The role ofNatural killer (NK) cells as APCs,Cytotoxicity, Immunoregulatory in Innate and Adaptive immuneas Potential Therapeutic and Preventive Target in Cancer and Autoimmunity

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Abstract: Natural killer (NK) cells are lymphocytes of the innate immune system that are critical in host defense and immune regulation. They are activated or inhibited through the ligation of germline-encoded receptors and are involved in mediating cytotoxicity, in producing cytokines and in providing co-stimulation to cells of the adaptive immune system.

NK cells have receptors that recognize Class I major histocompatibility complex (MHC), and their function is tightly integrated with other cells in the innate and adaptive immune systems.

Effective immunity requires coordinated activation of innate and adaptive immune responses. NK cells are principal mediators of innate immunity, able to respond to challenge quickly and generally without prior activation. The most acknowledged functions of NK cells are their cytotoxic potential and their ability to release large amounts of cytokines, especially IFN-c.

The activities of NK cells are regulated by the interaction of various receptors expressed on their surfaces with cell surface ligands. While the role of NK cells in controlling tumor activity is relatively clear.

In this article, I discus theRegulation of effector cells by NK cells, cytotoxicity effect, and in producing cytokines, Natural killer cells and immunoregulation, mechanisms of action of NK cells, NK cells and adaptive immunity; moreover, therapeutic applications of NK cells in cancer and autoimmunity.

Key Words: NK cells, Cytotoxicity, Immunoregulatory, Innate immune, Adaptive immune Cancer and Autoimmunity

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1. Introduction

Natural killer (NK) cells the first line of innate defense against viral infection, and they rapidly and directly kill infected cells in the absence of antigen presentation and recognition. In response to stimuli from diverse sources, including infections, cytokines, stresses and other immune cells, NK cells exert the following distinct functions: (i) secrete perforin and granzyme to directly kill target cells; (ii) release cytokines to regulate immune responses; and (iii) couple death-inducing receptors to target cells and induce apoptosis(1),(2). NK-deficient individuals are highly susceptible to a variety of viral infections, illustrating the key role of NK cells in the defense against viral infection(3).Natural killer (NK) cells are a heterogeneous group of immune cells that share severalcommon identifying properties. Morphologically, NK cells are large granular lymphocytes with abundant cytoplasm with azurophilic cytoplasmic granules.(4). Cell surface markers detected by flowcytometry and immunohistochemistry are now standard to identify NK cells, which are defined by the presence of an isoform of the neural cell adhesion molecule, CD56, and the absence of CD3, the pan-T-cell marker(5).With these phenotypic criteria, NK cells are present in significant numbers, comprising up to 29% of circulating lymphocytes(6), and 5% to 17% of mononuclear cells in hematolymphoid organs (7).Phenotypic NK-cell subsets have differing functions and anatomic distributions. For example, NK cells can be broadly divided into CD56bright and CD56dim subsets. Briefly, CD56bright NK cells have low resting cytotoxic activity, are preferentially found in secondary lymphoid tissue, and may represent a precursor to the more cytotoxic CD56dim NK cells that are found circulating in the peripheral blood (7).Subsets of NK cells can also express CD16, a low-affinity Fc immunoglobulin G receptor that allows NK cells to participate in antibody-dependent cellular toxicity (8). Other less-defined and smaller NK-cell subsets have been described, and as new surface markers are discovered, the definition and functional characteristics of NK cells will be further refined. NK cells were first discovered to recognize and lyse cells lacking major histocompatibility complex (MHC) without prior sensitization (9),(10). Later, NK cells were shown to lack germline T-cell receptor gene rearrangements and antigen-specific cell surface receptors.However, viruses have evolved various strategies to evade the NK cell recognition

and destruction during acute and persistent viral infections.An array of activating or inhibitory receptors on the surface of NK cells recognize the ligands of target cells, and the relative expression of these receptors and the outcome of their signal cascades determines NK cell activation and cytotoxicity(11).Numerous activating or inhibitory NK cell receptors have been identified in NK cells; the activating receptors recruit adaptors that contain the intracellular immunoreceptor tyrosine-based activating motif (ITAM), whereas the inhibitory receptors contain the immunoreceptor tyrosine-based inhibitory motifs (ITIM), consequently, they transduce activating orinhibitory signal cascades, respectively(12).A cluster of inhibitory receptors specifically binds to major histocompatibility complex (MHC) class I molecules, such as the inhibitory Ly49s family members in mice, the killer-cell immunoglobulin-like receptors (KIR) in humans, and the heterodimeric CD94-NKG2A receptor in both species that recognizes non-classic MHC class I molecules. These molecules allow NK cells to beregulated by self-MHC recognition and restrain the NK cell hyperactivity(12).Therefore, the NK cells preferentially kill the infected cells in which the surface expression of MHC molecules and the antigen presentation are inhibited by viruses(13).Four types of activating NK receptors recognize the different ligands: CD16 enables NKcells to exert antibody-dependent cell cytotoxicity; natural killer group 2 member D (NKG2D) recognizes a family of stress-induced ligands; natural cytotoxicity receptors (NCRs) are able to recognize pathogen-derived or induced ligands and tumor ligands; and the other receptors, including 2B4 (CD244), NKG2C, DNAM1 (CD226) and NKp80, recognize self-molecules(11). All receptors recognize a variety of ligands on the surface of target cells, and the major ligands include atypical major MHC class I, MHC class I-related chain A (MICA), MHC class I-related chain B (MICB), UL16 binding proteins 1–6 (ULBP1–ULBP6) and some viral proteins(12),(14).Upon the association between receptors and ligands, the receptors activate Syk (spleen tyrosine kinase) or ZAP70 (zeta-chain associated protein kinase 70 kDa) tyrosine kinases through the adapters DAP12, Fc"RI (also known as FcR) or CD3, or they activate phosphatidylinositol-3-kinase (PI3K) through the adaptor DAP10(12).During their development and maturation, NK cell receptors recognize self-ligands to obtain self-tolerance for normal and healthy cells through the processes of selection and education(15),(16).During viral infection, the balance of NK activating or inhibitory receptors shifts toward NK cell activation and increased cytotoxicity, whereas viruses employ complex mechanisms to reverse NK cell activation and maintain NK cell quiescence. Downregulation of MHC class I molecules by viruses prevents antigen presentation and reduces the immune response; however, it increases the susceptibility to NK cell recognition and destruction(13).Viruses possess more effective and distinct strategies to escape from NK cell immunity, including stimulating the inhibitory receptors and disrupting the activating receptors.Several viruses are able to inhibit NK cell activation through inhibitory receptors. Murine cytomegalovirus (MCMV) encoded MHC-I-like m157 in infected cell surfaces acts as a ligand of inhibitory Ly49C receptor, and their binding hampers NK cell activation. This outcome results in the evasion from NK cell clearance during MCMV infection in mice (17),(18). In humans, human leukocyte antigen (HLA)-C is capable of inhibiting NK cell cytotoxicity via inhibitory KIR receptors in human immunodeficiency virus type 1 (HIV-1) infection(19).HLA-C presents HIV p24 epitopes to KIR receptors and engages KIRs on NK cells; therefore, it inhibits NK cell function(20).Additionally, the epitopes of human cytomegalovirus (HCMV) glycoprotein UL40 are presented by HLA-E to NK cells via CD94/NKG2A receptor, by which protects the infected cells from NK cell killing(21).The natural selection of variations provides a novel viral escape through inhibitory NK cell receptors(21),(22).Here, we review the impairment of NK cell-activating receptors and ligands by viruses and further discuss the

unique aspects of viral evasion of NK cell recognition and destruction, which provides novel insights on the struggles between NK cells and viruses during persistent viral infection.

2. NK cell functions

NK cells are distinct from T cells or B cells and have distinct morphologic, phenotypic and functional properties. As suggested by their name, NK cells occur naturally, i.e., they are part of innate immunity and, unlike T cells or B cells, do not require sensitization for the expression of their activity. Morphologically, most NK cells are large granular lymphocytes in that they are bigger than normal lymphocytes and have more cytoplasm. Phenotypically, NK cells have several unique markers on their surface but are most traditionally characterized by being CD3_, CD56+. They are distinct from NKT cells which express CD3, rearrange their germline DNA T cell receptor genes (though with a limited repertoire) and are reviewed elsewhere(23).NK cell functions can be classified in three categories:

3. Cytotoxicity

NK cells can kill certain virally infected cells and tumor target cells regardless of their MHC expression(24).NK cells possess relatively large numbers of cytolytic granules, which are secretory lysosomes containing perforin and various granzymes. Upon contact between an NK cell and its target, these granules traffic to the contact zone with the susceptible target cell (the so-called immunological synapse), and the contents are extruded to effect lysis. Perforin-dependent cytotoxicity is the major mechanism of NK cell lysis, although NK cells can also kill in a perforin-independent manner utilizing FAS ligand, TNF or TNF-related apoptosis-inducing ligand (TRAIL), albeit less efficiently and in a slower time kinetic.

4. Cytokine and chemokine secretion

NK cells are best noted for their ability to produce IFN-g but also produce a number of other cytokines and chemokines including TNF-a, GMCSF, IL-5, IL-13, MIP-1 (a and h) and RANTES(25),(26),(27).Killing and cytokine secretion are probably mediated by two different subsets of human NK cells characterized by the intensity of expression of CD56 on their surface.

5. Contact-dependent cell co-stimulation

NK cells express several costimulatory ligands including CD40L (CD154) and OX40L, which allow them to provide a costimulatory signal to T cells or B cells(28),(29). Thus, NK cells may serve as a bridge in an interactive loop between innate and adaptive immunity. Dendritic cells (DC) stimulate NK cells which then deliver a co-stimulatory signal to T or B cells allowing for an optimal immune response.

6. Regulation of NK cell functions

The intrinsic cytotoxic capacity of NK cells raises the question as to why they do not kill autologous cells; this observation led to the ''missing self-hypothesis'(30)' This hypothesis states that NK cells are inherently capable of killing autologous cells, but that they are actively

prohibited from doing so by inhibitory receptors. More specifically, since ''self'' is defined by MHC, the hypothesis states that self-MHC engages inhibitory receptors on the surface of NK cells preventing them from delivering a lytic signal. A corollary of this hypothesis is NK-susceptible cells either might lack the molecules that ligate NK inhibitory receptors or might have molecules that engage NK activating receptors. This hypothesis was substantiated by discovery of inhibitory killer cell immunoglobulin receptors (KIR)(31),and several families of activating receptors(32).In contrast to T cell or B cell receptors, the specificities of these receptors do not require genetic recombination events. The current model for NK cell activation and inhibition is one based upon a balance of function between specific activating and inhibitory receptors. If the balance favors inhibitory signaling, then intracellular events leading to cell function will not progress. If the balance favors activation signals, NK cells can then progress through a series of intracellular stages and checkpoints to exert their function(33).

7. NK-cell-dependent regulation of DCfunction

DC is antigen-presenting cells that initiate and regulate immune responses.
In humans, both immature and mature human DC can induce resting NK-cell activation. Indeed, numerous studies have found that DC are capable of activating NK cells(34). However, DC–NK cell interactions are not one-sided affair, but rather, involve reciprocal interactions whereby NK cells can influence the function of DC and vice versa. That cross-talk between NK cells and DC is required for the generation of an appropriate immune response is implied by the findings that NK cells are found in close association with DC in both the lymph node and in inflamed skin(35),(36), (37),(38). Furthermore, depletion of NK cells has been found to affect both the number and activation state of DC in the lymph nodes(35),(39).Studies have attempted to define how activated NK cells influence the function of DC. The ability of activated NK cells to lyse immature DC (iDC) has been documented in a number of settings(40),(41),(42),(43),(44),(45),(46).NKcell- mediated killing of iDC is proposed to function as an editing mechanism. This theory suggests that only mature DC that have appropriate levels of MHC and costimulatory molecules, and are thus able to prime an effective immune response, will survive an encounter with an activated NK cell. The ability of NK cells to kill iDC, at least in vitro, is limited to a subset of cells expressing CD94/NKG2A, but lacking killer Ig-like receptors(47).Therefore, killing of iDC may not be mediated by all NK cells, but is potentially limited to a specialised subset of NK cells. The mechanism by which NK cells eliminate iDC is another important issue that still requires clarification. Killing of DC in a transplantation model(35),and in vitro (44), [42] is dependent on perforin. By contrast, in vivo, adoptively transferred iDC are eliminated by NK cells in a TRAILdependent manner(46).Thus, while the hypothesis that NK cells fine-tune immune responses by eliminating iDC is intriguing, definitive evidence that this process operates in vivo and how is still lacking.

8. NK cells regulate T-cell priming

Early studies revealed that NK cells can promote the generation of TH1 responses(48),(49),(50).In mice, NK cells are rapidly recruited to lymph nodes following Leishmania major infection and are a source of the IFN-g required for the induction of TH1 polarisation(51).A similar effect has been observed in humans, where NK-cellderived IFN-g was

found to enhance the activation of CD4+ T cells (52).Importantly, human tonsilar, but not peripheral NK cells were required for the expansion of IFN-g producing CD4+ T cells(52).The specificity observed here is due to the fact that the cytokine producing CD56hiCD16 NK-cell subset is enriched in secondary lymphoid organs, such as the tonsils. These results reinforce the notion that NK cells are not homogeneous, and that the nature of the NK-cell subset involved can profoundly influence the outcome of an immune response. The pro-inflammatory cytokines produced by NK cells might promote a TH1 response via a number of mechanisms. Naı¨ve T cells require an exogenous source of IFN-g for TH1 polarisation, which can be produced by NK cells in vivo(51).In addition, NK cells may indirectly promote TH1 polarisation by enhancing the maturation of DC. In vitro, NK-cellmediated maturation of DC requires cell–cell contact and the production of TNF-a and IFN-g by NK cells(45),(53),(54).The ability of NK cells to activate DC may also be essential for the initiation of immune responses to tumours or pathogens that do not directly activate DC. Some support for this theory comes from the observation that recognition of MHC class I low tumour cells by NK cells activates DC resulting in the induction of a CD8+ T-cell response(55).In human and mouse bone marrow transplantation systems, donor NK cells have been shown to play a protective role in graft outcome by killing the allogeneic recipient antigen-presenting cells responsible for priming alloreactive T cells and initiating GVH disease(56).In an allogeneic cardiac graft model, long-term graft survival was achieved by inhibiting NK cells in a setting where CD28 co-stimulation was lacking (CD28/ mice)(57).Interestingly, neither intervention alone was sufficient to improve graft survival. These findings led to the suggestion that NK cells might deliver help to T cells. Thus, after infiltrating the grafts, NK cells synthesise cytokines that circumvent the CD28 deficiency and provide the critical help required for CD8 T-cell priming. Since these studies were conducted using the anti-NK1.1 antibody to remove NK cells, the possibility that the observed effects are mediated by other cells carrying this determinant, particularly NKT cells, needs to be taken in consideration. In skin graft models, TH2 polarisation can be achieved by the numbers of DC that accumulate in the absence of NK-cell activation. In contrast, the regulation of donor DC by blood-borne NK cells recruited in the lymph nodes has been shown to favour a TH1 response(35),(58).Thus, in autologous systems, the DC maturation state is crucial in determining whether the DC will survive the encounter with NK cells, while TH1 polarisation appears to depend mainly on cytokine production. On the other hand, in transplantation settings, NK-cell activation following interaction with allogeneic DC seems to occur regardless of DC maturation. This is likely due to failure to engage inhibitory NK-cell receptors specific for self-MHC I by the allogeneic DC. The duration of DC persistence will then control the strength of the priming and the subsequent polarisation of the T-cell response. The impact of NK cells on DC functionality during immune responses has been largely inferred from in vitro studies. Perhaps, the best evidence that NK cells influence the function of DC in vivo has come from studying MCMV infection. Resistance to MCMV in C57BL/6 mice is mediated by Ly49H+ NK cells that recognise the virally encoded m157 protein(59),(60).During MCMV infection maintenance of the CD8a DC population is dependent on Ly49H+ NK cells(61).A recent report has suggested that the ability of Ly49H+ NK cells to maintain splenic DC populations is mediated by an indirect mechanism.

The report proposes that the Ly49H+ NK-cell-mediated early control of MCMV replication in the spleen of resistant mice prevents the release of immunosuppressive levels of IFN-ab(62).Administration of exogenous IFN-a to resistant mice was found to induce loss of DC from the spleen, and a slight and very transient delay in the activation of antigen- specific T cells(62).Alternatively, it has been proposed that the rapid control of viral replication by Ly49H+ NK cells may promote the maintenance of splenic DC by preventing the destruction of the splenic architecture(63).In addition to potentially influencing the function of DC, NK cells have recently been shown to induce the differentiation of CD14+ monocytes into DC(64).This process was found to require the production of GM-CSF by CD56bright NK cells and direct cell–cell contact. While the process was proposed to contribute to the maintenance of chronic inflammatory diseases, it is conceivable that it could also operate to expand the pool of DC during immune responses to pathogens and thereby impact on the outcome of subsequent T-cell responses. Together the published data provide evidence that NK cells can indirectlyinfluence DC-induced T-cell priming,however, evidence that NK cells directlyinfluence the functions of DC in vivoremains elusive.

9. Regulation of effector cells by NK cells

In addition to their potential role in regulating antigen presentation, NK cells may influence the outcome of the immune response by acting directly on effector cells. As mentioned previously, activation of naı¨ve T cells is dependent on IFN-g produced by NK cells(51).NK cells have also been reported to stimulate autologous CD4+ T cells, an effect that is dependent on the expression of OX40 ligand and CD86 by activated NK cells(65),A role for NK cells in the activation of B cells and the promotion of isotype class switching has also been noted(66),(67),(68).The ability of NK cells to restrain the immune response has also been observed in a number of settings. NKG2D-dependent killing of activated T cells by syngeneic NK cells has been reported(69).Furthermore, expression of Qa-1–Qdm by activated CD4+ T cells is required to prevent lysis by NKG2A+ NK cells(70).An implication of these results is that NK cells may be crucial for the termination of an immune response and consequently prevent the development of immunopathology.

Direct evidence for this proposition comes from studies of mice deficient in either perforin or granzymes. Replication of MCMV is enhanced in mice deficient in either perforin or granzymes AB(71). However, granzymeABdeficient mice survive infection while perforin-deficient mice develop a fatal haemophagocyticlymphohistiocytosislike syndrome(71).The haemophagocyticlymphohistiocytosis-like syndrome observed in perforin-deficient mice was induced by the accumulation of TNF-a producing CD11b+F4/80+ mononuclear cells and T cells(71).In wild-type mice NK cells were found to prevent immunopathology by eliminating the TNF-producing cellsin a perforin-dependant manner. A protective role of NK cells has also been reported in autoimmune diseases. In Fas-deficient mice, NK cells can suppress autoreactive B lymphocytes, while NK-cell depletion increases the severity of an autoimmune disease with features similar to those of systemic lupus erythematosus(72).NK cells have also

been shown to play a protective role in diabetes; treatment of NOD mice with CFA prevented the disease in an NK-cell-dependent manner(73).Collectively, the available data indicate that NK cells serve a dual purpose in that they can provide help and promote the initiation of an immune response, but can also curb the activity of immune effectors and thereby prevent immunemediated damage to the host.

10. Natural killer cells and immunoregulation

The ability of NK cells to kill cells and release immunomodulatory cytokines and chemokines allows NK cells to modulate the innate immune response and mold the development of the adaptive immune response. For example, human NK cells promote dendritic cell (DC) maturation and DC production of cytokines such as TNFα and IL-12 (74),(45),(55).Interestingly, NK cells can kill immature DCs, while mature DCs are resistant to killing as a result of their upregulation of MHC class I molecules(40),(75).Cytokine-activated human NK cells can also directly kill both activated macrophages (76), and T cells(77),(78), secondary to the upregulation of NKG2D ligands on these cells. NK cells are also able to provide costimulatory signals for CD4 T cells and augment their proliferation(79).Additionally, NK cell-derived cytokines (including IFNγ and IL-10(80),(81),(82),influence the diff erentiation(51),(52),and the proliferation of CD4 T cells(82).Impaired NK cell functional responses are frequently observed in patients with autoimmune disorders (discussed below). Th e importance of NK cell cytolytic function in immunoregulation is highlighted in hemophagocyticlymphohistiocytosis, a life-threatening disorder with uncontrolled immune activation and excessive T-cell production of cytokines leading to unrelenting phagocyte activation. Th is disorder results from a failure of cytolyticlymphocytes (CD8 T cells and NK cells) to kill infected cells and/or persistently activated T cells(83),(84).Patients with hemophagocyticlymphohistiocytosis uniformly have decreased NK cell cytolytic responses. Mutations in several proteins required for cytolytic granule release or function have been identified in hemophagocyticlymphohistiocytosis, including perforin, MUNC13-4, syntaxin 11, and syntaxin binding protein 2 (STXBP2)(83),(84).Mutations in STXBP2 directly implicate defective NK cell cytolysis in this disorder since STXBP2 expression is substantially higher in NK cells than in CD8 T cells and defects in degranulation have been observed in STXBP2-defi cient NK cells but not in STXBP2-defi cient CD8 T cells(85).As illustrated by hemophagocyticlymphohistiocytosis, NK cell functionalresponses must be carefully regulated to prevent damage to normal tissues or dysregulation of the adaptive immune res ponses (for example, dsyfunctional cytolysis resulting in persistent T-cell and macrophage activation or indiscriminate release of IFNγ resulting in inappropriate immune activation).

11. NK cell development

NK cells develop from a common lymphoid progenitor resident in the bone marrow but diverge from other lymphocyte lineages fairly early in development(86).They require c-KIT, FLT-3 and IL-15 and acquire specific cell surface markers as they progress through their developmental stages(87). It is currently not known whether there is a defined selection process analogous to thymic selection of T cells. A useful and unique setting for evaluating this particular question has been hematopoietic stem cell transplantation (HSCT)(88),, where the requirements and effects of specific receptor ligand matches and interactions have demonstrated a possible process of NK cell selection (see below). Insight into human NK cell

biology was gained from studying patients with severe combined immunodeficiencies. Mutations of the common gamma chain (gc), required for the function of IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, result in failure of both T and NK cell development. Similarly, mutations of Janus kinase-3 (JAK3) utilized by the gc result in failure of T and NK cell development. In contrast, humans with an IL-7Ra mutation are T cell deficient, but NK cell replete (89), demonstrating that IL-7-is not necessary for NK cell development. Some mutations of gc prevent T cell development but allow NK cell development(90).. For example, the A156V gc mutation results in an inhibition of IL-4 and IL-7 function but has no effect on IL-15 response. This result suggests that IL-15 is necessary for human NK cell development(91), a conclusion confirmed by IL-15 knockout mice(92).The significance of IL-15 is further illustrated by the description of a patient with absent expression of IL-15Rh chain who had no NK cells(93).Once NK cells have developed within the bone marrow, they exit and circulate in the peripheral blood where they comprise 5–20% of peripheral blood lymphocytes. The percentage of NK cells in the peripheral blood varies with age(94),(95).The proportion of NK cells in the peripheral blood is high at birth (20% on average) but reaches a nadir between 5 and 9 months of age (5% on average) after which it climbs steadily until late adolescence(95).NK cells can be demonstrated in several organs including the liver, lung, spleen and uterusIn contrast, NK cells are relatively scarce in the lymphatic fluid and in lymph nodes. Upon stimulation, however, NK cells rapidly home to, and accumulate in, the draining lymph nodes(96).

12. **NK cell inhibitory receptors**

NK cell inhibitory receptors maintain an inactive state within NK cells through the recognition of constitutively expressed "self- molecules" on potential target cells. There are three major types of inhibitory receptors: killer immunoglobulin receptors (KIRs), CD94/NKG2A, Ly49 and Siglecs. Most NK cell inhibitory receptors have immunoreceptor tyrosine-based inhibition motifs (ITIMs) located within their cytoplasmic tails. Most KIRs are inhibitory, in that their recognition of the major histocompatibility complex (MHC) suppresses the cytotoxic activity of their NK cell. KIRs (15 genes) are encoded in the leukocyte receptor complex (LRC) on human chromosome 19q13.4 where other Ig-like receptors are also encoded. Their nomenclature is based on whether the receptor has two or three Ig-like external domains (KIR2D or KIR3D) with short (S; without ITIM) or long (L; with one or two ITIM sequences) cytoplasmic domains (97).The S forms are activating receptors associated with DAP12 (immunoreceptor tyrosine based activation motif, ITAM, positive adapter molecule), whereas L forms are inhibitory receptors that contain ITIMs. Different KIRs have different specificity for HLAs. KIR2DL1 (CD158a) and KIR2DL2 (CD158b) are both specific for HLA-C; whereas, KIR3DL1 (originally called NKB1) and KIR3DL2 (previously named P140) are specific for HLA-Bw4 and HLA-A, respectively. CD94/NKG2A CD94/NKG2A is a family of C-type lectin receptors that are expressed predominantly on the surface of NK cells and a subset of CD8+ T-lymphocyte. The CD9/NKG2 family includes seven members: NKG2A, B, C, D, E, F and H. Genes encoding these receptors are clustered in the natural killer complex (NKC) on human chromosome 12 and mouse chromosome 6 together with Clr (C-lectin related) genes. CD94/NKG2A is capable of being either inhibitory or activating depending on the members of the complex. NKG2 receptors are transmembrane type II and specifically dimerize with the CD94 molecule to form heterodimers. CD94 contains a short cytoplasmic domain and it is responsible for signal transduction. Receptors of the CD94/NKG2 family bind non classical MHC class I glycoproteins (HLA-E in human and Qa-1 molecules in the mouse).

12.1. Ly49, Siglecs and other NK cell receptors

The Ly49 is an NK cell receptor more prominent in mice than in humans. The Ly49 family of genes is encoded in the NKC on mouse chromosome 6. The Ly49a receptor was originally identified on a mouse T cell tumor cell (98). Ly49b recognizes MHC class I molecules H-2Dd, H-2Dk and H-2Dp and Ly49c binds to H-2Kb. Non classical inhibitory receptors, include the LILR family of genes (also called LIR, ILT and CD85) and the CD33-related sialic acid binding Ig-like lectins (CD33rSiglecs); in particular human CD33rSiglec-7 (p75, adhesion inhibitory receptor 1 or AIRM1)(99),(100).Only one of the LILR genes, LILB1 (ILT2/LIR1), encodes an inhibitory receptor on NK cells. LILB1 expression is variable on peripheral NK cells, ranging from negligible to about 75%(101),(102),(103),(104),(105).These receptors (regardless of MHC restriction) have inhibitory motifs (ITIMs) in their cytoplasmic domains which blunt activation signals. CD33-related Siglecs are largely inhibitory and widely expressed on human and mouse NK cells, dendritic cells, neutrophils, monocytes, eosinophils, basophils and B cells (106).There are ten human CD33-related Siglecs: Siglec-3 (CD33), Siglec-5 (CD170), Siglec-6 (CD327), Siglec-7 (CD328), Siglec-8, Siglec-9 (CD329), Siglec-10, Siglec-11, Siglec-14 and Siglec-16. In contrast, mice have five CD33-related Siglecs: Siglec-3 (CD33), Siglec-E, Siglec-F, Siglec-G and Siglec-H(107),(108).

12.2. NK cell activating receptors

NK cells also express a variety of activating receptors which can be grouped into several categories. The main activating receptor groups on NK cells include CD16, NKR-P1 (NK1.1, CD161), NKG2D (KLRK1, CD314), NCR (NKp30, NKp44, NKp46, NKp80); and activating isoforms of human KIRs. These molecules function as activating receptors because they lack ITIMs and instead have ITAM positive adaptor molecules (DAP12). The first and best characterized activating receptor identified on NK cells is CD16, a low affinity Fc receptor for IgG (FcgammaRIII) (109).NK cells can mediate antibody-dependent cellular cytotoxicity through FcgammaRIII, which binds the Fc portion of IgG coating a target (110).Although there are several Fc receptors for IgG, NK cells express only FcgammaRIII. In addition, despite their ability to initiate antibody-dependent cell-mediated cytotoxicity (ADCC), CD16-CD3- human NK cells can still mediate natural killing(111).

12.3. NKR-P1 (NK1.1 and CD161)

NKRP1 (Kirb1) belongs to a family of lectin like molecules with type II orientation encoded in mice (NK1.1) (112),(113). Its expression is relatively selective for NK cells. NKR-P1A or CD161 is classified as a type II membrane protein because it has an external C terminus. NKR-P1A, the receptor encoded by the KLRB1 gene, recognizes lectin like transcript-1 (LLT1) as a functional ligand. In humans, there is only a single gene (NKRP1A) expressed on a subpopulation of NK cells.

12.4. NKG2D (KLRK1 and CD314)

The NKG2D receptor binds to ligands structurally homologous to MHC class I (e.g. human ligands MICA, MICB and mouse ligands RAE-1alpha, RAE-1beta(114).NKG2D is expressed as a

disulfide-linked homodimer on all human and mouse NK cells. It is distinct from other NKG2 molecules in that it shares very little homology (28% instead of 70%) and does not hetero-dimerize with CD94. In both mice and humans NKG2D expression is not restricted to NK cells. In humans it is also found on gamma delta TCR+ T cells and CD8+ T cells. In mice it is found on most NKT cells and on activated CD8+ T cells (Bauer et al. 1999). NKG2D does not have a cytoplasmic motif and preferentially associates with the signaling chain DAP10 via an YxxM motif for recruitment of PI3K (115),suggesting that NKG2D, when associated with DAP10 acts as a co-stimulatory molecule. In mice, there are two isoforms of NKG2D, a long form (NKG2D-L) and a short form (NKG2D-S). Although both forms are present on resting NK cells, the longer form is predominately expressed and preferentially associates with DAP10. Other NK activation receptors of NKG2 are heterodimeric NKG2A-CD94 and NKG2E-CD94(116).

12.5. NCR (NKp30, NKp44, NKp46)

NCRs are type I TM receptors that, unlike T cell receptors (TCRs) and immunoglobulins, do not undergo recombination in order to become functionally active. NCRs possess ITAMs which activate NK cells while NKp44 also has an ITIM. Originally identified as receptors with the ability to mediate the killing of tumor-transformed cells NCRs have also been implicated in the control and elimination of several pathogens (117). NCRs also have a role in immune homeostatis by regulating the expression of several immune cell types. The ligands for these receptors include self-derived molecules as well as pathogen components(118).

12.6. Adhesion receptors

For NK cells to efficiently carry out their effector functions, they must be able to migrate to the site of injury. Adhesion receptors are a key group of molecules that contribute to this function, by increasing their levels of expression.

13. Mechanisms of action of NK cells

NK cells can lyze virally infected cells and tumor cells without prior sensitization. This lysis or cytolytic function is controlled by inhibitory NK receptors that specifically bind to MHC (HLA) molecules on healthy cells and NK cell activation receptors that detect stressed cells. When MHC class I molecules are down regulated or lost on tumor cells or in viral infections, inhibitory signals from inhibitory receptors are lost resulting in NK cell activation. This is called "missing-self" triggered NK activation. NK cell activation receptors (e.g. NKG2D) can detect self-molecules up regulated at higher levels on damaged cells. This is called "stress-induced self- recognition." Cell surface receptors control inhibition and activation; proliferation and effector functions (cytotoxicity and cytokine production)(119),(86). Once activated by NK cell receptors NK cells can use several methods to exert their cytotoxic effects. These include

cytolytic granule mediated cell apoptosis and ADCC. When activated by cytokines or interferons NK cells secrete interferon gamma and TNF alpha which promote phagocytosis.

14. NK cells and adaptive immunity

The ability to generate memory cells following a primary infection and the consequent rapid immune activation and response to succeeding infections by the same antigen is fundamental to the role T and B cells play in the adaptive immune response. For many years, NK cells have been considered to be a part of the innate immune system. However, recently, increasing evidence suggests that NK cells can display several features that are usually attributed to adaptive immune cells (e.g. T cell responses) such as expansion and contraction of subsets, increased longevity and a form of immunological memory, characterized by a more potent response upon secondary challenge with the same antigen. The role of NK cells in both the innate and adaptive immune responses is becoming increasingly important in both basic research and targeted drug development.

15. Therapeutic Applications of NK Cells in various disease Conditions

NK cells play a crucial role in attacking tumor cells in our bodies, and are considered a promising tool for cancer therapy. Treatment range over the past two decades has included IL-2 administration to activate the endogenous NK cells or to adoptively transfer IL-2 activated NK cells(120),(121),(122),(123),(124).Autologous NK-cell therapy has been experimented on for the treatment of renal cell carcinoma, malignant glioma, and metastatic breast cancer. However, it was soon recognized that autologous adoptive NK-cell therapy may have certain drawbacks and thus may not be efficacious. The drawback is mostly attributed to the inhibition of NK cells by self-MHC I molecules expressed on the tumor cells. This has led to the use of allogeneic NK cell therapy in trials. In a pioneering study, Ruggeri et al. demonstrated that alloreactive NK cells given to patients with acute myelogenous leukemia (AML) could eliminate relapse, graft rejection, and protect them against graft-vs-host disease (GvHD) (125). Later, adoptive cellular transfer of allogeneic NK cells from haploidentical donors was also attempted for treatment of renal cell carcinoma, metastatic melanoma, refractory Hodgkin's disease, and refractory AML.(126). They were also found to be useful against several solid tumors such as neuroblastoma, renal, colon, gastric, and ovarian cancers,(127),(128). The trials concluded that NK-cell transfer was safe and efficacious. Similar trials were also conducted recently in patients with recurrent metastatic breast and ovarian cancer(129).The allogeneic NK cells have the advantage of being derived from healthy donors and have more cytotoxic activity. Moreover, NK cells do not induce GvHD, unlike T cells. As discussed in the earlier section, the role of NK cells has been established not only in cancer but also in various other disease conditions. Adoptive NK cell therapy can thus be explored for diseases such as asthma, multiple sclerosis, diabetes, arthritis, etc. The effectiveness of NK cells in controlling HIV-1 infection has already been demonstrated in in vitro and in vivo experiments.NK cell therapy can be applied to patients who are refractory tostandard highly active antiretroviral therapy (HAART). Besides the option of using NK cells for adoptive transfers, understanding the role of NK cells and their receptors can open up other strategies to treat diseases. For example, during the developmental stages of Type 1 diabetes, the activation of NK cells can be prevented by the administration of specific antibodies for blocking the NKp46 activation receptor. Similarly, in rheumatoid arthritis where the role of NK cells can possibly be protective or disease-enhancing, therapy can be

considered accordingly. Inhibitory receptor NKG2A can be blocked, which will stimulate NK cells and thus control the disease. Where NK cells enhance the disease condition, the blocking of RANKL (receptor activator of NFKB ligand) and M-CSF (macrophage colony-stimulating factor), factors which mediate osteoclastogenesis and bone destruction, can help(130).
For the purpose of therapeutic applications, allogeneic NK cells can be sourced from umbilical cord blood (UCB), adult donor lymphapheresis products, or even from NK-cell lines such as NK-92. Recently, studies have shown successful in vitro derivation of functional NK cells from human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC)(131),(132),(133).. hESC and iPSC-derived NK cells have demonstrated potent anti-tumorigenic and anti HIV activity, and are phenotypically similar to those of peripheral blood origin. Moreover, they are considered superior to UCB-derived NK cells because they have higher levels of KIR expression, thus making them more potent. Pluripotent cell-derived NK cells can therefore be an unlimited source for the adoptive transfer of NK cells to treat a range of diseases.
However, safety of hESC and iPSC-derived NK cells in terms of potential tumorigenicity needs to be determined before they can be utilized in the clinical set up. The application of NK cells as immunotherapeutic agent requires several technical developments. NK cells need to be isolated and expanded in sufficient numbers for them to act as effector cells. Moreover, the activity of NK cells needs to be enhanced for better efficacy. Expansion of NK cells has been attempted using cytokines such as IL-2 and IL-15 (134),(135).These two cytokines can also help increase the survivability of the NK cells (136).IL-2 is also thought to potentiate the cytotoxic ability of NK cells. Co-culturing NK cells with accessory cells such as irradiated Epstein Barr Virus (EBV) transformed lymphoblastoid cells, HFWT (a Wilm'stumor derived cell line), and K562 has been reported to enhance NK cell proliferation (137),(138),(139).Activation of NK cells can be achieved by various genetic engineering techniques to augment activating signals and also to downregulate inhibitory signals (140),(141),(142),(143),(144).Similarly, the specificity of NK cells can be increased through genetic modification approaches such as the use of chimeric antigen receptors (CARs)(145),(146),(147).

16. Conclusion

NK cells exert their biological activity by a triad of functions: cytotoxicity, cytokine secretion and co-stimulation. NK cells need to be evaluated whenever an autoimmunity, immunocompetency or immunodeficiency investigation is undertaken. The expanding characterization of the biological roles of KIR hints at yet undiscovered roles for NK cells in health maintenance.Since NK cell–DC cross-talk clearly influences innate immune responses and can also impact on adaptive immunity, a better understanding of the mechanisms involved is critical and necessary if the ultimate aim is to develop protocols that will provide better immunity following vaccination, cancer immunotherapy and in transplantation settings.

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