The Importance of Measuring Natural Killer Cell Subpopulations, Including CD57, in Health and Disease

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Abstract

Natural killer (NK) cells and cytotoxic T ($CD8^+$) cells are two of the most important types of cells in our body that protect it from foreign invaders. Both are cytotoxic cells, but the natural killer cell is part of the innate immune system, while the cytotoxic T cell is one of the major components of adaptive immunity. The most important function shared by these two different cells is the destruction of pathogen-infected and tumor cells. They both do this by releasing bullet-like granules that penetrate the enemy cells, resulting in the death of the infected or cancerous cells. In this review, we look at both cytotoxic T cells and natural killer cells, but pay particular attention to different subpopulations of natural killer cells, including $CD56^+CD16^{dim}$, $CD56^{dim}CD16^+$, NKT cells, CD57 NK cells, $CD57^+CD16^+$ NK cells, and $CD8^+CD57^+$ T cells, which are a kind of hybrid between a cytotoxic $CD8^+$ T cell and a $CD57^+$ NK cell. Among these groups, the CD57 subpopulation has been gaining interest. Previously thought to be a marker of inability to proliferate and immunosenescence, CD57 is now regarded as a marker of higher cytotoxic capacity. CD57 cells are now thought of as highly-differentiated long-lived cells that are more efficient in killing their targets than other NK cell subpopulations. In particular, frequencies of CD57-expressing cells in blood and tissues have been correlated with clinical progress in chronic infections or various cancers and with human aging. Overall, the CD57 NK cell subset may play an important role in immune function and disease pathogenesis. We examine all these cells in relation to health and disease, review the mechanisms by which they kill their target cells, and show why it is important to measure them with regard to patients suffering from particular medical conditions, such as viral infections including COVID-19; long COVID; ME/CFS; cancer; autoimmune disease; toxic chemical exposure; chronic alcoholism; chronic pulmonary disease; chronic severe pain; transplantation; acute physical stress; and early pregnancy loss.

1 Introduction

Natural killer (NK) cells and cytotoxic T ($CD8^+$, CTL or TC) cells both originate from bone marrow (1, 2). Our bones produce hematopoietic stem cells which differentiate into common myeloid cells and common lymphoid cells. Common myeloid cells develop into cells of the innate immune system, while common lymphoid progenitors develop into cells of the adaptive immune system. While technically considered an innate immune cell like macrophages, neutrophils and the like, NK cells have been discovered to have adaptive properties as well, so that they are now considered a bridge between innate and adaptive immunity (3, 4).

In order for cytotoxic $CD8^+$ T cells to kill their enemy target cells, they first have to be activated or primed by antigen-presenting cells, which give them little pieces of the enemy material so that the $CD8^+$ T cells can identify their targets. Cytotoxic $CD8^+$ T cells are very efficient in the killing of pathogens, particularly viruses that live in the cell. Cells harboring such viruses express on their surface viral peptides bound to major histocompatibility complex 1 (MHC-1) molecules that are detectable by the cytotoxic T cell (5-8).

Conversely, NK cells kill their target cells without any priming or prior activation by other cells. Instead, NK cells basically have a form of friend-or-foe identification system. For the NK cell, the friend identifier is the MHC-1 molecule on the suspect cell's surface (9, 10). Unlike the $CD8^+$ cell, which detects the viral peptide bound to the MHC-1 to identify its target, if the NK cell finds an MHC-1 molecule on the surface of a suspect cell, it will not destroy that cell. If an NK cell does not find an intact MHC-1 on a target cell, it will go into kill mode and destroy that cell. However, there is a third scenario wherein a target cell still has an MHC-1 molecule, but also expresses a ligand, such as a viral antigen, that identifies it as an enemy cell. In this case, the ligand overrides the inhibitory "friend" signal from the MHC-1, and the NK cell goes into assassin mode on the target cell (9,10).

Thus, NK cells and $CD8^+$ T cells are complementary partners, cytotoxic sidekicks in kicking the butts of enemy cells (11).

2 Natural Killer (NK) Cells and Subpopulations

Natural killer (NK) cells are a subset of lymphocytes which are part of the innate immune system, and are critical for immune surveillance and defense against infection and cancer (9, 10). NK cells contain bullet-like materials called granules which are filled with different highly potent enzymes, earning them the name large granular lymphocytes. By releasing these granules, they can kill viral-infected cells and cancer cells, defending the body against pathogens and tumors (see Figure 1).

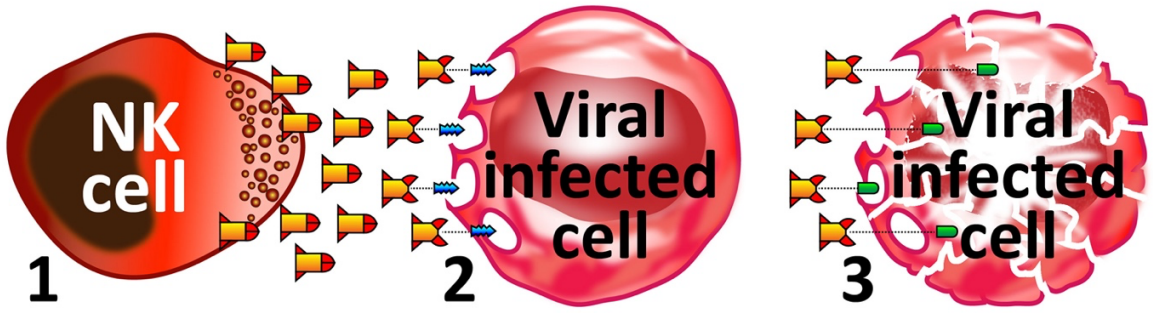


Figure 1. NK bullet attack. 1) The activated NK cell releases the granules (yellow bullets). 2) The granules release perforin (blue bullets), which blast holes into the surface membrane of the viral-infected or cancer cell. 3) The granules then release the granzymes (green bullets), which enter the cell through the holes made by the perforin and detonate inside the cell, causing apoptosis or cell death.

NK cells are involved in immunoregulation, and by producing different cytokines and mediators, they can shape adaptive immune responses (12, 13). Because of this immunoregulatory function, they play a significant role in autoimmunity. NK cells are capable of an array of functions that range widely from their classic anti-tumor and antiviral cytotoxic effector functions to their critical immunoregulatory roles. For example, NK cells activate dendritic cells, macrophages, T cells, T-helper cells, and $CD8^+$ cells. NK cells also stimulate plasma cells to produce antibodies (14). These different roles and functions require NK cells to interact with other cells, as shown in Figure 2.

NK cells compose 5 to 15% of peripheral blood mononuclear cells (PBMCs) in humans (9). Humans with deficient or dysfunctional NK cells are susceptible to infections that can potentially be life-threatening (15).

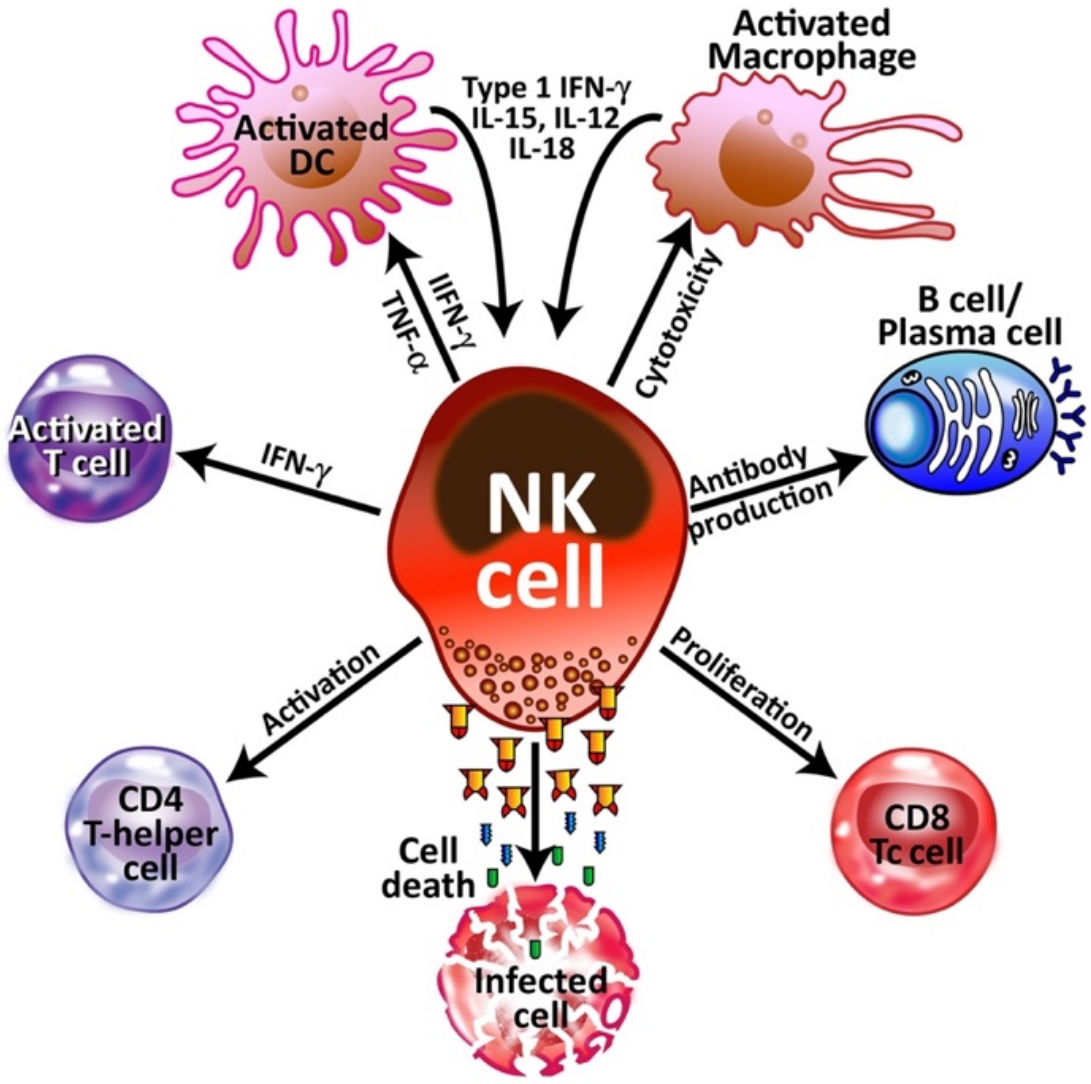
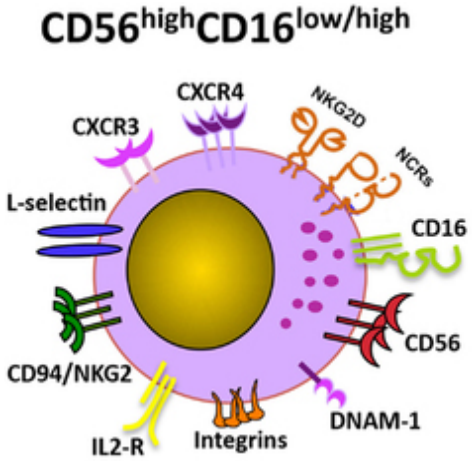


Figure 2. The multiple interactions of NK cells with other cells. NK cells perform various functions and roles that require them to interact with different cells in different ways, from shaping immune responses through the production of cytokines, to the outright killing or destroying of infected cells or cancer cells through the use of bullet-like granules.

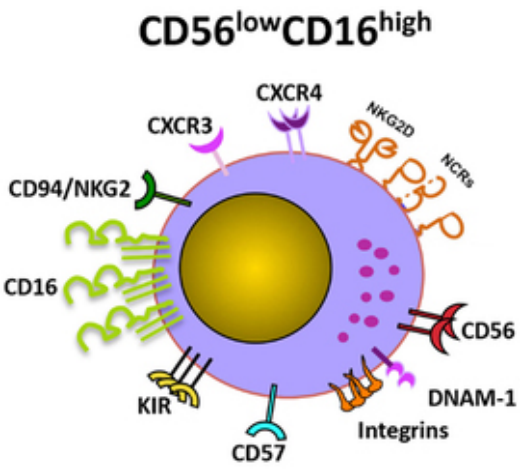
Among the different receptors found on their surfaces, NK cells carry two major cluster differentiation (CD) markers: CD16 and CD56. Based on the amount, strength, and concentration of the markers found on their cell membrane, NK cells can be classified into two basic subsets (16).

2.1 What are $CD56^{+}CD16^{dim}$ NK Cells?



$CD56^{bright}CD16^{neg/dim}$ or $CD56^{+}CD16^{-}$ NK cells make up about 10% of NK cells and are considered immature. These NK cells are found mainly in the lymphoid tissues. Despite the fact that they are only weakly cytotoxic, they are called regulatory NK cells because they are abundant producers of cytokines and chemokines such as interferon-γ, TNF-β, IL-10, and IL-13, giving them immuno-regulatory properties and a role in the shaping of the adaptive immune response. This cytokine-producing ability has been found to be negatively affected in people with myeloma. Phenotypic alterations of $CD56^{+}$ immune cell fraction have been reported in patients with various infectious autoimmune or malignant diseases (16-18).

2.2 What are $CD56^{dim}CD16^{+}$ NK Cells?



$CD56^{dim}CD16^{+}$ or $CD56^{low}CD16^{+}$ NK cells constitute about 90% of circulating NK cells and are considered mature. These are cytotoxic NK cells that are involved in the cytotoxicity of viral-infected cells and tumor cells. They are also involved in cytokine production. CD16 has been described as a receptor expressed on NK cells that facilitates antibody-dependent cellular toxicity (ADCC) by binding to the Fc portion of various antibodies on coated cells. For example, when an antibody binds to a receptor on a tumor cell, the $CD56^{low}CD16^{+}$ NK cell binds to the antibody, allowing the NK cell to release its granules and kill the cell. Likewise, if an anti-EBV antibody binds to a receptor on an EBV-infected cell, the $CD56^{low}CD16^{+}$ NK cell can bind to that antibody, induce the release of granules, and kill the infected cell (16, 17, 19).

NK cells are further divided into functionally distinct subsets of activating and inhibiting receptors which regulate NK cell activity. Among these are the killer-cell immunoglobulin-like receptors (KIRs), NKG2A, NKG2D, and natural cytotoxicity receptors (20-26).

2.3 What are CD57 Cells?

CD57 is a marker protein found on the surface of a subset of NK cells. They are a highly mature or developed discrete functionally distinct subpopulation of $CD16^{+}$ NK cells that are involved in immmunoprotection against pathogens and other environmental factors. Also called memory NK cells, they display high cytotoxic potential. They are highly-differentiated long-lived cells that are thought to be more efficient at killing their targets compared to other NK cell subpopulations. $CD57^{+}$ NK cells have become a matter of recent interest because they are now believed to play a unique role in immune function and have been implicated in a number of health conditions. The CD57 marker is also detected on neural cells, in which it acts as an adhesion molecule, and thus may be involved in the communications between the nervous system and the immune system. $CD57^{+}$ NK cells are

consistently associated with better outcomes in cancer and autoimmune disease. Low levels of $CD57^+$ are associated with lower overall survival in cancer (27).

CD57 is a very useful marker of NK cell maturation. Studies show that progression from $CD56^{bright}$ to $CD56^{dim}CD57^-$ to $CD56^{dim}CD57^+$ reflects a maturation pathway for NK cells (16). Acquisition of CD57 means a higher cytotoxic capacity, greater responsiveness to signaling via CD16 and natural cytotoxic receptors. $CD57^+$ cells identify the final stage of NK cell maturation, and their numbers increase with aging (20, 27-29). The numbers of $CD57^+$ NK cells also greatly increase in response to chronic exposure to antigens originating from tumor cells, bacteria, and viruses, particularly with EBV, CMV, HIV, hepatitis C and more. Increases in the detectable numbers of $CD57^+$ NK cells in blood are also associated with autoimmune disease (20, 30).

In different autoimmune diseases, $CD57^+$ NK cells fulfill an immunoregulatory role with their ability to delete autoreactive T cells that are chronically activated by viral antigens. Generally, increases in the population of autoreactive $CD57^+$ cells are associated with more severe autoimmune diseases such as Wegener's granulomatosis, multiple sclerosis, type 1 diabetes, Graves' disease, and rheumatoid arthritis. However, there are some instances where certain autoimmune diseases are consistently associated with reduced frequencies or absolute numbers of circulating $CD57^+$ NK cells and/or impaired cytotoxicity. In several autoimmune diseases such as atopic dermatitis, Sjögren's syndrome, IgA neuropathy and alopecia areata, a reduction of $CD57^+$ NK cells in peripheral blood was reported in comparison to controls (20).

$CD57^+$ NK cells are very important in early pregnancy failure. In fact, NK cell counts have been shown to be greater in a subgroup of patients who suffered recurrent pregnancy failure (3.42 ± 2.15) compared to controls (2.14 ± 1.42) (31).

In relation to many pathogens that induce or cause increase in $CD57^+$ cells, in 2001 it was reported that there was a reduction in the blood levels of $CD57^+$ NK cells in patients with chronic Lyme disease in comparison to those with acute Lyme disease and uninfected individuals (32, 33). However, a 2009 study found no differences between the NK cell counts of patients with chronic Lyme disease and those of controls (34). In fact, since the introduction of CD57 tests in the early 2000s, the first author (AV) has repeatedly stressed that low numbers for CD57 NK cells cannot be used as a specific biomarker for the diagnosis of Lyme disease. In agreement with this position, many authorities such as the CDC (35), North American medical experts (36), European science organizations (37), and the Royal College of Pathologists of Australasia (38) have explicitly warned against the use of CD57 and other unvalidated tests in the diagnosis of Lyme disease. Thus, we measure $CD57^+$ cells not for diagnosis of Lyme disease, but for indication of chronic exposure to antigens that originated from bacteria, viruses, tumor cells, and neoantigens formed in the body. The counting of $CD57^+$ cells is also recommended for patients with various autoimmune diseases, as a majority of them are induced by environmental factors (20). Finally, we recommend the measurement of $CD57^+$ cells for patients who suffered from recurrent pregnancy loss (31).

Overall, $CD57^+$ NK cells are a specialized subset of NK cells that may play an important role in immune function and disease pathogenesis (20).

2.3.1 Clinical Importance of Measuring $CD57^+$ NK Cells in Peripheral Blood

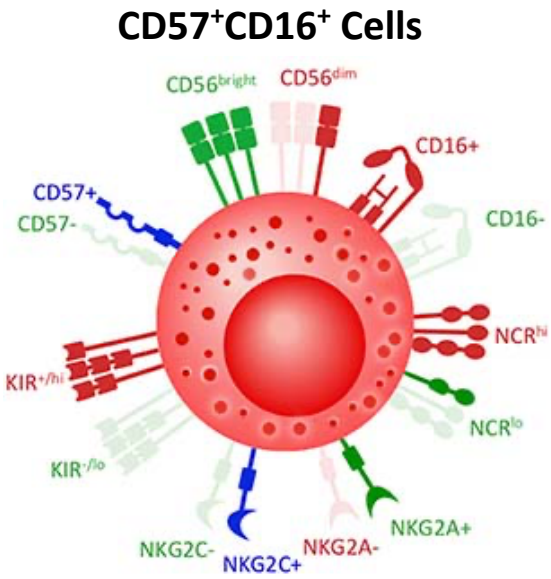
Persistent antigenic stimulation to various environmental factors, including bacteria, viruses, toxic chemicals and neoantigens results in the alteration of the number of $CD57^+$ NK cells.

Alterations in CD57+ cells have been observed in the following different major disorders (20, 29, 31, 39-44):

- Viral infections including COVID-19
- Long COVID due to viral persistence
- Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)
- Cancer
- Autoimmune disease
- Toxic chemical exposure
- Chronic alcoholism due to neoantigen formation
- Chronic pulmonary disease
- Chronic severe pain
- Transplantation
- Acute physical stress
- Early pregnancy loss

This is why the measurement of CD57+ NK cells clinically is highly, highly important.

2.3.2 What are $CD57^+CD16^+$ NK Cells?



$CD57^+CD16^+$ cells are a subpopulation of CD56dimCD16+ NK cells which carry CD57 markers of their surfaces. Cells that carry both CD57 and CD16 markers are highly, highly cytotoxic.

$CD57^+CD16^+$ cells contain the cytolytic enzymes granzyme A, granzyme B and perforin in their granules or bullet-like materials, enabling them to kill cancer cells and virus-infected cells with great efficiency. These cells are considered the most potent cells for combating acute and chronic viral infection (20, 27).

2.3.3 Importance of Measuring $CD57^+CD16^+$ NK Cells in Health and Diseases

1. Increase in CD57+CD16+ NK cells in chronic viral infection and other pathogens
   - Elevation in $CD57^+CD16^+$ cells is an indication of persistent antigenic stimulation, especially from viruses. Human CMV is one of the clearest examples of infection driving NK cell differentiation, particularly driving the expansion of $NKG2C^+$ NK cells, which preferentially acquire CD57. Mature CD57+ NK cells expand as a consequence of lifetime exposure to infections, including HBV, HCV, EBV, CMV,

hantavirus, and HIV. Their increase is an indication of ongoing or previous viral infections. Elevation in the levels of $CD57^+$ NK cells observed in patients with chronic viral infection correlates with slower disease progression (20, 27).

- Compared to healthy controls, in patients with severe COVID-19, high frequencies of $CD57^+CD16^+$ were detected despite the absolute number of these cells being decreased due to low WBC and low lymphocyte counts. Individuals with long COVID showed significantly increased levels of functional memory cells with high antiviral cytotoxic activity, including NK cells with the $CD56^+CD57^+NKG2C^+$ phenotype. The hypothesis that a persistent memory cytotoxic response against SARS-CoV-2 could be the cause of long COVID is supported by NK cells expressing both memory (CD57) and activation (NKG2C) markers that did not go away even after the initial SARS-CoV-2 infection was cleared (45-49).

2. Increase in CD57+CD16+ NK cells in cancer
   - $CD57^+$NK cells are reported in cancer patients. Increased frequency of $CD57^+$ NK cells has been correlated with less severe disease and improved survival of patients who suffer from carcinoma, lymphoma, and leukemia. $CD57^+$ cells can kill tumor cells through mechanisms that include perforin/granzyme-mediated cytolysis (Figure 2) and TRAIL- or Fas-mediated apoptosis (Figure 3). This killing of tumor cells by NK cells is initiated after the binding of the Fas ligand (FasL) or CD95 on the NK cells to the Fas receptor on a tumor cell, thus activating caspases which induce programmed cell death (Figure 3). CD57+ and CD16+ cells were assessed in patients with oral squamous cell carcinoma (OSCC). High level of $CD57^+$ NK cells correlated with longer overall survival. High levels of tumor-associated $CD57^+$ NK cells have also been associated with better outcomes in esophageal carcinoma, breast carcinoma, gastric carcinoma, and metastatic carcinoma (50).

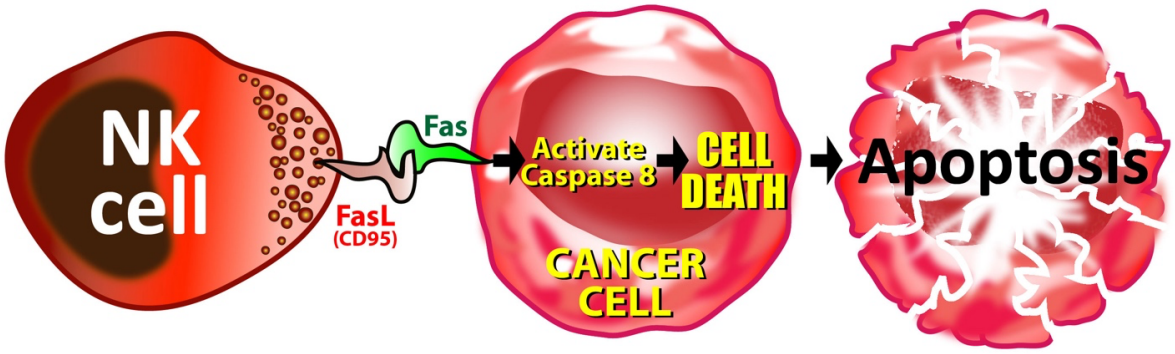


Figure 3. The caspase-mediated pathway to apoptosis. The binding of the CD95 Fas ligand (FasL) on the NK cell to the Fas on the tumor cell initiates a chain of events that activates Caspase 8, ultimately leading to the death or apoptosis of the cancer cell.

3. Changes in CD57+CD16+ NK cells in autoimmune diseases
   - Reduced frequencies or absolute numbers of circulating CD57+ NK cells and/or impaired NK cell cytotoxicity are consistently associated with autoimmune disease. This supports the regulatory role of cytotoxic $CD57^+$ NK cells in preventing or suppressing autoimmune

disease. For example, the blood level of CD57+ CD16+ cells is depleted in inflammatory and some autoimmune disorders, such as psoriasis, atopic dermatitis, rheumatoid arthritis, and type 1 diabetes. However, an increase in the number of $CD57^+$ CD16+ cells has been shown in tissues, such as skin, joints and pancreas, of affected individuals. Increased oxidative stress in patients with SLE may have caused preferential apoptosis of mature CD56dimCD57 + NK cells, perhaps contributing to the impairment of the ability to eliminate pathogenic $CD4^+$ T cells seen in SLE (16, 51).

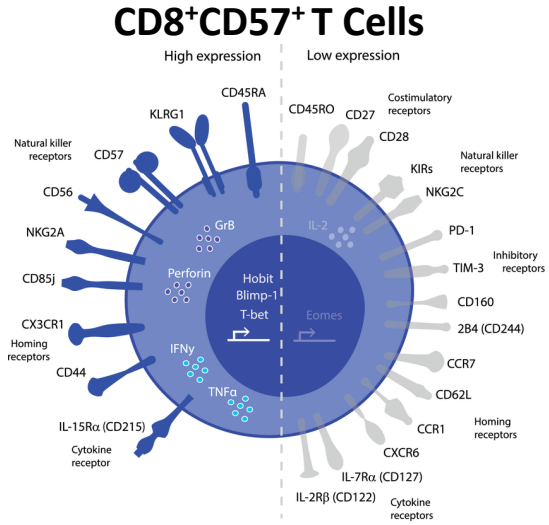
4. Increase in CD57+CD16+ NK cells in toxic chemicals
   - A significantly higher number of $CD57^+$ cells was detected in workers who were chronically exposed to hazardous chemicals such as aromatic amines (benzidine and beta naphthylamine). This increase in $CD57^+$ cells in workers exposed to toxic chemicals was associated with a decrease in the number of $CD4^+$ lymphocytes (52).

There is another important cell in the body's immune arsenal that partners with the NK cell like a superhero and his sidekick.

3 Cytotoxic $CD8^+$ T cells or cytotoxic T lymphocytes ($CD8^+$, CTL, TC)

As previously mentioned, $CD8^+$ T cells, like NK cells, originate in the bone marrow. However, what makes these cells different is that they migrate to the thymus and develop into maturity there, which is where the "T" in their name comes from. They include cytotoxic T cells, which are the main cellular warriors of the adaptive immune system, specializing in killing virally infected or malignant cells. They are the adaptive counterparts of the innate NK cells (5). They are called cytotoxic lymphocytes because they express cytotoxic molecules such as granzyme A, granzyme B and perforin. They also produce IFN-γ and TNF-α. These functions and properties together enable them to kill viral-infected cells and cancer cells with a high degree of efficiency. They are considered the most potent cells for stopping the spread of pathogens and tumor cells.

3.1 What are $CD8^+CD57^+$ T Cells?



$CD8^+$ $CD57^+$ T cells are a critical subpopulation of $CD8^+$ T cells that are mediators of adaptive immunity. CD8+ is formidable enough alone, but putting $CD8^+$ together with $CD57^+$ to get $CD8^+CD57^+$ is like combining two master killers into one super assassin. There is growing evidence that the $CD8^+CD57^+$ T-cell population plays a significant role in various diseases or conditions, associated with chronic immune activation such as cancer, chronic intracellular infections, chronic alcoholism, some chronic pulmonary diseases, autoimmune diseases, allogeneic transplantation, as well as has a great influence on age-related changes in the immune system status (7).

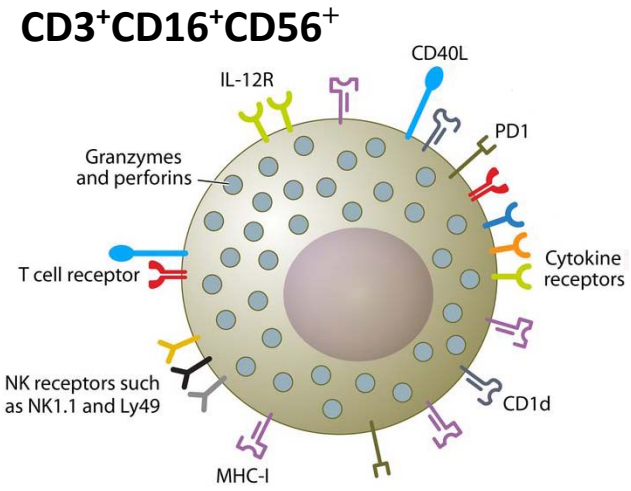
3.1.1 Importance of Measuring $CD8^+CD57^+$ T Cells in Health and Diseases

As a result of lifetime exposure to common antigens such as pathogens, neoantigens and autoantigens, the numbers of $CD8^+CD57^+$ cells increase with age. This is because persistent exposure to these antigens induce the expansion of $CD8^+CD57^+$ cells (53, 54).

1. Increase in $CD8^+CD57^+$ cells in chronic viral infection and other pathogens
   - An increase of the $CD8^+CD57^+$ T-cell population is observed in individuals with chronic infections such as EBV, CMV, measles, hepatitis C, parvovirus, HIV, SARS-CoV-2, toxoplasma, and more.
2. Increase in $CD8^+CD57^+$ cells in cancer
   - An increase in the numbers of $CD8^+CD57^+$ T cells has been observed in the blood of patients with different malignancies, including melanomas, head and neck cancer, and hemato-oncological diseases.
3. Changes in $CD8^+CD57^+$ cells in autoimmune diseases
   - Quantitative changes of the $CD8^+CD57^+$ T-cell population are observed in different autoimmune diseases, such as multiple sclerosis, type 1 diabetes, Graves' disease, ankylosing spondylitis, and rheumatoid arthritis. In Graves' disease, ankylosing spondylitis, polymyositis, dermatomyositis and rheumatoid arthritis, increased $CD8^+CD57^+$ T cells are also associated with severity of the disease. In rheumatoid arthritis, after treatment with Abatacep, a decrease of $CD8^+CD57^+$ T cells correlated with clinical response. In other autoimmune diseases, including lupus, type 1 diabetes and multiple sclerosis, decreases in the number of $CD8^+CD57^+$ T cells was observed.
4. Increase in $CD8^+CD57^+$ cells in chronic pulmonary disease
   - The number of $CD8^+CD57^+$ T cells is increased in patients with chronic obstructive pulmonary disease (COPD). This increase is associated with chronic antigenic stimulation coming from pathogens and inhalants which induce injury to epithelial and endothelial cells due to dysregulated immune response.
5. Increase in $CD8^+CD57^+$ cells in transplantation
   - In organ or bone marrow transplantation, the number of $CD8^+CD57^+$ T cells increase significantly to exert immunosuppressive activity. This increase in $CD8^+CD57^+$ T cells in transplant recipients is associated with better graft acceptance and stable function.
6. Increase in $CD8^+CD57^+$ cells in acute physical stress
   - In the case of acute physical stress, including high-intensity physical activity, $CD8^+CD57^+$ T cells are mobilized from peripheral tissue into the blood stream. However, during exercise recovery, these cells will return to their original tissues.
7. Increase in $CD8^+CD57^+$ cells in chronic alcoholism
   - The number of $CD8^+CD57^+$ T cells increases in chronic alcoholism. This increase is most likely caused by hepatocyte damage and chronic antigenic exposure to neoantigens or acetaldehyde-protein adducts after liver parenchymal necrosis. The expansion of $CD8^+CD57^+$ T cells could also be associated with antigens from chronic infections that are characteristic of heavy drinkers.

We have looked at NK cells and CTL cells, but there is a peculiar cell that seems to be half of one and half of the other.

3.2 What are $CD3^+CD16^+CD56^+$ NKT Cells?



$CD3^+CD16^+CD56^+$ cytotoxic natural killer T cells or NKT cells constitute a unique and very rare subset of CD1d-restricted T cells that serve as a bridge between innate and adaptive immunity because they carry receptors characteristic of both T cells and NK cells. They play a significant role at the interface between the innate and adaptive immune systems. They have major modulating effects on immune responses via secretion of cytokines. NKT cells are considered important players in tumor immunosurveillance, and have been shown to play a role in immune protection against microbial pathogens, different types of cancers, and in the control of autoimmune diseases (55, 56).

4 The Stages of the Cell Death Process

There are many important cell types in the human body, each with a specific function. However, one of the most important cells in our body that protects us against exposome factors is the natural killer (NK) cell. The primary job of the NK cells is the destruction of pathogen-infected cells as well as cancerous cells. They do this by releasing bullet-like materials or cytotoxic granules, which in turn release their own contents to kill the enemy cells.

This cytotoxic action of the NK cell is divided into three main stages:

Stage 1 – Target cell identification

NK cells have been shown to have many antenna-like structures or surface receptors that receive signals indicating there is an enemy nearby. Immediately these surface receptors are activated, not only to identify the signals generated from the surface of the pathogen-infected or cancerous cells, but also to alert the NK cell to prepare its ammunition for the upcoming battle.

Stage 2 – NK cell contact with target cells and formation of immunological synapses

Following target cell identification, the next phase of NK cell action involves NK cell contact with the enemy cells and the formation of cell-to-cell junctions with the target cells. This junction formation between the NK cell and target cells is very important because it ensures that only the harmful enemy cell is destroyed and not an innocent healthy cell. During this synapse formation, the activating signals induce the accumulation and movement of the granules through microtubules formed between the NK cell and the target cell.

Stage 3 – NK-cell-induced target cell death

The last phase of the NK cell's action is the destruction of pathogen-infected cells, cancer cells, cells modified by hazardous chemicals, or other stressed cells. This process which began with the immunological synapse formation is called the degranulation process or release of the bullets. Following the docking process, the granules release perforins that cause pore formation in the surface membrane of the target cell, granzymes, as well as other enzymes, such as serine proteases. Using the pores opened by the perforin, these enzymes gain entry into the enemy cells, where they activate caspase molecules that induce the death of the target cell, also known as apoptosis or the kiss of death. This mechanism by which NK cells kill pathogenic or cancerous cells is shown in Figure 4.

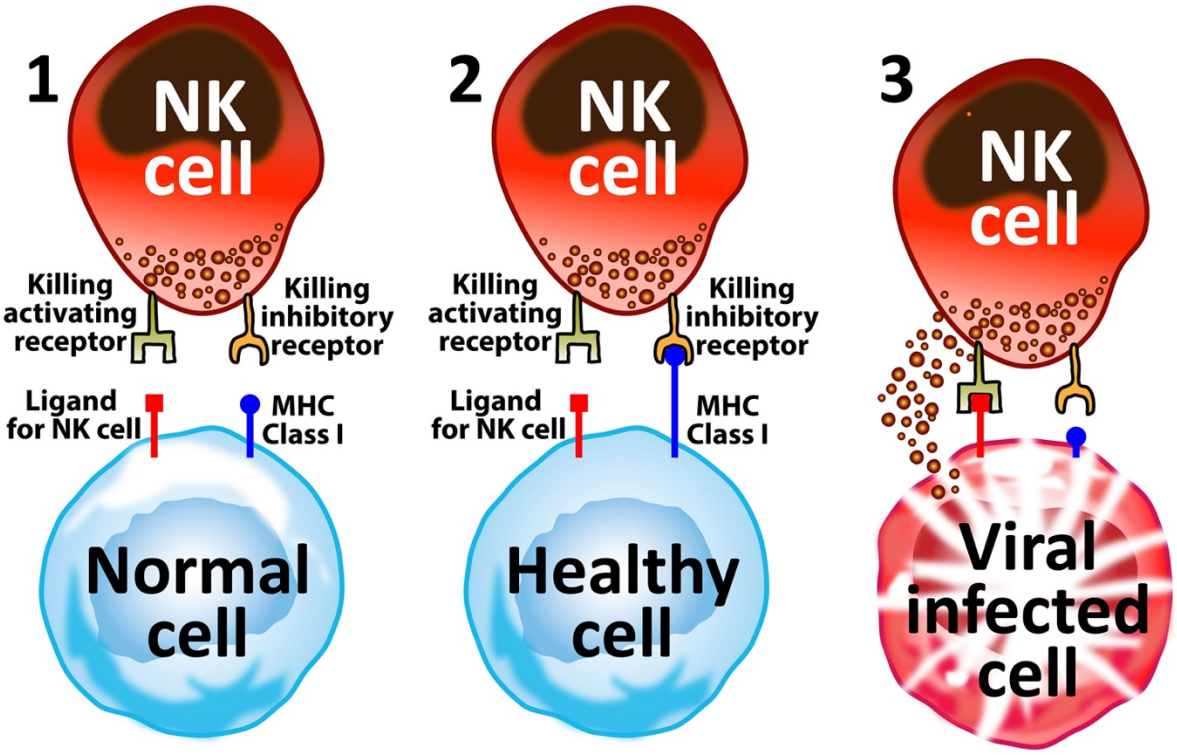


Figure 4. Why NK cells kill viral-infected cells and tumor cells, but not healthy cells. 1. Two different receptors on NK cells and their ligands on healthy cells ensure that NK cells do not attack normal cells. **2.** Healthy cells express a significant level of MHC Class I which engages with the killing inhibitory receptor (KIR). Because the ligand for NK cells does not engage the killing activating receptor (KAR), the NK does not kill the healthy cell. **3.** In viral-infected and cancer cells, the MHC Class I is significantly reduced, preventing the KIR from engaging with it. But the KAR on the NK cell binds strongly to the ligand for NK cells on the viral-infected/cancer cell, causing the release of destructive missiles from the NK cell, resulting in the killing of the viral-infected cell or tumor cell (see Figures 1, 3).

4.1 How NK cells kill target cells

First, some basics. NK cells are very specific killers. They will only kill infected, harmful or damaged cells. This is possible because NK cells have both a killing inhibitory receptor (KIR) and a killing activating receptor (KAR) (see Figure 4). On the other hand, the body's regular cells have on

the surface membrane a molecule called major histocompatibility complex 1 (MHC-1). These are basically name tags that identify a cell as belonging to you, the host.

If an NK cell comes in contact with a tissue cell and finds a name tag or MHC-1 molecule that identifies it as belonging to the host, the NK cell will leave that cell alone.

But if an NK cell comes in contact with another cell and finds a diminished or no MHC-1 due to viral or tumor damage, then that will be the signal for the NK cell to kill this target cell.

Below are the 5 main ways NK cells use to kill their target cells:

1. **Perforin**

   To perforate means to make a hole, and that's just what perforin does. Once a target cell is identified, the NK cell releases bullet-like materials called cytotoxic granules towards the target. The granules in turn release perforin, which lives up to its name by punching holes into the target cell.

2. **Granzymes**

   The NK cell's cytotoxic granules also release granzymes, which now enter the target through the holes made by the perforin. Once inside the target cell, the granzymes initiate the cell death process by activating the caspases. Think of the caspases as cellular scissors, cutting the DNA, RNA, and vital proteins inside the cell.

3. **Fas ligand (FasL)**

   NK cells also have FS-7-associated surface antigen (Fas) proteins on their surface. If the NK cell's Fas ligand (FasL) comes in contact with a Fas receptor on a target cell, this will initiate the same cell death process that the granzymes do, activating caspases to cut up the insides of target cells.

4. **Tumor necrosis factor alpha (TNF-α)**

   TNF-α is a cytokine, an immune product used by cells to communicate with each other. Similar to FasL, TNF-α can also bind to its own receptor and start the cell death process, destroying the contents of the target cell.

5. **Interferon gamma (IFN-γ)**

   IFN-γ actually does not directly kill harmful cells. However, it can be considered an accessory to cell murder, because what it does is boost immune antibodies and cells like macrophages to turn them into better killing machines. Life Science Identifiers

5 Why Do We Need to Measure $CD57^+$ NK Cells, $CD57^+CD16^+$ NK Cells, and $CD8^+CD57^+$ T Cells as Part of the Comprehensive LMAP™ at Cyrex?

- It is recommended for patients who would like to assess their immune system on a yearly basis.

- Individuals with repeated cold and flu and other viral infections.
- Individual who contracted COVID-19, was fully vaccinated, but is still repeatedly contracting the disease.
- Patients exhibiting symptoms of long COVID and/or myalgic encephalomyelitis/chronic fatigue syndrome.
- Person with abnormal blood levels of IgG, IgA, IgM (GAM) and IgG subclasses.
- Person with broken essential barriers (lungs, gut, blood-brain barriers) who are constantly exposed to antigenic stimulation.
- People who are exposed to hazardous chemicals, neoantigens, molds and mycotoxins.
- Individuals in early stages of autoimmunity and full-blown autoimmmune disease.
- People suffering from different types of cancer.
- Someone suffering from chronic alcoholism with repeated cold and flu.
- A patient with chronic pulmonary disease.
- Women who have suffered early pregnancy loss.
- Individuals suffering from stress, including acute physical stress.

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