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6 Human mucosal NK cells

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Abstract

Mucosal tissues are exposed to many different pathogens, and innate immune defenses are ready to provide a rapid first line of defense to protect these tissues. Natural Killer (NK) cells contribute to host defenses against tumors and infections, particularly with intracellular pathogens. Human NK cells in the blood can be divided into two major subsets based on their expression of CD56, $CD56^{bright}$ and $CD56^{dim}$. The $CD56^{dim}$ NK cells are the classic cytolytic NK cells and mediate rapid cellular cytotoxicity and cytokine production. They can also function with antibodies to mediate antibody-dependent cell-mediated cytotoxicity

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(ADCC). CD56^{bright} cells provide assistance to generate immune responses via cytokines, and they are present in secondary lymphoid tissue. The different mucosal tissues face unique pathogens, and the role of NK cells in mucosal tissues is relatively unknown. Data indicate that specific subsets of NK cells may be present in some mucosal tissues but not in others. In this review, we summarize what is known about NK cell subsets and their functions in different human mucosal tissues.

Introduction

Natural killer (NK) cells are an important part of the innate immune system. NK cells are lymphocytes and account for about 10% of peripheral blood mononuclear cells in humans (1). NK cells do not rearrange or express T or B cell receptor genes, but they express an array of cell surface receptors that allow them to recognize self-proteins and non-self proteins on other cells. Some of these receptors induce NK cell activation, while other receptors inhibit NK cell function. It is believed to be the balance of activating and inhibitory signals that NK cells encounter that determines whether and how NK cells respond (2). The receptor signals are spatially separated from one another, such that a particular NK cell can interact with two cells at the same time. While one cell may inhibit NK cell activation, the other cell may provide activation signals and result in NK cell cytotoxicity (3). Although cell cytotoxicity is the hallmark of NK cells and the basis for their name as 'natural killer' cells, NK cells are able to produce several different cytokines (4). Many of these cytokines are proinflammatory cytokines, such as Interferon (IFN)-γ, Tumor Necrosis Factor (TNF)-α, or Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF). However, NK cells have also been shown to produce Interleukin (IL)-5, IL-10, and IL-13 under certain conditions. They are also known to produce Transforming Growth Factor (TGF)-β, which is known to inhibit many lymphocyte functions.

NK cells are found in a number of different tissues, including spleen, lymph nodes, lung, liver, intestine, and uterus. The exact role and function of NK cells in these different tissues depends on the local environmental stimuli. They are believed to contribute to host defenses against tumors and infections, particularly with intracellular pathogens (5). There are significant clinical data that individuals with defects that alter NK cell activity suffer from an increase in infections, especially with herpes-type viruses (6-8). However, it is important to note that many of the molecular defects affect several different cell types, so it is not always clear that NK cells are responsible for the clinical manifestations. There has been one well-documented case of NK cell deficiency that resulted in recurrent, severe herpes-virus infections (9). Most of the work performed with human NK cells has been done using NK cells

derived from blood. Yet, most infections occur at mucosal surfaces and little is known about the function of NK cells located within these mucosal tissues. This review summarizes the data on human NK cells at mucosal sites. Animal studies are noted as well, as these studies have provided mechanistic understandings of NK cell function in these tissues. However, there are distinct differences between human and animal NK cells, so not all findings in animal models can be extrapolated to humans.

NK cell subsets in human blood

Most NK cells share the general ability to mediate cellular cytotoxicity and cytokine production, and there are a number of blood NK cell subsets with specialized functions (4). Human NK cells can be divided into more than forty different subsets based on the expression of various cell surface markers, but they can be divided into two major groups based on the expression of CD56 (10). CD56 is neural cell adhesion molecule (N-CAM) and has no known function on human NK cells. $CD56^{bright}$ NK cells account for 10% of blood NK cells, few of these cells express CD16, and they do not express CD57. This NK cell subset expresses high amounts of CD94 but few cells express killer cell immunoglobulin-like receptors (KIRs). The $CD56^{dim}$ NK cells account for 90% of blood NK cells, and express KIRs, CD57, and CD16. These are the prototypic cytotoxic NK cells that kill K562 target cells and mediate 'NK cell activity'. Because of their high expression of CD16, $CD56^{dim}$ NK cells mediate ADCC. In contrast, resting $CD56^{bright}$ NK cells have little spontaneous cytotoxicity but they produce higher amounts of cytokines compared to $CD56^{dim}$ NK cells on a per cell basis (11, 12). $CD56^{bright}$ NK cells also express CCR7 and CD62L, and these molecules allow this subset of NK cells to localize to secondary lymph nodes (13). NK cells account for about 1% of cells in lymph nodes, and all of the NK cells in human lymph nodes are $CD56^{bright}$ NK cells (14, 15). It has been suggested that $CD56^{bright}$ NK cells are the most common type of NK cell throughout the body because of their selective localization in lymphoid tissues (16).

Due to their ability to produce cytokines, such as IFN-γ and TNF-α, it has been proposed that the $CD56^{bright}$ NK cell subset is involved in the initial activation of adaptive immunity and promotion of Th1-type immunity and cytotoxic T lymphocyte (CTL) generation (4, 12). $CD56^{bright}$ NK cells are the NK cells found at sites of chronic inflammation, such as joints in rheumatoid arthritis or colon in colitis (17). Their potential to produce cytokines may lead to an exacerbation of these diseases. It is unclear whether NK cells contribute to the early disease process, or if they are recruited to these sites because of ongoing inflammation, where lymphoid aggregates and tertiary lymphoid tissue may have formed.

NK cells in mucosal tissues

NK cells are found in a variety of mucosal tissues in humans. NK cells have been reported in the lungs, the digestive system, and the uterus. Many studies have involved tissues isolated from patients with chronic inflammation, infection, or cancer, so it can be difficult to determine whether the NK cells found at these sites are contributing to the disease process or have been recruited as a consequence of the underlying pathology. However, there are several reports that involve the use of tissue distal to known pathology or biopsies from healthy volunteers, which provide an important source of understanding of the basic biology of NK cells in human mucosal tissues. Parts of the mucosal system are constantly exposed to microorganisms, such as the colon, vagina, and upper respiratory tract. Other parts of the mucosal tissues, such as the endometrium or lower airways, have a much lower constitutive microorganism load, although they are exposed to different viral or bacterial pathogens from time to time. Thus there may be unique NK cell functions required at different sites and influenced by anti-microbial responses within each tissue. In this review, we will examine data on the phenotype and function of NK cells within different mucosal tissues.

NK cells in the female reproductive tract

NK cells have been studied extensively in the endometrium and decidua compared to other mucosal sites. NK cells account for a large proportion of the leukocytes in secretory endometrium and during the first trimester of pregnancy in the decidua of humans (18, 19). The large number of NK cells present, their possible involvement in the tolerance to parental antigens, and the ease of obtaining tissues likely accounts for the large number of studies on NK cells in human endometrium. These studies have provided some vital insights into NK cells at this mucosal site and tissue NK cells in general.

NK cells in the uterus, called uNK cells, have a unique phenotype compared to those found in the blood. UNK cells express high levels of CD56, most express CD94, and few express CD16 or CD57 (20-22). These characteristics are similar to the $CD56^{bright}$ cells found in blood. However, a high proportion of uNK cells express KIRs, which are normally expressed to a much greater extent on $CD56^{dim}$ blood NK cells. UNK cells also express CD9 and CD69, and these molecules are not normally expressed on resting blood NK cells (20). Cells are typically isolated from tissue using a set of enzymes to help digest the tissue matrix and release isolated cells, yet blood NK cells do not express CD9 after a similar enzyme digestion (our unpublished data). Thus, CD9 is a useful marker to distinguish uNK cells from potential blood NK cell contaminants. These data and the observation that uNK cells have an increased cell size compared to blood NK cells suggests that uNK cells

represent cells that have differentiated or been activated within the uterine environment. Further molecular analysis supports this view. A study of the gene expression profile of decidual NK cells showed that they differentially expressed 278 genes compared to blood NK cells (23). The gene expression analysis of decidual NK cells also indicated that decidual NK cells were more similar to $CD56^{bright}$ NK cells in the blood than $CD56^{dim}$ NK cells. It has been a matter of debate as to which subset of blood NK cells are recruited to the endometrium, or whether there is a unique NK subset destined to go to the endometrium or developed locally from undifferentiated stem cells.

Different female reproductive tract (FRT) tissues have distinct subsets of NK cells and this may be accounted for by local conditions. Little analysis has been done on the presence of NK cells in the FRT outside of the endometrium. NK cell activity, that is, cytotoxicity of K562 tumor cells, has been reported in other FRT tissues (24). We have used multi-color flow cytometry to analyze the different FRT tissues and have found NK cells (CD45+, CD56+, CD3-) in Fallopian tube, cervix, ectocervix, and vaginal mucosa (our unpublished data). These NK cells all express CD9 like NK cells in the endometrium and unlike blood NK cells. Furthermore, our data suggest that NK cells in the upper FRT (Fallopian tube, endometrium and cervix) do not express CD16, while those in the lower FRT (ectocervix and vaginal mucosa) express CD16 (see Table 1).

Table 1. Phenotype of human NK cells.

Tissue	Cell surface molecules			
	CD56	CD9	CD16	CD94
Respiratory tract	+[a]	?[b]	+	?
Gastrointestinal tract	+	?	+/-	+/-
Secondary lymphoid tissue	+	?	+/-	+
FRT Upper[d]	+	+	+/-	+
Lower[e]	+	+	+	-[c]
Blood: $CD56^{dim}$	+	-	+	-
$CD56^{bright}$	+	-	+/-	+

[a] expressed
[b] unknown
[c] not expressed
[d] Fallopian tube, endometrium and cervix
[e] Ectocervix and vaginal mucosa

The finding that different subsets of NK cells are present in different locations may be due to specific local factors, such as chemokines, squamous versus columnar epithelial cells, or exposure to different amounts or types of microorganisms. The NK cells in the upper FRT are more like $CD56^{bright}$ blood NK cells that are potent producers of cytokines, while the lower FRT NK cells are more similar to $CD56^{dim}$ blood NK cells that mediate ADCC and typical NK cell mediated cytotoxicity. These regional differences in NK cell function may reflect specialized needs of the local innate immune defenses.

NK cells in the uterine endometrium have been shown to be involved in recruitment of trophoblasts and local rearrangement of blood vessels (25-27). These functions are not traditionally thought of as NK cell activities. NK cells are known to be a prominent cell type during the first trimester of pregnancy. Human trophoblasts have been shown to produce specific chemokines that can recruit NK cells, such as CXCL12 and CCL3 (28, 29). A recent study has shown that NK cells from the decidua produce chemokines that can recruit trophoblasts and endothelial cells (30). There is also an association between KIR and human leukocyte antigen (HLA) genotypes and susceptibility to preeclampsia (31). It has been documented that fetal growth restriction is associated with reduced numbers of NK cells in the decidua during pregnancy (32). Taken together, these data suggest that NK cells may be responsible for the optimal interactions between invading trophoblasts and endothelial blood vessels that results in a proper blood supply for the developing fetus. Data from animal studies indicate that uNK cells can produce IFN-γ and other factors that support angiogenesis (25). Animals deficient in NK cells do produce offspring, but there are abnormalities in the blood vessel structure of the placentas. Collectively, these data suggest that uNK cell angiogenic activity is an important part of NK cell function during pregnancy.

NK cells in the lungs and upper airways

The lungs and upper respiratory tract are constantly exposed to bacteria and viruses, and the innate immune system is an active component that helps to prevent infection. Bronchoalveolar lavage (BAL) has been used to investigate the cells and inflammatory mediators within the lungs. Robinson et al. described the isolation of large granular lymphocytes (LGLs) from the BAL fluid (33). These LGLs expressed CD57 and were able to bind to tumor cells, although they were not able to kill the tumor cells. The addition of IL-2 increased the lytic activity of these lung NK cells. In this study they demonstrated that alveolar macrophages or fluid was able to inhibit NK cell lysis of tumor cells, and this was confirmed in another study where pulmonary macrophages were shown to suppress the development of lymphokine-activated killer (LAK) cell activity in human lung lymphocytes (34). Weissler and colleagues examined NK cells in BAL fluid and lung tissue and determined that these cells expressed CD16 (35). Most of the CD16+ cells were located in the interstitium (86%) compared to the airways, while CD57+ LGLs could be found in the large airways and alveoli. Although CD57+ LGLs were present, all lytic activity was due to the CD16+ LGLs. This study describes normal human lung NK cells, as they used samples obtained within hours of death due to trauma or sudden death and not from patients undergoing surgery for lung cancer or other lung disease. They demonstrated that enriched

lung NK cells had similar cytotoxic activity compared to enriched blood NK cells. A recent study confirmed these findings that lung NK cells and blood NK cells have similar proportions of $CD56^{bright}$ and $CD56^{dim}$ NK cells (36). Meyer and Soergel examined differences in BAL lymphocytes between young (<36 years) and older (>64 years) healthy volunteers (37). Differences were observed with CD4+ T cell population, but the NK cells were similar and represented 3.1% to 3.4% of BAL cells and 12% of blood PBMCs. The NK cells from lung expressed CD56 and CD16. Taken together, these data indicate that normal human lung contains NK cells of the $CD56^{dim}$ subset and most of the NK cells are located within the interstitium.

A variety of studies have documented NK cells in the lungs of animal models. NK cell function was identified in murine and rat lungs (38, 39). Prichard et al. demonstrated that NK cells were present in guinea pig lungs and they became lytic once adherent cells are removed, similar to the findings from human lung NK cells (35, 40). More recently, NK cells have been identified in murine lung tissues in situ using a variety of staining techniques (41-44). These studies show that NK cells are found throughout the murine lung and can become activated during infection. How NK cells get into the lung is unknown, but CXCR3 deficient mice do not have NK cells in the lungs (45). These mice also have deficiencies of NK cells in other peripheral tissues suggesting that CXCR3 may be involved in NK cell recruitment to different locations.

NK cells can be activated and increase in number during lung infection. Although NK cells are important for the overall defense against herpes-virus infections, the activity of lung NK cells may not be critical. Protection of mice during intranasal infection with murine gammaherpesvirus (MHV-68) is not dependent on NK cells (46). NK cells are important in the early response against murine cytomegalovirus (MCMV) when the virus is given i.p., but not when the virus is given intranasally (47). It is possible to selectively deplete NK cells in the murine lung by using an intratracheal injection of anti-ASGM1 antibodies (48). The splenic NK cell activity and ability to reject bone marrow allografts remains intact. Using local depletion of NK cells, it was demonstrated that the lung clearance assay, an assay that measures retention of radiolabeled tumor cells in the lungs, is dependent upon splenic or blood NK cell activity and not lung NK cell activity (48).

Although $CD56^{dim}$ NK cells naturally reside in human lungs, $CD56^{bright}$ cells are recruited during infection or chronic inflammation. Schierloh et al. show that there is an increase in the $CD56^{bright}$ NK cells in pleural effusions from tuberculosis (TB) and parapneumonic infection patients (49). These NK cells have high expression of CD94, NKG2A, CD62L, but little CD16 or perforin expression, which is typical for $CD56^{bright}$ NK cells. They also express activation markers CD69 and HLA-DR. Their data also indicate that enriched NK cells from TB patients produced IFN-γ in response to irradiated Mycobacterium. In

sarcoidosis patients there is an increase in the $CD56^{bright}$ NK cells in the BAL compared to normal controls (36). Sarcoidosis is a granulomatous disease of unknown origin that usually affects the lungs. The increase in $CD56^{bright}$ NK cells from patients was also confirmed by an increase in CD94+ NK cells and a decrease in KIR+ NK cells. Thus local chronic inflammation may lead to recruitment of different NK cell subsets to the lungs.

NK cells have also been identified in the human nasal mucosa. In one study, CD16+, CD56- cells from the nasal mucosa were isolated and they accounted for about 2.7% of total cells (50). These data suggested the presence of $CD56^{dim}$ NK cells. Pawankar et al. compared lymphocytes from patients with house-dust mite perennial allergic rhinitis (PAR) and chronic infective rhinitis (CIR) (51). The study showed an increase in NK cells (CD16+ cells) in the CIR samples compared to the PAR samples. The increase in NK cells was from 8% to 15%. PAR is an IgE-mediated allergic disease that is characterized by sneezing, nasal congestion, and rhinorrhea, while CIR is bacterially or virally induced infective rhinitis with symptoms of nasal obstruction, mucus discharge and paranasal sinusitis. CIR is usually associated with *Hemophilus influenzae* or Streptococcus infection. The authors suggest that the NK cells may be recruited to mediate ADCC or provide local IFN-γ to help fight the infection.

Collectively, these studies indicate that NK cells are present in the human lungs and respiratory tract, and suggest that NK cells can take an active part during responses to infection. The NK cells normally present in human lung are predominantly $CD56^{dim}$, CD16+ NK cells, while data indicate that $CD56^{bright}$ NK cells can be found during chronic inflammation or infection.

NK cells in the gastrointestinal tract

NK cells can be found throughout the human digestive tract. Most of the NK cells have been identified in the lamina propria; while there have been reports of NK cells or NK-like cells among intraepithelial lymphocytes (IELs). One difficulty with evaluating the data on NK cells in the digestive tract is the presence of unusual T cell subsets that can have a LGL morphology, express NK cell receptors and markers, and mediate NK cell-type cytotoxicity. Most IELs are T cells, and there are greater numbers of IELs in the jejunum than in the ileum, with the fewest IELs being found in the colon (52). Almost all tissues examined were isolated from surgery as a treatment for inflammatory diseases or tumors, but most studies have analyzed tissues some distance (e.g. 5 cm) from the pathology. Although it can be difficult to rule out that the underlying pathology does not alter the presence or function of local immune cells, collectively these studies show a coherent picture of what types of NK cells are present in the human digestive tract.

Early studies provide evidence for NK cells in the human intestinal mucosa. The NK cells identified did not express CD57, and lytic activity could be increased by addition of IL-2 containing conditioned medium and reduced by depletion of CD16+ cells (53, 54). The NK cell activity was found among cells isolated from the lamina propria, and there were no NK cells (CD57+) among the IELs (53, 55). NK cell cytotoxic activity has also been identified in the intestines of rodents (56). A study that compared mononuclear cells from histologically normal tissue from inflammatory bowel disease (IBD) and colon cancer specimens showed NK cell lytic activity in both samples, and there was higher activity from ileum (IBD) samples than from colon (cancer patients) (57). The activity was due to CD56+ cells and not CD16+ cells. Another study reported NK cell activity as higher in samples from Crohn's patients than from ulcerative colitis patients (58). Crohn's disease is associated with a Th1-type inflammatory response, while ulcerative colitis has been associated with IL-13 and natural killer T (NKT) cell activation (59). Although disease status may contribute to the overall NK cell lytic activity in cells from these samples, the increased activity may have been due to the different tissues isolated.

An extensive marker analysis by Pang et al. showed that CD56+ cells were present in the lamina propria of human duodenum but not as IELs and were capable of killing K562 tumor cells (60). These cells express CD45, CD2, CD7, CD18, CD122 and CD69, but they did not express CD57, CD3, CD16 or CD8. Another study examined human NK cells from a variety of tissues including colon and found these colon NK cells to be CD56+, CD3-, and CD16+/-, which is consistent with them being of the $CD56^{bright}$ phenotype (61). Caballero and colleagues examined the lamina propria and IELs from the colon and rectal biopsies from different pathologic conditions or from healthy controls (62). They used CD57 as their NK cell marker and found ten times more CD57+ cells in the lamina propria from rectal biopsies than from colon biopsies among all samples; very few CD57+ cells were found in the colon and in the lamina propria. Leon et al. used histologically normal biopsy samples from children and adults undergoing endoscopic investigation where the final diagnosis excluded involvement of the intestine (63). In this study, only the IELs were isolated and enriched by percoll gradients. They reported cells that expressed intracellular CD3, but not cell surface CD3, and these cells were CD56+, CD16+/-, CD2+, perforin+, fasL-. Upon stimulation with phorbol myristic acetate (PMA) and ionomycin, the cells produced IFN-γ, TNF-α, IL-2, but not IL-4 or IL-10. The IELs with intracellular CD3+ produced a much greater amount of the Th1 type cytokines compared to the CD3- IELs. This study describes these as possible innate lytic effector cells in the human intestine, although not all may be true NK cells and some of the IELs may be T cells. One group has reported on NK cells in pediatric patients from duodenum and jejunum. They reported a population of CD3-, CD45+ cells that were

CD122+, CD7+, CD5-, and had variable expression of CD56, CD16+/-, CD2, and CD94 (64). Most of these cells express CD161 and contain perforin granules. These cells did not express CD57 or KIRs (65). All NK cells were CD69+ and CD103+. CD69 and CD103 are also expressed on decidual NK cells, so these markers may be common among mucosal NK cells (23). The study reported that this NK cell population was around 40% of leukocytes in control colon samples but was reduced to 2% in celiac disease patients. The number of NK cells varied between the donors, but it was higher than other reports where adult tissues have been examined. Thus there may be differences between NK cells in the gastrointestinal tract of children compared to adults.

Yun et al. examined NK cell responses to *H. pylori* in human gastric mucosa, and biopsies were taken from the antrum and duodenum (66). They found that 15% of lymphocytes from antrum biopsies were NK cells (CD56+, CD3-) in healthy volunteers and only 6% were NK cells in those having been infected with *H. pylori*. This apparent decrease in NK cells was due to an influx of B cells and CD4+ T cells, and the numbers of NK cells remained unchanged. There was no difference in the percent of NK cells in the duodenum between the two groups. The NK cells in these samples have a high expression of CD56 suggesting these cells are derived from the $CD56^{bright}$ NK cell subset. Their data also suggest that blood NK cells produce IFN-γ in response to killed *H. pylori* lysates, especially in the presence of IL-12. Using a transwell system, they show that *H. pylori* stimulated epithelial cells will result in blood NK cell production of IFN-γ. Incubation with the lysates also increased the NK cell perforin and granzyme B gene expression. These data are in line with a study that *H. pylori* pulsed dendritic cells (DCs) could stimulate human NK cells to produce IFN-γ (67). A murine model of helminth infection using *Trichinella spiralis* showed an IL-13 dependent intestinal inflammation (68). The pathology was similar in wildtype and in mice with severe combined immune deficiency (SCID) or athymic mice, and the authors suggest that intestinal tissue restructuring during infection is due to NK cell cytokine production. Thus, IL-13 production by NK cells and NKT cells may be involved in the development of ulcerative colitis (69).

The published studies are consistent with the idea that $CD56^{bright}$ NK cells are present in the gastrointestinal tract and few $CD56^{dim}$ NK cells are normally present. Although tissue is often taken for a clinical disease, there have been many studies, where some included the analysis of biopsies from healthy volunteers, and overall they indicate that $CD56^{bright}$ NK cells populate the lamina propria of the digestive tract. Whether there is selective recruitment of $CD56^{bright}$ NK cells from the blood or local differentiation of NK cells in the mucosa from progenitor cells is unknown. $CD56^{bright}$ NK cells express different cell surface markers, such as CD62L and CCR7, so they may be selectively recruited to the digestive tissues to help in the innate defense. A recent study

claimed to find hematopoietic stem cells (HSCs) among the IELs and lamina propria cells (70). These cells express CD45 and CD34. Many of these HSCs express CD7 and some express CD56. This population represents 6-7% of IEL CD45+ cells and lamina propria CD45+ cells. However, this study did not demonstrate that these HSCs can give rise to NK cells. It has been hypothesized that there may not be selective recruitment of NK cell subsets as much as selective development in mucosal tissues, in an analogous manner as has been proposed for $CD56^{bright}$ cells in human lymphoid tissues (71). Distinct stages of NK cell maturation have been described for progenitor cells isolated from in human secondary lymph nodes (72). Thus, it is possible that NK cells may be able to differentiate under local conditions in various tissues.

NKG2D ligand expression in mucosal tissues

NK cells express many different activating receptors, and it is believed to be the balance between the signalling from activating receptors and inhibitory receptors that determines whether an NK cell becomes activated when engaging a particular target cell (2). One important activating receptor is NKG2D. NKG2D is expressed by human NK cells, γδ T cells, CD8+ T cells, and a small subset (<2%) of CD4+ T cells (73, 74). In NK cells, NKG2D associates with DNAX activation Protein (DAP) 12 and mediates a primary activation signal leading to release of cytotoxic granules. The ligands for NKG2D are expressed on many different types of tumor cells, including carcinomas, lymphomas, and melanomas (75, 76). Ligands for human NKG2D are MHC Class I Chain Related Protein (MIC)A, MICB, and a set of UL16 Binding Protein (ULBP) binding proteins (1-4) (77). Murine ligands are Retinoic Acid Early inducible -1 gene (RAE1), Murine UL16-Binding Protein-Like Transcript 1 (Mult1), and H60. Although found on many different types of tumor cells, NKG2D ligands are not expressed on most normal tissues. They are found in differentiated gut epithelium, although the protein is not expressed on the cell surface under normal circumstances (75). NKG2D ligand expression is regulated by DNA damage response pathway, so that stresses that result in activation of DNA repair pathways lead to expression of NKG2D ligands (78).

NKG2D ligand expression has been associated with celiac disease in humans (79, 80). Although a recent study has shown that NKG2D ligands are not the primary target in this disease (81). Meresse and colleagues have shown that NKG2C/CD94+, CD8+ T cells mediate lysis of HLA-E expressing epithelial cells. The study demonstrated that NKG2C associated with DAP12, and cross-linking induced phosphorylation of Zeta-chain-associated protein kinase 70 (Zap70) and specific lysis of target cells. The epithelial cells also express NKG2D ligands, and it is thought that NKG2D on the CD8+ T cells may be involved in costimulation of these T cells. Thus, T cells that differentiate to

express NK receptors, including NKp46 and NKp44, are the primary mediators of cellular damage in celiac disease. Whether the expression of NKG2D ligands is an early or late event in this disease is unclear. However, these data highlight the fact that NK-like activity is not per se evidence of NK cell function, and that mucosal tissues have T cell subsets that upon chronic inflammation may differentiate to have NK-like activity.

It has been shown that lipopolysaccharide (LPS) will induce NKG2D ligands on human blood monocytes in vitro (82). One possibility is that this provides a means for monocyte/macrophages to activate NK cells to produce IFN-γ and increase macrophage anti-bacterial activity. A study using intracellular protozoans of the genus Cryptosporidium, a major cause of diarrheal illness worldwide, showed that epithelial cell lines and human ileal tissue have increased expression of NKG2D ligands upon infection with *C. parvum* (83). Upon infection, human lung cells may be induced to express ligands for NKG2D. Borchers et al. show that normal human airway epithelial cells do not express surface ligands for NKG2D, but that upon stress, the ligands can be expressed on the cell surface (84) In a follow up paper, they report that exposure to *Pseudomonas aeruginosa* led to expression of NKG2D ligands on murine airway epithelial cells and macrophages (85). Moreover, blocking of the NKG2D receptor *in vivo* led to decreased clearance of the bacteria and reduced Th1 type cytokines and nitric oxide. It was not shown in this study whether NK cells or NKG2D+ T cells were the effector cells.

Our own studies indicate that NKG2D ligands can be found in the human female reproductive tract epithelial cells. We have found that primary epithelial cells in the endometrium and other FRT tissues express MICA, although MICA may not be expressed on the cell surface under normal conditions (our unpublished data). These data combined with those from other mucosal tissues suggest that perhaps NKG2D ligands are stored intracellularly as proteins and are only put on the cell surface during infection or stress as a means to induce innate immune cell activation. It has been shown that MICA has a basolateral sorting motif and may be preferentially expressed on the basolateral surface of polarized epithelial cells (86). NK cells and other immune cells have the ability to interact with epithelial cells via small pores in the basement membrane. Thus ligands for NK cell receptors may play an important role in the innate defenses against infection in human mucosal tissues and inflammatory diseases.

Summary of mucosal NK cells

Mucosal tissues are exposed to many different pathogens and innate defenses have been constructed to provide a rapid first line of defense to protect these tissues. The $CD56^{dim}$ cells are the classic cytolytic NK cells and

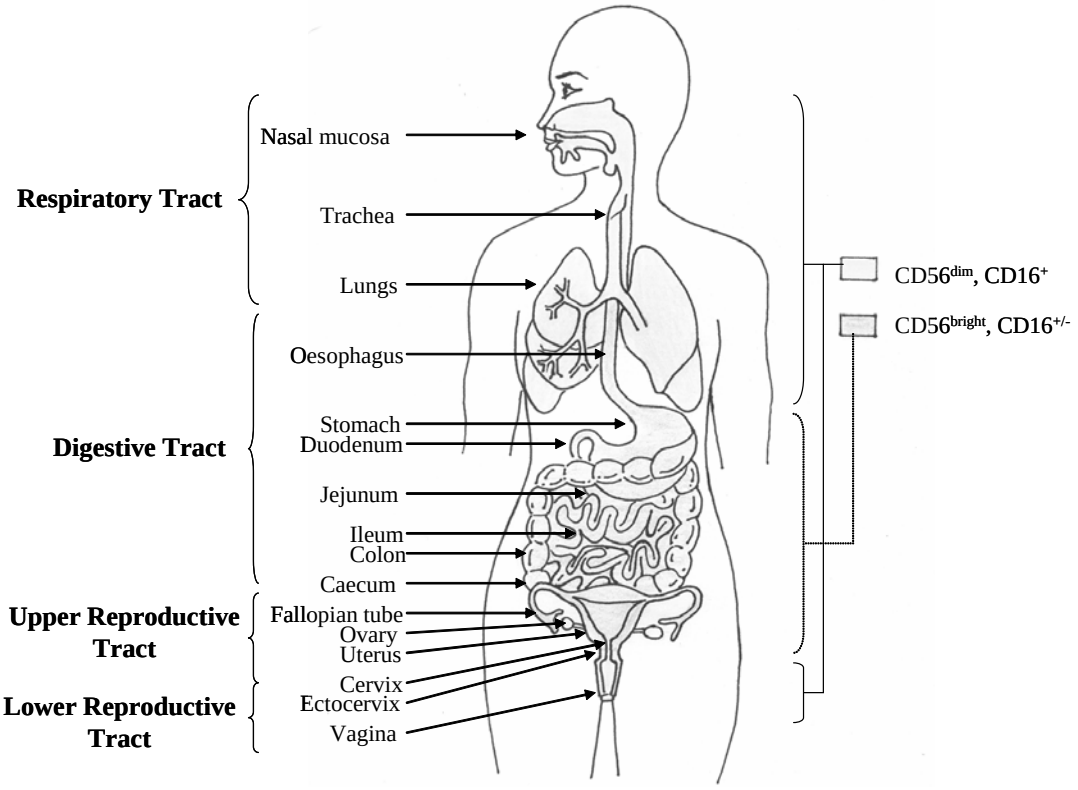

Figure 1. NK cell distribution in the human mucosa. $CD56^{dim}$, CD16+ NK cells are found in the respiratory and lower reproductive tract. $CD56^{bright}$, CD16+/- NK cells are found in the gastrointestinal tract and upper female reproductive tract.

mediate rapid cellular cytotoxicity and cytokine production. They can also function with antibodies to mediate ADCC. $CD56^{bright}$ cells are the 'helper' type of NK cells and provide assistance to generate immune responses via cytokines, and they are present in lymphoid tissue. $CD56^{bright}$ NK cells are found in lymphoid areas, upper FRT and gastrointestinal tract, while $CD56^{dim}$, CD16+ NK cells are in blood, lung, and lower FRT (see Figure 1). One possible reason for this distribution of NK cells may be the differential nature of pathogen exposure in those tissues directly exposed to the external environment. The distribution of NK cell subsets to specific areas within the human mucosa suggest that constant exposure to pathogens, as found in the respiratory tract and lower FRT, may benefit from having $CD56^{dim}$ NK cells present. In contrast, those mucosal areas that are more protected may benefit from the presence of $CD56^{bright}$ NK cells that can produce a variety of cytokines upon activation. In order to understand the immune responses at mucosal sites, it will be important to determine how pathogens and local epithelial cells induce specific recruitment and activation of NK cells as part of the larger innate immune defense network.

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