

LECTURE: 14

Title: NATURAL KILLER CELL FUNCTIONS AND SURFACE RECEPTORS

LEARNING OBJECTIVES:

The student should be able to:

- Describe the general morphology of the NK-cells.
- Enumerate the different functions of the NK-cells.
- Describe how can NK-cells functionally distinguished from T, and B-lymphocytes.
- Identify the NK-cell surface receptor that helps in binding to Fc portion of the IgG.
- Enumerate the different types of cytokines secreted by NK-cells in order to regulate both haemopoiesis, and immune responses.
- Enumerate the different types of NK-cell surface receptors, and identify their major functions such as:
 - CD16 (FcyRIII).
 - CD56.
 - CD57.
 - IL-2R (β -chain).
 - CD 94.
 - CD158 (the killer inhibitory receptor).
- Activation of NK, and the role of the INF- γ that produced by the NK cells.
- Identify the main function of Fas ligand (FasL)
- Explain the role of NK cell in the cooperation with the elements of the innate immune system.

LECTURE REFERENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE
IMMUNOLOGY. 6th edition. Chapter 2. pp. 21-22.

2. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter 1. .pg 4, 11, 12f, 17, 19t. Appendix 495.

3. TEXTBOOK:LAUREN SOMPAYRAC. HOW THE IMMUNE SYSTEM WORKS. PP 24-26.

NATURAL KILLER CELL FUNCTIONS AND SURFACE RECEPTORS

INTRODUCTION

Natural killer cells are classified as lymphoid cells, it thought to be derived from lymphoid cell progenitors in the bone marrow, and they are characterized by LGL morphology. NK cells express a large number of azurophilic than T cells. They represent about 15% of blood lymphocytes, 10-15% of bone marrow lymphocytes, 1-5% of the lymphocytes in the spleen, < 1% of the lymphocytes in the thymus, and < 1% of the lymphocytes in the lymph node. Natural killer cells express neither TCR nor BCR antigen receptors (**Figure-1**).

NATURAL KILLER CELL FUNCTIONS

Several functions can be performed by these types of cells, these are:

1. Functionally NK-cells are distinguished from T and B cells by their ability to lyse certain tumour cell lines (but not fresh tumours) *in vitro* without prior sensitization.
2. NK-cells recognize and kill virally-infected cells.
3. NK-cells can bind and kill targets coated with IgG antibodies via their receptor for IgG (Fc γ RIII: CD16). This property is referred to as antibody-dependent cellular cytotoxicity (ADCC).
4. NK-cells release interferon- γ (IFN γ) and other cytokines (e.g., IL-1 and GM-CSF) when activated, which may be important in the regulation of haemopoiesis and immune response.

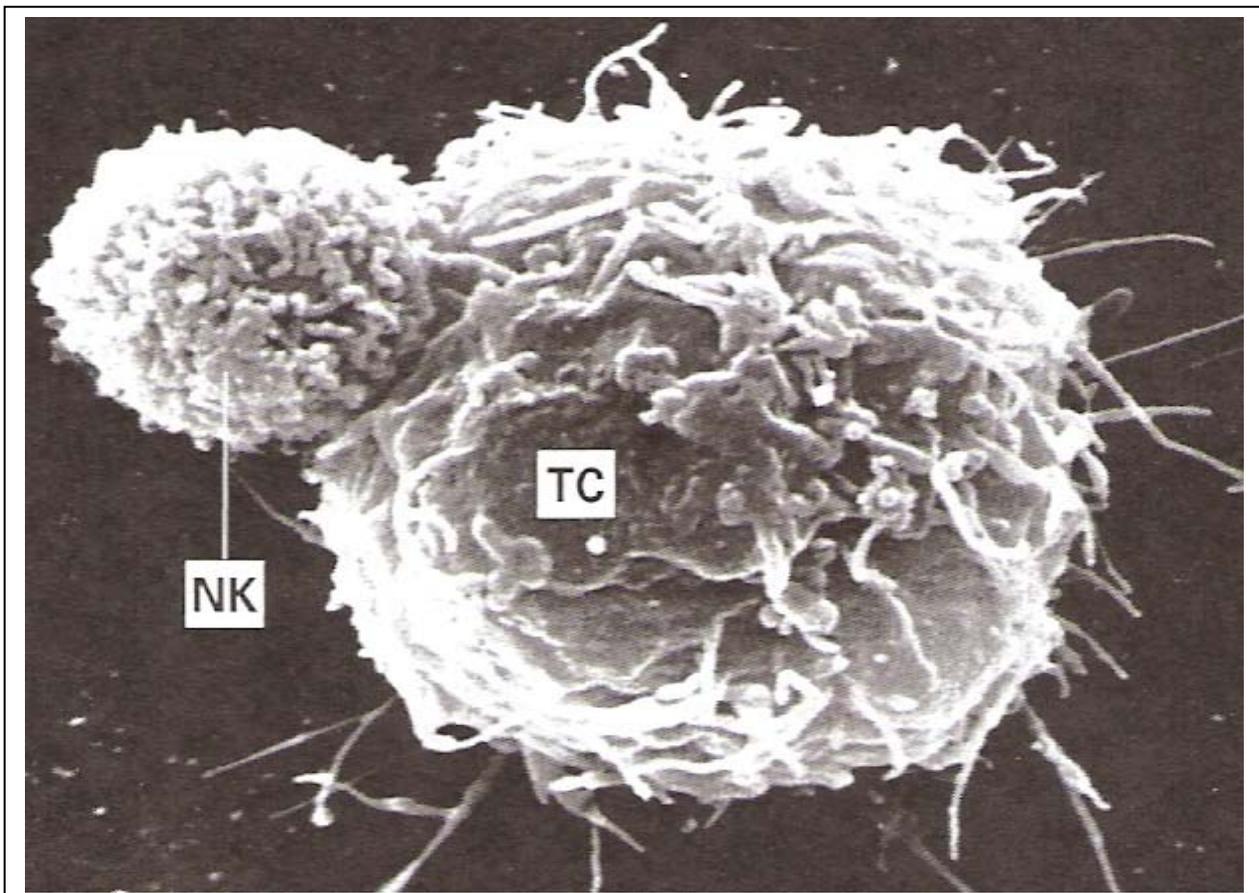


Figure-1 A natural killer cell (NK-cell) attached to a target cell (TC).

Phenotypic markers of NK cells

Most the cell surface markers of the NK cell are shared with T cells or monocytes/macrophages. The major marker of human NK cell is:

| <u>Marker</u> | <u>Shared specificities</u> |
|----------------------|---|
| CD16 (Fcγ RIII) | minority of T cells, granulocytes, some of the macrophage |
| CD11b | granulocytes, monocytes, some T cells |
| CD 38* | activated T cells, plasma cells, haemopoietic precursor |
| CD 2* | All T cells |
| CD 7 | All T cells |
| CD 8* | Some of T cells |
| CD 56 | minority of T cells |
| CD 57 | Some of T cells |
| IL-2R (β chain, P70) | activated T cells |
| p58 family | Some T cells |
| CD 94 | Some T cells |

* expressed in 10-80 % of NK cells

CD 16 (Fcγ RIII) is used to identify the NK cells in the purified lymphocytes population. CD 16 is found on some macrophages, neutrophils, and small population of TCR-1⁺ T cells. CD 56 molecule is homophilic adhesion molecule of the immunoglobulin superfamily receptor. **The absence of CD3 and the presence of CD56 and /or CD16 is currently in use as a definitive marker for NK cells in man.** Resting NK cell express the α chain of the IL-2 receptor (70 kDa). All T cells that show LGL morphology (TCR-1⁺ cells and proportion of TCR-2⁺ cells) also express this 70 kDa receptor. These cells express neither TCR nor BCR antigen receptors. But most surface antigens detectable on NK-cells by monoclonal abs are shared with T cells or monocytes/macrophages. CD16 (FcγRIII) are commonly used to identify NK-cells in purified lymphocytes populations. This marker is identified by monoclonal abs. CD16 is expressed also on neutrophils, some macrophages, and Some T cells (mainly TCR-1⁺ cells). CD56 molecules, a homophilic adhesion molecule are another important marker for NK-cells. The absence of CD3, but the presence of CD56 and /or CD16, is used as a definitive marker of NK cells. The IL-2 receptor is expressed on the resting NK cells. This receptor is expressed on all T cells that display LGL morphology, namely TCR-1⁺ cells respond to IL-2 by acquiring non-specific cytotoxic functions, and are known collectively as lymphokines activated killer (LAK) cells. LAK cells kill fresh tumour cells.

Activation markers on natural killer cells

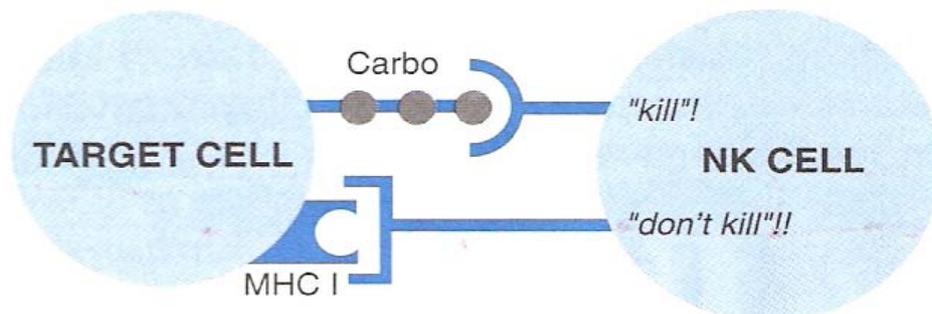
These activation markers include;

- * **Adhesion** molecules (allow efficient interaction with other cells)..
- * **Receptors** for growth and differentiation factors (required for continued proliferation and maturation). For example, **IL-2 receptor** which expressed following T cell **activation** is composed of three subunits. Resting T cells have the β unit (a low-affinity receptor of 55 kDa (CD25), and the γ unit. On activation the α subunit (70 kDa) is induced resulting in heterotrimeric high affinity IL-2 receptor. The **gp39 (CD71)** is a receptor for transferrin, it transiently expressed on the activated T cell, and it is important for **proliferation**. **Class II MHC molecules** are present on human T cell as a **late activation marker**. **CD29** is expressed on the T cells and the memory cells as a **very late activation marker**. **Activation markers on the NK cells include MHC class II molecules.**

ACTIVATION OF NATURAL KILLER CELLS

In addition to the complement system and the professional phagocytes, there is a third important player on the innate immune system team, the natural killer cells (NK) cell. This has been a difficult cell for immunologists to study, because there are different kinds of NK cells with some what different properties. These cells were originally called large granular lymphocytes, because, like the professional phagocytes, they are full of granules that contain chemical and enzymes. Although NK cells are descended from stem cells just like the rest of the blood cells, it is still uncertain just where NK cells fit on the family tree. The most recent evidence suggests that they are in the same family as the lymphocytes (T and B cells), but they also have some similarities to macrophages

Like neutrophils, NK cells use the "roll, stop, exit" strategy to leave the blood and enter tissues at sites of infection. Once in the tissues, NK cells are quite versatile. They can kill tumor cells; virus-infected cells, bacteria, parasites, and fungi, and they have at least two methods of killing. First, they can bore a hole in a target cell by secreting perforin molecules to form a membrane attack complex on the surface of the target. NK cells can then secrete enzymes that enter the target cell and cause it to commit suicide. As their second weapon of destruction, NK cells can use a protein called **Fas ligand (FasL)** that is expressed on the NK cell surface. FasL can interact with a protein called Fas on the surface of the target, and when these two proteins connect, they can signal the target cell to commit suicide by apoptosis. One of the mysteries about NK cells is how they identify which cells to kill. Their method of target recognition is quite different from that of T cells, which recognize their targets through their T cell receptors. NK cells have no TCRs, so they must be looking at something besides peptides displayed by MHC proteins. The latest thinking is that killing by NK cells actually requires two signals, a "kill" signal and the absence of a "don't kill" signal.



The "don't kill" signal seems to be the expression of MHC-I molecules on the surface of the potential target, because cells that express MHC I usually can't be killed by NK cells. The "kill" signal is thought to involve interactions between proteins on the surface of the NK cell and special carbohydrates on the surface of the target. Presumably, the carbohydrates molecules act as flags that indicate the cell has been infected with a virus or has become a tumor cell, but this part is still poorly understood. The best current synthesis of this two-signal system is that the balance between the "kill" and the "don't kill" signals determines whether NK cells will kill a target cell.

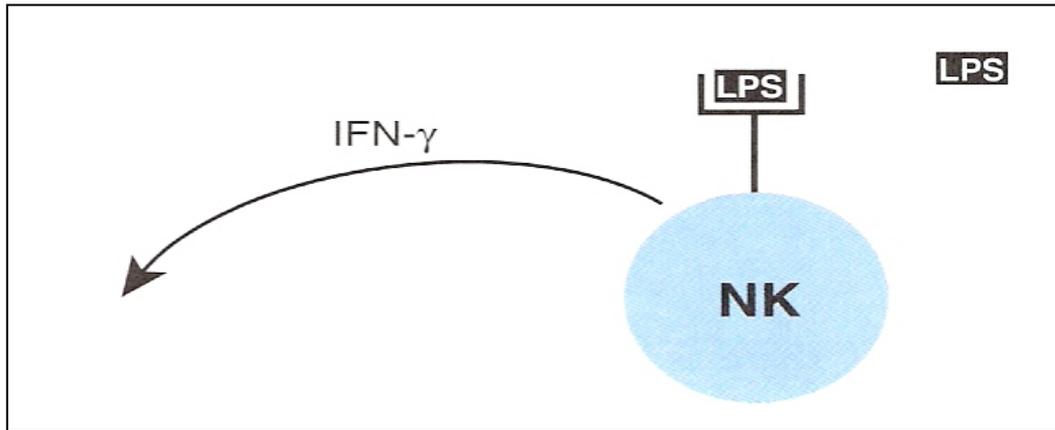
Now, why do you think it would be a good idea to have NK cells kills targets that do not express MHC molecules? Well, you remember from the introduction that killer T cells (CTLs) recognize foreign peptides presented by MHC proteins. So suppose some clever virus of cancer cell decides to turn off expression of these MHC molecules. Then that virus-infected cell or cancer cell would be invisible to the CTL, right? Well, it turns out that this exactly what many viruses and cancer cells do downregulate MHC expression. So, in those cases, wouldn't it be great to have another weapon that would kill virus-infected or cancer cells that don't have MHC on their surfaced? You bit it would. And that's something NK cells do: they specialize in killing cells that lack MHC molecules on their surfaces.

NK cells have a couple of other interesting features. First, in contrast of T cell, which need to be educated not to attack self, NK cells are genius cell that don't need this education. Somehow, probably through recognition of the "kill" carbohydrate, the NK cell knows an invader when it sees one, It is also interesting that NK cells are rather like CTLs and helper T cells all rolled into one. NK cells use perforin and FasL to kill, just like CTLs, but in addition, NK cells can function as cytokine factories, much kike Th cells. Indeed, NK cells are one of the major suppliers of IFN- γ .

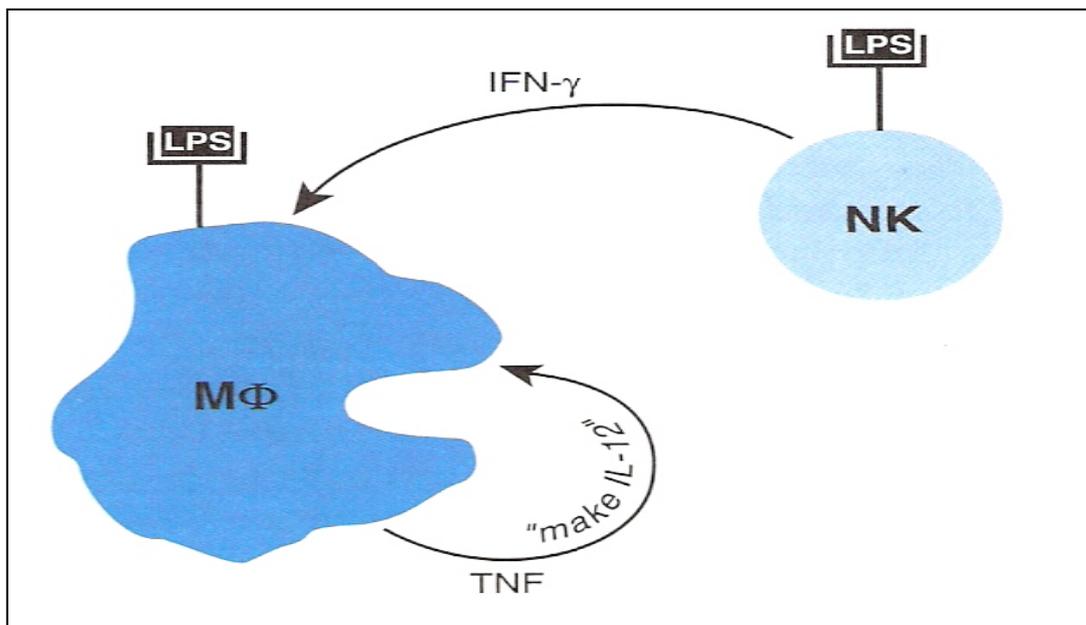
In some ways, NK cells also resemble macrophages. They contain lots of lysosome-like granules, and they can exist in several stages of readiness. Resting NK cells are able to produce some IFN- γ and can kill, but they produce more IFN- γ and kill a lot better if they are activated. So, what activates these killers? Several signals have been identified that can activates NK cells. One is the bacterial cell wall component; LPS that we learned earlier could activate macrophages. In addition, NK cells can be activated by the alarm interferons, IFN- α , and IFN- β , that cells give off when they are under viral attack. Certainly, other signals will be discovered that can activate NK cells, but you get the idea: NK cells are activated by signals that indicate an attack is on.

THE INNATE IMMUNE SYSTEM A COOPERATIVE EFFORT

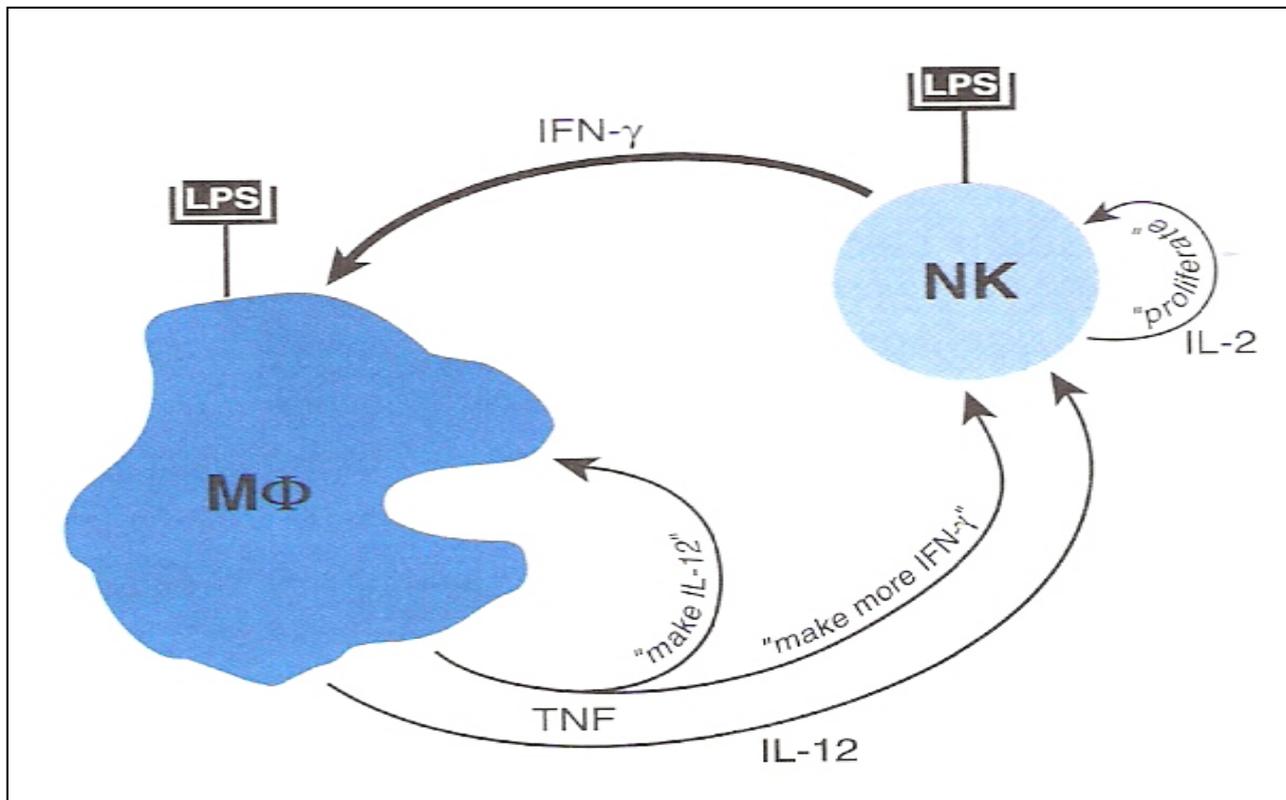
To make the innate system work efficiently, there must be cooperation between players on the innate system team. For example, there are two cytokines that help determine how much IFN- γ NK cell give off: TNF and interleukin 12 (IL-12). Whereas signals like LPS tell the NK cells to shift to a higher state of activation, TNF and IL-12 actually act like accelerators, the more TNF and IL-12 NK cells receive, the more IFN- γ they make. And where do the accelerating factors, TNF and IL-12, come from? Why, from activated macrophages. so the IFN- γ needed to prime macrophages come from NK cells, and the TNF and IL-12 required to increase the amount of IFN- γ made by NK cell come from macrophages. As a result of this interdependence, macrophages and NK cells work together to get each other fired up. Here's how it works: During a bacterial infection, molecules like LPS bind to receptors on the NK cell surface, and this signals that an attack is on. NK cells respond by producing significant amounts of IFN- γ .



The IFN- γ produced by NK cells can prime macrophages, which can then be hyperactivated when their receptors also bind to LPS.



When a macrophage is hyperactivated, it produces lots of TNF. The macrophage also has receptors on its surface to which this cytokine can bind. When the TNF produced by the hyperactivated macrophage binds to these receptors, the macrophage begins to secrete another cytokine, IL-12. Together, TNF and IL-12 cause NK cells to produce even more IFN- γ , so that even more macrophages can be primed.



Now, there is something else neat going on here. IL-2 is a growth factor that is produced by NK cells, and which can cause these cells to proliferate. Normally, however, NK cells don't express the receptor for IL-2, so they are unable to respond to this cytokine, even though they are making it. Fortunately, macrophages can fix this problem, because TNF from the macrophage upregulates expression of IL-2 receptors on the surface of the NK cells. Consequently, NK cells can now respond to the IL-2 they make and begin to proliferate, and as a result of this proliferation, there will soon be many more NK cells to defend against the invader.

So here you have these two cells, the macrophage and the NK cell, cooperation got set up a positive feedback loop. This positive feedback loop produces a "snowball" effect that help the innate system respond quickly and strongly to destroy invaders. This interaction between macrophages and NK cells is an excellent example of cooperation between the innate system players. Another example is the cooperation between the complement system and the professional phagocyte. As you learned earlier, complement protein fragments such as C3b can opsonize invaders for phagocyte ingestion. But complement opsonization can also play a role in activating macrophage, because when C3b binds to its receptor on the surface of a macrophage, it can provide an activation signal similar to that provided by LPS. This is a good idea, because there are invaders that can be opsonized by complement, but which do not make LPS. Cooperation between the complement system and the phagocytes is not a one-way street. Activated macrophages actually produce several of the most important complement components: C3, factor B, and Factor D. This can be a big advantage, because in the heat of battle, complement proteins may be depleted, and macrophages can help resupply the complement system. In addition, in an inflammatory reaction, macrophages secrete chemicals that can increase the permeability of nearby blood vessels. As a result, these vessels become leaky. And what do you think leaks out into the tissues? More complement proteins. In fact, in most cases, the adaptive system will not respond to an invader unless the innate system has already recognized that there is danger.

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