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REVIEW

Natural killer cells in malignant hematology: A primer for the non-immunologist

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ABSTRACT

Natural killer cells were first described over 40 years ago, but the last 15 years has shown tremendous progress in our understanding of their biology and our ability to manipulate them for clinical therapeutic effect. Despite the increased understanding by clinicians and scientists investigating these cells, their biology remains a confusing subject for many because of the wide array of receptors, complex interactions, multiple models of predicting function, and contradictory data in the literature. While they are microscopically indistinguishable from T cells and share many of the same effector functions, their mechanisms of target recognition are completely distinct from yet complimentary to T cells. In this review we provide a basic understanding of NK cell biology and HLA recognition as compared and contrasted to T cells using a metaphor of border patrol and passports. We conclude with a summary of the evidence for NK cell effects in hematologic malignancies and describe new advances in NK cell immunotherapy aimed at improving these effects.

1. Introduction

1.1. The emerging role for NK cells in the new world of immunotherapy

The field of immunotherapy for cancer has seen a renaissance in recent years as our knowledge of the immune system- and our ability to manipulate it with therapeutic intent- has expanded. Antibody-targeted therapy has become standard for many malignancies, antigen-specific adoptive T cell therapy is showing great promise for solid tumors [1,2] and viruses [3,4], immune checkpoint blockade has rapidly become frontline therapy for melanoma [5,6], and genetically-modified T cells have demonstrated high potential against CD19 + B-cell malignancies [7–9]. Adoptive immunotherapy with natural killer (NK) cells, however, has lagged behind that of T cells in part because of a delay in our fundamental understanding of their biology and function and a lack of robust methods for their application as an effective therapeutic tool. In this review, we present a simplified metaphor for understanding human NK cell biology, describe the recent clinical data that provide evidence for NK cell activity against hematologic malignancies, and discuss some of the ways in which this new knowledge is generating therapeutic optimism as it becomes translated to the clinic.

1.2. What are NK cells?

NK cells represent 5–15% of peripheral blood lymphocytes [10], but comprise 85% of the large granular lymphocyte population [11]. Having both cytotoxic and regulatory activity, NK cells recognize virus-infected or malignant cells that express danger signals (e.g., stress ligands, viral proteins, antibodies). Their participation in recognizing cancer through antibody-dependent cell cytotoxicity (ADCC) [12] is a major mechanism of the therapeutic benefit of anti-cancer antibodies. To control reactivity against self, NK cells have inhibitory receptors for self Human Leukocyte Antigen (HLA), and therefore have heightened sensitivity to cells that lack class I HLA [13].

NK cells were first described in the 1970s based on their functional ability to kill tumor cells without prior sensitization [11]. In their resting state they mainly circulate in the blood and hematopoietic tissues, but NK cells can also be isolated from tissues such as the liver, peritoneal cavity, and placenta. Upon activation, they extravasate into surrounding tissue to effect killing of pathogen-infected or neoplastic cells. Their recognition of target cells is not restricted by presentation of antigens through HLA, and is not antigen specific, differentiating them from T cells. Until the 1980's they were identified as lymphocytes lacking the

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receptors of B cells or T cells, giving rise to the name "null cells." Therapeutic use was largely in the context of heterogeneous activated lymphocyte populations, as they are a key active component of lymphokine-activated killer cells (LAK) [14,15], and cytokine-induced killer cells (CIK) possess NK cell-like phenotype and function [16]. About a decade after their initial discovery these cells were identified as CD3- lymphocytes (i.e., not T cells) that express CD56. This remains the most common definition for human NK cells, although CD56 is expressed by several other tissues in humans and is not expressed by NK cells of other species [17]. NKp46, an activating receptor broadly expressed on mature NK cells, may define NK cells more uniformly across species [17].

1.3. NK cells in control of viral disease

The study of NK cells in controlling murine cytomegalovirus (MCMV) has played a major role in our understanding of NK cell function in infection [18,19]. In humans, NK cells also play a major role in the immune response against viral infections [20], particularly those in the *herpesviridae* family [21] and influenza [22], and may have been involved in the control of ancient endogenous retroviruses [23,24]. NK cells have receptors specific for common viral protein motifs (e.g., hemagglutinin), and early recognition of viral-induced stress proteins is essential for establishing the cytokine-driven microenvironment that recruits and primes the adaptive immune system for more specific and definitive control of infection [24]. NK cell function associated with a "memory" phenotype expressing NKG2C is associated with control of human CMV infection, especially in the context of CMV reactivation after HSCT [25].

1.4. NK cells in cancer

NK cells also play a central role in tumor immunity through many of the same mechanisms as viral immunity. Similar to their role in viral infections, NK cells exhibit direct cytotoxicity against malignant cells that is critical to the priming of the adaptive immune response by releasing tumor antigens that can be processed and presented to T cells. In addition, release of pro-inflammatory cytokines such as IFNγ by NK cells recruits T cells to the tumor site, promotes T-cell and dendritic cell activation, and enhances humoral responses by promoting B cell maturation [26].

Adoptive immunotherapy with NK cells has been evaluated across a wide number of malignancies, most commonly by collecting and activating peripheral blood NK cells from an allogeneic donor [27]. More recently, a variety of ex vivo expansion and activation methods have been developed to increase both number and function of adoptively-transferred NK cells [28–31]. In addition to their anti-tumor activity when delivered as adoptive cell therapy in non-transplant settings [32–36], NK cells delivered in the allogeneic stem cell transplant setting may have potential for moderating infection, immune reconstitution, and graft-vs-host disease [37–41].

2. NK cell biology

2.1. NK cell activation as a balance between activating and inhibitory signals

T-lymphocyte activation is highly restricted to an antigenic peptide presented in the groove of Major Histocompatibility Complex (MHC) proteins, the complex of which is recognized by the T cell receptor. Potentially self-reactive T cell clones are deleted early in development. In contrast, NK cell activation is not antigen-specific, thus control mechanisms must be employed in mature cells to avoid auto-reactivity against normal cells. These two critical functions for a healthy immune system (activation and tolerance) are accomplished through different sets of receptors in NK cells — a wide variety of activating receptors for recognition of danger, balanced with an equally wide variety of inhibitory receptors that identify self. The balance between these signals determines whether NK cells will activate their effector function [11,42]. The three main receptor families expressed on NK cells include: natural cytotoxicity receptors (NCR's), C-type lectin receptors (CD94/NKG2), and killer cell immunoglobulin like receptors (KIRs) [33,40].

Whereas NCRs are all activating receptors, C-type lectin and KIR families include both activating and inhibitory receptors (Table 1). Most of these receptors have known ligands, while the ligands for some are still unknown. Some of the known ligands are found commonly on tumor cells as a result of cell stress and their presence is critical for NK cell recognition of these cells as abnormal. Inhibitory receptors provide control for NK cell activity against healthy tissue. This role was first recognized through the identification of MHC class-I deficient targets, which have heightened sensitivity to NK cell killing. This discovery led to the "missing self" hypothesis [43], which states that the presence of MHC Class I, which is ubiquitously expressed among healthy cells, provides NK cells with a "self" signal that is recognized by NK cell inhibitory receptors and thus prevents NK cell self-reactivity [36,43]. MHC Class I molecules in humans are present in three closely-linked genes of the human leukocyte antigen (HLA) family — HLA-A, HLA-B and HLA-C. NK cells primarily recognize HLA-B and -C, and despite the high allelic polymorphisms in these genes, they are grouped into three primary families with regard to NK cell recognition- Group C1, Group C2, and Bw4 (discussed in more detail below).

This recognition of MHC Class I by NK cells is in sharp contrast to that of T cells. Although peptides can disrupt the recognition of HLA by NK cells, [44] they mostly recognize the MHC Class I protein itself, rather than the antigenic peptide presented. If we consider HLA (the human MHC) to be analogous to a passport in which the peptide antigen "picture" of an individual cell is pasted, this critical difference between the way NK cells and T cells recognize targets may be illustrated by two ways of identifying dangerous individuals. We may imagine two very different ways of achieving this task- that of an assassin finding his target in a crowded city (Fig. 1) or that of a border control agent screening individuals entering the country (Fig. 2). T cells function similar to an assassin who has studied the information about one specific target sufficient to pick that target out of a crowd (Fig. 1) and are singularly focused on finding and eliminating that single target. Although T cells may have low affinity non-cytotoxic interactions with self during surveillance [45,46] or high-affinity cytotoxic interactions with normal tissue that bears a similar antigen (autoimmunity), T cells recognize a single antigen with high specificity (Fig. 1). For instance, T cells that are trained to recognize varicella through vaccination will not recognize the common cold you pick up later that month.

In contrast, NK cells function more like the border patrol agent screening passengers disembarking from an international flight (Fig. 2). The border patrol agent's job is not to identify or single out any one specific person as they pass through immigration. To the agent at the desk, an individual person's identity is not as important as whether they carry a valid passport appear safe and nonthreatening (Fig. 2). If a person appears to be nonthreatening and has a valid passport, they are granted entry (Fig. 2, #1). if the traveler does not have a valid passport (Fig.2, #2–3), entry is automatically and unceremoniously denied and further investigation of their origin and potential threat is assessed. However, if the traveler appears dangerous they may be taken in for questioning even if they have a valid passport (Fig. 2, #4). Similarly, NK cells use their inhibitory receptors (i.e., KIRs and NKG2A) to assess for the expression of valid passports (i.e., self proteins such as HLA), while the activating receptors assess for a wide variety of potential danger signs.

2.2. Basic killer immunoglobulin-like receptors (KIRs) nomenclature and function

As mentioned above, upon recognition of valid passports (HLA) in healthy cells, inhibitory KIRs send signals that shift the balance towards