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The Natural Killer Cell - 'Missing-Self' Recognition Strategy

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Abstract

The immune system is a built-in defense mechanism, which continuously monitors invading pathogens in all living organisms and keeps them physiologically fit. Both the innate and adaptive arms of the immune system are involved in this process of tolerating 'self' and targeting 'non-self' cells and their respective cellular antigens. Innate immunity is the first line of host defense and it is specialized in counteracting cancer cells and virally infected cells. In this line, natural killer (NK) cells form an important component. The 'self' is bestowed on the organism by embedding MHC (Major Histocompatibility Complex) molecules on the cell membrane. The loss or down-regulation of self-MHC class I molecules is sufficient to induce NK cell sensitivity. This is a simple rule for 'one' of the recognition strategies of NK cells according to the ' *missing-self hypothesis'*. It provided a basic platform of predictions for further conceptual development in the area of NK cell biology.

Introduction

The evolutionary success of a species depends upon the strategies adopted by its members in their struggle for existence as expounded by *Charles Darwin*. The struggle among the members of a population within a species and in between the species, not only aims at searching for mates, shelter and food, but also includes within an organism *viz*., combating against invading pathogens and eliminating obsolete or worn out cells. Through organic evolution, the biological system is endowed with increasingly complex assemblies of cells adept to a wide range of environments. Multicellular organisms strive to preserve the order and integrity through intricate but precisely defined interactions. Maintaining such an equilibrium, a process commonly termed as homeostasis, is vital for life and requires a sophisticated regulation. Organisms have developed a wide variety of genetically programmed defense reactions to counter threats to their existence. This is a function of one of the organ systems, namely immune system, to ensure biological fitness. A prerequisite in the defense reaction is to identify an untoward event as a threat to the well-being of an organism. This surveillance mechanism operates by two strategies: a positive identification strategy, which constitutes an activation step upon identification of 'disfigured-self or missing-self' molecules in the target cells where self molecules are MHC class I glycoproteins and negative or no response strategy which is an inactivation step (tolerance) upon recognition of cells presenting 'self' molecules thereby preventing auto reactivity against self cells.

In the present article, an attempt has been made to elucidate the recognition strategy adopted by one of the immune cell types of the lymphoid progeny, namely natural killer (NK) cells. They are vital for the elimination of foreign, infected and malignant cells from within the body of a mammal. The application of the NK cell '*missing-self hypothesis'* in therapeutics is also highlighted.

Immune Competent Cells

The hematopoietic microenvironment among mammals is the site for perpetual replenishment of hemocytoblasts (stem cells). Under the influence of respective cytokines/lymphokines, they get differentiated either as leukocytes or lymphocytes or erythrocytes or platelets (1). The **myeloid progeny** yields leukocytes; they constitute monocytes, basophils, eosinophils and neutrophils. These cells are phagocytic as they are endowed with TLRs (Toll-Like Receptors) and PRRs (Pattern Recognition Receptors/Pathogen Recognition Receptors). These receptors facilitate the recognition of pathogen-associated molecular patterns (PAMPs), characteristics of groups of pathogens. Thus, leukocytes are involved in innate immunity, protecting the host from infection by identifying conserved non-self molecules. It is the first line of defense in devouring pathogens, processing antigens from pathogens and presenting them to T-helper or cytotoxic T cells.

On the other hand, the **lymphoid progeny** yields lymphocytes. They are NK cells, B-cells and T-cells.

Both B- and T-cells are tuned towards adaptive immunity, the process that elucidates the unique mechanism in the retention of immunological memory. However, NK cell does participate differently in immune surveillance. The strategy adopted by NK cells invariably differs from that of T-cells. T-cells recognize foreign **antigens** presented by HLA-I or II molecules, whereas NK cells recognize target cells with either lost or down-regulated HLA-I transmembrane molecules (2, 3).

Self - Recognition Molecules are none other than MHCs

Invariably all healthy cells of mammalian organ systems possess transmembrane immunological molecules namely MHC (Major Histocompatibility Complex) molecules. Their counterparts in humans are HLAs (Human Leukocyte Antigens). In immunological parlance, both these acronyms are used synonymously. They are ubiquitously distributed among nucleated cells. MHC class I and II differ in their structure, participation, and at times in their distribution among varied cells. Nevertheless, both these molecules share a few common attributes *viz*., spatial location on trans-cell membranes and presentation of antigens of pathogens to immune competent cells. MHC class I molecules dock viral or tumor antigens and are presented to **CD**8+ T cells, whereas the processed bacterial antigens are presented through MHC class II molecules to **CD**4+ T cells. Thus, MHC molecules, through their presentation, make the immune competent cells alert and initiate a cascade of events to set up a designated and customized adaptive immunity.

In addition, transmembrane MHCs are designated as **self-recognizing** molecules. The possessors (cells) of these molecules in an organism are tolerated by their own immune competent cells and thus protected from auto-attack. Quintessentially, NK cells, T-cells and B-cells perpetually depend upon MHCs for executing their function; however, the strategies adopted by them invariably vary. Therefore, the presence of MHC molecules on nucleated cells of an individual serve as a benchmark or diagnostic tool for immune competent cells to assess them either as self or healthy or infected or foreign or worn out cells.

Natural Killer (NK) Cells

NK cells provided ample scope for immunologists for more than 30 years to unfold intricacies of 'killing cellular machines'. These cells were first identified in mid-1970s by virtue of their ability to rapidly lyse tumor cells *in vitro* without previous 'priming' (2, 4). Since then, much knowledge has been acquired with respect to their origin, differentiation, receptor repertoire and effector functions, as well as their ability to shape adaptive immune responses (4-7). NK cells represent a unique subset of lymphocytes, distinct from T- and B-cells. Further, their participation is well documented in host antimicrobial and antitumour defense reactions (8, 9), defense against bacterial and viral infections (10-12), control of **hematopoiesis** (13-15), *in vivo* rejection of tumor cells (16-18), prevention of tumor metastasis (18-21) and **allogeneic resistance to bone marrow grafts** (22). Thus, NK cells constitute the first line of innate immune defense. They are normally found in peripheral blood and constitute around 15% of lymphocytes.

NK cells are studded with several surface receptors (described in the following section). They facilitate the recognition and instant lysis of **allogeneic cells**, infected cells and malignant cells **(**23, 24) by binding to cognate ligands expressed by these target cells (Fig.1). Unlike NK cells that detect the absence of MHC-I molecules in a target cell, the viral antigens presented by MHC class I molecules of the infected cell are recognized by cytotoxic-T cells and in effect lyse the infected cell. If the target is an allogeneic cell, certainly it possesses incompatible MHC antigens or if the target is a malignant or an infected cell, it is most commonly associated with the loss or down- regulation of MHC class I molecules. Therefore, the incompatible and lost MHC class I antigens of the target cell enable NK cells to recognize and execute target cell lysis. That is to say that the lytic program of NK cell is prompted by **negative cues** from the target cell, namely the absence or loss of self-MHC class I molecules. In other words, NK cells detect the **missing-self antigen** in allogeneic and malignant cells (25).

NK Cell Receptors And Recognition of Target Cells

Living cells perpetually interact with neighboring cells within an organism, just as we interact with our neighbors through sensory cues; it may either be bacteria or protozoa or moving corpuscles in metazoans. That is the reason why **immune synapse** and **neural synapse** are comparable. To facilitate interaction, cells are equipped with transmembrane molecules namely receptors. Principally, the immune

competent cells are profusely equipped with receptors that recognize and bind to cognate ligands present on target cells, and are thus obliged to defend the body against the invading microorganisms and malignant cells. Furthermore, the immune system needs to keep a track of future encounters apart from just recognizing foreign cells. Interaction among immune competent cells is comparable to sharing of information amongst themselves about the detection of target cells, a process that is mediated through cytokines and receptors. As highlighted in the previous section, NK cells recognize the presence of self-MHC class I ligands and binding of these ligands to their corresponding receptors prevent (Fig.1a) target cell lysis. The inhibitory receptors carry immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails that initiate inhibitory signals in NK cells. Further, NK cells make use of their inhibitory KIRs (Killer Immunoglobulin-like Receptors) to protect (tolerate) its host self-corpuscles from being targeted. On the other hand, loss or down-regulation of MHC class I molecules and the presence of activating ligands on the target cell that bind to corresponding activating receptors on NK cells, will render it susceptible to NK cell attack (Fig.1b). To put it more simplistically, when an NK cell finds a susceptible target, *ex*: a tumor cell, it deploys a tentacle with venom sacks and attaches to the target cell. It then reaches out with another tentacle to search for an 'off switch', a flag that tells the NK cell to back off and not to kill the cell. If such a flag is not found, the NK cell blasts the target cell with particles, penetrating its outer shell and injects venom into the target cell. The venom pulsates in and out of the cell until it disintegrates into small pieces. This drama is reenacted almost 10,000 times every day in a pathological situation wherein cancer cells are formed and destroyed in a vulnerable person's body. If this activity stops, the cancer cells are free to grow and become a clinical case of cancer disease.

The Missing-Self Concept

As elaborated in the previous sections, all the three lymphocytes namely NK, T- and B-cells rely on MHC molecules for recognition and to elicit functional responses against targets. Of course, their dependence on target MHC molecules varies. NK cells discretely detect the lack of expression of (missing) MHC class I molecules on the target cell. Further, the presence of activating signals prompts an NK cell to initiate its lytic function (Fig.1b). It is quite unusual to detect a target cell on the basis of missing display of self-molecules in the biological system. Generally, the presence of molecules generates sensory cues. The major breakthrough in understanding NK cell recognition, and its function to detect and eliminate cells that fail to express normal self-markers, came from the *"missing-self hypothesis"* proposed by Klas Kärre and collaborators (25-28). According to this hypothesis, NK cells detect the information that is missing on the target but present in other cells of the host as opposed to T cells and this can be expressed as 'activation upon recognition of the unexpected and inactivation upon recognition of the expected'. More specifically this hypothesis postulates that the absence or incomplete expression of a complete set of host MHC class I molecules will be sufficient to render a diseased or infected or worn out host cell susceptible to NK cell attack (25). Two models proposed to explain how NK cells detect missing MHC molecules are described below.

(1) **Effector inhibition model**: In essence, this model envisages that receptors of NK cell transduce a negative signal after recognizing the self-MHC class I molecule (Fig.1a). Upon encountering a target, there is a molecular interaction between NK cell and its target. In normal course, this would cause the lytic mechanism to initiate. However, soon after the detection of MHC class I molecules on the target cell, the effector function (lysis) of NK cell is inhibited (Fig.1a). Thus, 'effector inhibition' is an outcome of the presence of 'self' molecules on the target.

(2) **Target interference model**: This model does not focus on negative cues. However, it envisages that the triggering receptors on NK cells recognize their ligands on the target cell upon contact. Further, the presence of MHC class I ligand on the target interferes with the interaction and thus impedes lytic mechanism.

The above two models mainly emphasize the function of NK cell in tolerating 'self' corpuscles.

Importance of Missing-Self Concept

Since the time the '*missing-self hypothesis*' was first proposed, much has been learnt about NK cell surface receptors, their role in the molecular basis of missing-self recognition and the mechanisms underlying NK cell tolerance (29-31). The most obvious importance of this concept in its validity lies in the understanding how autoimmune dysfunction is prevented in human body *i.e.,* normal cells are protected from the destruction by NK cells. One of the current interests in the NK field is the use of NK cells

in **KIR-ligand (MHC) mismatched allogeneic HSCT** (Hematopoietic Stem Cell Transplantation). This has a most direct application of the '*missing-self*' concept in a clinical perspective. In the KIR-ligand mismatched HSCT, **donor-derived licensed** (functional) **NK cells** are infused into patients whose MHC class I ligands are incompatible to the donor NK cell inhibitory KIRs (since each individual has a different MHC expression profile which arises due to MHC polymorphism). This type of NK cell-mediated therapy resulted in enhanced clearance of tumors with improved outcome for patients with almost no GvHD (Graft versus Host Disease). This **alloreactive NK model** has already become a clinical tool in high-risk leukemic pathological conditions (32). Yet, another case study (33) revealed that patients with acute myelogenous leukemia (AML) having high number of NK cells in their peripheral circulation showed speedy recovery compared to patients with low NK cell numbers.

Conclusion

NK cell biology represents a fascinating facet in the evolutionary struggle between intracellular adversities and the host immune system. Its regulation is remarkably unique in terms of recognition strategies and genomic diversity. Increasingly, more evidence is being accumulated showing the importance of NK cells in tumor surveillance, reproduction, maternal-fetal interface, HSCT and many other conditions. The '*missing-self hypothesis*', has attracted much attention in the scientific community; it provided a basis for Kärre and many other groups to search for mechanisms, receptors and ligands that paved the path for significant contributions and discoveries during late eighties and early nineties. These discoveries eventually became a strong edifice for the current understanding and advancement in the NK cell research and have no doubt provided the basic platform for wonderful cues in today's NK cell research. More and more studies have to be performed meticulously to seek answers to many other fundamental questions in NK cell biology (34-37). These studies would provide clinically relevant insights since NK cells are now believed to be relevant to future therapies against human cancer, either alone or in combination with other therapies.

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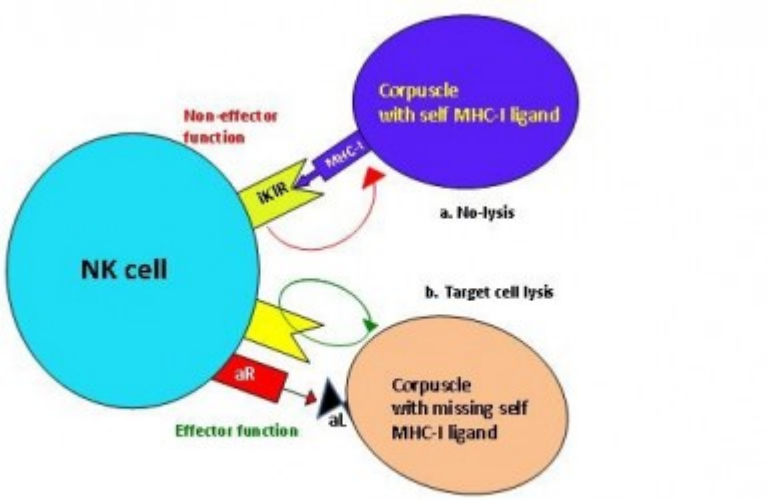
Illustrations

Illustration 1

Fig.1. A simplified schematic diagram showing that the lytic function of NK cell depends on missing self-MHC-I ligand and presence of activating signals. iKIR: inhibitory Killer Immunoglobulin Receptor, aR: activating Receptor, aL: activating Ligand.



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