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MINIREVIEW

Role of Human Natural Killer Cells in Health and Disease

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Natural killer (NK) cells, the $CD3^-$ $CD56^+$ $CD16^+$ subset of peripheral blood lymphocytes, have long been known to be involved in non-major histocompatibility complex-restricted natural immunity to virally infected and malignant target cells. The association of abnormalities in NK cell numbers or functions with a broad spectrum of human diseases has been more clearly defined in recent years as a result of the improved knowledge of NK cell physiology and advances in monitoring of NK cell functions in health and disease. The ability to reliably measure changes in NK activity and/or numbers during the course of disease or response to treatment has focused attention on the role of the NK cell in disease pathogenesis. The improved understanding of NK cell deficiency in disease has opened a way for therapies specifically designed to improve NK cell function. The therapeutic use of biologic response modifiers capable of augmenting NK cell activity in vivo and of adoptive transfer of highly enriched, activated autologous NK cells in diseases such as cancer and AIDS is being evaluated. The importance of NK cells in health and the consequences of NK cell deficiency or excess are likely to be more extensively monitored in the future.

Natural killer (NK) cells are a subset of lymphocytes with the distinct morphologic features of large granular lymphocytes (LGL) and important biologic functions. They are distinguishable from T and B lymphocytes by surface phenotype, cytokine profile, and the ability to mediate spontaneous cytotoxicity, without prior sensitization, against a broad range of targets, including tumor cells and virally infected targets (22, 63). In human peripheral blood, NK cells account for about 5 to 15% of circulating lymphocytes, but in some organs, e.g., the liver, they represent up to 45% of tissue-infiltrating lymphocytes (19).

While it has been widely recognized that NK cells mediate natural immunity, their role in human health has generally been underestimated. Current evidence indicates that decreased or absent NK cell numbers or activity is often associated with the development or progression of cancer, acute or chronic viral infections, autoimmune diseases, immunodeficiency syndromes, and psychiatric illness. The ability to reliably measure NK activity in human body fluids or tissues and to enumerate cells which express NK cell-associated surface markers has contributed considerably toward a better definition of NK cell involvement in human diseases and their pathogenesis.

From recent evidence, it appears that the NK cell participates either directly or indirectly in multiple developmental, regulatory, and communication networks of the immune system. Today, the NK cell is increasingly frequently viewed as a remarkably efficient effector cell which is not only equipped for killing but is also capable of rapid response to exogenous or endogenous signals by producing a variety of cytokines and factors involved in interactions between immune and nonimmune cells. In this review, we summarize the functional characteristics and the physiologic role of human NK cells and evaluate potential therapeutic approaches based on NK cell upregulation or their adoptive transfer to patients with cancer or other diseases.

Morphologic and phenotypic characteristics of NK cells. NK cells, which belong to a subset of lymphocytes referred to as LGL, have average diameters of 7 to 8 μm in the resting state and 10 to 12 μm in the activated state. On May-Grünwald-Giemsa-stained smears of peripheral blood mononuclear cells (PBMNC), LGL are easily recognizable not only by the reniform nucleus but also by the presence in the cytoplasm of numerous azurophilic granules. To enumerate NK cells in blood or body fluids, it is not sufficient to depend on morphology, however, because activated T lymphocytes may acquire the same appearance. To distinguish NK cells from other lymphocytes, it is necessary to use monoclonal antibodies (MAbs), which recognize distinctive surface markers expressed on NK cells, for staining and flow cytometry. Mature, circulating NK cells express the $CD3^-$ $CD56^+$ $CD16^+$ $CD2^{dim}$ phenotype and are distinguishable from T cells by the lack of the T-cell receptor or of rearranged T-cell receptor genes, which retain the germ line configuration in mature NK cells (28). Unlike B cells, NK cells do not express surface immunoglobulin (Ig); however, by virtue of expressing FcγRIII, the Fc receptor for IgG, NK cells may be positive for surface-bound Ig (40). Surface markers expressed on NK cells or activated NK cells include interleukin-2 (IL-2) receptors (41); other types of Fc receptors (45, 46); β_1 and β_2 integrins (74); various activation antigens, e.g., HLA-DR, transferrin receptor (CD71), CD69, and the activation-inducing molecule Leu23 (47); and putative clonotypic NK receptor(s) for binding to target cells (38). The nature of this NK receptor remains undefined and controversial today.

Many of the surface molecules expressed on NK cells are present on other hematopoietic cells, and therefore, what distinguishes NK cells from other PBMNC is a unique combination of several markers, e.g., CD56, CD16, and CD2, as well as the absence of certain other markers, such as CD3, CD14, and surface Ig. Not all NK cells express the consensus phenotype defined above, and subsets of, for example, $CD56^+$ $CD16^-$ or $CD56^-$ $CD16^+$ NK cells have been recognized and

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TABLE 1. Expression of β_1 and β_2 integrins on human resting NK cells and NK cells activated in vitro by IL-2[a]

Integrin	Surface antigen	Mean % positive cells ± SEM (mean fluorescence intensity ± SEM)	
		Fresh NK cells (n = 9)	NK cells + IL-2 (n = 10)
β_1 (VLA)	CD29 (β_1)	85 ± 3 (85 ± 11)	89 ± 6 (175 ± 30[b])
	CD49b (α_2)	6 ± 1 (NA[c])	87 ± 3[b] (165 ± 39)
	CD49c (α_3)	8 ± 2 (NA)	91 ± 5[b] (123 ± 20)
	CD49d (α_4)	94 ± 2 (141 ± 15)	98 ± 1 (249 ± 12[b])
	CD49e (α_5)	85 ± 4 (89 ± 8)	83 ± 7 (148 ± 19[b])
	CD49f (α_6)	43 ± 5 (61 ± 6)	9 ± 4[d] (84 ± 8)
β_2	CD11a (α_L)	96 ± 1 (343 ± 34)	99 ± 1 (927 ± 137[b])
	CD11b (α_M)	94 ± 5 (349 ± 43)	97 ± 3 (287 ± 25)
	CD11c (α_x)	85 ± 5 (369 ± 32)	85 ± 3 (341 ± 39)
	CD18 (β_2)	93 ± 2 (257 ± 56)	98 ± 1 (428 ± 49[b])

[a] NK cells were purified by negative selection with magnetic beads from the peripheral blood of normal donors. They were incubated in the presence of IL-2 (300 IU/ml) for 6 days, stained with fluorescein isothiocyanate-conjugated MAbs to the surface antigens listed, and evaluated by flow cytometry. The data were provided by H. Rabinowich, Pittsburgh Cancer Institute.

[b] Significantly increased ($P < 0.002$) compared with fresh (resting) NK cells.

[c] NA, not available.

[d] Significantly decreased ($P < 0.04$) compared with fresh (resting) NK cells.

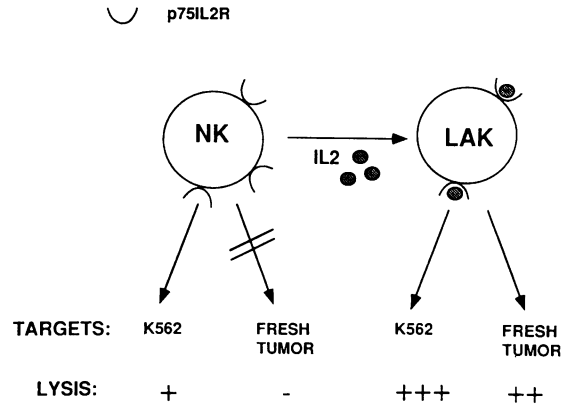

FIG. 1. NK cells obtained from the peripheral blood of normal individuals are able to kill NK-sensitive tumor cell lines such as K562 but generally do not kill freshly isolated tumor cells. NK cells constitutively express IL-2Rβ (p75) and, in the presence of IL-2, develop into lymphokine-activated killer (LAK) cells capable of killing a broad array of fresh or cultured tumor cell targets.

may represent functionally distinct subsets of NK cells (29, 40). Surface receptors on NK cells are up- or downmodulated, depending on the cellular activation state. Among various classes of surface molecules expressed on NK cells, three have been particularly extensively investigated in recent years: receptors for IL-2 (IL-2R), Fc receptors (FcR), and adhesion molecules.

The IL-2R is a complex of at least three distinct polypeptide chains, α (p55), β (p75), and γ (p64), expressed on the surface of lymphoid cells (62). Each of these component chains can bind IL-2 independently of the others, but the interaction of the β plus γ or α plus β plus γ chains leads to the formation of the high-affinity (10^{-9} to 10^{-11} M) receptors. Most resting NK cells (>50%) constitutively express the β (p75) chains of IL-2R, and only a small subset (<10%) of NK cells ($CD56^{bright}$ $CD16^-$) in peripheral blood express functional high-affinity IL-2R (68). Shortly after activation with exogenous IL-2, however, most NK cells rapidly upregulate expression of the β chain and express IL-2Rα de novo (9). In the presence of IL-2, α chains (p55) are rapidly shed from the NK cell surface (soluble IL-2R), so that mostly intermediate-affinity (β) IL-2R are detectable on NK cells exposed to IL-2 in vitro or in vivo in patients receiving IL-2 therapy (68). While IL-2Rβ plays an initial role in the IL-2-induced intracellular signalling pathway(s), IL-2Rγ is essential for IL-2 internalization, IL-2-induced signal transduction, and control of the rate of dissociation of IL-2 from the receptor complex (62, 65).

Among the surface molecules involved in signal transduction on NK cells are the FcR CD16, CD32, and the receptor for IgM (36, 40, 44). The presence on NK cells of the latter two types of FcR has been confirmed recently (36, 44). It is known that CD16 in association with the zeta chain is an essential signal-transducing complex similar to the T-cell receptor-CD3 complex in T cells (4). Upon ligand binding, the CD16-zeta complex induces transcription of genes that encode proteins relevant to NK cell functions (3). The FcR are responsible for antibody-dependent cellular cytotoxicity mediated by NK cells.

A broad range of cellular adhesion molecules (CAMs) are detectable on circulating NK cells (Table 1). Among these CAMs, the β_2 integrins have been shown to be important in signal transduction and activation of NK cells (51), while the B_1 integrins, specifically VLA-4 and VLA-5 (receptors for fibronectin) and VLA-6 (a receptor for laminin), participate in NK cell binding to solid substrates, extracellular matrix (ECM) components, and cell targets (15). NK cells also express adhesion proteins like laminin and fibronectin, and antibodies to these proteins have been reported to inhibit NK cytotoxicity without affecting NK-target cell binding (57). If confirmed, these findings would suggest that antifibronectin MAbs interfere with the lytic process at a postbinding stage (57). Other β_1 integrins are not expressed on fresh NK cells but are induced after cellular activation (Table 1). We have recently demonstrated that the receptor for vitronectin ($\alpha\beta_3$ integrin), which mediates cell adhesion to the ECM protein vitronectin, is present and serves as a signal-transducing molecule on NK cells (48).

Functional characteristics of NK cells. The ability to spontaneously lyse a broad range of virally infected targets or tumor cells is the best-known functional attribute of NK cells. The mechanisms and molecular basis of NK cell target recognition and interactions between NK cells and their targets are still poorly understood. Lysis of targets by NK cells involves several steps occurring in sequence as follows: (i) recognition of target cells by as yet unknown mechanisms; (ii) binding of NK cells to targets (conjugate formation), probably involving various CAMs on both effectors and targets; (iii) NK cell activation, leading to rearrangements of cytoplasmic granules and release of pore-forming enzymes (degranulation); and (iv) injury and lysis of target cells. The NK cell is a selective killer which does not harm normal "self" but eliminates NK-susceptible targets without a need for antigen processing or presentation by major histocompatibility complex (MHC) molecules (63).

The selective target cell repertoire of NK cells is not completely understood. For example, as illustrated in Fig. 1, only activated NK cells (e.g., lymphokine-activated killer cells) kill fresh tumor cells, while the cytotoxic repertoire of resting NK cells is restricted to certain tumor cell lines. It has been thought that NK cell-mediated killing is not MHC restricted, but this concept has been challenged recently (38). Moretta and colleagues have provided evidence that NK cells show alloantigen specificity and that, by using MAbs EB6 and GL183, it is possible to define distinct alloantigen-reactive

clones of NK cells (37). Thus, receptors specific for alloantigens encoded by the HLA-C or a closely linked gene are thought to be present on NK cells (37). The role of the MHC class I complex in the susceptibility of targets to NK-mediated lysis has been controversial. Transfection of MHC tumor cells with the MHC class I gene confers resistance to lysis by NK cells (59), and this resistance has been mapped to the α_1 and α_2 domains of HLA-A2 molecules (60). It thus appears that expression of class I MHC molecules on target cells confers protection against NK cell lysis, while the absence of MHC molecules enhances susceptibility to lysis, as if NK cells were able to sense this absence ("missing-self hypothesis"). The possibility that a negative signal is delivered to NK cells by class I MHC molecules or MHC-associated ligands, inducing protection from NK-mediated lysis, provides an explanation of why NK cells are not harmful to self targets (i.e., normal tissue cells) and is consistent with the presence of alloantigen receptors on NK cells.

While immunosurveillance depends on the ability of the NK cell to recognize and kill its target, other functions of NK cells may be biologically and physiologically even more important. Among lymphocytes, NK cells are the first to respond to activation by IL-2 and possibly other signals. In response to IL-2, a small subset of NK cells (10 to 30%) rapidly acquire the ability to adhere to plastic or other solid surfaces (67) and upregulate surface expression of IL-2R and other activation antigens as well as expression of mRNA for a variety of cytokines (64). We have named these cells A-NK cells, A for the adherence and activation that characterize this early response to IL-2 (67). While all NK cells undergo activation and expansion in the presence of IL-2 and/or other cytokines (IL-4, IL-6, or IL-12), A-NK cells proliferate significantly better than nonadherent NK cells, perhaps because they receive a double stimulatory signal, i.e., IL-2 and adherence to plastic, and achieve very high levels of antitumor activity in vitro (67). The optimal conditions for NK cell proliferation in culture have not been defined so far, and IL-2 and IL-12 (natural killer stimulatory factor), both of which support NK cell growth in vitro, are not sufficient for optimal proliferation of NK cells (52). Apparently, costimulatory signals delivered by, e.g., leukocyte-conditioned medium, irradiated B-lymphoblastoid cell lines, or as yet undiscovered new NK cell growth-promoting factors, are required for optimal NK cell proliferation in culture (52).

Activated NK cells, especially the subset of A-NK cells, become highly mobile and develop membrane structures called podosomes, which facilitate their movement along solid surfaces and binding to tissue cells or ECM (35). Activated NK cells show significantly increased integrin-mediated adhesion to fibronectin- or laminin-coated plates (49) and binding to human umbilical vein-derived endothelial cells cultured on type I collagen (1). Associated with this increased adhesion and mobility of activated NK cells are changes in expression of activation markers and CAM on the cell surface (47, 49), upregulation of mRNA levels for cytokines (64), increased adhesiveness to human umbilical vein-derived endothelial cells and transendothelial migration (74), as well as highly augmented cytotoxicity (67). It has been demonstrated that not only do IL-2- or phorbol ester-activated NK cells adhere better to ECM components or cell surfaces but these changes are associated with changes in the phosphorylation of the α subunits of the β_1 integrins VLA-4, -5, and -6 (25). Signal transduction via integrins on NK cells is not always associated with increased expression of these CAMs on the cell surface; in fact, it may only be associated with conformational changes of CAMs, which are sufficient for induction of an activation signal (25). Alterations in CAMs on activated NK cells are under intense investigation at this time, in order to elucidate the interactions of NK cells with ligands on vascular endothelium, ECM, tumor cells, and other tissue cells. These interactions determine both the entry and effectiveness of NK cells in tissue and for those reasons are of great biologic importance.

TABLE 2. Cytokines, cellular enzymes, and factors known to be produced by activated NK cells

Type	Factors	Examples
Cytokines	Interleukins	IL-1, IL-2, IL-3, IL-4, IL-6
	Hematopoietic cell growth factors	Granulocyte macrophage or macrophage colony-stimulating factors; stem cell factor type 1
	Tumor necrosis factors	TNF-α, TNF-β
	Interferons	IFN-α, IFN-γ
	Transforming growth factor β	
	Natural killer cytotoxic factor	
	Other growth factors	Platelet-derived growth factor
Enzymes	Proteases and peptidases	
	Phospholipases	A2, $C\gamma_1$, $C\gamma_2$, D
	Serine esterases	Granzymes A and B
Other	Perforin	
	Proteoglycans	Chondroitin sulfate A
	Osteopontin (glycoprotein rich in RGDS)	
	Arachidonic acid	
	C-reactive protein	

Activated NK cells produce a spectrum of cytokines (Table 2). This ability of NK cells to produce hematopoietic cell growth factors, interferons, interleukins, tumor necrosis factors α and β, transforming growth factor, and other growth factors (43, 64), coupled with their ability to respond rapidly to exogenous signals by upregulating mRNA production for various cytokines within minutes (67) and increasing migration to tissue sites (50, 74), is responsible for the importance of the NK cell as a mediator or effector of the intercellular communication network. Upon activation, NK cells also express and upregulate the receptors for a variety of chemotactic factors, cytokines, growth factors, and hormones, including neuropeptides (33), which allows them to remain responsive to signals generated not only within the immune system but also elsewhere in the body. As indicated in Table 2, NK cells are also capable of producing many enzymes, some of which are associated with the cellular membrane and are likely involved in cell-to-cell interactions; others, which are intracellular, may be released during NK cell-mediated lysis. One cytokine produced during this process is natural killer cytotoxic factor, which is probably released from intracytoplasmic granules and participates in the lysis of NK cell-sensitive targets (6). Overall, the NK cell has the potential to register changes rapidly and respond spontaneously to signals generated in its immediate environment or at distant locations in the body without a need for antigen presensitization.

The interactions of NK cells with Ig, mediated by the FcR, probably play a crucial role in regulation of NK cell functions. Both stimulatory and inhibitory signals can be received and transduced via the FcγIIIRs (10, 61), depending on whether monomeric, cross-linked, or antigen-complexed IgG molecules are involved, and the intracellular pathways engaged in pro-

cessing and directing these signals are now under intense investigation (56). The possibility that different types of FcR expressed on NK cells cooperate with each other as well as with other receptors on NK cells in the handling of exogenous signals has to be considered one explanation for the cell's ability to channel various stimuli into a response which best reflects the requirements imposed by its external microenvironment. Binding of serum IgM to the FcμR on NK cells, which has been shown to result in downregulation of gamma interferon (IFN-γ) mRNA expression (74), may serve the essential regulatory function of inhibiting the nondiscriminatory capacity of NK cells to kill a variety of cellular targets, including normal hemopoietic and other tissue cells. The ability of NK cells to produce IFN-γ in response to IL-2 or viral challenge (64) is important in host defense against infectious agents. It may be physiologically desirable to downregulate this function in the absence of such agents, and serum IgM may be responsible for this downregulation via the FcμR on NK cells. In the presence of target-bound antibodies, especially those of the IgG3 isotype, expression of FcR on the NK cell surface provides it with an additional opportunity for binding to the target and initiating the lytic process.

The majority of circulating and tissue NK cells in healthy humans are in a resting state, i.e., they are not in cycle or proliferating. However, these resting NK cells are prepared to respond immediately to signals and thus are remarkably well suited to mediate the first line of defense against various pathogens (17, 63). While this swift responsiveness to antigen-independent stimuli is advantageous for the effector cell, it also requires a regulatory "check and balance" system. The functions of NK cells have to be carefully regulated because of the potential for inappropriate cytokine release or damage to normal cells or tissues. Both autocrine and paracrine types of regulatory mechanisms are probably involved, and the functions of NK cells may be controlled at the level of development, differentiation, activation, and availability of NK cells in the microenvironment as well as via the modulatory influences of other mononuclear cells (73). For example, the development of NK cells from lymphoid cell precursors in the bone marrow into NK cell precursors depends on soluble factors produced by the stromal cells in the marrow (17) and various cytokines produced by T or B lymphocytes (32). NK cell maturation from NK cell precursors present in the blood, spleen, and other lymphoid organs is also dependent on a mixture of cytokines derived from T and B lymphocytes, including IL-2, which has been shown to interact with colony-stimulatory factors, a group of hematopoietic growth factors supporting the proliferation and differentiation of precursor cells into mature blood cells (43, 63, 73).

The presence of NK cells at the site of tissue injury is a function of their availability, i.e., the number in the environment and the ability to proliferate and migrate. The latter is clearly determined by cytokines and chemotactic factors produced locally (74). The ability of NK cells to proliferate appears to depend not only on the local concentration of IL-2 but also on the putative NK cell proliferation-inducing factor(s), which is thought to be distinct from IL-12 (natural killer stimulatory factor). The production of proliferation-inducing factor by activated human PBMNC has been inferred from the observation that irradiated concanavalin A-activated PBMNC or lymphoblastoid B cell lines are necessary for NK cell proliferation in culture (52). There is a general agreement that neither recombinant IL-2 nor IL-12 is sufficient to optimally support the proliferation of purified human NK cells in culture (47, 52) and that a unique growth factor may be required for NK cell growth. Finally, evidence has accumulated that monocytes are involved in the regulation of NK activity. Thus, human monocytes maintained in short-term culture have been shown to strongly modulate the NK activity of fresh autologous or allogeneic PBMNC (11). On the other hand, monocytes cultured for 5 to 7 days acquire the morphologic and phenotypic features of macrophages and significantly and consistently upregulate NK activity. This modulation of NK activity was dependent on macrophage viability, cellular integrity, and ability to synthesize RNA and proteins, indicating that a macrophage-derived cytokine might be responsible for NK cell activation (11).

A number of cytokines and various subsets of mononuclear cells as well as other cells present in a particular tissue environment modulate NK functions. In vivo, NK cell development, growth, activation, and proliferation may be orchestrated by events which occur at a particular tissue site. The ability of NK cells to promptly and efficiently respond to such events qualifies them as excellent mediators of regulatory and defense mechanisms.

Biologic role of NK cells. NK cells are involved not only in defense against pathogens and elimination of metastases but in a variety of other biologically significant interactions (53, 63, 73). The antiviral activities of NK cells have been well documented in animal models of viral pathogenesis (69). In humans, changes in systemic NK activity after viral challenge are consistent with the pattern of initial activation of NK cells within the first 2 days and suppression of this activity around day 5 to 7 after infection, followed by a return to the baseline level (58). There is evidence that NK cells participate in eliminating tissue cells infected by mycoplasma or bacteria (13, 73). NK cells also participate in regulatory interactions between immune cells and nonimmune cells. For example, NK cells are capable of directly upregulating polymorphonuclear leukocytes to kill *Candida albicans* (13). NK cells produce neutrophil-activating factors, which allow polymorphonuclear leukocytes to more effectively kill *C. albicans* and possibly other infectious organisms (13).

The involvement of NK cells in the regulation of hematopoiesis has been investigated extensively. Because the NK cell is able to produce a spectrum of cytokines, including colony-stimulating factors, it plays a role in regulation of hematopoietic differentiation (12). NK cells may also play an important role in determining the outcome of bone marrow transplantation and generation of the graft-versus-host disease (39, 76). Although controversy has long existed about the beneficial versus the detrimental effects of human NK cells on bone marrow progenitors, recent studies indicate that the transfer of donor IL-2-activated NK cells enhances engraftment after allogeneic transplantation, possibly because these cells serve as a source of multiple cytokines necessary for immunologic reconstitution (39). On the other hand, it has been generally expected that transferred allogeneic NK cells would not contribute to the graft-versus-host process and, in fact, might ameliorate it via the release of immunosuppressive cytokines (39). However, in a recent report, graft-versus-host disease-like lesions were induced by xenogeneic transplantation of human IL-2-activated NK cells into mice with severe combined immunodeficiency (76). The role of NK cells in graft-versus-host disease remains unclear at present and is under careful scrutiny in both experimental and clinical transplantations.

NK cells are known to be present in the decidua in the first trimester of pregnancy, and although their role in reproduction is not well understood, they may play a trophic and/or regulatory role in the growth of the fetal-placental unit.

Substantial evidence for the involvement of NK cells in interactions of the immune system with the neuroendocrine

axis has accumulated. NK cells express receptors for neuroendocrine hormones as well as neural adhesion molecules on their surface, and it is likely that they play an important role in modulating behavioral changes that accompany stressful life events (30). The current view is that NK cells participate either directly or indirectly in multiple developmental, regulatory, and effector functions of the immune system. In addition, they appear to be responsible for activities at the interphase between the immune system and other systems, e.g., reproductive or neurologic. The biologic role of NK cells is not restricted to immune surveillance against infectious agents or tumor metastasis; rather, it is viewed as a much more broadly based involvement in a variety of essential biologic processes ranging from reproduction to senescence (73).

Measurements of NK cells. In humans, the number of NK cells and NK activity are generally measured in the peripheral blood. The measurement of NK cell number involves staining PBMNC with MAbs to mark NK cells and then determining the percentage of positive cells by flow cytometry (71). The percentage is then converted into the absolute number of NK cells by comparison with the simultaneously obtained differential lymphocyte count. NK activity is measured in a short-term in vitro assay with ^{51}Cr-labeled K562 leukemia cells as targets and PBMNC as effector cells. These assays have, in the past, required isolation of PBMNC from peripheral blood, but today, both can be, and probably should be, performed on unseparated peripheral blood. Whole-blood NK cell assays probably provide a more precise measurement of the effects of various factors present in the blood on NK activity than do conventional assays performed with isolated PBMNC. Both types of NK cell assays have been described previously (14, 70), and they can be reliably performed in a clinical laboratory but only when sufficient quality control measures are taken to establish reproducibility and to minimize daily variability. This becomes particularly important when serial measurements of NK activity are needed, as in monitoring of patients during the course of their disease or during immunotherapy. The criteria for acceptability of the NK cell assay have been reviewed (71).

Although the number and activity of NK cells are generally determined in the peripheral blood, NK cells are widely distributed in human tissues. The spleen, liver, and lungs appear to contain considerable numbers of NK cells (73). In contrast, the lymph nodes contain relatively few NK cells, as determined by flow cytometry with anti-NK cell MAbs, and the NK activity of cells freshly obtained from lymph nodes is generally much lower than that of NK cells in the blood and is often undetectable (73). Little is known about the distribution of NK cells in other human tissues. Tumor-infiltrating lymphocytes from certain tumors, e.g., glioblastoma, renal cell or ovarian carcinoma, and some others, appear to contain more NK cells (10 to 20%) than those from melanoma, for example (unpublished observations). The bone marrow, which is the source of NK precursor cells, contains very few mature NK cells. Cells with NK surface markers have been detected in the human thymus, although NK activity is usually not detectable and their function at this site of T-cell maturation remains unknown (73). All lymphoid tissues and bone marrow appear to contain NK cell precursors, because considerable NK activity can be induced from lymphoid tissue cells after incubation with IL-2. A new MAb, 8A2, which might detect NK cell precursors and thus be useful in localization of these precursors in various human tissues, has recently been described (66). NK activity and the number of NK cells in tissues can be measured, but such measurements require dissociation of these tissues with enzymes and separation of mononuclear cells. Both the percentage of NK cells and level of NK activity appear to be higher in human liver than in peripheral blood, and NK cells isolated from this organ are predominantly in an activated state (19). In contrast, NK cell activity measured in fresh mononuclear cells separated from a variety of human tumors, including those in the liver, is low or undetectable, suggesting that NK cells might be absent or functionally suppressed in the tumor microenvironment (75).

Both the level of NK activity and number of NK cells vary substantially among normal individuals, and normal ranges for both need to be established for every clinical laboratory by testing a large population of normal volunteers. In our laboratory, the normal range of NK activity, defined in terms of 80% middle range, is 55 to 350 $LU_{20}/10^7$ effector cells, based on assays performed with many hundreds of normal donors (unpublished data and reference 71). The percentage of circulating NK cells defined by two-color flow cytometry as $CD3^-$ $CD56^+$ lymphocytes in the circulation of these healthy donors is 12% ± 6% (mean ± standard deviation). Enumeration of NK cells by flow cytometry cannot substitute for the assessment of cytotoxic activity. The correlation between the number of circulating NK cells and NK activity for normal individuals is significant but not particularly strong (71), probably because NK cells may vary considerably in their state of activation. For this reason, assessments of both the number of NK cells and their activity are necessary to adequately evaluate natural immunity or monitor its changes during disease or therapy.

In general, NK activity is a stable trait for a given individual, and normal individuals fall into groups with low, moderate, or high NK activity, based on repeated (at least three) NK activity measurements over time. It thus appears that among normal individuals, it is possible to define low and high responders, and although this distinction is not clearly understood, it might be biologically important because of indications that individuals with persistently low NK activity may be prone to more frequent upper respiratory infections and less able to deal with stressful events (71). Serial measurements of NK activity have not been widely performed in the past because of a requirement for rigorous control of day-to-day reproducibility to ensure that the changes observed reflect true biologic activity and not differences in the assay. Nevertheless, serial measurements of NK activity are necessary to be able to correlate changes in NK activity with, e.g., disease progression or response to treatment. Also, if NK cells are to be examined for their biologic importance in human disease and to determine whether NK activity can be used as a prognostic or even diagnostic parameter for patients with various diseases, longitudinal NK measurements must be reliably performed.

NK cells in disease. The role of NK cells in human disease has been reviewed by us recently (71). Briefly, human diseases with an associated NK abnormality can be categorized into those with low or absent NK activity (i.e., NK cell deficiency) and those in which NK activity appears to be excessive. In either category, abnormalities in NK activity can be transient or persistent. Transient decreases or increases in NK activity relative to the normal baseline level defined for a given individual accompany a variety of events and diseases, e.g., circadian variations, exercise, stressful situations, common colds, and more severe viral infections (71). A normal NK cell response to a viral infection, for example, appears to be a rapid and significant rise which occurs within 24 h of viral challenge, followed by a decrease on days 5 to 7 and a gradual return to the baseline level (58). Frequently, but not always, the number of circulating NK cells parallels changes in NK activity (71). Thus, transient changes from the baseline in NK cell activity appear to be physiologically normal responses to life events. On the other hand, persistently low or high levels of NK

TABLE 3. Examples of abnormalities in NK activity or cell number associated with clinical symptoms or increased disease risk in humans

NK abnormality	Condition	Associated symptoms or risks
Persistently low activity/number	Acquired or congenital immunodeficiencies, including AIDS	Higher incidence of cancer and increased frequency and severity of infections
	Chediak-Higashi syndrome	Increased risk for lymphoma
	Deficiency of the CD11/CD18 CAM family	Increased susceptibility to viral infections
	Cancer	Dissemination of metastases
	Familial cancer	Higher than normal incidence of malignancy
	Leukemia	Precedes relapse
	X-linked lymphoproliferative syndrome	Increased susceptibility to Epstein-Barr virus
	Breast cancer (at diagnosis)	Poor prognosis
	Head and neck cancer (prior to therapy)	Poor prognosis
	Cytoreductive therapy	Higher rates of recurrence
	Viral infections[a]	Higher frequency, severity, and duration
	Other viral/bacterial infections	Higher frequency, severity, and duration
	Autoimmune diseases	Possibly more active disease, increased frequency of infections
	Behavioral disorders	
	Low NK syndrome	Fatigue, dullness, fever
	Chronic fatigue syndrome	Extreme fatigue, listlessness, increased frequency of viral illness
	Depression	More severe symptoms
	Chronic stress	Unable to cope with daily hassles, fatigue
Activity absent[b]		Variable, including severe, disseminated, and life-threatening viral infections; recurrent warts, CIS,[c] and pulmonary abnormalities; erythrocyte aplasia
Persistently high activity/number	NK cell lymphoproliferation	
	Chronic LGL proliferation	LGL lymphocytosis, cytopenia, splenomegaly
	Acute LGL proliferation	Consistent with aggressive leukemia/lymphoma
	Hepatic disease	Not known

[a] e.g., cytomegalovirus, Epstein-Barr virus, herpesvirus.
[b] Rare; only three cases described.
[c] CIS, carcinoma in situ.

activity are likely to be associated with disease. NK activity appears to be a more sensitive marker of disease progression than the absolute number of NK cells.

A considerable amount of evidence has accumulated to substantiate the involvement of the NK cell in many human diseases (53, 71). Table 3 is a list of human diseases which have been associated with persistent alterations in NK activity and/or NK cell number. It is essential to realize that while the association between low or high NK activity and disease has been convincingly established in many cases, for the most part, such an association does not imply that the NK cell abnormality is related to the pathogenesis. It is likely that abnormally high or low NK activity is a result of disease rather than its cause in many cases and that it may be an epiphenomenon not at all related to the disease process itself. On the other hand, it is necessary to recognize that, in some instances, experimental evidence has been obtained for a direct and, perhaps, causal relationship between low NK activity and disease. For example, mice depleted of NK cells by treatment with the anti-NK cell antibody anti-asialo GM_1 after surgical removal of a tumor developed significantly and overwhelmingly more lung metastases than control animals which were not treated with the antibody and had a normal number of NK cells (16). The susceptibility of mice to cytomegalovirus infection increases significantly in the absence of NK cells, and resistance to cytomegalovirus can be restored with adoptively transferred NK cells (7). In humans, a positive correlation has been observed between sensitivity to infections and depressed NK activity (2). Also, a patient whose persistently low or absent NK activity was accompanied by insidious, severe, and frequent viral infections has been described (5). However, to be able to causally associate the deficit in NK activity with the disease pathogenesis, it will be necessary to demonstrate not only that the deficit precedes disease onset and that the degree of deficit can be linked to disease severity, but that correction of the deficit leads to amelioration of symptoms or recovery. Thus, if therapeutic transfers of NK cells to animals bearing established tumor metastases could be demonstrated to result in regression of these metastases and prolonged survival, then a strong argument could be made for the role of NK cells in the control of metastatic disease. Studies ongoing in our laboratory indicate that therapeutic intrasplenic transfers of IL-2-activated NK cells and IL-2 to nude mice bearing established hepatic metastases of human gastric carcinoma indeed result in nearly complete elimination of liver metastases and in significantly prolonged survival of the animals (unpublished data).

As shown in Table 3, chronically low levels of NK activity occur not only in cancer, particularly when there are large tumor burdens or disseminated metastases, but also in a variety of immunodeficiency syndromes, severe, life-threatening viral infections, autoimmune diseases, and behavioral disorders (71). Chronically high levels of NK activity are associated with lymphoproliferative syndromes, including aggressive LGL leukemia (26), and in some hepatic disorders (18). In addition,

low NK activity may not only be a risk factor for malignancy but have a prognostic significance in predicting relapse, response to treatment, and survival time free of metastasis in patients with cancer (71). In general, patients with low NK activity appear to be at higher risk of infections, to have more prolonged diseases, or to suffer more severe symptoms than those whose NK activity remains normal. On the other hand, excessive levels of NK activity are relatively rare, and the biologic significance of chronically high NK activity is not clear at this time.

NK cells in therapy. From the evidence reviewed above that chronically low levels of NK activity or number in patients with cancer or other diseases may be associated with more severe symptoms or increased risk of disease progression, augmentation of NK activity in disease may be of benefit to the patient. Therapy aimed at augmenting NK activity could be particularly advantageous for patients with cancer or immunodeficiencies. Such therapy is available today, and it generally consists of attempts to increase NK activity in vivo by the administration of agents with known NK-potentiating activity or adoptive transfer of activated autologous NK cells either locally or systemically to patients deficient in NK activity.

A variety of agents, generally referred to as biologic response modifiers (BRMs), are known to increase the activation, proliferation, or cytotoxicity of existing NK cells; other BRMs promote NK cell extravasation and accumulation in tissues, e.g., lung and liver, resulting in higher local cytotoxic activity. A partial list of commonly used BRMs includes a spectrum of cytokines such as interferons, IL-2, and IL-12; bacterial products such as OK432 (picibanil); plant lectins such as lentinan; MAbs that bind to triggering structures on NK cells (e.g., anti-CD16 MAb); and interferon inducers such as polyribonucleotides. The effects of BRMs, especially cytokines, on signal transduction in NK cells, their mobility in tissues or ECM, and their ability to lyse tumor cells or virally infected targets are under intense investigation at this time.

Among the best-known activators of NK cells are IL-2 and IFN-α (20, 21). These two cytokines have been used extensively not only for patients with malignancies but also for those with viral infections. For example, IFN-α has been therapeutically effective in a proportion of patients with chronic hepatitis B infection (24), and patients with human immunodeficiency virus have received systemic IL-2 therapy in combination with zidovudine (34). For patients with advanced malignancies unresponsive to other treatments, both IL-2 and IFN-α therapies have resulted in durable responses (27, 55). Systemic or locoregional therapy with IL-2, even at low or moderate doses, leads to well-documented increases in the number of circulating NK cells and NK activity (8). Although the extent of this IL-2-induced stimulation of NK activity in vivo could not be directly linked to clinical response, it may be at least partly responsible for the antitumor effectiveness of cytokine-based immunotherapy.

Immunotherapy with adoptively transferred activated NK cells and cytokines has been used mainly for patients with advanced malignancies in the hope of capitalizing on the well-documented antimetastatic activity of NK cells (23, 72). The earliest clinical trials were performed with lymphokine-activated killer cells and high-dose IL-2 in patients with metastatic melanoma or renal cell carcinoma (54). The results of these trials have been only mildly encouraging in that the rate of objective clinical responses achieved was about 20 to 30% (54). However, it is important to note that these responses, some of long duration (>2 years), were achieved in patients with metastatic disease unresponsive to conventional therapies. Lymphokine-activated killer cells are obtained by incubating PBMNC in the presence of 6,000 IU of IL-2 per ml and generally contain a substantial proportion of activated NK cells in addition to activated T lymphocytes (42). More recently, clinical trials with purified NK cells, selected and expanded in vitro from patients' PBMNC, have been done on the hypothesis that transfer of a selected subset of highly activated antitumor effector cells may be therapeutically more effective and require fewer cells and possibly lower doses of cytokines to support their viability and in vivo activity. The results of two preliminary phase I trials with patients with metastatic melanoma or renal cell carcinoma demonstrated that immunotherapy with selected, highly purified, and in vitro-expanded subsets of NK cells is feasible, well tolerated, and, in some cases, effective for metastasized disease (23, 72). Since the mechanism of the antitumor activity of systemically transferred, in vitro-activated NK cells is not known and may depend on the ability of these effector cells to reach or localize to tumor metastases, locoregional delivery of purified activated NK cells together with IL-2 to the liver via the hepatic artery is currently being evaluated at the Pittsburgh Cancer Institute for patients with colon cancer metastatic to the liver.

Increasingly often, bone marrow transplantation or peripheral stem cell transfer has been used for the treatment of malignancies. The transfer of activated NK cells after transplantation is based on the rationale that NK cells have potent antitumor effects and thus can help eliminate minimal residual disease. Purified and activated human NK cells are being used for the therapy of patients with cancer after high-dose chemotherapy and peripheral blood stem cell transplantation (31). Preliminary results from clinical trials evaluating this therapy suggest that systemic transfer of A-NK cells plus IL-2 within 2 to 3 days after peripheral blood stem cell transplantation may not only help eliminate minimal residual disease but facilitate hematopoietic recovery (31).

While both in vivo augmentation of NK activity with BRMs and adoptive transfer of activated NK cells are promising new therapies, additional clinical and basic studies are needed to build on the progress achieved to date and to acquire a better understanding of the interactions between activated NK cells and their targets in vivo.

Summary. The NK cell plays a major role in human health and disease. Although persistent abnormalities in NK cell activity or number appear to be associated with a wide spectrum of human diseases, evidence for the causal association of abnormally low NK activity with pathogenesis is so far available only from a limited number of experimental models. For diseases characterized by NK cell deficiency, therapy with adoptively transferred purified and in vitro-activated subsets of NK cells or with BRMs, which can restore or augment NK activity in vivo, offers an opportunity to demonstrate that the NK cell contributes to immunopathogenesis. Future research and clinical efforts should be directed at achieving a better understanding of the interactions between the NK cell and its various targets, dissecting the functional repertoire of the NK cell, improving the monitoring of various NK cells in tissues, organs as well as the peripheral blood, and devising clinical trials in which hypotheses relevant to the role of the NK cell in various human diseases can be reliably evaluated.

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