

# Human Uterine NK Cells Interact with Uterine Macrophages via NKG2D upon Stimulation with PAMPs

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## Keywords

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## Introduction

Living organisms are exposed constantly to microorganisms present in the environment and need to cope with invasion of these microorganisms. Immune cells in the female reproductive tract (FRT) maintain a critical balance, which allows for a response to pathogenic challenge and development of a semi-allogeneic fetus. Innate immune cells recognize conserved microbial structures known as pathogen-associated molecular patterns (PAMPs). In the presence of these PAMPs, cells produce an array of inflammatory cyto-

## Problem

The initiation of an immune response often involves the cooperation of various innate immune cells. In the human endometrium, uterine natural killer (uNK) cells and uterine macrophages are present in significant numbers and in close proximity, yet how they cooperatively respond to infectious challenge is poorly understood.

## Method of study

Primary autologous uNK cells and macrophages were co-cultured to determine functional interactions after stimulation with pathogen-associated molecular patterns.

## Results

After stimulation by polyI:C, human uNK cells interact with autologous uterine macrophages and produce interferon- $\gamma$  in an NKG2D-dependent manner. Stimulated primary uterine macrophages up-regulated the expression of MHC Class I chain-related protein A (MICA), but expression of the cognate receptor NKG2D remained unchanged on uNK cells, even in the presence of cytokines.

## Conclusion

This study demonstrates that the NKG2D-MICA interaction is an important molecular mechanism that is involved in the innate immune response to microbial signals in the human uterine endometrium.

kines and chemokines, leading to autocrine and paracrine stimulation of both the innate and adaptive immune systems. Many cells in the FRT express Toll-like receptors (TLRs), which enable them to recognize PAMPs on bacterial, viral, and fungal pathogens. There are eleven known human TLRs, and uterine NK (uNK) cells are known to express several of these including TLRs 2, 3 and 4.<sup>1</sup> TLR2 binds to microbial products, including peptidoglycan (PGN) and zymosan. TLR3 recognizes dsRNA associated with viral replication; thus, polyI:C, which is a synthetic mimetic for dsRNA, can induce TLR3 signaling. Previous

studies have shown that ligation of TLR3 by polyI:C results in the activation of antigen-presenting cells (APCs), as well as the production of inflammatory cytokines by uNK cells.<sup>1</sup>

Uterine macrophages and NK cells are important cellular components of the innate immune defense system in the human FRT. Uterine macrophages constitute approximately 10% of the leukocytes in the uterus, while NK cells constitute from 10% up to 70% of the uterine leukocyte population.<sup>2</sup> NK cell activation is greatly increased in the presence of sub-optimal doses of certain cytokines, such as interleukin (IL)-1 $\beta$ , IL-12, IL-15, or IL-18.<sup>3,4</sup> These cytokines are produced by a number of cells in the reproductive tract, including epithelial cells and macrophages.<sup>5–7</sup> When activated by the monokines IL-12, IL-15, and IL-18, blood NK cells promoted TNF- $\alpha$  production by CD14<sup>+</sup> monocytes in a cell contact-dependent manner.<sup>8</sup> Conversely, CD14<sup>+</sup> monocytes synergized with monokines to promote interferon- $\gamma$  (IFN- $\gamma$ ) production by blood NK cells.<sup>8</sup>

Natural killer cells use various activating and inhibitory receptors to detect alterations in virally infected cells.<sup>9,10</sup> Some NK-cell activating receptors directly recognize pathogen-encoded proteins expressed on infected cells, as is the case with the murine NK receptor Ly49H, which recognizes M157 encoded by murine cytomegalovirus (CMV).<sup>11</sup> Alternatively, NK cells recognize host proteins expressed upon infection. For instance, CMV-infected human fibroblasts up-regulate ligands that bind to the NK receptor NKG2D.<sup>12</sup> NKG2D is an NK-cell activating receptor that is also expressed by  $\gamma\delta$  T cells, NKT cells and CD8 T cells, including those that lack the CD28 co-receptor.<sup>12,13</sup> Thus, NKG2D mediates the activities of various immune cells and can act as regulator of local immune responses in various mucosal systems.

A number of NKG2D ligands have been identified in humans and mice. In humans, these are the MHC class I polypeptide-related sequences A and B (MICA and MICB) protein family and the cytomegalovirus UL16-binding protein (ULBP; also known as RAET1 proteins) family, which consists of five members (ULBP1-4 and RAET1-G).<sup>13</sup> The NKG2D receptor/ligand system is involved in the recognition of 'stressed' cells and tumors, as the ligands are almost undetectable in normal tissues, but expression can be induced by transformation, infection, or cellular stress, such as DNA damage.<sup>14</sup> In mucosal systems, such as the respiratory tract, cell surface expression of NKG2D ligands has been detected under condi-

tions of oxidative stress and viral infection.<sup>12,15</sup> Studies using blood monocyte-derived macrophages have shown that TLR signaling can lead to up-regulation of NKG2D ligands that can be recognized by autologous blood NK cells.<sup>16</sup> However, uterine macrophages develop in a specialized microenvironment, and their interactions with other cellular components within the human FRT, such as uNK cells are not understood. One of the primary difficulties in understanding the function of human immune cells is the limited number of studies that examine functional interactions between human cells taken from primary tissues. Most infections begin at mucosal sites, not in the blood, yet we know little about how the immune cells within these sites interact to develop an immune response. The overall goal of this study was to test the hypothesis that upon pathogenic challenge, uNK cells interact with CD14<sup>+</sup> uterine macrophages in a reciprocal fashion, thereby initiating or amplifying the local inflammatory response. In this study, we determined one molecular mechanism that is involved in how primary macrophages and NK cells from the human endometrium work together to generate an immune effector response.

## Materials and methods

### Isolation of Macrophages from Tissue Samples

Endometrial tissue specimens were obtained immediately following surgery from patients undergoing hysterectomy for various gynecological disorders. Preliminary diagnoses included fibroids, pelvic pain, menorrhagia, prolapse, and uterine mass. The sections used were distal to any sites of pathology and were determined to be unaffected by disease at the gross anatomical level.<sup>17</sup> Tissues were from women in age ranging from 38 to 60 years. Tissues were frozen in OCT for sectioning. Single cell suspensions were prepared from tissues using the protocol as in Eriksson et al.<sup>1</sup> Any contaminating RBCs were eliminated from endometrial cells by lysis with NH<sub>4</sub>Cl/Tris-HCl for 5–10 min at room temperature. Positive selection with anti-CD14<sup>+</sup> MicroBeads (Milenyi Biotec, Auburn, CA, USA) was used for purification of macrophages from human endometrial tissues. Uterine macrophages were incubated in complete medium [RPMI 1640 supplemented with 2-mercaptoethanol (50  $\mu$ M), penicillin (100 U/mL), streptomycin (100  $\mu$ g/mL), HEPES (10 mM), sodium pyruvate (1 mM), non-essential amino acids

(0.1 mM) and 10% FBS] for stimulation with PAMPs or frozen for use in future experiments. All human studies were done with the approval of the Dartmouth College Institution Review Board.

#### Generation of Macrophages from Blood Monocytes

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood with Ficoll Hypaque (density = 1.077). Monocytes were purified from mononuclear cell fractions, as described by Mentzer et al.<sup>18</sup> They were matured into macrophages by incubation with either 100 ng/mL of macrophage colony stimulating factor (M-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF).<sup>19</sup> The cells were cultured in 5% CO<sub>2</sub> at 37°C in complete RPMI medium for 10 days and media was switched every third day. Matured adherent macrophages were plated at  $4 \times 10^5$  per well in a 12-well plate and stimulated with PAMPs (in triplicate) for 48 hr. Cells were harvested and analysed for MICA expression by flow cytometry.

#### Generation of Polyclonal Expanded or Clonal Uterine NK Cells

After macrophage isolation, endometrial cells were cultured in NK cell medium [RPMI 1640 supplemented with 2-ME (50 µM), penicillin (100 U/mL), streptomycin (100 µg/mL), sodium pyruvate (1 mM), non-essential amino acids (0.1 mM), and 5% human serum supplemented with 500 u/mL of IL-2] for 2–3 days to expand NK cells. Cells were harvested and uNK cells isolated using the Human NK Cell Enrichment Kit (StemCell Technologies, British Columbia, CA, USA). uNK cells were expanded in IL-2 for up to a total of 14 days (polyclonal expanded uNK cells) or cloned using standard NK-cell cloning procedures.<sup>1</sup> Culture experiments with autologous macrophages were performed with IL-2-expanded autologous uNK cells. For the generation of uNK cell clones, isolated uNK cells were plated at 1–3 cells/well in U-bottom 96-well plates together with irradiated (100 Gy) feeder cells ( $5 \times 10^4$  allogeneic PBMCs and  $5 \times 10^3$  RPMI 8866 cells) in NK clone media supplemented with 1 µg/mL Phytohemagglutinin (PHA) and IL-2 (500 u/mL). After 10 days, the wells were examined for growth of cells, and cell phenotypes analysed by flow cytometry. CD56<sup>+</sup> CD3<sup>-</sup> cells were expanded further and maintained in NK clone medium.<sup>20</sup>

#### Antibodies and Reagents

The following Abs were used: FITC-conjugated anti-CD45 and TriColor-conjugated anti-CD45; R-PE-conjugated anti-CD56, FITC-conjugated anti-CD56 and biotinylated anti-CD56 (Caltag Laboratories, La Jolla, CA, USA); Allophycocyanin-conjugated anti-CD3 and TriColor-conjugated anti-CD3; mouse anti-human MICA (R&D Biosystems, Minneapolis, MI, USA); R-PE-conjugated anti-human NKG2D or purified mouse anti-human NKG2D; biotinylated anti-human CD14; mouse IgG2b control (Caltag laboratories), mouse IgG1 control (BD Pharmingen, San Jose, CA, USA) and biotinylated mouse IgG1 control and Allophycocyanin-conjugated goat anti-mouse Ig. The anti-MICA Ab does not cross-react with rhuMICA. A FACS Calibur (BD Biosciences, San Jose, CA, USA) was used for flow cytometric analysis. Human rIL-12 (10 ng/mL) and rIL-15 (100 ng/mL) were obtained from PeproTech (Rocky Hill, NJ, USA). Human FcR blocking reagent (Cohn's fraction) was purchased from Sigma-Aldrich (St. Louis, MO, USA). PGN (10 µg/mL), LPS (100 ng/mL), and polyI:C (50 µg/mL) were obtained from InvivoGen (San Diego, CA, USA). Vehicle controls were: water (for polyI:C and LPS) and 0.02% ethanol (for PGN).

#### ELISPOT Assay

IL-2-expanded uNK cells were plated at  $1-1.5 \times 10^4$  cells/well with Cohn's fraction followed by the blocking antibodies anti-NKG2D, anti-NKp30, or mouse IgG1 (isotype control for both antibodies). Antibodies were added at 1.5 µg/well, so final concentration in each well was 15 µg/mL. Uterine NK cells were incubated with autologous macrophages in complete media at ratios of 3:1 or 10:1. After 48 hrs of culture, cells were tested for IFN-γ production in ELISPOT assays (Mabtech, Cincinnati, OH, USA). Cells were cultured in triplicates in 5% CO<sub>2</sub> at 37°C. Number of spots/well was counted, and means and S.D. calculated.

#### Immunohistochemistry

Cryostat sections (8 µm) were fixed in cold acetone and washed with phosphate-buffered saline (PBS). Sequential blocking was performed with 0.03% hydrogen peroxide, 10% FBS and 1X Cohn's Fraction in PBS followed by use of the Avidin/Biotin Blocking Kit (VECTOR, Burlingame, CA, USA). Serial sections

were incubated with biotinylated mouse anti-human CD14 antibodies, biotinylated mouse anti-human CD56 (Caltag) or isotype IgG. Signal was developed using the VECTASTAIN® Elite ABC Kit (VECTOR). Sections were counterstained with hematoxylin, acid-rinsed, dehydrated, and mounted with Permount (Fisher, Pittsburgh, PA, USA). The human monocytic leukemia cell line THP-1 was obtained from the American Tissue Type Collection. THP-1 cells were treated for 48 hr with 50 ng/mL PMA to differentiate them into adherent M $\phi$  and these cells were used as a positive control for CD14 staining.<sup>21</sup>

### Statistics

Differences between groups were analysed using the Student's *t*-test. *P* value < 0.05 was considered significant.

### Results

#### MICA is Up-regulated on Human Uterine Macrophages after TLR3 Stimulation

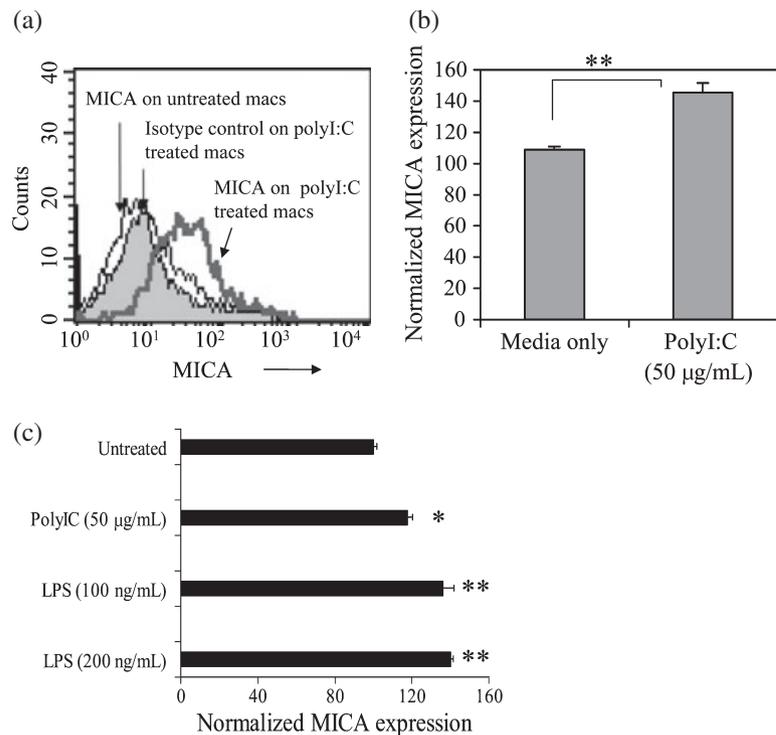
To determine whether MICA is expressed on uterine macrophages, we analysed MICA expression by flow cytometry. Primary CD14<sup>+</sup> macrophages isolated

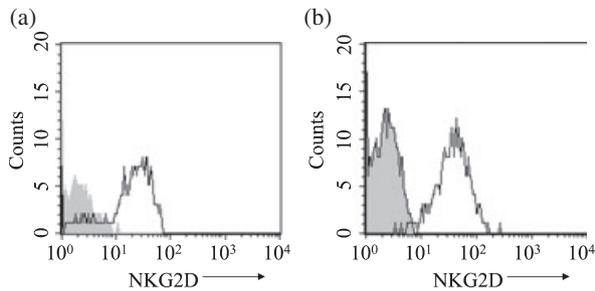
from human endometrium did not constitutively express MICA on the cell surface. PolyI:C stimulated macrophages up-regulated MICA expression by up to 50% (Fig. 1b). Previously, it has been shown that human blood monocytes matured to macrophages by an adhesion method express MICA after LPS stimulation.<sup>16</sup> Blood monocytes were matured to macrophages with M-CSF or GM-CSF to develop the macrophages.<sup>19</sup> These cells up-regulated MICA on the cell surface after exposure to polyI:C (Fig. 1c). The increase in MICA expression on the cell surface of uterine macrophages exposed to polyI:C was higher than that observed on blood-derived macrophages. Similarly, studies with murine peritoneal macrophages have reported that Rael is expressed on the cell surface after polyI:C stimulation.<sup>10</sup> The expression of MICA on the cell surface may allow uterine macrophages to interact with NKG2D-expressing cells, such as NK cells and T cells, present in the human endometrium.

#### Resting and Activated Human Uterine NK Cells Express NKG2D

All blood NK cells express NKG2D, but significant molecular differences have been noted between blood- and decidual NK cells.<sup>22,23</sup> To examine

**Fig. 1** Macrophages up-regulate MHC Class I chain-related protein A (MICA) expression after stimulation with PolyI:C. (a) Uterine macrophages were isolated by digestion of tissue and exposed to PolyI:C (50  $\mu$ g/mL) for 48 hr and analysis of MICA expression was done by flow cytometry. Histograms show representative data. (b) Bar graphs indicate MICA expression normalized to isotype control  $\pm$  S.D. *n* = 3, \*\**P* value < 0.005. (c) Human blood monocytes were treated with macrophage colony stimulating factor (M-CSF) or GM-CSF for 10 days to mature them into macrophages and subsequently stimulated for 48 hr with PAMPs. Bar graphs indicate values normalized to the untreated macrophages  $\pm$  S.D., *n* = 3. Data are representative of monocytes that had been treated with M-CSF. \**P* < 0.05, and an \*\**P* < 0.005.



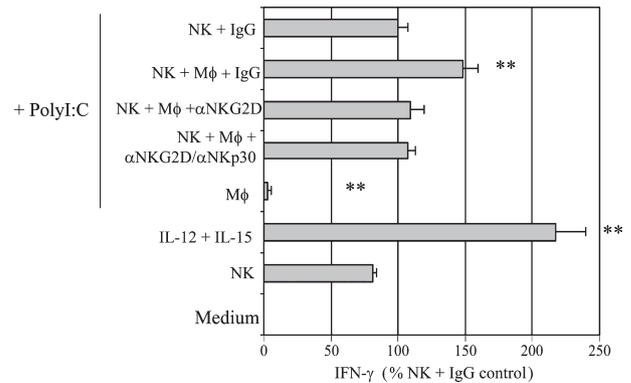


**Fig. 2** Uterine natural killer (uNK) cells express NKG2D (a) Freshly isolated uterine NK cells were isolated by digestion of tissue and stained for NKG2D expression (open) or appropriate isotype control (filled). The histograms shown are gated on CD45<sup>+</sup>, CD56<sup>+</sup>, CD3<sup>-</sup> EM cells. (b) Uterine NK polyclonal cells grown for 7 days in IL-2 media express NKG2D at the cell surface. Histograms shown are representative of four individuals for fresh and activated uNK cells.

NKG2D expression on uterine NK cells, we analysed activated polyclonal NK cells and fresh uterine NK cells. As seen in Fig. 2b, polyclonal NK cell populations express NKG2D on the cell surface. As uNK cells are not consistently in an activated state under physiologic conditions, we also gated on NK cells in freshly isolated endometrial cells (Fig. 2a). Fresh uNK cells within the endometrium expressed the same amount of NKG2D as the activated polyclonal uNK cells. Hence, activation with IL-2 had no effect on NKG2D expression. These data show that freshly isolated human primary uterine NK cells and polyclonal activated uNK cells express NKG2D receptors on the cell surface, which could therefore interact with MICA expressed on activated macrophages.

#### IFN- $\gamma$ Production by PolyI:C Stimulated Human uNK Cells Cultured with Autologous Macrophages is NKG2D-dependent

We have previously shown that uNK cells produced IFN- $\gamma$  after polyI:C stimulation only in the presence of APCs.<sup>1</sup> To determine if human uterine macrophages activate NK cells via NKG2D, experiments were performed with primary uNK cells and autologous uterine macrophages. The data in Fig. 3 demonstrate that co-cultures of uNK cells and macrophages stimulated with polyI:C produced significantly more IFN- $\gamma$  than that by uNK cells alone. Blocking the interaction of NKG2D by pre-treatment of the uNK cells with anti-NKG2D mAbs resulted in decreased IFN- $\gamma$  production to the amount produced by stimulated uNK cells alone. NKp30 is involved in

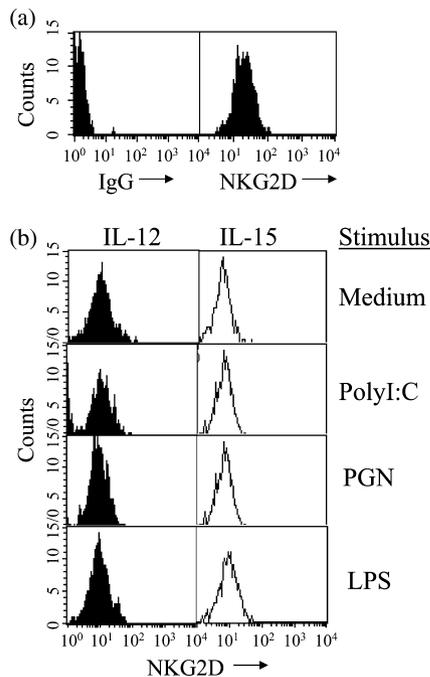


**Fig. 3** Blocking NKG2D interaction between uterine natural killer (uNK) cells with autologous macrophages inhibits IFN-gamma production by NK cells. Tissue macrophages were isolated and frozen while the uNK cells from the same tissue were expanded with IL-2. After pre-incubating uNK cells with blocking antibodies, the two cell types were cultured with polyI:C. Data shown are composite of two patients and are presented as % of NK + IgG control. \*\* $P < 0.01$  compared to NK + IgG control.

DC activation of NK cell IFN- $\gamma$  production,<sup>24</sup> so we tested whether NKp30 was involved in uterine macrophage-uNK interaction as well. Additional blocking with anti-NKp30 mAbs did not lead to any further reduction in IFN- $\gamma$  production. Uterine NK cells alone stimulated with polyI:C did not produce more IFN- $\gamma$  than unstimulated uNK cells, and polyI:C stimulation of macrophages did not result in production of IFN- $\gamma$  (Fig. 3). To test whether the uNK cells produced IFN- $\gamma$  upon stimulation by pro-inflammatory cytokines, polyclonal uNK cells were stimulated with IL-12 (10 ng/mL) and IL-15 (100 ng/mL), as these cytokines have been shown to be important for regulating NK-cell responses.<sup>20,25-27</sup> In accordance with previous results, uNK cells produce significantly higher amounts of IFN- $\gamma$  after stimulation with IL-12 and IL-15 (Fig. 3). Thus, NKG2D receptor recognition is necessary for the activation of human uNK cells with autologous uterine macrophages in response to polyI:C.

#### NKG2D is Not Up-regulated by uNK Clones Stimulated with PAMPs

The endometrial environment contains many cytokines, including IL-12 and IL-15.<sup>20,28,29</sup> The amounts of these cytokines can vary depending on the stage of menstrual cycle. To determine how these cytokines may alter the expression of NKG2D on human uNK cells, NKG2D expression on uNK cell clones



**Fig. 4** NKG2D is not up-regulated by uterine natural killer (uNK) clonal cells stimulated with pathogen-associated molecular patterns (PAMPs). (a) Clonal uNK cells in interleukin (IL)-2 media were stained for NKG2D expression or a suitable isotype control. (b) The same uNK cells in IL-2 media were stimulated for 5 days with PAMPs (as indicated) in the presence of suboptimal cytokines IL-12 (5 ng/mL) or IL-15 (10 ng/mL) and analysed for NKG2D expression (filled and unfilled histograms, respectively). The isotype controls for the stimulated population was equivalent to the unstimulated population and hence not shown here.

was analysed after treatment with IL-12 and IL-15 at suboptimal amounts as studies have demonstrated a requirement for suboptimal cytokines for NK cells to respond.<sup>30</sup> Suboptimal amounts of each cytokine alone is insufficient to trigger IFN- $\gamma$  production, but in combination with other cytokines or PAMPs, they can activate uNK cell clones, but not primary or IL-2-expanded uNK cells.<sup>30</sup> As shown in Fig. 4, the amount of NKG2D receptors on the cell surface did not change in the presence of the PAMPs (polyI:C, PGN, LPS) and cytokines (IL-12 or IL-15). These data indicate that under conditions where uNK cells were exposed to microbial stimuli, the amount of NKG2D expression on the uNK cells remained unchanged. Thus, a possible means of regulation of uNK cell-macrophage interactions is via alterations in the expression of MICA or other NKG2D ligands on the macrophages.

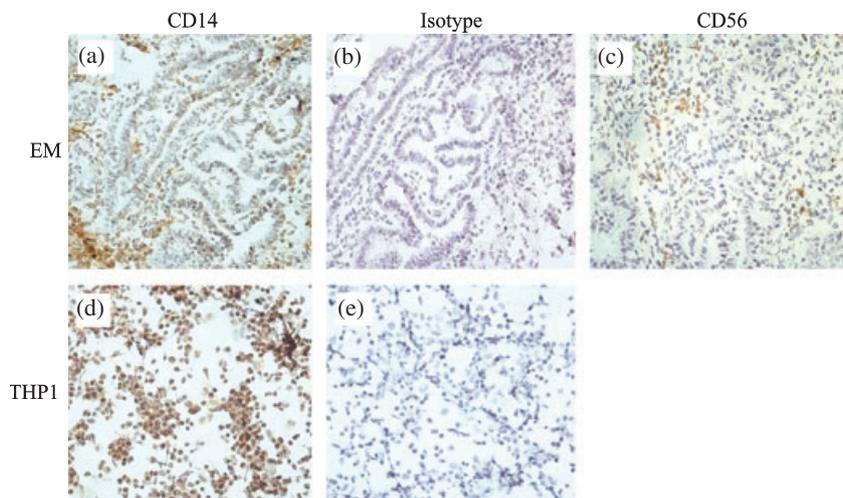
### uNK Cells are in Close Proximity to Uterine Macrophages in the Endometrium

For cells to interact *in vivo*, they must be at the same tissue locations at some point. Various studies have demonstrated the presence of NK cells and macrophages in human endometrium under conditions, including endometritis and pregnancy.<sup>31–34</sup> We sought to verify the location and presence of macrophages and NK cells within the non-pregnant human endometrial tissues that we had used for our studies. We performed immunohistochemical analysis of primary tissue sections. Analysis of CD14 (a macrophage marker) and CD56 (an NK-cell marker) in serial sections of human endometrium showed that both cell types are present abundantly in overlapping locations within the tissue stroma surrounding the glandular epithelium (Fig. 5). Uterine NK cells have also been seen in contact with glandular epithelial cells. Their proximity suggests that these immune cells can interact with each other in the human endometrium and are well-suited to respond to pathogens that may cross the epithelial cell layer.

### Discussion

The human endometrium (EM) is a complex mucosal tissue containing a variety of leukocytes in contact with both stromal and epithelial cells. Studies have shown that blood NK cells interact with human blood-derived macrophages and murine peritoneal macrophages.<sup>10,16</sup> However, there are no studies reported using primary uNK cells and autologous macrophages. It is known that tissue M $\phi$  are markedly heterogeneous and express very different phenotypes compared to blood monocytes, which reflects their functional specialization within particular microenvironments.<sup>35</sup> uNK cells are known to have a unique phenotype that is distinct from blood NK cells.<sup>20,36</sup> In the present study, we examined interactions between human uterine NK cells and uterine macrophages when exposed to PAMPs as pathogen mimetics. We found that TLR3 agonist stimulated uterine NK cells and autologous uterine macrophages interact in an NKG2D-dependent manner. Studies from our lab have shown that MICA is the primarily expressed NKG2D ligand in the human endometrium.<sup>37</sup> Hence, our studies on the endometrium focused on this molecule.

Uterine macrophages express TLR3 but it is unclear which cellular responses are regulated by TLR3 on



**Fig. 5** CD14<sup>+</sup> (macrophages) and CD56<sup>+</sup> (NK) cells are in human endometrium. Specific staining for CD14 (macrophages) (a,d) and CD56 (NK cells) (c) along with isotype control mAbs (b) are shown. EM sections (a,b,c) shown are at 200 $\times$  magnification. Specific and isotype controls shown are consecutive tissue sections. THP1 cells (d,e), used as a positive control for CD14 or with isotype control mAbs, are also shown at 200 $\times$  magnification. The slides were counterstained with hematoxylin.

macrophages (Pioli PA, unpublished data). Uterine NK cells express TLR2, TLR3, and TLR4. IFN- $\gamma$  production by uNK cells is observed in response to PAMPs only when the uNK cells were co-cultured with APCs or as activated uNK cell clones in the presence of cytokines.<sup>1</sup> These data suggest that cell–cell contact with APCs and/or their cytokines is essential for triggering fresh uNK cells. The requirement for cellular interaction may ensure that the uNK cell response is regulated such that it is triggered only when an innate immune response is already underway.

No increase in IFN- $\gamma$  production was observed on stimulation of isolated IL-2-expanded uNK cells with polyI:C alone. This is consistent with studies which show that NK cells require accessory cells for activation in the presence of polyI:C.<sup>1,30,38</sup> However, one study did report direct activation of NK cells via polyI:C.<sup>39</sup> Differences in the methods used for isolation and culture of the cells, numbers of cells used, or serum source may account for the reported differences. Our study also reports that the presence of autologous macrophages led to a significant increase in IFN- $\gamma$  production. IFN- $\gamma$  is involved in the regulation of nearly all phases of the immune and inflammatory responses, including the activation and differentiation of T cells, B cells and macrophages. IFN- $\gamma$  produced by NK cells also promotes DC antigen presentation, which is an important functional link between innate and adaptive immune responses and has implications in anti-tumor and antimicrobial immunity.<sup>40</sup>

The expression of NKG2D on uNK cell clones was not regulated by cytokines or PAMPs. As NKG2D is involved in uNK cell recognition of pathogen-acti-

vated or infected cells, one level for possible regulation of innate immunity would be alterations in NKG2D expression. Our data show that NKG2D expression on fresh or IL-2-expanded uNK cells was identical. This is relevant as co-culture experiments with autologous cells were performed with IL-2-expanded uNK cells. IL-2 stimulation may influence the cytolytic activity of uNK cells and thus these cells might not reflect the activity of resting uNK cells in the human endometrium. However, we have shown that freshly isolated uNK cells produced similar amounts of IFN- $\gamma$  as IL-2-expanded uNK cells in the presence (or absence) of PAMPs.<sup>1</sup> In addition, the cell surface phenotype of fresh and IL-2-expanded uNK cells is very similar. On the other hand, uNK cell clones can be quite different, and clones can be activated directly by PAMPs, which is likely to be a consequence of their initial activation with PHA and irradiated feeder cells. We have also shown that activated uNK cell clones by themselves produce IFN- $\gamma$  in response to PGN or polyI:C when a suboptimal amount of IL-15 is present.<sup>1</sup> Previous studies have shown that the IFN- $\gamma$  response of blood NK cells to TLR2 agonists was enhanced in the presence of suboptimal cytokines, such as IL-15, IL-12, or IL-1 $\beta$ .<sup>30</sup> Uterine macrophages produce IL-15, IL-12 and IL-1 $\beta$  (and Pioli, unpublished data).<sup>7</sup> IL-15 is present in the human endometrium and increases as the menstrual cycle progresses.<sup>28</sup> We analysed whether PAMPs also regulated uNK cell function by altering NKG2D expression. However, the expression of NKG2D was not changed after exposure to PAMPs and cytokines.

Cell surface expression of MICA can be induced on human monocytes and macrophages after

stimulation by PAMPs. Immunoblotting studies on human monocytes reported the presence of MICA protein in monocytes, but it was not detected at the cell surface.<sup>41</sup> We found that blood monocyte-derived macrophages express MICA on the cell surface after polyI:C stimulation. Transcription of MICA and MICB is known to be constitutive in resting and LPS-activated macrophages alike, but protein expression is only detected in macrophages exposed to high doses of LPS.<sup>16</sup> Up-regulation of NKG2D ligands upon LPS activation has also been reported for mouse macrophages.<sup>10</sup> Thus, activation by PAMPs or other inflammatory stimuli may drive the trafficking of intracellular MICA to the cell surface, triggering interaction with other immune cells expressing the NKG2D receptor. There have been no reports determining the effect of polyI:C on NKG2D ligand expression by human macrophages. Our data indicate that there was an up-regulation of MICA by human blood monocyte-derived macrophages, although the change in expression of MICA on these blood-derived macrophages was not as high as it was on uterine macrophages.

In summary, this study demonstrates that MICA is up-regulated on freshly isolated uterine macrophages in the endometrium after TLR3 stimulation. These macrophages can interact with proximally located uNK cells, which can result in uNK cells producing IFN- $\gamma$ . These findings indicate that the interaction of NKG2D receptors and their ligands are an important mechanism for triggering innate immune cell activation within the human endometrium. These data suggest that the innate immune response to viral infection in the human endometrium involves cellular interactions between the uNK cells and macrophages via the NKG2D receptor and MICA.

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