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, Suter M *et al.* Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. *Nat Med* 1999; **5**: 405–411.
3 Wehner R, Löbel B, Bornhäuser M, Schäkel K, Cartellieri M, Bachmann M *et al.* Reciprocal activating interaction between 6-sulfo LacNAc+ dendritic cells and NK cells. *Int J Cancer* 2009; **124**: 358–366.
4 Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. *Nat Rev Immunol* 2002; **2**: 957–964.
5 Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001; **22**: 633–640.
6 Moretta A, Bottino C, Mingari MC, Biassoni R, Moretta L. What is a natural killer cell? *Nat Immunol* 2002; **3**: 6–8.
7 Jacobs R, Stoll M, Stratmann G, Leo R, Link H, Schmidt RE. CD16-CD56+ natural killer cells after bone marrow transplantation. *Blood* 1992; **79**: 3239–3244.
8 Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008; **9**: 503–510.
9 Jacobs R, Hintzen G, Kemper A, Beul K, Kempf S, Behrens G *et al.* CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. *Eur J Immunol* 2001; **31**: 3121–3127.
10 Schäkel K, Kannagi R, Kniep B, Goto Y, Mitsuoka C, Zwirner J *et al.* 6-Sulfo LacNAc, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human dendritic cells. *Immunity* 2002; **17**: 289–301.
11 Schäkel K, Mayer E, Federle C, Schmitz M, Riethmüller G, Rieber EP. A novel dendritic cell population in human blood: one-step immunomagnetic isolation by a specific mAb (M-DC8) and in vitro priming of cytotoxic T lymphocytes. *Eur J Immunol* 1998; **28**: 4084–4093.
12 Schmitz M, Zhao S, Deuse Y, Schäkel K, Wehner R, Wöhner H *et al.* Tumoricidal potential of native blood dendritic cells: direct tumor cell killing and activation of NK cell-mediated cytotoxicity. *J Immunol* 2005; **174**: 4127–4134.
13 Tufa DM, Chatterjee D, Low HZ, Schmidt RE, Jacobs R. TNFR2 and IL-12 coactivation enables slanDCs to support NK-cell function via membrane-bound TNF-α. *Eur J Immunol* 2014; **44**: 3717–3728.
14 Jähnisch H, Wehner R, Tunger A, Kunze A, Oehrl S, Schäkel K *et al.* TLR7/8 agonists trigger immunostimulatory properties of human 6-sulfo LacNAc dendritic cells. *Cancer Lett* 2013; **335**: 119–127.
15 Schäkel K, von Kietzell M, Hänsel A, Ebling A, Schulze L, Haase M *et al.* Human 6-sulfo LacNAc-expressing dendritic cells are principal producers of early interleukin-12 and are controlled by erythrocytes. *Immunity* 2006; **24**: 767–777.
16 Shrikant P, Benos DJ, Tang LP, Benveniste EN. HIV glycoprotein 120 enhances intercellular adhesion molecule-1 gene expression in glial cells. *J Immunol* 1996; **156**: 1307–1314.
17 Etienne-manneville S, Chaverot N, Donny A. ICAM-1-coupled signaling pathways in astrocytes converge to cyclic AMP response element-binding protein phosphorylation and TNF- α secretion. *J Immunol* 1999; **163**: 668–674.
18 Smith A, Stanley P, Jones K, Svensson L, McDowall A, Hogg N. The role of the integrin LFA-1 in T-lymphocyte migration. *Immunol Rev* 2007; **218**: 135–146.
19 Bose TO, Pham Q-M, Jellison ER, Mouries J, Ballantyne CM, Lefrançois L. CD11a regulates effector CD8 T cell differentiation and central memory development in response to infection with Listeria monocytogenes. *Infect Immun* 2013; **81**: 1140–1151.
20 Lebedeva T, Dustin ML, Sykulev Y. ICAM-1 co-stimulates target cells to facilitate antigen presentation. *Curr Opin Immunol* 2005; **17**: 251–258.
21 Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009; **27**: 519–550.
22 Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, Cheever A *et al.* Caspase-1 independent IL-1beta production is critical for host resistance to mycobacterium tuberculosis and does not require TLR signaling in vivo. *J Immunol* 2010; **184**: 3326–3330.
23 Jayaraman P, Sada-Ovalle I, Nishimura T, Anderson AC, Kuchroo VK, Remold HG *et al.* IL-1β promotes antimicrobial immunity in macrophages by regulating TNFR signaling and caspase-3 activation. *J Immunol* 2013; **190**: 4196–4204.
24 Doitsh G, Galloway NLK, Geng X, Yang Z, Monroe KM, Zepeda O *et al.* Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014; **505**: 509–514.
25 Simmons D, Botting R, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacol Rev* 2004; **56**: 387–437.
26 Harizi H. Reciprocal crosstalk between dendritic cells and natural killer cells under the effects of PGE2 in immunity and immunopathology. *Cell Mol Immunol* 2013; **10**: 213–221.
27 Chatterjee D, Marquardt N, Tufa D, Beauclair G, Low H, Hatlapatka T *et al.* Role of gamma-secretase in human umbilical-cord derived mesenchymal stem cell mediated suppression of NK cell cytotoxicity. *Cell Commun Signal* 2014; **12**: 63.
28 Van Elssen CHMJ, Vanderlocht J, Oth T, Senden-Gijsbers BLMG, Germeraad WT V, Bos GMJ. Inflammation restraining effects of prostaglandin E2 on natural killer–dendritic cell (NK-DC) interaction are imprinted during DC maturation. *Blood* 2011; **118**: 2473–2482.
29 Müller C, Tufa DM, Chatterjee D, Mühlradt PF, Schmidt RE, Jacobs R. The TLR-2/TLR-6 agonist macrophage-activating lipopeptide-2 augments human NK cell cytotoxicity when PGE2 production by monocytes is inhibited by a COX-2 blocker. *Cancer Immunol Immunother* 2015; **64**: 1175–1184.
30 McDonald PP, Bald A, Cassatella MA. Activation of the NF-kappaB pathway by inflammatory stimuli in human neutrophils. *Blood* 1997; **89**: 3421–3433.
31 Newton K, Dixit VM. Signaling in innate immunity and inflammation. *Cold Spring Harb Perspect Biol* 2012; **4**: a006049.
32 Costantini C, Calzetti F, Perbellini O, Micheletti A, Scarponi C, Lonardi S *et al.* Human neutrophils interact with both 6-sulfo LacNAc+ DC and NK cells to amplify NK-derived IFN {gamma}: role of CD18, ICAM-1, and ICAM-3. *Blood* 2011; **117**: 1677–1686.
33 Rissoan CR, Soumelis V, Kadowaki N, Grouard G, Briere F, de Malefyt RW *et al.* Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999; **283**: 1183–1186.
34 Münz C, Steinman RM, Fujii S. Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *J Exp Med* 2005; **202**: 203–207.

35 Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC *et al.* Effector and regulatory events during natural killer-dendritic cell interactions. *Immunol Rev* 2006; **214**: 219–228.
36 Borg C, Jalil A, Laderach D, Maruyama K, Wakasugi H, Charrier S *et al.* NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood* 2004; **104**: 3267–3275.
37 Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Münz C. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. *J Exp Med* 2002; **195**: 343–351.
38 Krammer PH. CD95's deadly mission in the immune system. *Nature* 2000; **407**: 789–795.