TLRs Mediate IFN-γ Production by Human Uterine NK Cells in Endometrium[1]

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The human endometrium (EM) contains macrophages, NK cells, T cells, B cells, and neutrophils in contact with a variety of stromal and epithelial cells. The interplay between these different cell types and their roles in defense against pathogen invasion in this specialized tissue are important for controlling infection and reproduction. TLRs are a family of receptors able to recognize conserved pathogen-associated molecular patterns. In this study, we determined the expression of TLRs on uterine NK (uNK) cells from the human EM and the extent to which uNK cells responded to TLR agonist stimulation. uNK cells expressed TLRs 2, 3, and 4, and produced IFN-γ when total human endometrial cells were stimulated with agonists to TLR2 or TLR3 (peptidoglycan or poly(I:C), respectively). Activated uNK cell clones produced IFN-γ upon stimulation with peptidoglycan or poly(I:C). However, purified uNK cells did not respond directly to TLR agonists, but IFN-γ was produced by uNK cells in response to TLR stimulation when cocultured with APCs. These data indicate that uNK cells express TLRs and that they can respond to TLR agonists within EM by producing IFN-γ. These data also indicate that the uNK cells do not respond directly to TLR stimulation, but rather their production of IFN-γ is dependent upon interactions with other cells within EM. ***The Journal of Immunology,*** **2006, 176: 6219–6224.**

The human endometrium (EM)[3] is a complex mucosal tissue with a unique immune cell component, and it is regulated by sex hormones throughout the menstrual cycle (1, 2). The EM must be prepared to respond to potential pathogen challenges, yet be able to control immune cell responses to allow the development of a semiallogeneic fetus. In the nonpregnant state, there are a tightly controlled influx, spatial compartmentalization, and regulation of immune cells (3, 4). Unlike in the murine uterus, uterine NK (uNK) cells in the human uterus are found in large numbers spread throughout the EM with increasing numbers as the menstrual cycle progresses (5–7).

NK cells play an important part in the innate immune system and were first defined functionally by their ability to kill certain tumors and virally infected cells without a requirement for MHC restriction or previous immunization (8). NK cells produce immunoregulatory cytokines that contribute to early host defense against several types of viruses, bacteria, and parasites. In humans, ~10% of PBLs are NK cells (9). Human NK cells can be defined phenotypically by the expression of CD56 and the absence of CD3, and NK cells fall into two subsets according to their surface density of CD56. uNK cells account for a large percentage of leukocytes in the human EM; express high levels of CD56, low, or no CD16; and lack CD57 expression (10–12). In addition, uNK cells express other molecules not found on blood NK cells (12).

TLRs are used by the innate immune system to recognize microorganisms and their products. Eleven TLRs have been identified, and they recognize conserved pathogenic structures termed pathogen-associated molecular patterns. TLR2 binds to microbial products, including peptidoglycan (PGN) and zymosan, while TLR4 binds to LPS derived from Gram-negative bacteria. TLR3 recognizes dsRNA associated with viral replication; thus, poly(I:C), which is a synthetic mimetic for dsRNA, can induce TLR3 signaling. Several studies reported the expression of TLRs on blood NK cells, but conflicting results have been obtained in functional studies using blood NK cells depending on the effector functions examined and which TLRs were investigated (13–17). One controversial issue is the extent to which NK cells respond to specific TLR ligands. A direct stimulation of blood NK cells by TLR agonists has been reported by some groups (13, 14), yet others demonstrate an additional requirement for suboptimal cytokines in order for NK cells to respond (15). Both indirect and direct effects of TLR stimulation on effector functions, cell-cell contact dependence, and requirements for cytokines have been reported (16, 17). This study examines the expression of TLRs on uNK cells and the ability of uNK cells to produce cytokines in response to TLR agonists alone or in the presence of other EM cells.

Materials and Methods

Isolation of human endometrial cells

Endometrial tissue specimens were obtained from women undergoing a hysterectomy for various gynecological disorders. We used samples from 32 patients with an average age of 43 ± 6 years. Initial patient diagnosis included fibroids, pelvic pain, menorrhagia, endometriosis, cervical stenosis, and prolapse. Endometrial samples were staged as proliferative, secretory, or inactive/atrophic. The tissue samples that we used were distal to any pathological changes. We used a cell dispersion method that used treatment with an enzyme mixture composed of collagenase and DNase, followed by passage of cells through a mesh screen to facilitate cell dispersion (18). Any RBC present were eliminated from the endometrial cells by treatment with lysis buffer (NH_4Cl/Tris-HCl) for 5–10 min at room temperature. Blood cell contamination of these endometrial tissue cells was

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[3] Abbreviations used in this paper: EM, endometrium; Ct, cycle time; FRT, female reproductive tract; LPG, lipophosphoglycan; PGN, peptidoglycan; uNK, uterine NK.

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<2% (19). The isolated cells were cultured or used for experimental treatments directly. All human studies were done with approval of the Dartmouth Institutional Review Board.

Abs and reagents

The following Abs were used: FITC-conjugated or TriColor-conjugated anti-CD45, allophycocyanin-conjugated anti-CD3, and R-PE-conjugated anti-CD56 (MEM-188) (Caltag Laboratories); PE Cy7-conjugated anti-CD56 (B159), allophycocyanin-conjugated anti-IFN-γ (B27), and allophycocyanin-conjugated or PE-Cy7-conjugated mouse IgG1 control (BD Pharmingen); FITC-conjugated anti-TLR2 and biotin anti-TLR3 (eBioscience); biotin anti-TLR4 (Serotec); and FITC streptavidin (Jackson ImmunoResearch Laboratories). Human rIL-12 and rIL-15 were obtained from PeproTech. Human FcR blocking reagent (Cohn's fraction) was purchased from Sigma-Aldrich. PGN (10 μg/ml), zymosan (10 μg/ml), and poly(I:C) (50 μg/ml) were obtained from InvivoGen. Vehicle controls were: PBS (poly(I:C)), 0.2% ethanol (PGN), and 0.1% ethanol (zymosan).

Flow cytometry and cell sorting

A FACSCalibur (BD Biosciences) was used for flow cytometric analysis of cell surface staining of all samples. A FACSAria cell sorter was used for purifying uterine cells. Immune cell populations were sorted from EM cells using the following gating strategy: $CD45^+CD3^-CD56^+$ (uNK cells) and $CD45^+CD3^-CD56^-CD15^-$, high forward light scatter (APC). The purity obtained for uNK cells was typically higher than 99%. uNK cell clones were grown, as described, and their phenotype was confirmed by flow cytometry ($CD56^+$, $CD3^-$) (12). For intracellular IFN-γ analysis, freshly prepared endometrial cells were cultured at 3×10^5 cells/well in 96-well U-bottom microtiter plates for 18 h. Brefeldin A (10 μg/ml) was added to each well 5 h before harvesting to allow for accumulation of intracellular proteins. Cells were harvested and stained for CD3, CD45, and CD56; fixed; and permeabilized with saponin (0.1%). The cells were then stained intracellularly with anti-IFN-γ mAb or IgG control, washed, and analyzed by flow cytometry.

TLR stimulation of uNK cells and uNK cell clones

ELISA. Cells from enzymatic digestion of human endometrial tissue were seeded at 2×10^5 cells/well in microtiter plates in complete medium (RPMI 1640 supplemented with 2-ME (50 μM), penicillin (100 U/ml), streptomycin (100 μg/ml), sodium pyruvate (1 mM), nonessential amino acids (0.1 mM), and 5% human serum). uNK cell clones were seeded at 5×10^4 cells/well in complete medium. After stimulation by poly(I:C) (50 μg/ml), PGN (10 μg/ml), zymosan (10 μg/ml), medium only, or IL-12 (10 ng/ml)/IL-15 (100 ng/ml) in culture for 72 h, cell-free supernatants were harvested and analyzed for the production of IFN-γ.

ELISPOT. Freshly prepared endometrial cells were stained for CD45, CD3, CD8, and CD56. Immune cell populations were then sorted using the following gating strategy: $CD45^+CD3^-CD56^+$ (uNK cells) and $CD45^+CD3^-CD56^-CD15^-$, high forward light scatter (APC). Cells were then tested for IFN-γ production in ELISPOT assays (Mabtech). Briefly, cells were plated at $4–5 \times 10^3$ cells/well with or without APCs (NK cell: APC ratio = 1:1–1:3) and cultured in triplicate for each treatment in 5% CO_2 for 36 h at 37°C. Numbers are presented as the sum of spots for each triplicate.

Isolation of RNA and production of cDNA

Total RNA was prepared from sorted endometrial cells using a micro RNAeasy kit (Qiagen). DNA was removed and RNA was amplified up to 30 times using MessageAmpII technology (Ambion). Synthesis of first-strand cDNA was conducted using the First Strand cDNA Synthesis Kit from Fermentas, following the manufacturer's instructions. Control sample cDNA that served as a positive control was obtained from RNA derived from total EM tissue.

Quantitative real-time PCR

Duplicate samples containing 12.5 ng of cDNA, SYBR green mix (Applied Biosystems), and real-time PCR primers designed for the 3′ end of the TLR genes were mixed and analyzed on an ABI7700 thermal cycler (Applied Biosystems). To calculate the relative amount of gene product present in the sample, cycle time (Ct) was determined. The average Ct value was calculated from duplicate wells for each sample with each primer set. Most duplicate samples varied by <0.5 Ct. Nontemplate control samples were evaluated for each primer set. The relative gene expression for each individual cDNA sample was determined by calculating δ Ct values (ΔCt) by subtraction of the Ct value for β-actin primers from the Ct value for each TLR primer. The expression of each gene was determined in each experiment by the formula: expression = $10{,}000 \times 1/(2^{\Delta Ct})$.

Statistics

Statistical comparisons were done using a paired *t* test, as indicated. A value of $p < 0.05$ was considered significant.

Results

NK cells in the human uterine EM express TLRs

To determine whether uNK cells within the EM expressed TLRs, we used flow cytometric analysis of freshly prepared single cell suspensions of human endometrial cells. uNK cells expressed TLR4 and TLR3 (Fig. 1, *A* and *B*), but no surface expression of TLR2 was detected on uNK cells (Fig. 1*C*). Because previous reports have shown that TLR2 may be expressed intracellularly in the absence of surface expression (20), we also stained fresh EM cells for intracellular TLR2. Positive intracellular staining by TLR2 mAb on uNK cells indicated that TLR2 was expressed by uNK cells, but it was not localized on the cell surface (Fig. 1*D*).

To confirm the expression of TLRs by uNK cells indicated by the flow cytometry data, we analyzed the level of TLR mRNA in highly purified human uNK cells. Total RNA was isolated from freshly sorted or IL-2-activated uNK cells, and TLR gene expression was determined by quantitative real-time PCR using cDNA. All three TLRs were expressed by uNK cells (Fig. 2). TLR2 had the highest relative mRNA expression among the TLRs tested, followed by TLR4 and TLR3. In addition to the purified uNK cells, we also analyzed uNK cell clones and found that TLRs 2, 3, and 4 were expressed at levels that were comparable to the sorted fresh uNK cells (data not shown). Thus, uNK cells express TLR2, TLR3, and TLR4.

uNK cell clones respond directly to TLR agonists

The expression of a particular TLR is not always sufficient to determine whether a given cell will respond to an agonist for that receptor. We cultured uNK cell clones in the presence of agonists for TLR2 or TLR3 with and without suboptimal amounts of IL-15. Low amounts of cytokines have been shown to allow blood NK cells to respond to TLR agonists (15). We observed that uNK cell

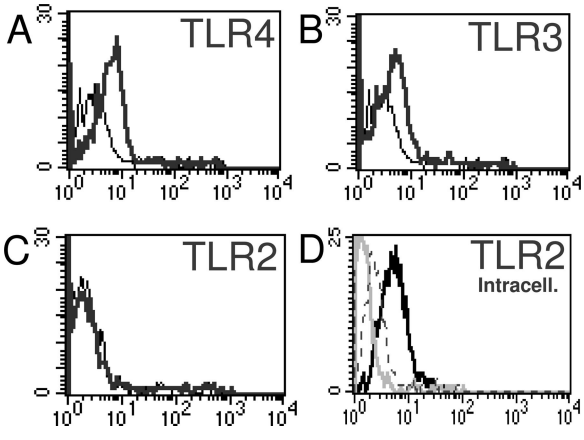

FIGURE 1. uNK cells express TLRs. Fresh human endometrial cells were isolated and analyzed for expression of TLRs. Following enzyme digestion of endometrial tissue, uNK cells ($CD56^+CD3^-CD45^+$) were stained for CD45, CD3, CD56, and TLR2 (*C*); TLR3 (*B*); or TLR4 (*A*). Panels show anti-TLR staining (thick line) and control staining (*A–C*) (thin line). *D*, Shows intracellular staining for TLR2 (thick line), intracellular control (broken line), and surface control (gray line). Data are representative of four experiments.

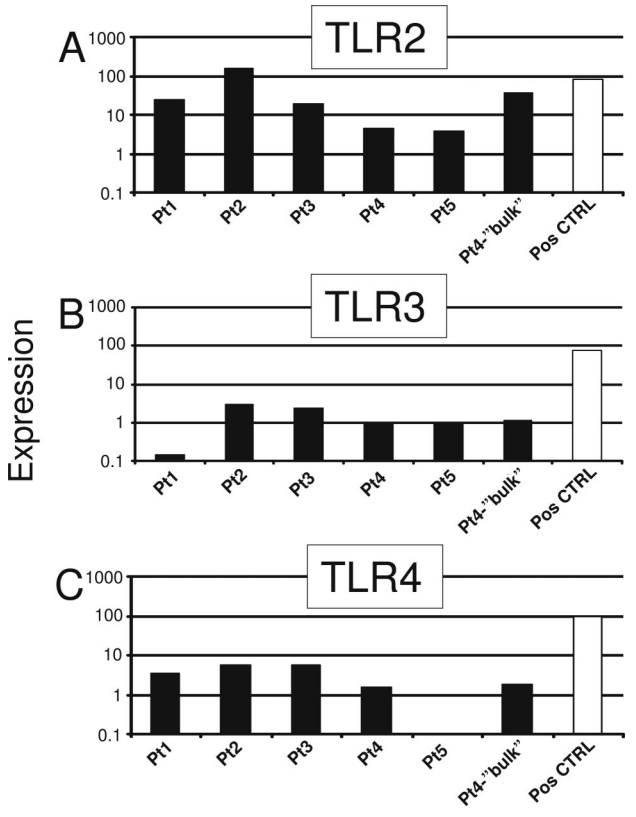

FIGURE 2. uNK cells express TLR mRNA. Purified uNK cell expression of TLR2 (*A*), TLR3 (*B*), and TLR4 (*C*) was determined by quantitative real-time PCR. Bars show relative expression levels of uNK cell TLR mRNA isolated and purified from five different patients (Pt1–5). A proportion of the uNK cells from Pt4 was cultured in IL-2 (500 U/ml) for 13 days before RNA isolation (Pt4-"bulk"). Positive control samples consisted of RNA from total EM tissue. Negative control samples showed no signal (data not shown).

clones produced IFN-γ when cultured with poly(I:C) (a TLR3 agonist) alone (Fig. 3). The low amounts of IL-15 (≤2 ng/ml) did not induce IFN-γ production above medium only amounts, but the combination of TLR agonist (poly(I:C) or PGN) and IL-15 resulted in a larger production of IFN-γ by the uNK cell clones. These data indicate that uNK cells express functional TLR2 and TLR3, and triggering of these receptors results in production of IFN-γ.

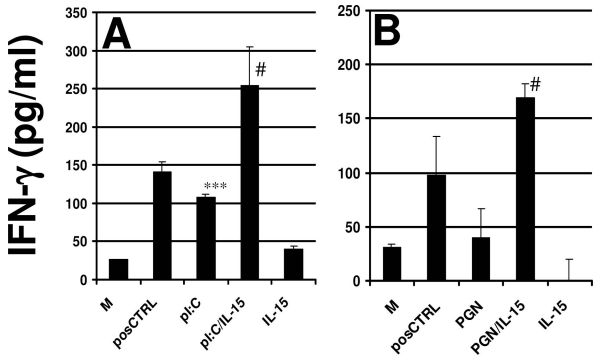

FIGURE 3. uNK cell clones respond directly to TLR agonists. uNK cell clones were cultured with poly(I:C) (*A*) or PGN (*B*) for 72 h, and supernatants were harvested and analyzed for IFN-γ production by ELISA. In some wells, a suboptimal dose of IL-15 (*A*, 2 ng/ml; *B*, 0.4 ng/ml) was included, as indicated (IL-15). Medium only (M) and IL-12/IL-15 (*A*, 10 ng/ml/100 ng/ml) or PMA/ionomycin (*B*, 10 ng/ml/1 μg/ml) were included as controls (posCTRL). These results are representative of seven different uNK cell clones tested. ($p < 0.001$: ***, compared with medium only; $p < 0.05$: #, compared with TLR agonist only).

Fresh EM cells produce IFN-γ in response to TLR stimulation

To determine whether human EM cells produce IFN-γ in response to TLR agonists, we analyzed the production of IFN-γ by total EM cells in response to TLR2 and TLR3 stimulation. Fresh human endometrial cells were cultured for 72 h in the presence of TLR agonists and then analyzed for IFN-γ production by ELISA. As shown in Fig. 4, poly(I:C) and PGN induced significant IFN production by EM cells compared with medium only. The highest IFN-γ production was seen after poly(I:C) stimulation (1442 pg/ml), and a high response toward PGN (1104 pg/ml) was found. We did not see a significant induction of IFN-γ production after stimulation with zymosan (479 pg/ml) compared with medium alone (320 pg/ml). These data indicate that total human endometrial cells can produce significant amounts of IFN-γ in response to selective agonists for TLR2 or TLR3.

Fresh uNK cells produce IFN-γ within the EM in response to TLR stimulation

To determine whether uNK cells within EM produce IFN-γ in response to TLR agonists, freshly prepared human endometrial cells were cultured in the presence of poly(I:C), PGN, or zymosan for 18 h, and analyzed for intracellular IFN-γ production by uNK cells (Fig. 5). IFN-γ production was observed in 6.4% of $CD56^{+}CD3^{-}$ uNK cells from endometrial cells cultured in the presence of poly(I:C) compared with 0.8% of uNK cells from endometrial cultures grown in medium without poly(I:C) stimulation. A total of 5.6% of the uNK cells produced IFN-γ in response to IL-12/IL-15. No IFN-γ-positive cells were observed in isotype control samples (Fig. 5*D*). A summary of data from five patients shows the percentage of uNK cells within a total EM population that produced IFN-γ in response to TLR2 or TLR3 agonists, medium alone, or IL-12/IL-15 stimulation (Fig. 5*E*). Consistent with

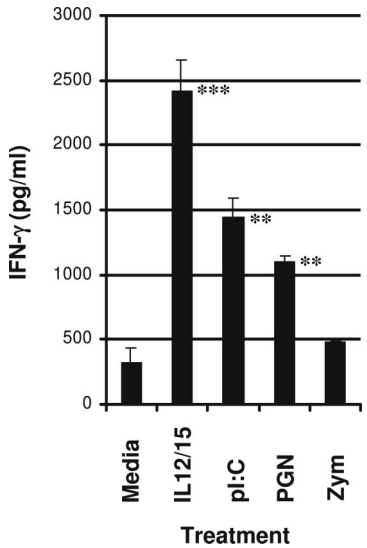

FIGURE 4. Cytokine production by EM cells in response to TLR agonists. Fresh human endometrial cells were cultured with TLR agonists for 72 h. Cell-free supernatants were assayed for IFN-γ by ELISA. Panels show IFN-γ release by EM cells activated by poly(I:C) (pI:C), PGN, or zymosan (Zym). IL-12/IL-15 and medium were included as positive and negative controls, respectively. Data presented are representative of three experiments ($p < 0.001$: ***; $p < 0.01$: **, compared with medium only).

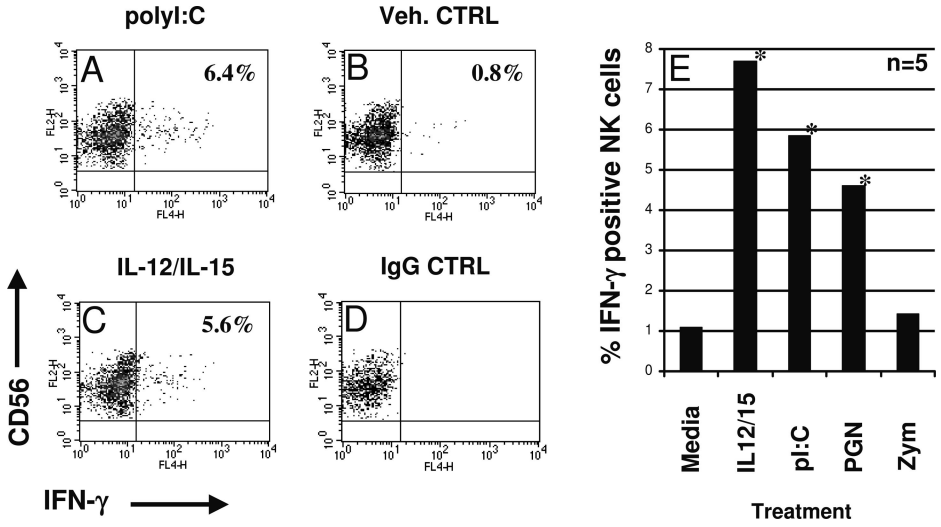

FIGURE 5. Agonists for TLR2 and TLR3 stimulate IFN-γ production by uNK cells. Fresh human endometrial cells were cultured for 18 h with poly(I:C) alone (*A*), together with IL-12/IL-15 (*C*), vehicle control without poly(I:C) (*B*), or control IgG1 (*D*) and analyzed for intracellular IFN-γ in uNK cells by flow cytometry. *E*, Shows the mean percentage of IFN-γ-producing uNK cells within total EM cells stimulated with poly(I:C) (pI:C), PGN, or zymosan (Zym). Results are shown as an average from five different patients ($p < 0.05$: ∗, compared with medium only).

the IFN-γ production by total fresh EM cells in response to TLR agonists, uNK cells produced IFN-γ in response to IL-12/IL-15, poly(I:C), and PGN stimulation. No significant increase was observed in the percentage of uNK cells that produced IFN-γ in response to zymosan compared with cells cultured in medium only. When zymosan was used at five times the dose, there was no IFN-γ production by uNK cells, demonstrating that the inability to respond to zymosan was not likely due to insufficient amounts of agonist (data not shown). Our initial data show that there was no IFN-γ production by uNK cells upon LPS stimulation, and <0.5% of T cells produced IFN-γ under these conditions (data not shown). These data show that uNK cells within EM respond to TLR agonists by producing IFN-γ, and that uNK cells are responsible for the IFN-γ produced within the EM after TLR stimulation.

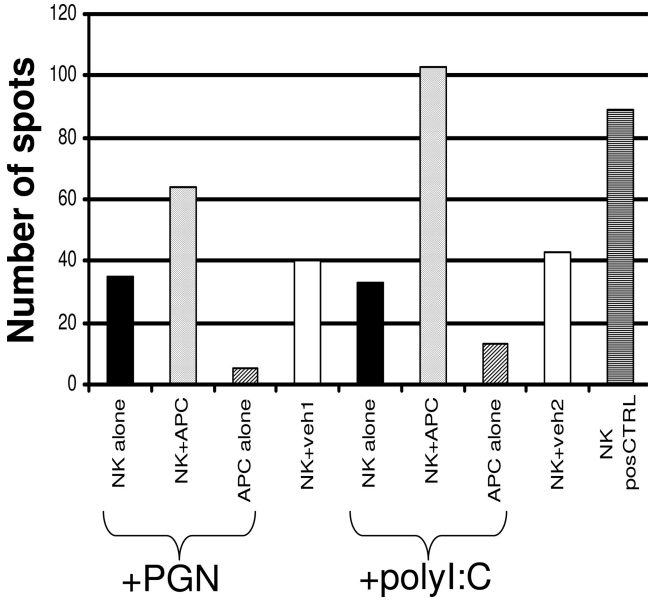

FIGURE 6. TLR agonists induce IFN-γ production by purified uNK cells in the presence of EM APCs. Freshly sorted uNK cells were cultured with PGN and poly(I:C) for 36 h, and IFN-γ production was determined by ELISPOT. Panel shows the results from uNK cells alone or cocultured with APC (1:1.5 ratio), or APC alone. IL-12/IL-15 (NKposCTRL) and vehicle only were included as controls. Data shown are representative of four experiments.

Freshly sorted uNK cells do not directly respond to TLR agonists

Although uNK cell clones were able to respond to TLR2 or TLR3 agonists, it was possible that fresh uNK cells may require additional signals to respond via these receptors. To determine whether uNK cells responded directly to TLR agonists, freshly sorted human uNK cells were cultured in the presence of TLR agonists PGN or poly(I:C) (Fig. 6). No significant differences in IFN-γ production were observed between uNK cells treated with poly(I:C), PGN, or zymosan (data not shown), compared with control samples (NK + vehicle). To determine whether IFN-γ production by uNK cells required the presence of other immune cells, we mixed purified uNK cells and purified APCs that were sorted from EM cells and stimulated these cultures with TLR agonists. We observed a significant increase in the number of IFN-γ-producing cells when uNK cells were cocultured with APCs: PGN, 1.8-fold and pI:C, 3.1-fold, respectively. We also tested whether cytokines or cell supernatants could substitute for cells. There was no significant increase in the IFN-γ production by purified uNK cells after TLR stimulation in the presence of suboptimal amounts of IL-15 or APC conditioned medium compared with those that were cultured with TLR agonist only (data not shown). This suggested that the synergistic effect of APCs could not be explained by soluble factors produced by APCs alone, but most likely required cell-cell contact or both cell-cell interactions in combination with locally produced soluble factors.

The percentage of purified uNK cells that produced IFN-γ spontaneously was 28% of the number of cells that responded in the positive control samples in the ELISPOT assays ($n = 7$), suggesting that some uNK cells were able to produce IFN-γ even in the absence of stimulatory treatment. Spontaneous secretion of IFN-γ by purified uNK cells is supported by a previous study in which IFN-γ, IL-4, and IL-10 were found to be spontaneously produced by NK cells isolated from early pregnancy decidua (21). In our study, there was little spontaneous IFN-γ production by purified uterine T cells (4.8% of control; $n = 6$) or blood NK cells (7% of control; $n = 2$). Collectively, these data demonstrate that uNK cells can respond with IFN-γ production to TLR2 or TLR3 stimulation, but require the presence of other cells.

Discussion

This study examines TLR function and TLR-mediated responses on human uNK cells. uNK cells express TLR2, TLR3, and TLR4,

and there was significant IFN-γ production by uNK cells in response to TLR agonists when examined as a contextual part of total EM cells. However, we found that uNK cells did not respond directly to TLR agonists, yet uNK cells did respond to TLR agonists when cocultured with APCs or as uNK cell clones in the presence of cytokines. These data suggest that cell-cell contact with APCs and/or their cytokines is essential for triggering fresh uNK cells. The requirement for accessory cell involvement may be an important regulatory component to ensure that uNK cells in the EM produce IFN-γ only after other innate responses have been stimulated. Samples were derived from women in the proliferative, secretory, or inactive/atrophic stages, and we observed similar uNK cell responses from these different samples, suggesting that the menstrual cycle was not a factor in determining the response of uNK cells under these conditions.

The data indicate that fresh, purified uNK cells do not respond directly to TLR3 stimulation, while activated uNK cell clones were able to respond to TLR stimulation alone. It is possible that cell-cell interactions enable fresh uNK cells to respond to TLR3 agonists directly or in combination with cytokines produced by other nearby cells. These results are consistent with results obtained with blood NK cells in that stimulation through TLR3 and TLR9 did not directly induce cytokine production by NK cells (15). This is also in agreement with other studies in which NK cell cytokine responses to poly(I:C) were dependent upon the presence of other accessory cells (16, 17), although one study did report direct activation of NK cells via poly(I:C) (13). Technical differences in cell purification, culture conditions, numbers of cells used, or serum source may also account for reported differences. As one component of the EM, uNK cells produce IFN-γ upon stimulation with poly(I:C). This finding suggests that uNK cells are an important part of the cell-mediated immune defenses in human EM.

Whereas poly(I:C) and PGN were able to induce IFN-γ by uNK cells within the EM, we did not observe significant uNK cell responses to zymosan stimulation. This finding is particularly interesting because both PGN and zymosan are TLR2 agonists. The differential response to these agonists could be due to unique features of how TLR2 recognizes these molecules. Differences in TLR responses depending on structural differences between ligands have been previously reported (22). This study showed that the levels of activation of NK cells were dependent on quantitative and qualitative changes that occur in phosphosaccharide repeat units of lipophosphoglycan (LPG) from *Leishmania major*. There was a variable potency between LPG from metacyclic promastigotes and procyclic promastigotes to stimulate blood NK cells, even though TLR2 is involved in binding both forms of LPG. The results suggest that blood NK cells are able to detect differences in the number of phosphosaccharide repeat domains or in the glycan side chains. It is possible that a similar distinction is made by the TLR2 on uNK cells or on accessory cells when recognizing PGN compared with zymosan in EM, thus explaining the differential effects resulting from treatment with these TLR ligands. PGN is derived from Gram-positive bacteria, while zymosan is derived from yeast, so one implication of this finding is that uNK cells may be more likely to produce IFN-γ in response to a bacterial infection rather than a yeast infection. Another study reported that zymosan was able to inhibit the cytotoxicity of blood NK cells, so the differential activation may be due to an inhibitory effect of zymosan on NK cells (23). In this study, IFN-γ production was the cytokine examined. It may be that zymosan is able to induce cytokines other than IFN-γ or other responses from uNK cells.

One study using a TLR2 agonist, KpOmpA, demonstrated direct activation of human blood NK cells (14). The IFN-γ response of blood NK cells to this molecule was greatly enhanced in the presence of suboptimal cytokines, such as IL-15, IL-12, or IL-1β. However, we did not observe direct activation of fresh uNK cells in the presence of PGN or zymosan, which are also TLR2 agonists. This may be due to differential expression of signaling components or other differences in TLR function between fresh uNK cells and blood NK cells. Interaction with nearby cells may therefore be more important for the regulation of uNK cells within the female reproductive tract (FRT) compared with NK cells in blood. Activated uNK cell clones did respond directly to PGN in combination with low amounts of IL-15. IL-15 is expressed in the human EM and increases throughout the menstrual cycle (24). Given the demonstrated effects of IFN-γ on the vascular remodeling and trophoblast invasion in the FRT, it is possible that IFN-γ production may be more restricted within EM so that uNK cells do not respond to the presence of pathogenic ligands without additional signals provided by other stromal cells. These EM cells could be APCs, as demonstrated in this study, or they could be other EM cells such as epithelial cells or fibroblasts.

The differential expression of TLRs within tissues of the FRT suggests that the responses to pathogenic challenge may be dependent on the site of entry of a particular pathogen (25). TLRs are expressed at higher levels in the upper FRT than in the lower reproductive tract. It is possible that NK cells in other tissues within the FRT may respond differently to TLR stimulation than uNK cells. Interestingly, uterine epithelial cells have been shown to respond to poly(I:C) by producing proinflammatory cytokines such as TNF-α, IL-6, GM-CSF, and G-CSF (26). Endometrial macrophages express both TLR2 and TLR3 and are potential candidates for accessory cells to uNK cells during TLR stimulation of EM. Macrophages triggered via TLR2 or TLR3 are a potent source of cytokines (27–29). However, it remains unclear whether there are sufficient cytokines to substitute for cell-cell contact during activation of resting uNK cells. It is likely that both cell-cell interactions and cytokines are involved in the activation of uNK cells during infection.

NK cells and other elements of the innate immune system are present in the EM, and are most likely involved in both successful pregnancy and defense against invasion by microorganisms. Our data demonstrate that uNK cells respond to TLR ligand stimulation within the EM by producing IFN-γ, and that accessory cells are required for triggering of resting uNK cells. NK cell IFN-γ may activate macrophages and promote B cell IgG responses to remove the bacteria (30). Taken together, these findings suggest that uNK cells can play a role in the immune defense within the EM following infection within the FRT.

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Disclosures

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