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Mini-review

Natural killer cells: The journey from puzzles in biology to treatment of cancer

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ABSTRACT

Natural Killer (NK) cells are innate immune effectors that are primarily involved in immunosurveillance to spontaneously eliminate malignantly transformed and virally infected cells without prior sensitization. NK cells trigger targeted attack through release of cytotoxic granules, and secrete various cytokines and chemokines to promote subsequent adaptive immune responses. NK cells selectively attack target cells with diminished major histocompatibility complex (MHC) class I expression. This "Missing-self" recognition by NK cells at first puzzled researchers in the early 1990s, and the mystery was solved with the discovery of germ line encoded killer immunoglobulin receptors that recognize MHC-I molecules. This review summarizes the biology of NK cells detailing the phenotypes, receptors and functions; interactions of NK cells with dendritic cells (DCs), macrophages and T cells. Further we discuss the various strategies to modulate NK cell activity and the practice of NK cells in cancer immunotherapy employing NK cell lines, autologous, allogeneic and genetically engineered cell populations.

Introduction

Cancer is a complex heterogeneous disease in which abnormal cells divide without control, able to invade other tissues and metastasize to distant sites. With about 8.2 million cancer deaths in 2012 and around 13.2 million cancer deaths projected to occur worldwide by 2030, cancer is clearly one of the most alarming health problems we face today [1]. The standard therapeutic procedures currently in practice for all types of cancers include surgery, radiation therapy and chemotherapy. Although these therapies are proven highly effective in eradicating the primary tumor, advanced metastatic disease will not be cured by these measures and patients eventually die of their disease [2]. Moreover, the increasing chemotherapeutic drug resistance, relapse of disease after achieving certain remission, ineffectiveness against cancer stem cells are the other limitations associated with these therapies which are of major concern today [3,4]. Therefore, new and more effective methods of treating these patients are urgently needed. Over the last decade, new paradigms for cancer treatment have evolved from relatively nonspecific cytotoxic agents. The identification of cancer genes and tumor related signaling pathways that are essential for tumor cell growth and survival has provided researchers with a better insight in tumor pathogenesis and development of new therapies, including targeted therapies and cancer immunotherapy [5]. Targeted approaches focus on the inhibition of tumor specific proteins or molecular pathways involved in tumor growth and progression; whereas immunotherapy is aimed at exploiting the destructive properties of the immune system to eradicate cancer cells [2].

Cancer immunotherapy is the targeted therapy designed to induce antitumor response against malignancies by harnessing the power of the immune system [6]. Active and passive immunotherapies are the two different approaches used to generate these antitumor responses. Active immunotherapy encompasses agents such as peptides or whole cell vaccines, recombinant virus encoding tumor associated antigens (TAAs) or DCs. Passive immunotherapy, also known as adoptive immunotherapy comprises effector molecules (antibodies) or effector immune cells (T and NK cells) and nonspecific immune stimulants [2]. Adoptive cell therapy (ACT) is the transfer of naturally occurring or genetically engineered cell populations that have been expanded *ex vivo* free from the potentially immunosuppressive tumor microenvironment that prevents them from proliferating, becoming activated and killing tumor cells. In the adoptive transfer setting, these cells are often infused with appropriate growth factors to stimulate their survival and expansion *in vivo*. In addition, manipulation of the host patient with lymphodepleting chemotherapy prior to transfer of antitumor cells will prevent rejection and also provide favorable environment for further cell expansion and activation by eliminating immunosuppressive regulatory T cells (Tregs) [7]. The distinct features viz. ability to kill tumor cells without the need to recognize tumor-specific antigen, ease to isolate and expand *ex vivo* and the short lifespan without

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the need to use a suicide vector to prevent overexpansion of transferred cells make NK cells attractive targets for cancer immunotherapy [3]. However, some recent findings indicate that long-lived NK cells are generated under certain circumstances. In situations like lymphopenia induced by certain viral infections or caused by chemotherapy and radiation treatment, NK cells undergo homeostatic proliferation resulting in the generation of a long-lived population of NK cells [8]. These homeostatic or space-driven long-lived memory NK cells reside for months in both lymphoid and non-lymphoid sites and can rapidly mount protective secondary responses when virus is reencountered. Thus, these homeostatically expanded NK cells undergo all four phases, namely the expansion, contraction, memory maintenance and recall response as memory T-cells [9]. In this review we present an overview on biology of NK cells, the interactions of NK cells with other immune cells, strategies to modulate NK cell activity and the use of NK cells in cancer immunotherapy.

Natural killer cells biology: Phenotypes, receptors and functions

NK cells are innate effector lymphocytes that recognize and lyse virus infected and malignant transformed cells without MHC restriction or prior sensitization [10]. The name "natural killer" indicates their natural occurrence and spontaneous ability to kill lymphomas and leukemia cells in non-immunized animals. NK cell activity was first identified in mice, where lethally irradiated mice without any prior sensitization rejected bone marrow (BM) allografts. In 1975, Herberman and Kiessling independently identified the natural cytotoxicity of these cells against syngeneic and allogeneic tumors, even when using effector cells from athymic mice [11–13]. These studies led to the discovery of BM derived cytotoxic cells, discrete from a T-cell and require no prior immunization to lyse a target cell, thus the name "Natural Killer" was born.

Phenotypes

NK cells develop in the BM from common lymphoid progenitor cells, and after development they distribute widely throughout lymphoid and non-lymphoid tissues, including BM, lymph nodes (LN), spleen, peripheral blood (PB), lung and liver [6]. These cells comprise 10–15% of all circulating lymphocytes and are defined by the surface expression of CD56, CD16 and absence of T-cell receptor (CD3). Human NK cells can be divided into two subpopulations CD56dim and CD56bright on the basis of surface density of CD56. CD56dim NK cells constitute approximately 90–95% of the NK cells in the PB and possess a high cytotoxic potential, whereas CD56bright cells make up approximately 5–15% of total NK cells, exhibit little cytotoxicity and mainly produce immunoregulatory cytokines upon activation [14]. These subsets also differ in the expression of a number of other cell-surface markers. For example, CD56bright subset is shown to exclusively express interleukin (IL)-2 receptor α chain (IL-2Rα/CD25), while they lack or express only at very low levels the FcγRIII (CD16). On the other hand, the CD56dim subset has high expression of CD16 and lacks CD25 expression [15]. These properties set very different roles to the different subsets with regard to antibody dependent cellular cytotoxicity (ADCC) and response to IL-2 stimulation. Furthermore, they differ in the expression of two major families of MHC class I binding receptors. While CD56dim NK cells express high levels of killer-cell immunoglobulin-like receptors (KIRs) and low levels of CD94/natural killer group 2 (NKG2) receptors, the opposite holds true for the CD56bright NK cells. These differences in receptor expression have been correlated with their differential ability to kill various tumor target cells and the specific alloreactive properties of CD56dim NK cells because of their high KIR levels.

NK cell subsets also clearly differ with respect to their tissue localization which may reflect their specialized function *in vivo*. CD56bright cells are better able to leave the vasculature and constitute the majority of NK cells in LN and also outnumber CD56dim NK cells in tonsils, lungs, mucosal sites and uterus as they are equipped with molecules such as chemokine receptors CCR7 and CXCR3 and the adhesion molecule CD26L [16]. The CD56bright NK cells present in the LN are believed to play an important role in shaping immune responses by regulating DCs and T-cell priming toward Th1. Therefore, NK cells are important for bridging innate and adaptive immune responses through secretion of pro-inflammatory cytokines and direct cell-to-cell interactions.

Receptors and functions

NK cells are important immune effector cells that are primarily involved in immunosurveillance to eliminate a wide range of abnormal cells (including malignantly transformed cells, virus infected cells, cells bound by antibody) as well as stress cells, without damaging the healthy and normal "self" cells. MHC-I molecules are virtually expressed on every cell in the body as markers of "self" and these cells are not affected by NK cell activity. Many abnormal tumor or virally infected cells decrease their cell surface expression of MHC-I in order to evade cytotoxic T lymphocyte (CTL) detection, but these abnormal cells inherently become susceptible to NK cell-mediated attack in the absence of the tolerizing MHC-I ligands [17]. This "missing self-recognition" hypothesis was first proposed by Ljunggren and Karre in the late 1980s, but the mechanism on how NK cells will detect the lost MHC-I was unclear at that time. Later, it was identified that NK cell inhibitory receptors were shown to detect this absence of MHC-I expression. In addition, NK cells can also recognize either pathogen-encoded molecules that are not expressed by the host, called 'non-self recognition', or self-expressed proteins that are upregulated by transformed or infected cells, called 'stress-induced self recognition' by means of their activating receptors [6].

"Dynamic equilibrium hypothesis" defines that NK cell activation is regulated by integration of signals emanating from germline encoded activating and inhibitory cell surface receptors [18]. The activating receptors expressed on human NK cells include immunoglobulin gamma Fc-region receptor III (FcγRIII), activating forms of KIR2DS and KIR3DS, NKG2D, natural cytotoxicity receptors (NCRs: NKp30, NKp44, NKp46), C-type lectin receptors (CD94/NKG2C, NKG2E/H and NKG2F), NKp80, DNAX accessory molecule (DNAM-1) and 2B4 [19,20]. Of these the NKG2D and NCRs are particularly important receptors for triggering NK cell responses toward tumor cells [21]. The inhibitory receptors encompass two distinct classes: the monomeric type I glycoprotein of the immunoglobulin superfamily (KIR2DL and KIR3DL), leukocyte immunoglobulin like receptors (ILT2), and the type II glycoproteins with a C-type lectin scaffold, CD94: NKG2A receptors [22]. The ligands for activating receptors are self-proteins which are usually rare on normal cells, but are up-regulated at the cell surface during either infection or malignant transformation [23]. For instance, NKG2D specifically recognizes MHC class I chain related (MIC) A, MICB and other non-MHC molecules such as UL16-binding proteins 1–6 (ULBPs) that are not expressed on normal cells but are up-regulated on stress cells, such as tumors [24,25]. The NCRs binds to heparin sulfate on the surface of tumor cells [26]. In addition, NKp30 recognizes human leukocyte antigen (HLA)-B associated transcript (BAT3) and B7H6 expressed on stressed and transformed cells. NKp46 and NKp44 have a role in the elimination of virus infected cells through recognition of viral hemagglutinins. KIRs recognize different allelic groups of HLA-A, HLA-B and mainly HLA-C molecules, whereas CD94/NKG2A binds to the nonclassical MHC HLA-E [27]. KIRs also promotes the maturation of functionally responsive NK cells, as studies