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Unique characteristics of NK cells throughout the human female reproductive tract

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Abstract In this study, we have analyzed the presence and subsets of NK cells throughout the tissues of the FRT. We demonstrate that there are NK cells in the various FRT tissues and that their phenotype and regulation are largely dependent upon the FRT tissue where they reside. NK cells in the Fallopian tube, endometrium, cervix, and ectocervix expressed CD9 while blood NK cells did not. We have also found that unique subsets of NK cells are in specific locations of the FRT. The NK cells in the lower reproductive tract did not express CD94, but they did express CD16. In contrast, NK cells in the upper FRT express high amounts of CD94 and CD69, but few NK cells expressed CD16. All of these FRT NK cells were able to produce IFN-γ upon stimulation with cytokines. Furthermore, the number of NK cells varied with the menstrual cycle in the endometrium but not in the cervix or ectocervix. These data suggest that unique characteristics of the tissues may account of specific localization of different NK cell subsets.

Introduction

Natural killer (NK) cells are an important part of the innate immune defenses [1]. NK cells have the ability to recognize and kill many types of tumor cells using several different receptors, and they are also able to produce a variety of cytokines, including IFN-γ, GM-CSF, and TNF-α [2–4]. In human blood, NK cells account for approximately 10% of mononuclear cells and have been divided into two major functional subsets based on bright or dim expression of CD56 [2]. $CD56^{bright}$ cells account for only 10% of blood NK cells, whereas in lymph nodes 100% of NK cells are $CD56^{bright}$. These NK cells express little CD16 and are less cytotoxic against tumor cells than $CD56^{dim}$ NK cells, yet they produce more cytokines per cell than $CD56^{dim}$ NK cells [5]. The $CD56^{bright}$ NK cells are believed to help direct immune responses and promote adaptive immunity through their production of cytokines, while the $CD56^{dim}$ NK cells express CD16 and are highly lytic cells against tumor cells. Thus, different subsets of human NK cells have unique functional

Abbreviations: FT, Fallopian tube; EM, endometrium; CX, cervix; ECX, ectocervix; FRT, female reproductive tract.

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capabilities [6]. Understanding the localization and properties of NK cells in different tissue environments is necessary to fully understand innate immunity and the development of adaptive immune responses.

NK cells are found in human endometrium (EM) where they account for a large percentage of total leukocytes in the later stages of the menstrual cycle [7–9]. The increase in NK cells is thought to be due to a combination of influx of NK cells from the blood and proliferation of NK cells within the EM. The key chemokines that recruit NK cells into the EM are ligands for CXCR3. CXCL10 and CXCL11 are regulated by estrogen and progesterone in human EM, while other chemokines, including CCL4 and CCL5, are not regulated by sex hormones [10]. The expression of CXCL9 and CXCL10 is higher in the secretory phase, and the amount of these chemokines correlates with the number of NK cells in EM [7,10]. Blood NK cell subsets are thought not to be recruited equally into the EM. The NK cells found in EM are more similar to the $CD56^{bright}$ subset, although uterine NK (uNK) cells have several distinct features [11–13]. UNK cells have high expression of CD56 and CD94, little CD16 or CD57, but they also express KIRs and CD9. These findings suggest that, as NK cells migrate into the EM, they appear to differentiate further to acquire unique characteristics that may be important for their role in EM.

The female reproductive tract (FRT) is a mucosal tissue that is adapted to allow the development of a semi-allogeneic fetus [14]. Mechanisms must exist that protect the developing fetus from the maternal immune response while allowing immune defenses to respond to potential pathogens that attempt to invade the FRT. Far from being a sterile environment, the FRT is host to a variety of commensal organisms. The lower FRT (vagina and ectocervix) are colonized by a considerable number of microorganisms, while the upper FRT (cervix, uterus, and Fallopian tube) has a much lower constitutive microorganism load. Although constantly in contact with microorganisms, the FRT is protected by a number of physical and innate immune defenses. The interaction of commensal microorganisms and the innate defenses of the FRT are very poorly understood, yet these interactions are vital for resistance to infection within the FRT. Each FRT tissue has unique, well-characterized functions in reproduction. The Fallopian tube is where fertilization takes place, implantation is in the uterus, and the lower tract epithelium serves as a barrier against invading organisms. Less clear is the organization and function of immune defenses in these tissues. NK cell activity, as evidenced by lysis of K562 tumor cells, has been shown for leukocyte populations from throughout the FRT [15]. However, NK cells could not be identified in these studies due to the isolation procedures that were used. NK activity, as measured by lysis of tumor cells, can be mediated by subsets of activated T cells and it can be increased by inflammation. Thus, lysis of K562 cannot describe the nature of the NK cells present in a particular sample.

In this study, we analyzed the presence and subsets of NK cells throughout the tissues of the FRT. We demonstrate that there are NK cells in the various FRT tissues and that their phenotype and regulation are largely dependent upon the tissues in which they reside. We have also reported the presence of unique subsets of NK cells that are localized to the lower FRT and the upper FRT. These data suggest that unique characteristics of the tissues may account for specific localization and function of NK cell subsets in the FRT.

Materials and methods

FRT samples

FRT specimens were obtained from women undergoing elective hysterectomy for various gynecological disorders at the Dartmouth Hitchcock Medical Center. We used samples from 37 patients with an average age of 51 (±13) years. The preliminary patient diagnoses included genital prolapse, fibroids, pelvic pains, and menorrhagia. Tissue samples used were distal to the pathological changes and processed as previously described [16]. Briefly, tissues were minced with sterile scissors/surgical blades, and single cell lymphocytes released by digesting the tissue with 0.1% type IV collagenase and 0.01% DNase I (both from Sigma-Aldrich) at 37 °C in DMEM-F12 for 1 h. Thereafter cells were passed through a 100 μm cell strainer to facilitate cell dispersion. There was minimal blood contamination, but if present, RBCs were eliminated by treatment with lysis buffer (NH_4Cl/Tris–HCl) for 5 min at room temperature. The resulting isolated single cell suspensions were used for flow cytometry staining. This study was conducted with approval of the Dartmouth Institutional Review Board.

Isolation of PBMCs

We obtained peripheral blood from healthy donors, and PBMCs were separated on lymphoprep gradients, according to protocols provided by the manufacturer (Axis Shield, Oslo, Norway). Sample collection was approved by Dartmouth College Institution Review Board.

Antibodies and reagents

The antibodies used in this study included: FITC-conjugated or Tri-color-conjugated anti-CD45, allophycocyanin-conjugated anti-CD3, FITC-conjugated anti-CD16, anti-CD69, R-PE-conjugated anti-CD56 (MEM-188) from Caltag Laboratories (LaJolla, CA); PE Cy7-conjugated anti-CD56 (B159), allophycocyanin-conjugated or PE-Cy7-conjugated, and FITC-conjugated anti-CD9, anti-CD94, allophycoyanin-conjugated anti-IFN-γ, and mouse IgG1 control (BD Pharmingen, San Jose, CA). Human FcR blocking reagent (Cohn's fraction), brefeldin A and saponin were purchased from Sigma-Aldrich (St. Louis, MO), and IL-12 and IL-15 were purchased from Peprotech, Rocky Hill, NJ.

Flow cytometry

FACSCalibur and FACSCanto flow cytometers were used for analysis of cell surface expression of samples. Before specific antibodies were used, an FcR blocking reagent (Cohn fraction II (Sigma)) was added to reduce non-specific staining prior to addition of specific antibodies. Cells were stained for CD3, CD45, and CD56, in combination with CD9, CD16, CD69, CD94, or IgG isotype controls. Single cell suspensions of FRT cells were gated on $CD45^{+}CD56^{+}CD3^{-}$

cells (NK cells) and analyzed for surface expression of specific cell surface molecules.

Intracellular IFN-γ staining

For intracellular IFN-γ analysis, fresh cells isolated from the endometrium, cervix, and the ectocervix were cultured in 500 U IL-2/ml. Following 4 days of culture in U-bottom 96 well plates, cells were stimulated with IL-12 (10 ng/ml) and IL-15 (100 ng/ml) for 13 h. Brefeldin A was added to each well 5 h prior to harvesting in order to allow for the accumulation of intracellular proteins. Cells were then harvested and stained for CD3, CD45 and CD56, fixed and then permeabilized with saponin (0.1%). Cells were thereafter stained with anti-IFN-γ or mouse IgG isotype control and analyzed by flow cytometry [11].

Statistical analysis

Statistical comparisons were done using a Student's *t*-test, and a value of $p<0.05$ was considered significant.

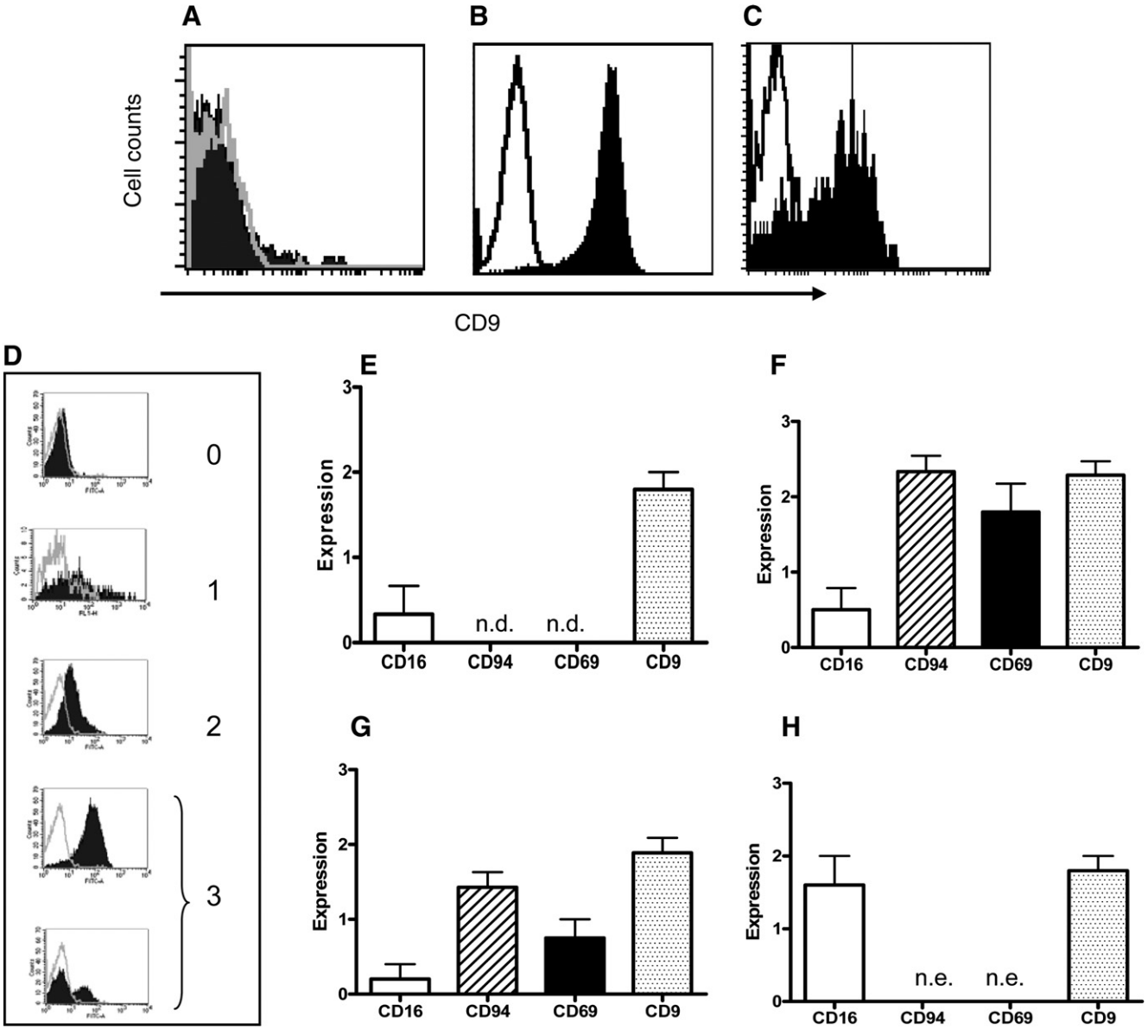

Figure 1 Subsets of NK cells differ between upper and lower FRT tissues. Expression of CD9 is shown for NK cells ($CD45^+$, $CD56^+$, $CD3^-$ cells) from (A) blood, (B) EM, and (C) FT. Closed histograms represent specific expression of CD9, and open histograms represent isotype control staining. The expression of different cell surface proteins was quantified on a three-point scale. (D) Examples of expression and scoring. Closed histograms represent specific staining, and open histograms represent isotype control staining. Expression was rated as 0 (no expression), 1 (low expression), 2 (medium expression) or 3 (high expression on all cells or a distinct subset). Expression of CD16, CD95, CD69, and CD9 was determined on NK cells ($CD45^+$, $CD56^+$, $CD3^-$ cells) in (E) Fallopian tube, (F) endometrium, (G) cervix, and (H) ectocervix. Error bars represent SEM. n.d.: not done, n.e.: no expression (rated 0 in all samples). There were between three and seven independent samples analyzed per data point.

Results

FRT NK cells express CD9, while CD16, CD69 and CD94 are differentially expressed on FRT NK cells

NK cells are found in large numbers within the endometrium, and "NK cell activity" has been reported from isolated cells taken from tissues of the female reproductive tract [15]. To distinguish the female reproductive tract (FRT) NK cell populations from those found in the blood, we analyzed the expression of different cell surface receptors. All NK cells from the FRT tissues express CD9 (Fig. 1), whereas blood NK cells do not express CD9. The cells are released from the FRT samples by the use of collagenase and DNAse treatment [16]. Using this approach, we determined that NK cells from PBMCs subjected to the same enzyme digestion do not express CD9, so the expression of CD9 is not a consequence of enzymatic activity (data not shown). The upregulation of CD9 has also been observed on decidual NK cells and may represent the consequence of NK cells migrating into the female reproductive tract or into mucosal tissues. NK cells in endometrium (EM) and cervix (CX) express CD69, with highest expression on EM-derived NK cells (Fig. 1). NK cells from the CX also expressed CD94 to a similar extent as EM NK cells. However, there was no expression of CD94 or CD69 on NK cells from ectocervix (ECX), but the ECX NK cells expressed high amounts of CD16. CD16 was absent or expressed at low amounts on CX, EM and Fallopian tube (FT) NK cells. These data indicate that FT and CX NK cells have a similar phenotype as the EM NK cells and may be derived from the $CD56^{bright}$ NK cell subset in the blood. The differential expression of these molecules on ECX NK cells is consistent with their resemblance to $CD56^{dim}$ NK cells in the blood. The $CD56^{dim}$ blood NK cells express little CD94 and high levels of CD16. Thus, NK cells in different FRT tissues, even within one individual at a given time point, express unique combinations of cell surface molecules.

NK cells throughout the female reproductive tract differ in size and granularity compared to blood NK cells

To characterize the NK cells throughout the FRT, we have analyzed single cell suspensions from FT, EM, CX, and ECX. CD45 is expressed on all leukocytes and is a good marker to distinguish leukocytes from other cell types in these cell suspensions. We identified $CD45^+$, $CD56^+$, and $CD3^-$ cells in each of these FRT tissues. As shown in Fig. 2, we have found that CD45 expression is lower on EM NK cells compared to EM T cells or blood NK cells. By gating on all $CD45^+$ cells, we were able to identify NK cells ($CD56^+$, $CD3^-$) and T cells ($CD56^-$, $CD3^+$) in the different FRT tissues. We have observed only a few $CD56^+$, $CD3^+$ T cells in the FRT, in contrast to peripheral blood. The size (FSC) and granularity (SSC) of NK cells in CX

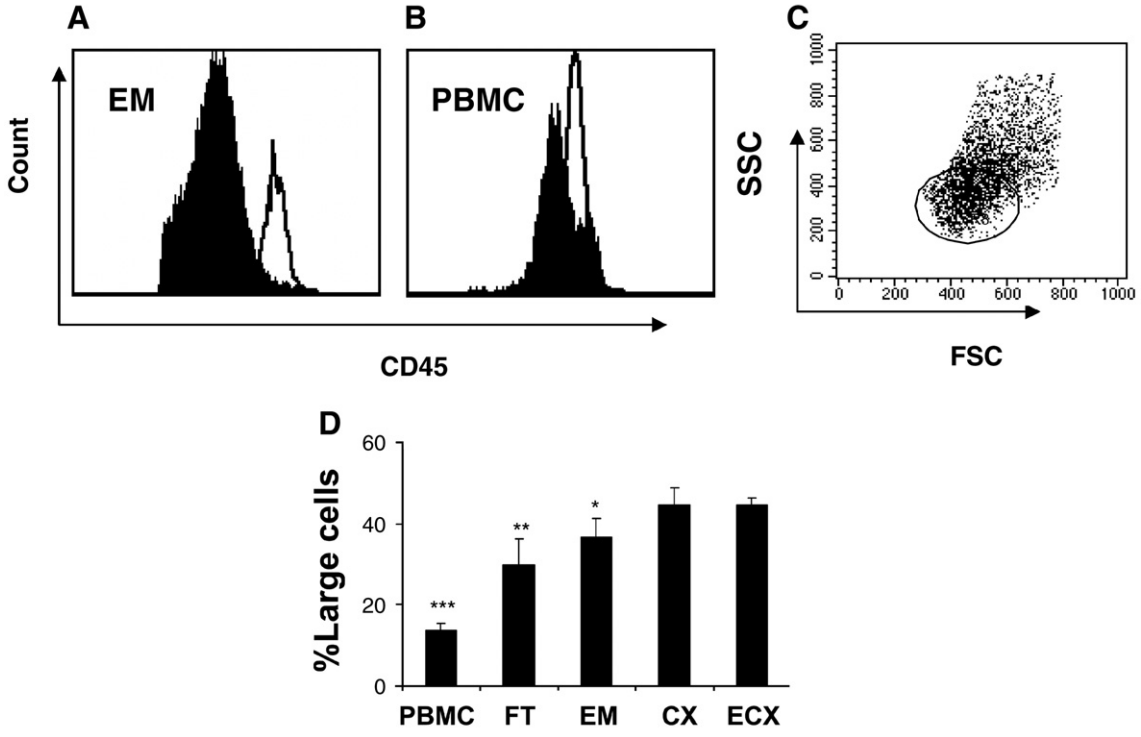

Figure 2 NK cells in the FRT are larger and more granular than blood NK cells. NK cells were identified in blood and tissues as $CD45^+$, $CD56^+$, $CD3^-$ cells. The expression of CD45 on EM (A) and PBMC (B) derived NK cells (filled histogram) and $CD3^+$ T cells (open histogram) is shown. (C) The size (FSC) and granularity (SSC) of NK cells in EM are shown. (D) The percentages of large cells (outside of the gate shown in C) were determined for NK cells from PBMC ($n=7$), FT ($n=5$), EM ($n=12$), CX ($n=11$), and ECX ($n=10$). A $^*p<0.05$ indicates that these NK cells were significantly different from NK cells found in ECX.

and ECX are greater than that of EM or FT NK cells. The majority (64%) of EM NK cells were small lymphocyte sized cells, while NK cells in the CX and ECX (45%) are larger and more granular suggesting that NK cells in these tissues were more activated.

NK cells of the FRT produce IFN-γ in response to IL-2 and IL-15 stimulation

The percent of total cells that express CD45 was highest in the FT and lower in other reproductive tissues (Fig. 3), and these data are consistent with reported findings [17]. The percentage of those leukocytes that were NK cells was lowest in FT (6%) and between 16% and 22% in other FRT tissues. In comparison, the percentage of T cells among leukocytes was similar for all tissues and slightly increased in ECX; this was similar to reported data [17]. To establish whether or not FRT NK cells could produce cytokines, we determined the production of IFN-γ by the EM, CX, and ECX NK cells following stimulation by cytokines. Freshly isolated EM, CX, and ECX cells were cultured in the presence of IL-2 for 4 days followed by IL-12 and IL-15 stimulation, and NK cells were analyzed for intracellular IFN-γ production. The data in Fig. 4 show that between 14% and 50% of NK cells from these tissues produced IFN-γ in response to IL-12 and IL-15 stimulation. These data indicate that NK cells from these FRT tissues are functional and that they can produce IFN-γ.

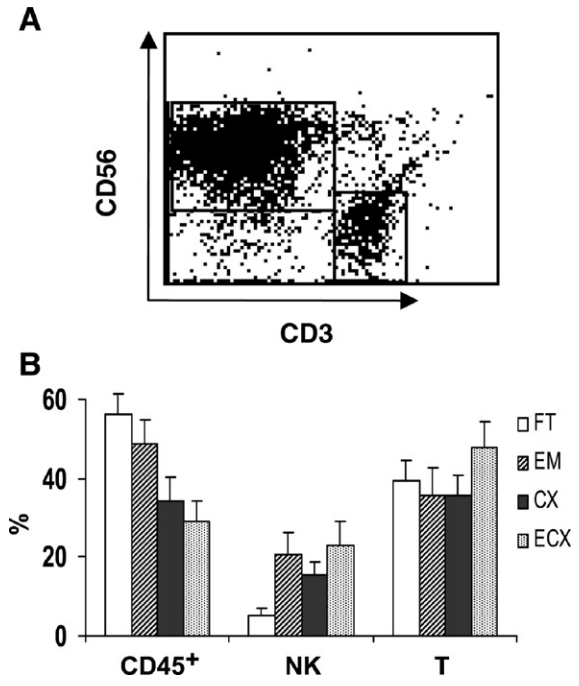

Figure 3 NK cell numbers throughout the FRT. NK cells were identified in blood and tissues as $CD45^+$, $CD56^+$, $CD3^-$ cells (A). In B, the percent of $CD45^+$ cells (of total cells), NK cells (of $CD45^+$ cells), and T cells (of $CD45^+$ cells) is shown from FT (open bars). EM (hatched bars), CX (filled bars), and ECX (dotted bars). Error bars indicate SEM. There were between nine and sixteen independent samples per data point, with the exception of FT ($n=6$).

NK cell numbers can differ between tissues within an individual and are regulated in the EM during the menstrual cycle

There were differences in NK cells between tissues of a given individual. For example, as shown in Fig. 5A, the percent of NK cells in CX and ECX from an individual was usually very similar, although the amount varied from patient to patient. However, the NK cells found in EM and FT varied considerably. The data in Fig. 5B show that whereas in some individuals the percentage of leukocytes that were NK cells were similar to those in CX (Pt 9), in other samples the NK cells made up a much higher (Pt 4) or lower (Pt 7) percent of total leukocytes. These data show that the number of NK cells appears linked for CX and ECX and that the recruitment of NK cells, survival and/or expansion in EM can vary within an individual. The percentage of NK cells in FT was lower than that of other FRT tissues and similar between different patients. These data suggest that tissue-to-tissue variations will lead to differences in the presence of NK cells and innate defenses within each tissue.

NK cell numbers in the EM are known to increase as the menstrual cycle progresses, and our data demonstrate that there were more NK cells in the EM samples isolated during the secretory phase compared to those samples taken during inactive or proliferative phases (Fig. 6). However, the percentages of NK cells in the CX and ECX were similar between samples, regardless of the stage of the menstrual cycle. We did not have sufficient numbers of FT samples from all menstrual stages to evaluate whether menstrual cycle may alter NK cells in FT. These data indicate that NK cell numbers in the EM varied with the stage of the menstrual cycle, but the NK cells in other FRT tissues were not regulated in the same manner.

NK cell numbers change with age in FRT tissues

The immune system is known to function less efficiently with increasing age. Although the total cell numbers isolated from a given tissue often drop with age, the percentage of the isolated cells that were leukocytes was very similar in CX and ECX between those under 60 and from those over 60 years of age (data not shown). In EM and FT, the percentage of leukocytes decreased after age 60. Although the tissue changes that occur during the menstrual cycle may be complete before the age of 60, we did not observe a difference in the percent of leukocytes or NK cells between individuals that were under 45 and those from 45 to 60 (data not shown). The NK cell population as a percent of total leukocytes increased slightly in both CX and ECX in those individuals that were over 60 years of age. As shown in Fig. 7, there was a decrease in the percentage of NK cells found in EM after 60. Not only was the percentage of leukocytes lower in individuals over 60 years, which may be expected, but the percentage of total leukocytes that were NK cells was only about 33% of the amount in women under age 60 resulting in much lower numbers of NK cells in the EM in older individuals. Our data indicate that NK cells decrease in the upper FRT after age 60, but the lower FRT tissues continue to have significant numbers of NK cells. Thus, the dynamics of

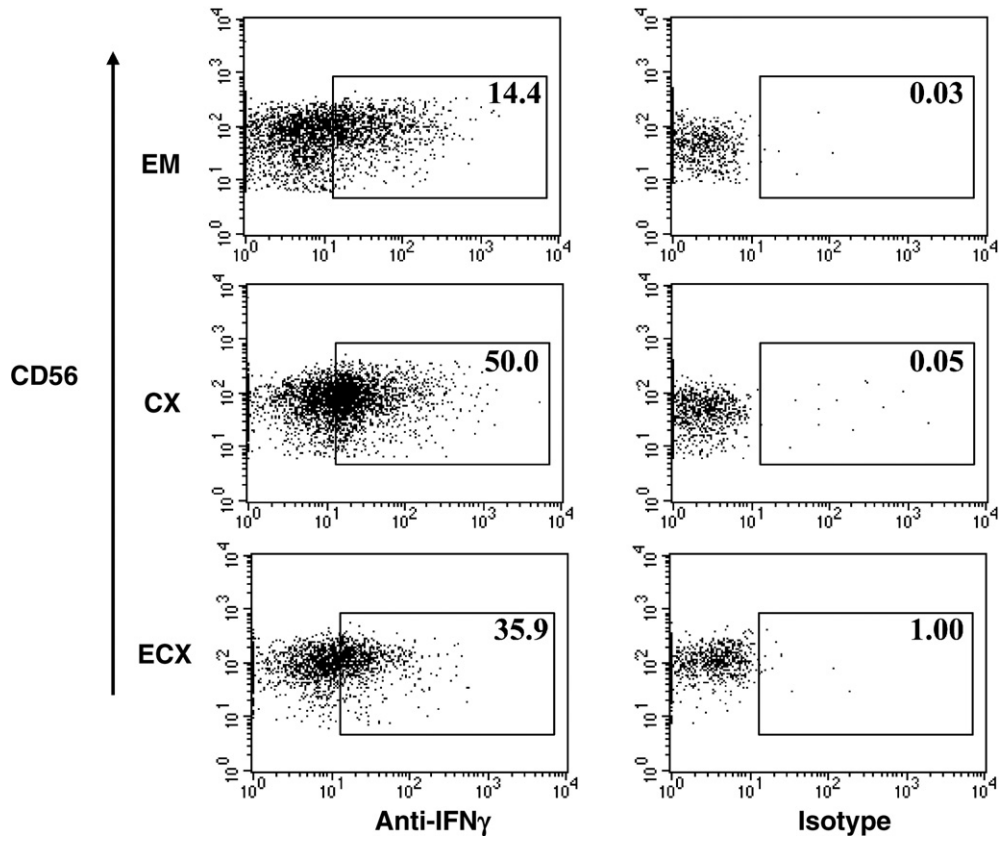

Figure 4 NK cells in the FRT produce IFN-γ. IFN-γ production by EM, CX, and ECX $CD56^+$, $CD3^-$ NK cells are shown. Single cell suspension of fresh FRT cells from the EM, CX, and ECX from the same individual were cultured in IL-2 containing media for 4 days, and thereafter stimulated with IL-12 and IL-15 overnight. $CD56^+$ NK cells were gated from the CD45 gate and analyzed for intracellular IFN-γ. This figure is a representative of three independent experiments.

NK cell recruitment and survival in FRT tissues changes with age and is different between EM, CX, and ECX.

Discussion

In this study, we investigated the nature of NK cells within the human FRT tissues. NK cells have the ability to produce proinflammatory cytokines and respond to tumor and virus-infected cells. Thus, they represent an important component of innate immunity in the FRT. NK cells are present throughout the tissues of the reproductive tract. Our studies indicate that the phenotype of FRT NK cells is different from blood NK cells, and the number of NK cells varies between FRT tissues. Furthermore, we demonstrate that the characteristics of NK cells vary with the site analyzed and we established that IL-12 and IL-15 stimulate IFN-γ production from the FRT NK cells.

Although NK cell "activity" has been observed in cells derived from several different FRT tissues, the identification of NK cells in these tissues has not been reported. The classic "NK activity" is cytotoxicity of K562 tumor cells. However, K562 cells can be recognized by a variety of receptors, some of which can be expressed on cells other than NK cells. The level of K562 cell lysis will also depend on the NK cell activation state and cannot be strictly linked to the number of NK cells present. One difficulty in studying tissue NK cells is the high autofluorescence background of cells within FRT tissues, in particular the cells in CX and ECX. To demonstrate the presence of NK cells, it was necessary to avoid the use of FITC- or PE-conjugated antibodies unless the antigens were expressed at very high levels, like CD45. Using a four-color staining protocol, we were able to identify NK cells within these tissues. We determined that all NK cells within the FRT express CD9, and this protein is not expressed on blood NK cells. This study provides clear evidence for the presence of NK cells in these tissues.

The lower FRT tissues (vagina and ectocervix) are lined with squamous epithelium and have a rather high microbial load of commensals and potential pathogens. The upper FRT tissues (cervix, endometrium, and Fallopian tube) are lined with columnar epithelium and have much less microbial exposure, although these tissues are not sterile. Aspects of the innate immune system, such as Toll-like receptors, are known to vary between upper and lower FRT tissues [18]. In this study, we report that upper FRT tissues contain NK cells that express CD94 and CD69, yet little CD16, and appear more similar to $CD56^{bright}$ blood NK cells. In contrast, lower FRT tissues have NK cells that express little CD94 or CD69, but highly express CD16, so these appear more like $CD56^{dim}$ blood NK cells. We did not have enough samples from vaginal mucosa to include this tissue in our analysis, but our initial analysis suggests that NK cells in vaginal mucosa are similar to those in ECX (our unpublished data). One possible expla-

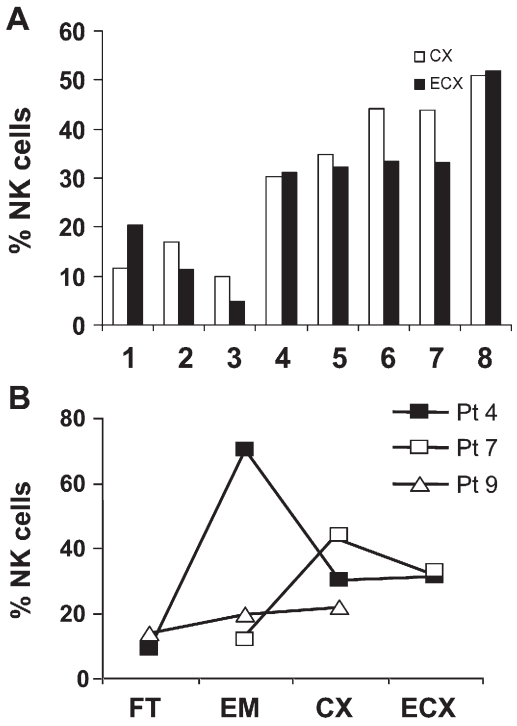

Figure 5 The numbers of NK cells vary between FRT tissues within individuals. (A) The percent NK cells ($CD45^+$, $CD56^+$, $CD3^-$) of total $CD45^+$ cells in the CX (open bars) and ECX (filled bars) of eight individual patients (#1–8) are shown. (B) The percent NK cells ($CD45^+$, $CD56^+$, $CD3^-$) of total $CD45^+$ cells in FT, EM, CX, and ECX from three individual patients (Pts 4, 7, and 9) are shown.

nation for such large differences in NK cell subsets within the FRT tissues is that different chemokines may be produced between the upper and lower FRT tissues that accounts for the recruitment of specific NK cell subsets to these tissues.

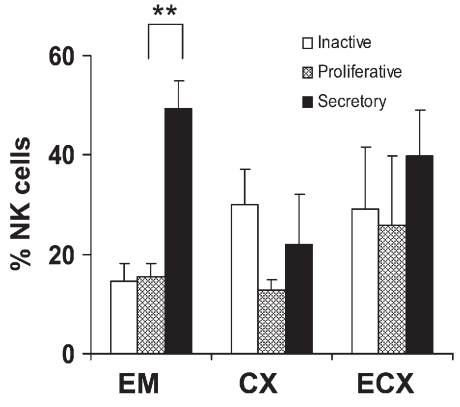

Figure 6 NK cells in EM are regulated by the menstrual cycle, while NK cells in CX and ECX are independent of the menstrual cycle. The number of NK cells ($CD56^+$, $CD3^-$) as a percent of total $CD45^+$ leukocytes is shown in EM, CX, and ECX. The samples were isolated from patients in the inactive (open bars), proliferative (hatched bars), or secretory (filled bars) phase of the menstrual cycle. Error bars represent SEM. $^{**}p<0.01$ indicates samples were statistically different. There were between three and six independent samples per data point.

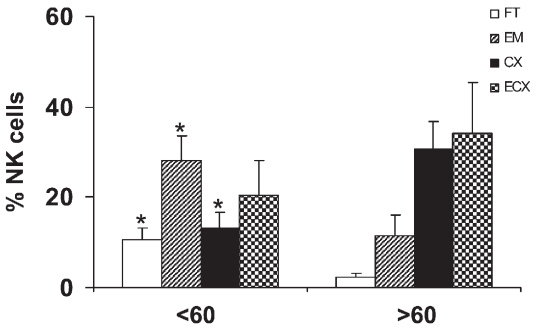

Figure 7 The percent of NK cells is reduced after the age of 60 in EM and FT, but not other FRT tissues. The percent NK cells ($CD45^+$, $CD56^+$, $CD3^-$) of total $CD45^+$ cells in the FT (open bars). EM (hatched bars), CX (filled bars), and ECX (dotted bars) are shown. Samples were separated based on age of individual patients, as under 60 and those 60 years and older. Error bars represent SEM. $^*p<0.05$ indicates a significant difference between samples from that tissue taken from patients under 60 years and those 60 years and older. There were between three and sixteen independent samples per data point.

Possible reasons for this may include differences in the nature of the epithelial cells, underlying stromal cells, or the presence of microorganisms. EM epithelial cells have been shown to produce chemokines that can recruit $CD56^{bright}$ NK cells, and chemokines in the EM are under control of sex hormones [7,10]. We recently reviewed the literature on human mucosal NK cells and found that NK cells in the lungs and upper respiratory tract are primarily $CD56^{dim}$, $CD16^+$ NK cells, while those found in the gastrointestinal tract and secondary lymph nodes are $CD56^{bright}$, $CD16^-$ NK cells [19]. Thus there may be a compartmentalization of human NK cell subsets to specific tissue regions in the human.

The FRT is a unique mucosal site that must be protected against potential pathogenic organisms but also maintain an environment for the successful development of a semi-allogeneic fetus. Thus, precise mechanisms must be used to control immune cells within these tissues. The $CD56^{bright}$ and $CD56^{dim}$ NK cells have been demonstrated to have different functional capabilities [5]. Although both subsets of NK cells have the ability to kill tumor cells and produce proinflammatory cytokines after activation, the $CD56^{bright}$ NK cells produce more cytokines compared to $CD56^{dim}$ NK cells, and $CD56^{dim}$ NK cells are better able to mediate lysis of tumor cells [2]. Due to differences in the expression of CD16, $CD56^{dim}$ NK cells are the primary NK cells that mediate antibody dependent cellular cytotoxicity (ADCC) [20]. Our data indicate that the upper FRT tissues recruit NK cells more like the $CD56^{bright}$ NK cells, while the lower FRT recruit NK cells more like the $CD56^{dim}$ NK cells. The primary role of NK cells in the lower FRT is presumably to fight against invading microorganisms. These NK cells could mediate their effects via NK recognition and cytotoxicity and utilize the recognition of pathogens coated with IgG via CD16. Although NK cells in the EM are able to mediate anti-microorganism functions, one of their primary roles is to help establish an environment suitable for development of the placenta and fetal growth. There is compelling evidence that NK cells in the uterus are

involved in blood vessel reorganization during trophoblast invasion [21]. Data also suggest that alterations in NK cell function may have a role in preeclampsia [22,23]. These functions may be best accomplished by cytokine secretion from CD56^{bright} NK cells, and this may be the benefit of recruiting this subset to the EM. Because this subset of NK cells does not express CD16, they are not able to kill IgG-coated target cells. Indeed, in this study we have demonstrated that FRT NK cells have the capacity to produce cytokines.

A previous study showed that CTL activity in the EM varied with the menstrual cycle and that this activity was inversely correlated with NK cell activity [15]. CTL activity was lower during the secretory phase than in the proliferative phase. Furthermore, the CTL activity was three-fold or higher in EM cells that were isolated from postmenopausal women [24]. Thus as NK cells decrease with age in the EM, one may speculate that there is a compensatory mechanism to increase the cytotoxic effector cells in EM.

The data in this study show that FRT NK cells express CD9 not found on blood NK cells. Thus, CD9 is a convenient marker to distinguish FRT NK cells from blood NK cells. CD9 is a surface molecule expressed by a wide variety of hematopoietic cells and other tissues, including platelets, basophils, eosinophils, and on activated T cells, and its function remains unknown [25,26]. These changes suggest that NK cells differentiate after they migrate into FRT tissues. Another interesting observation from this study is that, although NK cells are regulated by the menstrual cycle in the EM, they do not appear to be regulated by the menstrual cycle in other FRT tissues. It is known that specific CXCR3-binding chemokines are regulated in human EM by sex hormones [10]. However, other chemokines are likely involved in the recruitment of NK cells to other FRT tissues. It was striking that the percentage of NK cells in ECX and CX was linked, even though the phenotypic characteristics of NK cells in these tissues appear to be different. Another surprising observation was that NK cell numbers and phenotypes differed within the FRT tissues of individuals. Although anatomically and functionally linked, these data show that there is a dynamic and tissue-specific regulation of NK cells within human FRT mucosa. In conclusion, these data suggest hypotheses for how the interaction of epithelial cells, stromal cells, and the presence of commensal and pathogenic microorganisms may regulate the presence and function of NK cells within mucosal tissues. Understanding the role of stromal cells, epithelial cells, and microorganisms in the recruitment and maintenance of NK cells within FRT tissues will be important in order to understand innate immune responses within the FRT and other mucosal sites.

Acknowledgments

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References

[1] C.A. Biron, K.B. Nguyen, G.C. Pien, L.P. Cousens, T.P. Salazar-Mather, Annu. Rev. Immunol. 17 (1999) 189–220.
[2] M.A. Cooper, T.A. Fehniger, M.A. Caligiuri, Trends Immunol. 22 (2001) 633–640.
[3] L.L. Lanier, Annu. Rev. Immunol. 23 (2005) 225–274.
[4] C. Bottino, R. Castriconi, L. Moretta, A. Moretta, Trends Immunol. 26 (2005) 221–226.
[5] M.A. Cooper, T.A. Fehniger, S.C. Turner, K.S. Chen, B.A. Ghaheri, T. Ghayur, W.E. Carson, M.A. Caligiuri, Blood 97 (2001) 3146–3151.
[6] S.S. Farag, M.A. Caligiuri, Blood Rev. 20 (2006) 123–137.
[7] K. Kitaya, T. Nakayama, N. Daikoku, S. Fushiki, H. Honjo, J. Clin. Endocrinol. Metab. 89 (2004) 2470–2476.
[8] J.S. Hunt, Biol. Reprod. 50 (1994) 461–466.
[9] A. King, V. Wellings, L. Gardner, Y.W. Loke, Hum. Immunol. 24 (1989) 195–205.
[10] C.L. Sentman, S.K. Meadows, C.R. Wira, M. Eriksson, J. Immunol. 173 (2004) 6760–6766.
[11] M. Eriksson, S.K. Meadows, C.R. Wira, C.L. Sentman, J. Leukoc. Biol. 76 (2004) 667–675.
[12] S. Verma, A. King, Y.W. Loke, Eur. J. Immunol. 27 (1997) 979–983.
[13] J.N. Bulmer, L. Morrison, M. Longfellow, A. Ritson, D. Pace, Hum. Reprod. 6 (1991) 791–798.
[14] C.R. Wira, J.V. Fahey, C.L. Sentman, P.A. Pioli, L. Shen, Immunol. Rev. 206 (2005) 306–335.
[15] H.D. White, K.M. Crassi, C.R. Wira, in: A.J. Husband, K.W. Beagley, R.L. Clancy, A.M. Collins, A.W. Cripps, D.L. Emery (Eds.), Mucosal Solutions: Advances in Mucosal Immunology, University of Sydney, Sydney, 1997, pp. 385–391.
[16] M. Eriksson, S.K. Meadows, S. Basu, T.F. Mselle, C.R. Wira, C.L. Sentman, J. Immunol. 176 (2006) 6219–6224.
[17] A.L. Givan, H.D. White, J.E. Stern, E. Colby, E.J. Gosselin, P.M. Guyre, C.R. Wira, Am. J. Reprod. Immunol. 38 (1997) 350–359.
[18] P.A. Pioli, E. Amiel, T.M. Schaefer, J.E. Connolly, C.R. Wira, P.M. Guyre, Infect. Immun. 72 (2004) 5799–5806.
[19] C.L. Sentman, T.F. Mselle, S. Basu, in: L. Brossay (Ed.), Everything you always wanted to know about NK cells but were afraid to ask, Transworld Research Network, Kerala, India, 2007, pp. 99–115.
[20] G. Trinchieri, Adv. Immunol. 47 (1989) 187–376.
[21] A.A. Ashkar, G.P. Black, Q. Wei, H. He, L. Liang, J.R. Head, B.A. Croy, J. Immunol. 171 (2003) 2937–2944.
[22] S.E. Hiby, J.J. Walker, M.K. O'Shaughnessy, C.W. Redman, M. Carrington, J. Trowsdale, A. Moffett, J. Exp. Med. 200 (2004) 957–965.
[23] M. Sakai, K. Ogawa, A. Shiozaki, S. Yoneda, Y. Sasaki, K. Nagata, S. Saito, Clin. Exp. Immunol. 136 (2004) 114–119.
[24] H.D. White, K.M. Crassi, A.L. Givan, J.E. Stern, J.L. Gonzalez, V.A. Memoli, W.R. Green, C.R. Wira, J. Immunol. 158 (1997) 3017–3027.
[25] K.R. Park, T. Inoue, M. Ueda, T. Hirano, T. Higuchi, M. Maeda, I. Konishi, H. Fujiwara, S. Fujii, Mol. Hum. Reprod. 6 (2000) 252–257.
[26] C. Boucheix, P. Benoit, P. Frachet, M. Billard, R.E. Worthington, J. Gagnon, G. Uzan, J. Biol. Chem. 266 (1991) 117–122.