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NK cells interactions with dendritic cells shape innate and adaptive immunity

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1. ABSTRACT

While natural killer (NK) cells received their name from their ability to mediate spontaneous cytotoxicity, it has recently become clear that they require activation to target most transformed and infected cells. Dendritic cells (DCs) have been shown to mediate NK cell activation during innate immune responses. Surprisingly, this interaction was recently reported to be required to restrict infections by NK cells, and to take place in secondary lymphoid organs. Here we review these recent studies on NK cell interactions with DCs, discuss the molecular mechanisms underlying the cross-talk between these two innate lymphocyte populations, and out-line how DCs and NK cells synergize to enhance innate immunity against microbes and tumors as well as shape the adaptive immune system. Based on this better understanding, we propose that NK cells should be targeted for their protective functions and as an adjuvant during immunotherapy development.

2. INTRODUCTION

NK cells were originally identified as lymphocytes that could readily lyse cells, in sharp contrast to T cells that need to be primed to become cytotoxic (1, 2). However, NK cells are now recognized to require prior activation to efficiently kill infected or transformed cells. It was first reported by Laurence Zitvogel and colleagues (3) that DCs could affect NK cell function in immune surveillance of tumors. Since then, it has been shown that also human DCs can activate NK cells (4, 5, 6) and that NK cell activation by DCs is required for protective innate immunity mediated by these lymphocytes in mice (7, 8). Furthermore, it was shown in mice and humans that NK cells can polarize T cell responses. Therefore, the interaction between DCs and NK cells is not only important during innate immune responses, but also shapes adaptive immunity, and thereby extends the influence of this interaction far beyond the initial response of the immune system to infections.

Based largely on human *in vitro* studies, several types of NK cell interactions with DCs have been observed, in which the activation stage of the cells involved determines the outcome of the encounter. Activated NK cells have been described to be able to mature immature DCs or, under certain conditions, to lyse them. These interactions might be essential in editing or suppressing immune responses. Alternatively, mature DCs have been shown to activate resting NK cells to produce cytokines, proliferate, and increase their cytotoxicity via both cell-contact dependent and independent signals (9, 10).

Here we review recent studies shedding light on the different facets of DC/NK cell interactions and discuss the implications for both immunotherapy and innate immune responses

3. NATURAL KILLER CELLS

NK cells are lymphocytes, which have originally been characterized by their ability to kill target cells (11). This original definition has been extended, and in humans two main subsets that strongly differ in their functions have been characterized. The major subset in human peripheral blood, $CD56^{dim}CD16^{+}$ NK cells, readily lyses target cells after activation, but secretes low level of cytokines (12, 13). In contrast, $CD56^{bright}CD16^{-}$ NK cells produce large amounts of cytokines, such as IFN-gamma, upon stimulation, but acquire cytotoxicity only after prolonged activation (13, 14). Significant differences have also been found between these subsets with respect to expression of inhibitory and activating receptors as well as chemokine receptors, which regulate target cell recognition and homing of NK cells, respectively (15, 12). In addition, these NK cell subsets differ with regard to their organ distribution. 90% of human peripheral blood NK cells are $CD56^{dim}CD16^{+}$, whereas $CD56^{bright}CD16^{-}$ NK cells constitute less than 10%. However, in secondary lymphoid organs, the $CD56^{bright}CD16^{-}$ NK cells are markedly enriched (16, 14), and this subset constitutes 75% of NK cells present in lymph nodes and tonsils. As lymph nodes are suggested to harbor 40% of all human lymphocytes, whereas probably only 2% of all lymphocyte circulate through peripheral blood at any given moment, $CD56^{bright}CD16^{-}$ NK cells in secondary lymphoid organs constitute a remarkable pool of innate effector cells (17). Similar, functionally different NK cell subsets have not been identified in the mouse so far. However, some features of human $CD56^{bright}CD16^{-}$ NK cells are shared by mouse $CD127^{+}GATA\text{-}3^{+}$ NK cells of thymic origin, which are enriched in lymph nodes, have lower expression of inhibitory as well as cytolytic molecules and primarily respond by cytokine secretion to cytokine activation (18). In contrast, $Mac1^{high}CD27^{+}$ mouse NK cells seem to be superior to $Mac1^{high}CD27^{-}$ mouse NK cells in both cytokine production and cytotoxicity (19). Future studies will show if these NK cell subsets are indeed the functional counterparts of human $CD56^{bright}CD16^{-}$ NK cells in mice.

The developmental relationship between the two main human NK cell subsets is still debated. In this regard, it has been observed that NK cells from secondary lymphoid organs can acquire cytotoxic ability and the expression of inhibitory and activating NK cell receptors upon stimulation with cytokines such as IL-2, IL-12 and IL-15 (14) (20). Moreover, several independent research teams have recently demonstrated that $CD56^{bright}CD16^{-}$ NK cells can convert into cytotoxic $CD56^{dim}CD16^{+}$ NK cells with all phenotypical and functional features (21-22). This linear differentiation has also been shown *in vivo* upon transfer of $CD56^{bright}CD16^{-}$ NK cells into NOD-SCID mice (23). Therefore, $CD56^{bright}CD16^{-}$ NK cells might home to secondary lymphoid tissues after leaving the bone marrow, and might mature in lymph nodes and tonsils to cytolytic $CD56^{dim}CD16^{+}$ NK cells with the full repertoire of inhibitory and activating NK cell receptors. Taken together, these results support the idea of a linear differentiation model of human NK cells development and the concept that lymphoid organs are sites of NK cell maturation.

Cytolytic activity of fully matured NK cells is controlled by the balance between inhibitory and activating signaling pathways (24). To prevent killing of normal cells, most NK cells express an array of inhibitory receptors, many of which recognize major histocompatibility complex (MHC) Class I molecules, expressed by almost all nucleated cells (25, 26). This observation led to the missing-self hypothesis, whereby the postulated role of NK cells is to destroy cells that express decreased levels of MHC Class I molecules (27). Indeed, MHC class I molecules are often down-regulated in virally infected and cancer cells, supposedly a trait selected for avoiding cytotoxic T-lymphocyte (CTL) recognition (28). In humans, there are three types of major MHC Class I specific inhibitory receptors expressed by NK cells. KIRs and immunoglobulin-like transcripts (ILTs) bind classical and non-classical HLA class I molecules, whereas the C-type-lectin heterodimer CD94/NKG2A, and its alternatively spliced form NKG2B, bind to the non-classical MHC class I molecule HLA-E (29, 30). Interestingly, KIRs are encoded in a gene cluster, are extremely polymorphic, and each one is expressed in a different pattern creating a heterogeneous population of NK cell clones in every individual (31). KIRs are able to distinguish HLA class I allotypes, and HLA-C haplotype recognition by KIR2DL1 and KIR2DL2 is determined by amino acids in the C-terminal portion of the MHC class I α1 helix (32). The binding of MHC class I complexes to KIRs or to the heterodimeric CD94/NKG2A receptor initiates inhibitory pathways that can override activation signals (33). On the other hand, reduced expression of MHC Class I is not the only requirement for NK cell activation, and overexpression of activating ligands on target cells can also trigger NK cell function. Moreover, absence of MHC class I only translates into NK cell recognition if activating NK cell receptors are also engaged by activating structures on the MHC class I low target cell.

NK cells express a number of activating and co-activating receptors (24). The main activating NK cell receptors are the C-type lectin homodimer NKG2D, the immunoglobulin natural cytotoxicity receptors (NCRs), NKp30, NKp44, and NKp46, and CD16, mediating antibody dependent cellular cytotoxicity (ADCC) of

antibody opsonized targets. In addition, NK cells express a variety of activating co-receptors, including 2B4 (CD244), NTB-A, NKp80, and DNAM-1 (CD226), which contribute, but are not required for NK cell activation (34). Ligands for several but not all of those receptors are known. In the specific case of NKG2D, an array of cellular stress-induced molecules has been identified. They include non-classical MHC class I molecules (MHC class I polypeptide-related sequence A or B, MICA and MICB in humans), and the MHC class I-related UL-16 binding proteins (ULBP) 1-4 which are induced in viral infections, during transformation, and by DNA-damaging agents (35, 36). While the NCR ligands on tumor cells are still unknown, viral hemagglutinins have been suggested as ligands for NKp44 and NKp46 (37, 38). In addition, poliovirus receptor (PVR; CD155) and nectin-2 (CD112) have been identified as DNAM-1 ligands (39), whereas CD48 engages 2B4 (40, 41). These studies indicate that as for inhibitory NK cell receptors, NK cells receive activating signals from a panel of receptors, recognizing often multiple ligands, in order to detect a variety of transformation- and infection-induced changes during innate immune responses (42).

Our knowledge of the receptors and ligands that are essential for target cell recognition by activated NK cells has increased dramatically over the last years, but a number of experimental systems have also demonstrated that NK cells need to receive additional signals to protect the host during infection or immune surveillance. We will discuss these signals that are initially necessary to heighten NK cell responsiveness below.

4. DENDRITIC CELLS

DCs are generally regarded as sentinels of the immune system. They reside in all tissues in a immature or resting form, and get activated upon pathogen encounter, in inflammatory environments, and by other cells of the immune system (43, 44). Upon maturation or activation, they migrate at enhanced frequency to secondary lymphoid tissues, preferentially to the perifollicular T cell zones, in order to prime T cell responses, but also activate other innate lymphocytes residing in these areas (45, 46). With their migration two types of information are transmitted from the site of DC activation. First, DCs carry antigens that they have endocytosed at these sites, and present them on their cell surface after processing. The second signal is imprinted by the inflammatory environment leading to DC maturation. It is reflected by changes in costimulatory molecule expression, cytokine production, and chemokine receptor expression, enabling the activated DC to initiate the most effective immune response against the particular threat at the site of DC activation. In order to optimally transmit these two signals, DCs are equipped with a variety of receptors for antigen uptake and for sensing their environment. For example, microbial stimuli of invading pathogens are often sensed by toll-like receptors (TLRs) (43). TLRs are able to recognize a variety of pathogen constituents, ranging from DNA motifs, such as CpG oligonucleotides, double-, and single-stranded RNAs, to protein components of viruses and bacteria. This variety of sensors enables DCs to react to different pathogenic challenges, and, similar to NK cells, DCs are able to integrate signals from several receptors, and enhance their response accordingly (47). Intriguingly, it has been shown recently that antigens that engage simultaneously endocytic receptors and TLRs are more efficiently processed for MHC display than antigens that are just endocytosed (48). Therefore, both DCs and NK cells use a large repertoire of receptors to sense their environment, and, as we will discuss below, they do so not independently from each other, but feed back the information to each other via DC maturation by activated NK cells and NK cell activation by mature DCs.

5. INTERACTION BETWEEN NK CELLS AND DCS

Although DCs were mainly considered to be the classic antigen-presenting cells (APC) initiating and activating the adaptive immune response, they have been found to interact with other innate immune cells such as NK cells. These observations have highlighted, firstly, the importance and complexity of the interplay between innate lymphocytes under various conditions, and, secondly, the strong context-dependence of the outcome of this interaction. Indeed, nowadays it is recognized that the DC/NK cell crosstalk varies with the activation status of the cells involved, and that the outcome of this interaction shapes the following adaptive immune response and APC function. Here we want to discuss the location, underlying mechanisms, and functional relevance of DC/NK cell interactions.

5.1. Where does it take place?

The sites of DC encounter with NK cells had been postulated to be inflamed tissues and secondary lymphoid organs, but the main body of evidence that DC/NK cell interactions indeed take place in these organs has only been recently provided. The two different interaction sites might favor DC encounter with $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cells differently. With respect to homing to inflamed tissues, colocalization of NK cells and DCs has been observed in atopic skin lesions (49). Based on the chemokine receptors expression at their surface (CXCR1, CX3CR1 and ChemR23), the major blood subset $CD56^{dim}CD16^{+}$ is thought to home towards inflammatory chemokines, and could interact with DCs at peripheral sites of inflammation (15, 50). Interestingly, both plasmacytoid and myeloid DCs have also been reported to rely on ChemR23 to home to inflammation sites (51). However, the minor subset of $CD56^{bright}CD16^{-}$ NK cells might also reach inflamed areas because they express CXCR3 (52), and these cells indeed have been found to predominate in autoimmune inflamed and some tumor tissues (53, 54). Interestingly, DCs have been shown to preferentially induce the proliferation and IFN-gamma production of the later subset (10, 52, 13). With respect to DC/NK cell interactions in secondary lymphoid organs, both in humans and in mice, a close association of NK cells and DCs could be observed in perifollicular T cell zones of lymph nodes (10, 55, 56). Extending these previous findings, Lucas and colleagues have found that selective ablation of DCs prevents NK cell recruitment to local lymph nodes after TLR stimulation,

and that their interaction with DCs at these sites is crucial for the emergence of effector NK cells in the periphery (8). These studies in mouse and human document that DC/NK cell interactions take place at peripheral inflamed sites and in secondary lymphoid tissues. However, the difference between the two species seems to be that humans constitutively harbor a substantial amount of NK cells in secondary lymphoid tissues, which are enriched in the NK cell subset that preferentially responds to DC activation. On the other hand, NK cells are rare in murine secondary lymphoid tissue, but get recruited to these sites upon mature DC migration into secondary lymphoid tissues (8, 57).

5.2. How do NK cells and DCs cross-talk?

5.2.1. NK cell activation by DCs

Depending on the DC maturation conditions, several soluble factors have been suggested as essential for the activation of NK cells by DCs. Among the soluble factors, IL-12 has been repeatedly observed to induce IFN-gamma secretion and proliferation, and is probably the most pivotal signal that enhances NK cell effector function in humans and in mice (10, 58). In addition, IL-18, type I IFNs, and IL-15 have also been suggested to mediate NK cell activation by DCs (8-10, 59-61). In these studies it has become apparent that different cytokines mediate different aspects of DC-induced NK cell activation and steer the innate immune response to distinct NK cell effector functions. While IL-12 primarily triggers IFN-gamma secretion by NK cells, type I IFNs enhance NK cell cytotoxicity, and IL-15 has the capacity to trigger NK cell proliferation and survival (9-10). Some of these cytokines, such as IL-12 and IL-15, can trigger all of these NK cell effector functions, when they are present at high enough concentrations. Therefore, DC maturation stimuli might preferentially rely on one or several cytokines to enhance NK cell activity (9). Along the same line, different DC subsets are also known to differ in their capacity to produce these cytokines. Accordingly, it was found that plasmacytoid DCs with their exquisite ability to secrete large amounts of type I interferons mainly enhance NK cell cytotoxicity, whereas myeloid DCs are superior in stimulating IFN-gamma secretion and proliferation by NK cells (9, 10, 62). Furthermore, IL-2 might also play a role in NK cell activation by DCs under certain DC activation conditions (63), even though most DC maturation stimuli enhance NK cell activity in an IL-2-independent fashion (10, 64). Some of the very same cytokines involved in NK cell activation by DCs have been shown to differentiate $CD56^{bright}CD26^{-}$ NK cells into $CD56^{dim}CD16^{+}$ NK cells (14, 20). Therefore, it is feasible that DCs might assist NK cell differentiation. Since IL-15 and IL-15Ralpha have been described to be essential for NK cell development in mice (65, 66), especially, the superior ability of DCs to present IL-15 on their surface (8, 10) could facilitate NK cell differentiation. These studies suggest that DC subsets exposed to different maturation stimuli enhance NK cell activation and possibly NK cell differentiation by various means. These distinct pathways of NK cell stimulation activate different arms of NK cell effector functions, and thereby tailor the NK cell response to the needs of the particular immune response. However, common themes in NK cell activation by DCs are that activating NK cell receptors rarely play a dominant role and that this interaction is mainly mediated by cytokines that either work only over short distances and make this interaction transwell-dependent, or are secreted by DCs in abundance and appear transwell-independent.

Although NK cell activation by DCs is thought to be primarily cytokine-dependent, direct cell-to-cell contact enhances it (8, 10, 55, 56). Several studies have characterized the immunological synapse underlying the cross-talk between NK cells and myeloid cells in general (67-70). These studies started out from the substantial body of literature on synapses between activated NK cells and tumor cells which were either susceptible or resistant to NK cell lysis (71, 72). However, conjugates between mature DCs and resting NK cells do not seem to fit into the classical distinction between activating and inhibitory NK cell synapses, as mature DCs provide both activating and inhibiting signals to NK cells either through cytokine secretion into the synaptic cleft or MHC class I expression, respectively. Therefore, we suggested calling the DC/NK cell synapse regulatory as it transmits both inhibitory and activating signals simultaneously across the interaction site (70). With respect to activating signals mediated through the DC/NK cell synapse, Borg and colleagues showed that human NK cell activation by LPS-matured DCs required cellular contact and IL-12 was released towards the interface of the DC/NK cell conjugates in a polarized fashion (67). In addition, Semino and colleagues investigated aggregate formation between immature DCs and resting NK cells. They observed a lysosome-mediated secretion of IL-18 by DCs at the DC/NK cell interface, followed by secretion of high mobility group B1 (HMGB1) by NK cells, which in turn matured DCs (68). While these previous studies characterized DC/NK cell conjugates at late timepoints of 25min and 3h, we reported recently the rapid formation of a functional and long-lasting immunological synapse between resting NK cells and mature DCs already after 1min of incubation. Although inhibitory NK cell receptors were polarized to this synapse, and are known to protect mature DCs from NK cell lysis (6), activation signals, such as mobilization of intracellular calcium and CD69 up-regulation, were transmitted in parallel. As activating receptors, we found IL-15R, both its alpha- and beta-chain localized to the interface of the DC/NK cell conjugates. Three-dimensional reconstruction of the DC/NK cell synapse revealed that these inhibitory and activating signals seem to be transmitted by spatially separated domains at the synapse center providing the physical environment for NK cell activation without triggering DC cytolysis (70). Indeed blocking of synapse formation or IL-15Ralpha abolished NK cell survival mediated through DC/NK cell conjugates. Therefore, cell-contact dependency of NK cell activation by mature DCs might primarily result from optimal cytokine mediated NK cell stimulation via this synapse (67, 70). These studies suggest that mature DCs rapidly establish a regulatory synapse with NK cells through which they channel sequential signals for NK cell activation with early polarization of IL-15 and later IL-12 release, while at the same time protecting themselves from NK cell lysis, in

order to enter successively additional interactions with other NK cells or T cells.

5.2.2. DC maturation by NK cells

In addition to NK cell activation by mature DCs, activated NK cells can also trigger DC maturation. DC maturation has been reported after NK cell recognition of MHC Class I^{low} tumor cells and NK cell activation with IL-2 (4, 5, 73). NK cells mature DCs via TNF-alpha and IFN-gamma production, and cell-to-cell contact-dependent signals (4, 5). NK-matured DCs display up-regulation of CD86 and secrete IL-12. Because DC maturation might be confined to the site of innate lymphocyte activation, it is potentially a local and very early event (44). However, this interaction in turn expands and activates innate lymphocytes and initiates T cell immunity. Indeed, the interplay between DCs and NK cells can completely replace $CD4^+$ T cell help in the induction of anti-tumor $CD8^+$ T cell responses (74). Taken together, the DC/NK cell cross-talk enables the immune system to use the entire repertoire of germline-encoded receptors on DCs and NK cells for the initiation of immune responses. If DC receptors sense invaders or cellular transformation, they will activate NK cells, and vice versa if activating NK cell receptors are engaged efficiently, this will translate into DC maturation. The superior ability of NK cells to recognize virus-infected or transformed cells via NKp46 and NKG2D, especially after virus-induced MHC class I down-regulation, might make NK cells important accessory cells for DC-initiated immune responses.

5.3. What are the functional consequences of DC/NK interactions in the regulation of immune responses?

5.3.1. DC editing by NK cells to avoid graft-versus-host (GVHD) disease

Because they express normal levels of MHC Class I, autologous cells are usually spared from NK cell-mediated lysis. However, immature DCs are an exception and can be lysed by activated NK cells even in an autologous setting (75, 76). Up-regulation of MHC class I molecules protects mature DCs from NK cell lysis (6). A major component of this MHC class I- mediated protection of DCs from NK cell lysis is the expression level of the non-classical MHC class I molecule HLA-E, the ligand for the inhibitory receptor CD94/NKG2A on NK cells. Indeed, CD94/NKG2A positive NK cells have been identified as the main NK cell subset targeting immature DCs (77). Interestingly, CD94/NKG2A is the only inhibitory receptor found so far on the surface of NK cells in secondary lymphoid organs (14). As activating NK cell receptors, NCRs have been identified to mediate NK cell lysis of immature DCs with the help of DNAM-1 (6, 78-80), whereas NKG2D might be involved in macrophage killing by NK cells (69) and targeting of influenza-infected DCs (81). Depending on the myeloid DC subset, NKp30 alone or with the contribution of NKp46 seem to mediate NK cell lysis of immature myeloid DCs (6, 78, 79). Therefore, activated NK cells can both mature or kill DCs, depending of the ratio between these two cell types (4). Low amounts of activated NK cell primarily mature DCs, but activated NK cells can also kill immature DCs rapidly when they outnumber them. This could reflect different stages of NK cell homing to inflamed tissues. At the beginning of an immune response, few NK cells, homing to sites of inflammation, might assist in DC maturation, while later on, when large numbers of NK cells have accumulated, they could eliminate immature DCs to avoid tolerance induction by incompletely matured DCs.

Although the killing of DCs is quite intriguing per se, and the editing of DCs by NK cell lysis might ensure that only matured DCs are involved in antigen presentation and T cell priming during activation of adaptive immunity (17, 82-85), recognition of DCs by NK cells might also have direct therapeutic implications. Along these lines, NK cell-mediated killing of immature DCs and other myeloid cells might be of relevance in patients suffering from acute myeloid leukemia (AML), and could be exploited for their treatment. In this disease, NK cells of AML patients were found to express decreased levels of NKp30, and displayed poor cytolytic activity against immature DCs or leukemic blasts (86, 87). One way to restore NK cell activity is repopulation of these innate lymphocytes after bone marrow transplantation. However, recipients of foreign bone marrow are threatened by the development of graft-versus-host disease (GVHD). Indeed, GVHD is a common and life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT), used currently to cure malignant disorders such as AML Generally the risk for developing GVHD decreases with the degree of HLA-matching between donor and recipient; the greater the match, the smaller the risk (88). Although HLA-matching is crucial, 75% of patients do not have an HLA-identical sibling, and haploidentical (that is, matched for one haplotype and unmatched for the second one) transplantation becomes a viable option. In haploidentical bone marrow transplantation, reconstituting NK cells of donor origin might develop with a mismatch between their inhibitory receptors and the MHC Class I molecules at the surface of recipient cells, especially when the HLA mismatch occurs in HLA-C alleles, which can be distinguished by KIR2DL inhibitory NK cell receptors. In AML, these alloreactive NK cells might prevent leukemia relapse and, at the same time, GVHD. After analyzing 112 patients, Ruggeri and colleagues published recently that transplantation of alloreactive NK cells is associated with significant lower relapse rates (3% vs 47%), a better event-free survival (34% vs 6%), and remission (67% vs 18%) (89, 90). These promising results have now been repeatedly observed by other groups, refining the model with respect to the importance of a donor KIR – recipient HLA-C allele mismatch (91, 92). As alloreactive donor-derived T cells activated by recipient APCs cause GVHD, it has been suggested that donor-derived alloreactive NK cells kill not only tumor cells but also recipient DCs preventing them to activate the T cell response involved in GVHD (88). These studies might suggest a therapeutic benefit of DC editing by activated NK cells.

5.3.2. Polarization of T cell responses after DC/NK cell interaction

Maturation not only protects DCs from NK-mediated lysis, but also enables DCs to migrate to secondary lymphoid tissues, where they efficiently present

antigens to T-lymphocytes (93). Notably, $CD56^{bright}CD16^{-}$ NK cells are preferentially found in T cell zones of secondary lymphoid organs in close proximity to DCs (10, 55, 56). Both, NK cells and DCs probably reach these sites by virtue of their CCR7 expression (17). Recently, we reported that $CD56^{bright}CD16^{-}$ NK cells from secondary lymphoid tissues enhanced the priming of IFN-gamma producing alloreactive $CD4^{+}$ Th1 cells. NK cells in secondary lymphoid tissues supported Th1 polarization more efficiently than blood NK cells by their superior ability to produce IFN-gamma after stimulation with DCs, matured with the double-stranded RNA mimic poly (I:C) (94). Similarly, mouse NK cells were shown to support Th1 polarization after interaction with DCs *in vivo* (55, 57, 95). As already discussed above, T cell polarization was mediated by a NK cell subset constitutively present in human secondary lymphoid tissues, whereas it required NK cell recruitment to secondary lymphoid organs in mice. These results suggest that NK cell activation by DCs and its polarizing effect on adaptive immune responses should be harnessed for vaccine development. Namely, adjuvants that enable DCs to activate NK cells might preferentially achieve Th1 polarization.

5.3.3. Enhanced innate immunity to microbial infection after DC/NK cell interaction

NK cells have been implicated in the innate resistance to numerous pathogens (96). In this review we will, however, focus on their importance for the immune control of herpesvirus infections. Indeed, lack of immune control of this virus family is a hallmark of NK cell deficiency (97, 98).

The most prominent example of innate immune control of a herpesvirus by NK cells is the murine cytomegalovirus (MCMV), a β-herpesvirus. Innate resistance to this virus is mediated by NK cells that carry the Ly49H activating NK cell receptor (99, 100). Mouse strains susceptible to fatal MCMV infection lack Ly49H, and Ly49H transgene expression confers resistance to MCMV in these mice (101). Not unlike NKp46, this receptor selectively recognizes a viral surface protein on infected cells called m157 (102, 103). However, as discussed above, activation of innate immune control of MCMV requires $CD8^{+}$ DCs (104). DCs sense MCMV infection at least in part via TLR9 stimulation, and then activate NK cell after maturation (105, 106). These studies demonstrate that NK cell activation by DCs leads to an essential innate immune response in mice that is crucial for surviving MCMV infection.

With respect to human herpesviruses, there is evidence to support the role of innate lymphocytes in limiting the early infection of Epstein Barr virus (EBV), a γ-herpesvirus (107). Male patients with X-linked lymphoproliferative disease (XLP), who frequently succumb to primary EBV infection by developing EBV-associated lymphomas, have a mutation in the SAP gene leading to defective recognition of EBV-transformed B cells by NK cells (108-110). Lack of NK cell function is not the only immune deficiency in XLP patients, but most likely contributes to loss of EBV specific immune control. Furthermore, EBV-induced B cell transformation is restricted by IL-2-activated peripheral blood NK cells *in vitro (*111-113). Moreover, NK cell-depleted PBMCs were less efficient in controlling tumor development after transfer of EBV-transformed B cells in SCID mice (114). In addition, lytically EBV replicating B cells were found to be more susceptible to activated NK cell lysis (115). Finally, EBV-driven lymphoproliferative disease was found to be associated with a novel primary immunodeficiency affecting NK cell function (98). These studies indicate that innate immune control of EBV is at least in part mediated by NK cells. We could recently demonstrate that this innate immunity mediated by NK cells causes restriction of EBV infection *in vitro*, and requires NK cell activation by myeloid DCs. Human myeloid DCs mature in response to EBV particles and are then able to efficiently activate NK cells. Transformation of B cells by EBV in culture was inhibited by NK cells after activation by mature DCs. Tonsilar NK cells, purified from the primary site of EBV infection, were most efficient in limiting B cell transformation by EBV due to their superior ability to secrete high levels of IFN-gamma after activation by DCs. IFN-gamma by DC activated NK cells was able to inhibit the establishment of EBV transformation program (116). In addition to myeloid DCs, EBV has been found to activate plasmacytoid DCs in a TLR9-dependent manner, and these cells limit outgrowth of EBV-transformed B cells *in vivo* (117). Therefore, both myeloid and plasmacytoid DCs sense EBV infection and probably contribute to NK cell activation for innate immune control of EBV.

6. PERSPECTIVE

Due to the studies discussed above, a new understanding of NK cell biology has emerged in recent years. This has revised our previous concept of NK cell function. From solitary killers, they have become team players interacting early with the main antigen-presenting cells, DCs, to reach their full functional potential. DC-induced NK cell activation should be harnessed in immunotherapies against viruses and tumors, both for direct innate immunity and for polarization of adaptive immune responses. Along these lines, adjuvants for immunotherapy via DC activation should be chosen to allow NK cell activation in addition to efficient T cell priming by DCs. Vaccine formulations that stimulate NK cells in addition to T cells could ensure maintenance of MHC class I restriction elements on the targeted tissues and benefit from direct anti-viral and anti-tumor effects of NK cells responses as well as the assistance of NK cells in T cell priming. Such comprehensive immunotherapies, harnessing both innate and adaptive lymphocyte compartments, would mimic much better successful immune responses in humans (Figure 1).

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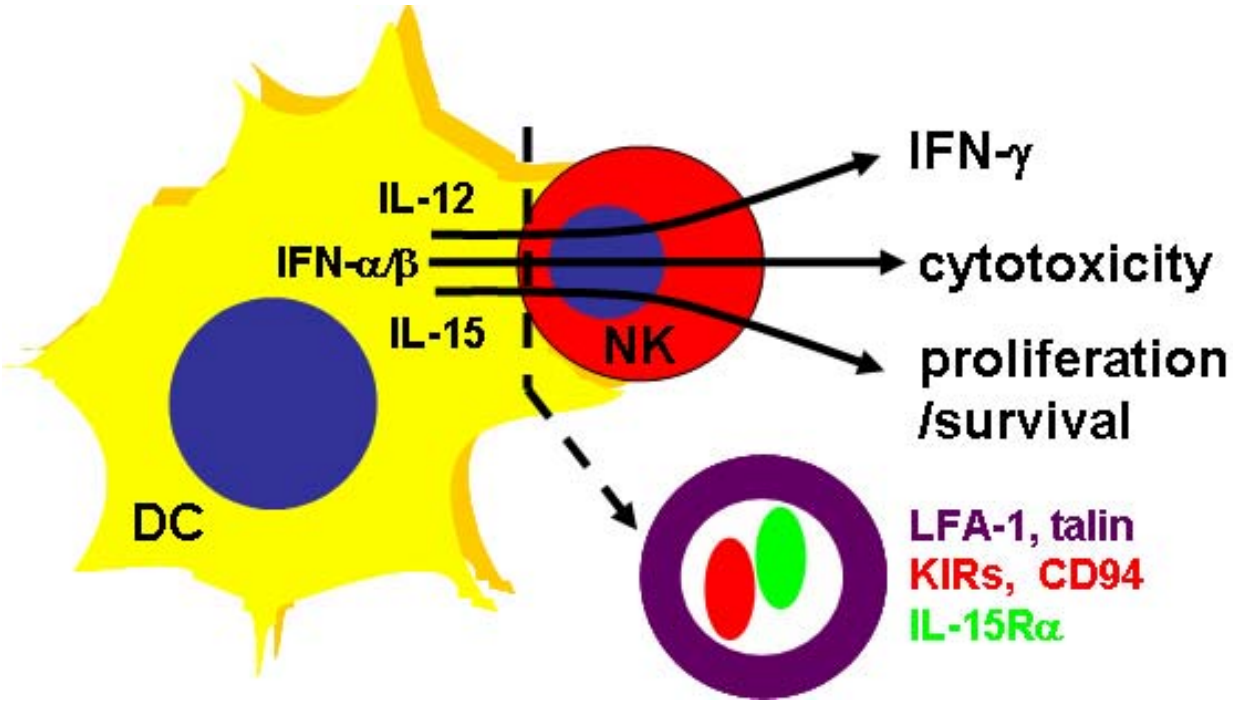

Figure 1. NK cell activation by DCs is mediated through conjugate formation. In this interaction mature DCs stimulate IFN-gamma secretion by IL-12, cytotoxicity by type I IFNs, and proliferation/survival by IL-15. NK cell activating signals are transmitted via cytokine receptors in domains spatially separated from inhibitory signals, preventing DC lysis, and are located at the center of the DC/NK cell synapse.

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