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REVIEW ARTICLE

Biology and Clinical Relevance of Human Natural Killer Cells

By Michael J. Robertson and Jerome Ritz

NATURAL KILLER (NK) cells comprise 10% to 15% of human peripheral blood lymphocytes (PBL) and most have the morphology of large granular lymphocytes (LGL). NK cells are defined functionally by their ability to lyse target cells without deliberate prior sensitization and without restriction by major histocompatibility (MHC) antigens. NK activity was first described in the 1970s, when it was observed that lymphocytes freshly isolated from normal unimmunized hosts could lyse allogeneic tumor cell lines. Subsequent studies showed that such NK activity was mediated by "null cells," lymphocytes devoid of characteristic T-cell and B-cell surface antigens. Use of monoclonal antibodies (MoAbs) and techniques of molecular biology have allowed the unequivocal identification of NK cells as a discrete population of cytolytic effectors (Table 1). NK cells have been implicated in several activities in vivo, including destruction of tumor cells, resistance to viral infections, and regulation of hematopoiesis. Although NK activity was originally described in the mouse and in humans, effectors resembling murine and human NK cells have been isolated from many vertebrates, including monkeys, rats, hamsters, cats, dogs, birds, and fish. Furthermore, NK-like effectors have been described in animals as primitive as starfish and earthworms. Because the latter do not possess cells analogous to vertebrate T and B lymphocytes, the development of NK-like activity probably preceded evolution of the adaptive immune system. Although NK cells can be isolated from spleen, bone marrow, liver, lung, and intestine, the present review will focus on peripheral blood NK cells. A summary of all aspects of NK cell biology is beyond the scope of this article; other recent reviews discuss areas not considered here.

SURFACE ANTIGENS OF HUMAN NK CELLS

NK cells can be partially purified by discontinuous Percoll density gradient centrifugation. However, only 60% to 80% of Percoll-enriched large granular lymphocytes (LGL) exhibit NK activity or characteristic NK cell surface antigens, and some NK cells do not demonstrate typical LGL morphology. Obtaining highly purified populations of NK cells has generally required positive selection by flow cytometry or immune rosette techniques, or negative selection by elimination of other lymphocyte subsets using complement lysis or immunomagnetic beads. In the quest for "NK-specific" antigens, the reactivity of many monoclonal antibodies (MoAbs) with NK cells has been determined. No single surface antigen described to date unambiguously identifies all human NK cells. The antigens used most extensively as NK cell "markers" for clinical and basic research purposes are CD56 (NKH-1, Leu-19) and CD16 (Fcy R III). The CD56 antigen is expressed by virtually all human peripheral blood cells capable of non-MHC-restricted cytotoxicity. It appears to be identical to the neural cell adhesion molecule (NCAM), a well-characterized structure mediating homotypic adhesive interactions between neural and muscle cells. NCAM has at least three isoforms, generated by differential splicing of the RNA transcript from a single gene located on human chromosome 11. The core polypeptide of the CD56 antigen appears to be the 140 Kd isoform of NCAM, which is variably glycosylated and sialylated to produce mature species with molecular weights ranging from 175 to 220 Kd. The CD56 antigen itself appears not to participate directly in NK killing of most target cells, although a recent report suggests that CD56 can mediate homotypic adhesion of NK cells to CD56+ tumor cell lines.

A small fraction (<5%) of normal peripheral blood lymphocytes (PBL) expresses both CD56 and CD3 and is capable of non-MHC-restricted cytotoxicity. CD3 is an invariant complex associated with the T-cell receptor (TCR); CD3 is not present on NK cells, which do not rearrange TCR genes nor express T-cell receptor heterodimers. Some investigators have proposed the term "non-MHC-restricted T cells" be used to describe these CD56+ CD3+ lymphocytes. Furthermore, CD3+ human thymocytes cultured in interleukin 2 (IL-2) can be induced to express CD56 in parallel with their acquisition of NK activity. Although CD56 expres-
cells in an MHC-unrestricted fashion, not all CD56+ cells express CD56.\(^7,24\)

### Table 2

**Surface Antigens of Human Natural Killer Cells**

<table>
<thead>
<tr>
<th>CD Cluster</th>
<th>Antigen</th>
<th>% of NK Cells Expressing Ag*</th>
<th>Other Cell Types Expressing Ag</th>
<th>Typical Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>SRBC receptor</td>
<td>~70-90</td>
<td>T cells, thymocytes</td>
<td>T11, Leu-5b, 9,6</td>
</tr>
<tr>
<td>CD7</td>
<td>p40</td>
<td>80-90</td>
<td>T cells, thymocytes</td>
<td>3A1, Leu-9, G3-7</td>
</tr>
<tr>
<td>CD8</td>
<td>MHC class I ligand</td>
<td>30-40</td>
<td>CTL, suppressor T cells</td>
<td>T8, Leu-2</td>
</tr>
<tr>
<td>CD11a</td>
<td>LFA-1</td>
<td>&gt;95</td>
<td>All leukocytes</td>
<td>2F12, MHM24</td>
</tr>
<tr>
<td>CD11b</td>
<td>C3bi receptor (CR3)</td>
<td>80-90</td>
<td>Monocytes, PMN</td>
<td>Mo-1, OKM1, Leu-15</td>
</tr>
<tr>
<td>CD11c</td>
<td>p150</td>
<td>~30-60</td>
<td>Macrophages, PMN</td>
<td>Leu-M5</td>
</tr>
<tr>
<td>CD16</td>
<td>Fc (\gamma) receptor III</td>
<td>80-90</td>
<td>Macrophages, PMN</td>
<td>G8, B73.1, Leu-11</td>
</tr>
<tr>
<td>CD18</td>
<td>(\beta) chain of CD11a-c</td>
<td>&gt;95</td>
<td>All leukocytes</td>
<td>10F12, MHM23</td>
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<tr>
<td>CD38</td>
<td>p45</td>
<td>~60-80</td>
<td>Activated T, plasma cells</td>
<td>T10, H97, Leu-17</td>
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<tr>
<td>CD54</td>
<td>ICAM-1</td>
<td>~30-60</td>
<td>Many activated cells</td>
<td>RR1/1, OKT27</td>
</tr>
<tr>
<td>CD56</td>
<td>NKH-1 (NCAM)</td>
<td>&gt;95</td>
<td>T cell subset</td>
<td>NKH-1, Leu-19</td>
</tr>
<tr>
<td>CD57</td>
<td>HNK-1</td>
<td>50-60</td>
<td>Many cells</td>
<td>L186, Leu-7</td>
</tr>
<tr>
<td>CD58</td>
<td>LFA-3</td>
<td>85-95</td>
<td>Many cells</td>
<td>TS2/9, BRIC5</td>
</tr>
</tbody>
</table>

**Table 1. Characteristics of NK Cells and Other Cytolytic Effectors**

<table>
<thead>
<tr>
<th>Surface Antigen</th>
<th>NK Cells</th>
<th>T Cells</th>
<th>Monocytes</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>70-90</td>
<td>&gt;95</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>CD3</td>
<td>0</td>
<td>&gt;95</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD8</td>
<td>30-40</td>
<td>30-40</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>CD11b</td>
<td>80-90</td>
<td>10-15</td>
<td>&gt;90</td>
<td>&gt;90</td>
</tr>
<tr>
<td>CD15</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>60-80</td>
<td>&gt;95</td>
</tr>
<tr>
<td>CD16</td>
<td>80-90</td>
<td>&lt;5</td>
<td>10-15(\S)</td>
<td>&gt;95</td>
</tr>
<tr>
<td>CD56</td>
<td>&gt;95</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

**Functional attributes**
- ADCC: Yes No Yes Yes
- NK activity: Yes No No No
- Phagocytosis: No No Yes Yes
- Immunologic memory: No Yes No No
- Proliferative capability: Yes Yes No No

*Approximate % of cells from each lineage that express antigen; data summarized from references 4, 5, 18, 29, 31, 36, 48, 58-61, 78, and 232.

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derived by techniques similar to those used to clone T lymphocytes.\textsuperscript{41-43} NK clones were initially defined solely by their cytolytic activity against irrelevant allogeneic targets, and a number of cell lines originally described as NK clones have actually proved to be of T-cell lineage.\textsuperscript{41-44} Some of these “NK-like” T-cell clones are probably derived from the rare peripheral blood T cells that express CD56 and exhibit spontaneous non-MHC-restricted cytotoxicity.\textsuperscript{17,24} As with T cells in general, most of these CD56+ T cells express typical \(\alpha/\beta\) T-cell receptors. Furthermore, CTL clones with NK activity can arise from the small subset of T cells that have \(\gamma/\delta\) T-cell receptors.\textsuperscript{45,46} Although most freshly isolated peripheral blood \(\gamma/\delta\) T cells do not express CD56 or exhibit NK activity, the majority can be induced to kill without MHC restriction by short-term culture in IL-2 alone.\textsuperscript{47}

Although T-cell clones with NK activity are interesting in their own right, the term NK clone should probably be reserved for cell lines lacking CD3 and T-cell receptors; a germline configuration of TCR genes has been demonstrated for some NK clones as well.\textsuperscript{25} CD56 expression by clonal cell lines does not necessarily indicate NK cell lineage, because antigen-specific T-cell clones can acquire CD56 after in vitro culture.\textsuperscript{48}

Prolonged propagation of NK clones has been difficult, although selected clones have been grown for several years by frequent subcloning with irradiated feeder cells; these NK clones have demonstrated a stable surface antigen phenotype and cytolytic activity.\textsuperscript{4,41,43} Clonal NK cell lines have proved invaluable for studies of NK cell antigen expression, cytolytic activity, and cytokine production. However, such clones represent relatively activated NK effectors, and single clones do not reflect the known heterogeneity of polyclonal NK cell populations.\textsuperscript{4} Optimally, studies using polyclonal NK cells should complement those performed on NK clones.

**ORIGIN AND DIFFERENTIATION OF NK CELLS**

Considerable data indicate that NK cells are derived from bone marrow precursors. Marrow grafts can reconstitute NK cells in lethally irradiated laboratory animals; the regenerating NK cells have been shown to be of donor origin.\textsuperscript{233-235} Engraftment of NK cells has also been demonstrated in patients undergoing allogeneic marrow transplantation for hematologic malignancies.\textsuperscript{182-184} Furthermore, lymphocytes with an NK surface phenotype and typical NK activity can be generated in vitro from bone marrow precursor cells.\textsuperscript{110,236,237} Several cytokines appear to regulate the differentiation of mature NK cells in such in vitro cultures.\textsuperscript{110,237} The precise relationship of NK cells to other hematopoietic cell types remains controversial. Detailed characterization of leukocyte differentiation antigens has not permitted an unambiguous attribution of lineage because NK cells share surface antigens with both T cells and myeloid cells (Tables 1 and 2). Marrow grafting experiments in mice have shown that NK cell precursors can be transplanted from mutant strains lacking T- and B-cell progenitors\textsuperscript{235} or myeloid progenitors.\textsuperscript{24} Thus murine NK cells probably constitute a third lymphoid lineage comparable to T cells and B cells.

It is not certain whether NK cells differentiate entirely in the bone marrow, but most data suggest that thymic processing is not required.\textsuperscript{233-237,250} After being released from the marrow, most NK cells appear to circulate in peripheral blood or migrate to the spleen; very few are detectable in the thymus or lymph nodes of healthy persons.\textsuperscript{5,14,29} Factors influencing NK cell migration and localization are poorly understood. Furthermore, the lifespan of NK cells has not been well-characterized; lifespans ranging from a few days to several months have been reported for laboratory animals.\textsuperscript{5,250} Like other lymphocytes, but unlike fully differentiated myeloid cells, at least some mature peripheral blood NK cells retain proliferative capability.\textsuperscript{105,106}

**CYTOLYTIC ACTIVITY OF NK CELLS**

For several years NK cells could be defined only functionally as lymphocytes capable of mediating the spontaneous killing of target cells. Such NK activity is characterized by several features that clearly differentiate it from the typical cytolytic activity of T cells: 1) it occurs within hours of target cell exposure without deliberate prior immunization; 2) it does not require target cell expression of appropriate self-MHC antigens; 3) it does not appear to involve a clonally distributed specificity.\textsuperscript{4,5,10} NK activity is usually determined by measuring the release of radiolabeled chromium from target cells that have been exposed to effector cells.\textsuperscript{50} Tumor cell lines such as K562 (derived from a patient with chronic myelogenous leukemia in blast phase), Molt-4 (derived from a patient with acute lymphoblastic leukemia), or Daudi (derived from a patient with Burkitt’s lymphoma) are commonly used as targets in such assays; virus-infected cells or fresh tumor cells may also be used. Because it is very sensitive to NK cytolysis and does not express MHC antigens, K562 is frequently the target cell of choice for standard NK activity assays.\textsuperscript{50} Although cell lines are often described as “NK-sensitive” or “NK-resistant,” these should be considered relative terms; sensitivity to NK killing represents a spectrum and varies depending on experimental conditions (presence of stimuli, effector-to-target ratio, duration of the assay, etc).

Lack of restriction by syngeneic MHC antigens is one of the defining characteristics of NK cytotoxicity. Indeed, sensitivity to NK killing appears to be inversely related to target cell expression of MHC glycoproteins.\textsuperscript{51} Mutant cell lines with diminished surface MHC expression are more sensitive to NK cytolysis than are their parental cell lines\textsuperscript{51,52}; NK resistance can be restored by transfection of normal MHC or \(\beta_2\) microglobulin genes.\textsuperscript{53,54} Furthermore, “masking” of target cell MHC antigens using MoAbs increases target cell susceptibility to NK effectors.\textsuperscript{55} It has been suggested that MHC glycoproteins might inhibit the recognition of other surface antigens by NK cells or could interact with NK surface structures that transmit a negative signal for cytotoxicity.\textsuperscript{51,52}

NK cells are capable of dual cytolytic activity; in addition to spontaneous antibody-independent non-MHC-restricted cytotoxicity (NK activity), they can mediate antibody-dependent cellular cytotoxicity (ADCC).\textsuperscript{5,56,57} In ADCC, antibody-coated target cells are lysed by effector cells bearing Fc receptors for immunoglobulin; effector cells expressing Fc receptors and capable of ADCC include NK cells,
monocytes, and granulocytes. Because the “ADCC receptor” of NK cells is well-characterized, it will be considered first, followed by a discussion of the more elusive “NK receptor.”

**Antibody-dependent cellular cytotoxicity.** The CD16 antigen (Fcγ R III) is a 50 to 70 Kd heavily glycosylated polypeptide expressed by 80% to 90% of NK cells, essentially all neutrophils, some eosinophils, a small fraction of resting peripheral blood monocytes and T cells, and most activated monocytes and tissue macrophages. CD16 binds the Fc region of human IgG1 and IgG3 with low affinity (Kₜ ≈ 5 × 10⁵ M⁻¹); it does not bind IgG2 or IgG4 detectably. Of interest, the CD16 present on granulocytes is biochemically and serologically distinct from that of NK cells. CD16 is attached to the membrane of neutrophils via a phosphatidylinositol glycan moiety, whereas the CD16 of NK cells is an integral transmembrane protein. These extremely homologous molecules are encoded by two separate but tightly linked genes on human chromosome 1. The longer cytoplasmic domain of NK cell CD16 may participate in NK cell triggering during ADCC. Such triggering may also involve the ζ chain, a 32 Kd homodimer implicated in activation through the T-cell receptor. Although they express neither CD3 nor T-cell receptors, most NK cells do express the “TCR” ζ chain. The ζ chain has been shown to associate with CD16 on NK cells, and activation through CD16 results in tyrosine phosphorylation of ζ. Crosslinking of NK surface CD16 induces a rapid rise in cytosolic free calcium ion concentration and the production of inositol-1,4,5 trisphosphate, both important biochemical messengers during lymphocyte activation. Triggering of NK cells through CD16 ultimately results in the expression of activation antigens and the secretion of cytokines. CD16 appears to be solely responsible for the ADCC of NK cells, because CD16 blocking with immune complexes or CD16 modulation with MoAbs or phorbol esters abrogate this cytolytic activity.

**Spontaneous non-MHC-restricted cytotoxicity and the NK receptor.** Activation through CD16 can thus trigger NK cells to kill antibody-coated targets. Despite intensive investigation, the receptors mediating antibody-independent NK activity remain elusive. Conjugate formation between NK cells and target cells is necessary but not sufficient for NK activity; it is largely mediated by the binding of NK cell surface LFA-1 (CD11a/CD18) and CD2 to their target cell ligands, ICAM-1 (CD54) and LFA-3 (CD58) respectively. ICAM-1 on NK cells may also be involved in adhesion to target cells. Activation of T cells through CD2 involves phosphorylation of the TCR ζ chain, and ζ may also participate in the triggering of NK cells through CD2 or other NK cell surface structures. Indeed, the NK ζ chain is associated with proteins other than CD16, and these are being evaluated as possible NK receptors.

Other cell adhesion molecules might also subserve receptor-like functions under certain circumstances. We have recently characterized the expression of several adhesion molecules by NK cells and have demonstrated that NK cell target specificity can be regulated by changes in adhesion molecule expression. Others have shown that laminin-like molecules are expressed by both human and murine NK cells. Heteroantisera or MoAbs to these molecules inhibit the cytolytic activity of both resting and stimulated NK effectors, possibly by inhibiting both adhesion and triggering. One group has developed MoAbs that recognize surface structures common to human, rat, and catfish NK effectors and also MoAbs to target ligands present on human and murine NK-sensitive cell lines as well as fish parasites. Because all MoAbs that blocked NK cytolytic activity did so by inhibiting conjugate formation, these MoAbs would appear to define adhesion molecules involved in NK cytotoxicity that have been highly conserved during evolution. However, it remains to be demonstrated that any known NK surface structure can trigger NK activity after binding its physiologic target cell ligand.

**Terminal cytolytic effector mechanisms.** Although the receptors for NK activity and ADCC are probably distinct, these two cytolytic pathways would appear to involve common terminal effector mechanisms. Commensurate with their LGL morphology, resting NK cells have preformed cytolytic granules that can be discharged immediately after exposure to sensitive target cells. Granule exocytosis releases perforins, serine esterases, and chondroitin sulfate proteoglycans into areas bounded by the close apposition of effector cell and target cell plasma membranes. Perforins (also called cytolyins or pore-forming proteins) are inserted into the target cell membrane as monomers, subsequently polymerizing to form cylindrical transmembrane pores that permit osmotic lysis of the target cell. Membrane lesions produced by NK cells and CTL appear to be identical, and closely resemble those produced by the membrane attack complex of complement. Perforin-mediated membrane damage is not the sole mechanism of NK cytosis, however. NK cells also secrete soluble toxins, collectively called NK cell cytotoxic factors or NKCF (Table 3). Tumor necrosis factor (TNF-α) is a major mediator of the soluble lytic activity of NK cells, but other proteins are probably involved as well. TNF-α appears to activate a target cell endonuclease that degrades genomic DNA into 180 to 200 base pair fragments. This process, called apoptosis or programmed cell death, involves the active participation of TNF-sensitive target cells, unlike the osmotic lysis caused by perforins or complement. CTL can also induce such target cell “suicide,” possibly through secretion of TNF-α or TNF-β.

The function of the serine esterases and sulfated proteoglycans found in the granules of NK cells remains unclear. The serine esterases could be involved in the activation of perforins or other lytic effector molecules, and at least one esterase (granzyme A) has been implicated in target cell
NATURAL KILLER CELLS

Table 3. Cytokine Production by NK Cells

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Resting NK Cells</th>
<th>Activated NK Cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>0</td>
<td>0</td>
<td>92, 93, 247, 248</td>
</tr>
<tr>
<td>IL-2</td>
<td>0</td>
<td>0</td>
<td>5, 94</td>
</tr>
<tr>
<td>IL-3</td>
<td>0</td>
<td>+</td>
<td>92</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>?</td>
<td>+</td>
<td>92, 95</td>
</tr>
<tr>
<td>G-CSF</td>
<td>0</td>
<td>+</td>
<td>92</td>
</tr>
<tr>
<td>M-CSF</td>
<td>0</td>
<td>+</td>
<td>92</td>
</tr>
<tr>
<td>IFN-α or β</td>
<td>0</td>
<td>?</td>
<td>96, 97</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0</td>
<td>+</td>
<td>72, 73, 92, 96</td>
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<tr>
<td>TNF-α</td>
<td>0</td>
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<td>72, 73, 92, 96</td>
</tr>
<tr>
<td>TNF-β</td>
<td>0</td>
<td>+</td>
<td>96</td>
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</tbody>
</table>

Various stimuli were used to activate NK cells in vitro, including IL-2, anti-CD16 MoAb, combinations of calcium ionophore and phorbol ester, and exposure to target cells. Negative results do not necessarily prove that NK cells cannot produce a given cytokine, since other stimuli might be able to induce production.

Abbreviations: IL, interleukin; CSF, colony stimulating factor (G, granulocyte and M, monocyte); IFN, interferon; TNF, tumor necrosis factor; 0, none detected; +, detected; ?+, conflicting results reported.

*The preponderance of the data suggests that CD3 + LGL, but not CD3 - NK cells, can produce IL-1.

DNA fragmentation induced by CTL. It has been suggested that the proteoglycans bind other granular components or help protect the effector cell from its own lytic factors. Further work is needed to test these hypotheses.

REGULATION OF NK CELLS: THE CENTRAL ROLE OF IL-2

Several cytokines have been shown to affect NK cell proliferation or cytotoxic activity (Table 4). Of these, the interferons (IFN) and IL-2 have been the most extensively studied. The interferons were initially identified as proteins, secreted by virus-infected cells, that inhibited viral replication. The biochemistry, serology, and genetics of these important immunomodulatory cytokines have subsequently been well-characterized. Interferons α and β comprise at least 15 related proteins that appear to interact with the same cell surface receptor; IFN-γ is a structurally distinct protein that binds to a different receptor. All three types of interferon can augment the cytolytic activity of NK cells, although the potency of this effect differs among the various molecular species that have been tested. In general, the α and β interferons stimulate NK activity to a greater degree than does IFN-γ. Enhanced NK cytolytic activity is evident within 4 to 6 hours of IFN exposure, requires RNA but not DNA synthesis, and is transient. Although early studies suggested that IFN could augment only the killing of targets already sensitive to NK activity, subsequent work demonstrated that IFN could induce the lysis of cells relatively resistant to unstimulated NK cells. Interferon by itself does not cause NK cells to proliferate; indeed, they appear to inhibit the NK proliferation induced by IL-2. On the other hand, IFN and IL-2 can synergistically augment NK cytotoxicity.

Shortly after IL-2 was isolated and characterized, it was shown that peripheral blood NK cells could proliferate and exhibit enhanced cytolytic activity in response to IL-2 alone. The past few years have seen a remarkable increase in our understanding of the IL-2 receptor and the role of IL-2 in the biology of NK cells. The IL-2 receptor is now known to consist of at least two subunits. The low affinity receptor or CD25 (Tac antigen, IL-2Rp55) is a polypeptide of ~55 Kd that binds IL-2 with an equilibrium dissociation constant (Kd) of 1.7 × 10^−8 mol/L, whereas the intermediate affinity receptor (IL-2Rp75) has a M, of 70 to 75 Kd and a Kd of 1.0 × 10^−9 mol/L. IL-2Rp75 has a relatively large cytoplasmic tail and can transduce functional signals in the absence of the p55 chain; IL-2Rp55 has a short cytoplasmic segment and has not been shown to transmit signals alone. Expressed together on the cell surface, the p55 and p75 chains can associate to form a heterodimer with high affinity for soluble IL-2 (Kd, 2.6 × 10^−11 mol/L).

The majority of peripheral blood NK cells constitutively express only the intermediate affinity IL-2 receptor, and relatively high concentrations of IL-2 (~1 nmol/L) are required to stimulate these lymphocytes. However, the small subset of CD56−CD16− NK cells constitutively expresses high affinity IL-2 receptors as well as an excess of free IL-2Rp75 chains. These NK cells respond...
to 10- to 100-fold lower IL-2 doses than those needed to stimulate CD56<sup>dim</supificate+ CD16<sup>+</sup> NK cells. NK cells demonstrate enhanced target cell killing within 4 to 6 hours of IL-2 exposure, but optimal stimulation of cytolytic activity requires the presence of IL-2 for at least 18 to 24 hours. Antibodies to the IL-2Rp55 chain that disrupt high affinity IL-2 receptor<sup>114</sup> do not inhibit the augmentation of NK cytotoxicity by IL-2; thus the activation of NK cytolytic activity appears to be mediated by the intermediate affinity IL-2Rp75. By contrast, NK cell proliferation may require expression of the high affinity IL-2 receptor. Continuous exposure to IL-2 for 3 to 4 days is generally required to induce NK cell synthesis of IL-2Rp55.<sup>115,116</sup> Furthermore, IL-2 has been shown to induce NK cell synthesis of IL-2Rp55.<sup>115,116</sup> Thus it is possible that NK cell proliferation in response to IL-2 occurs predominantly through high affinity IL-2 receptors formed by the association of newly synthesized IL-2Rp55 with the IL-2Rp75 that is constitutively expressed by NK cells. Alternatively, additional factors may be required to induce proliferation of NK cells expressing only IL-2Rp75.

The intracellular biochemical events induced by binding of IL-2 to its cell surface receptor are essentially unknown. IL-2 stimulation of T and B lymphocytes does not appear to involve the calcium-dependent phosphoinositol and protein kinase C pathways<sup>3,13</sup> or the participation of cyclic AMP or GMP as second messengers. After IL-2 exposure, activated T cells exhibit rapid phosphorylation of several cellular proteins, including tyrosine phosphorylation of the IL-2Rp75 chain. However, it is not clear that such phosphorylation accompanies signal transduction through the intermediate affinity receptor expressed by freshly isolated NK cells. Synthesis of c-myc mRNA can be detected in NK cells within two hours of IL-2 exposure. Although the product of this cellular protooncogene appears to be involved in IL-2-induced lymphocyte proliferation, its possible role in regulating NK cell cytolytic function has yet to be fully defined.

The effects of IL-2 that ultimately produce augmented cytolytic activity are also poorly understood. Initial reports<sup>15,16</sup> that IL-2 exerted its effects on NK cells by stimulating autocrine secretion of IFN-γ have proved erroneous. IL-2 induces increased expression of some NK cell surface adhesion molecules, apparently mediating in part the induction of cytotoxicity for NK-resistant targets. An increase in the number and size of NK cell cytolytic granules has also been demonstrated following IL-2 activation. Furthermore, IL-2 stimulates NK cell expression of mRNA for some serine proteases. Because all of these changes require several days of IL-2 exposure, they cannot account for the earliest increase in NK cytolytic activity. Elucidation of the mechanisms of NK cell activation by IL-2 remains an area of active investigation.

**ROLE OF NK CELLS IN NORMAL PHYSIOLOGY**

**Resistance to microbial pathogens.** Of the various physiologic functions ascribed to NK cells, antiviral activity has perhaps been documented most persuasively. Numerous studies have demonstrated that NK cells can selectively lyse virus-infected target cells while sparing uninfected cells. IFN secreted by accessory cells or NK cells themselves in response to viral infection potentiates the cytolytic activity of NK cells, whereas IFN has been shown to protect normal cells preferentially from NK cytolytic activity. IFN activity thus partially accounts for the efficient and selective lysis of virally infected cells. However, IFN is not solely responsible, or even absolutely necessary, for NK killing of virus-infected cells. The expression of viral antigens or other surface structures by infected cells appears to render them more sensitive to NK cytolytic activity.

Mammalian cells infected by many different viruses (including herpes, vaccinia, measles, mumps, and influenza viruses) can be lysed in vitro by NK cells. However, the bulk of the data supporting a role for NK cells in resistance to viral infection in vivo are from studies of murine herpesvirus infections. Strains of mice with relatively low NK activity are more susceptible to herpes simplex and cytomegalovirus infections. Adoptive transfer of marrow from virus-resistant strains to lethally irradiated virus-sensitive mice confers resistance to viral infections, whereas treatment with reagents that inhibit NK activity abrogates such resistance. Furthermore, infusions of anti-asialo GM<sub>1</sub>, which inhibit NK function in vivo, increase the severity of influenza pneumonia in mice or hamsters. Murine hepatitis virus infections are also more severe in mice treated in vivo with anti-asialo GM<sub>1</sub>. The extreme rarity of isolated human NK cell deficiency has made it difficult to demonstrate the importance of NK antiviral activity in man. Patients with Chediak-Higashi syndrome or leukocyte adhesion deficiency disease demonstrate impaired NK function, but their clinical course is generally determined by the concomitant granulocyte abnormality. Biron et al have described the complete and apparently isolated deficiency of NK cells in a teenage girl. This patient experienced a series of life-threatening viral infections, including primary disseminated varicella, primary cytomegalovirus pneumonia, and severe primary cutaneous herpes simplex infection. The PBL of this patient expressed neither CD56 nor CD16 and demonstrated no NK activity even after stimulation with IL-2 or IFN-α. Limited in vitro testing disclosed normal neutrophil, T cell, and B cell function. With antiviral therapy and supportive care, the patient eventually recovered from each infection; a normal specific T-cell response and a rise in specific antibody titers were documented during her convalescence from the varicella infection. This case provides the best direct evidence that NK cells play a crucial role in human defenses against herpesvirus infection. Presumably, NK effectors hinder viral dissemination and limit the total burden of pathogen during the early phases of infection, whereas the more delayed, antigen-specific humoral and T-cell immune responses mediate ultimate control of the virus.

NK cells can kill other infectious pathogens in vitro, including both gram-positive and gram-negative bacteria, the fungus *Cryptococcus neoformans*, and the protozoa *Toxoplasma gondii* and *Trypanosoma cruzi*. NK bactericidal activity has been shown to involve the secretion of...
soluble factors, whereas direct cell-mediated lysis appears to underlie the killing of fungi and protozoa. Activated NK cells have also been reported to lyse preferentially monocytes infected by intracellular bacteria. The relevance of these findings to normal physiology in vivo remains to be determined.

**Regulation of hematopoiesis.** The phenomenon of hybrid resistance in mice first suggested that NK cells might be involved in the regulation of hematopoiesis. Lethally irradiated F hybrids were observed to reject parental hematopoietic grafts even though other parental grafts were tolerated. This hybrid resistance is genetically distinct from classical allogeneic immune responses associated with the MHC complex. NK cells were subsequently shown to be the predominant effector cells responsible for hybrid resistance. Some data suggest that other effector cells also participate in this phenomenon.

The effects of NK cells on human hematopoiesis in vitro have been studied by several investigators. Typically, hematopoietic progenitor assays have been performed using normal bone marrow or peripheral blood that has been exposed to NK effectors or their supernatants. The data are somewhat confusing; both stimulation and inhibition of various colony forming units (CFU) by NK cells have been described. Partially purified NK cells have been shown to promote the erythroid burst-forming unit (BFU-E) activity of autologous peripheral blood. Furthermore, supernatants from highly purified NK cells (~80% to 95% pure) cultured in medium alone exhibit colony-stimulating activity for early myeloid progenitors from allogeneic peripheral blood or bone marrow. Activation of NK cells with IL-2 or IFN-γ does not enhance the colony-stimulating activity, whereas this activity is diminished but not abolished by neutralizing MoAbs to GM-CSF. The supernatants of similar NK cell preparations activated by exposure to an NK-sensitive cell line, on the other hand, exhibit both colony-stimulating and colony-inhibiting activity; the latter was shown to be due almost entirely to the presence of TNF. Degliantoni et al. have also described the colony-inhibiting activity of activated NK cells; the supernatants of CD16+ PBL stimulated with allogeneic bone marrow mononuclear cells or NK-sensitive target cells contained such inhibitory activity. TNF appeared to mediate both the colony-inhibiting activity and NK soluble cytotoxic activity in these supernatants. The ability of NK cells to produce cytokines was studied more rigorously by Cuturi et al. Polyclonal NK cells were expanded in culture by exposure to irradiated B-lymphoblastoid cells and then enriched to >98% purity by negative selection. Northern blot analysis of total cytoplasmic RNA failed to detect any constitutive expression of GM-CSF, G-CSF, M-CSF, IL-1, or IL-3 by these highly purified NK cells. However, NK cells could be induced to express mRNA for GM-CSF, M-CSF, or IL-3 using various stimuli (Table 3). NK cells stimulated with IL-2 and anti-CD16 MoAbs also secrete soluble inhibitory factors, including IFN-γ and TNF.

Contamination of the purified NK cell fractions by small numbers of non-NK effectors is a concern in these studies. Clonal populations of NK cells have the obvious advantage of being free of all other cell types. Such NK clones were found to inhibit the generation of CFU-Mix (CFU for mixed granulocyte, erythrocyte, monocyte, and megakaryocyte, colonies [CFU-GEMM]), CFU-GM, BFU-E, and CFU-E from allogeneic bone marrow; a period of close contact between the NK cells and the marrow cells was required for the effect. Different NK clones inhibited the different CFU variably, but in a clonally stable manner. Supernatants from NK clones stimulated by normal marrow cells or an NK-sensitive target cell line could also inhibit CFU formation, and this soluble inhibitory activity appeared to be due in part to IFN-γ. However, Niemeyer et al. could demonstrate no inhibitory effect on marrow CFU-Mix, CFU-GM, CFU-G, CFU-M, or BFU-E by highly purified polyclonal CD16+ autologous PBL. Differences in the activation states of the NK effectors might account in part for this discrepancy; NK clones are continuously stimulated by the conditions required for their propagation.

Despite apparent contradictions in the results summarized above, it is clear that NK cells can affect the growth of normal human hematopoietic progenitors in vitro. Under different experimental conditions, NK cells can secrete soluble factors with either stimulatory or inhibitory activity (Table 3). The preponderance of the data suggests that activation of NK cells is required for either activity; contact with early myeloid progenitor cells seems to be an adequate stimulus. Although NK cells could also regulate hematopoiesis by direct lysis of myeloid progenitor cells, there is currently little evidence that this is important for the effects described.

**Elimination of neoplastic cells.** The theory of immune surveillance postulates that immune effectors can recognize and destroy spontaneously arising malignant tumor cells. Early investigations designed to test this hypothesis focused on cytotoxic T lymphocytes; such studies were largely disappointing and evoked some skepticism over the validity of this theory. However, considerable experimental evidence suggests that NK cells, rather than T cells, may mediate the destruction of autologous tumors. Studies in mice revealed a direct correlation between the level of NK activity and resistance to transplanted syngeneic tumors. Immunosuppressive doses of cyclophosphamide render rodents more susceptible to injected malignant cells, and this sensitivity can be reversed by the adoptive transfer of either NK clones or polyclonal lymphocytes highly enriched for NK activity. Neither polyclonal T cells nor CTL clones on the other hand, confer tumor resistance under identical conditions. Furthermore, unfractionated syngeneic splenocytes have been shown to mediate resistance to pulmonary metastases in cyclophosphamide-treated mice, whereas similar splenocytes depleted of NK cells did not exhibit this activity.

It has been shown that NK effectors can inhibit malignant tumor growth in vivo under experimental conditions. It has been much more difficult to demonstrate that NK cells are involved in immune surveillance against spontaneously arising autologous tumors in man. Indirect supportive evidence comes from studies of patients with deficient NK cell function. Patients with the Chediak-Higashi or X-linked lymphoproliferative syndromes demonstrate abnormally low
NK activity but relatively normal T-cell and B-cell function. These patients are particularly susceptible to malignant lymphoproliferative disorders, possibly as a result of their NK deficiency. However, this susceptibility could also reflect inadequate NK antiviral activity with subsequent EBV-induced lymphoproliferation. Deficient NK activity has also been found in the members of families with an apparent genetic predisposition to melanoma or other cancers. Numerous retrospective studies have documented subnormal NK cell function in patients with a variety of malignant tumors. The degree of NK impairment seems to correlate with disease status, being most marked in patients with advanced cancers. Depressed NK activity could be an obvious consequence of the untreated or uncontrolled malignancy, rather than an antecedent state predisposing these patients to cancer. This issue can be addressed adequately only by a prospective, longitudinal study comparing the incidence of cancer in persons with high NK function with that in otherwise normal persons exhibiting low NK activity. Such a study is unlikely to be undertaken because of practical considerations.

Participation in the graft-versus-leukemia effect after bone marrow transplantation. Bone marrow transplantation (BMT) provides curative treatment for some patients with hematologic malignancies. BMT permits the use of chemotherapy and radiotherapy in doses that would be lethal without marrow grafting, and the high-dose preparative regimens undoubtedly contribute to the antitumor efficacy of BMT. Nonetheless, substantial data suggest that immune effector cells may also be important for the control of hematologic malignancies after BMT. Retrospective analyses of relapse rates after transplantation have provided indirect evidence for this "graft-versus-leukemia" (GVL) effect. Despite receiving similar preparative regimens, patients with acute or chronic leukemias who receive marrow grafts from identical twins relapse much more frequently than patients with nonidentical sibling donors. Furthermore, patients undergoing allogeneic BMT who have no graft-versus-host disease (GVHD) relapse more often than patients with GVHD; relapse rates are inversely related to the severity of GVHD. Finally, depletion of T cells from bone marrow allografts can prevent GVHD but has also been associated with a higher risk of relapse in several series. Because mature alloreactive T cells mediate GVHD in humans, these data imply that T cells can exert an antileukemic effect after allogeneic BMT. However, a clinical trial attempting to enhance this effect by deliberately promoting GVHD proved prohibitively toxic and did not demonstrate better leukemic control.

Laboratory studies indicate that NK cells can also have GVL activity. Similar to their effects on normal early myeloid progenitors, NK cells can inhibit the growth of clonogenic tumor cells from patients with myeloid leukemias. Unstimulated NK cells can readily lyse cultured leukenic cells, and tumor cell lines derived from patients with lymphoid and myeloid leukemias are prominent among the classic "NK-sensitive" targets. Although fresh leukemic blasts are generally resistant to NK cell killing, they can often be efficiently lysed by activated NK effectors. Furthermore, lymphocyte clones derived from the peripheral blood of patients undergoing allogeneic BMT can mediate GVL activity. In one study of a patient with acute lymphoblastic leukemia (ALL), all clones with cytolytic activity against cryopreserved autologous blasts expressed CD56 but not CD3 and had other characteristics of NK cells; cytogenetic analysis confirmed that these NK cells were of donor origin. Indeed, NK cells have been shown to engraft quickly after BMT, and can represent the majority of PBL during the first few weeks posttransplant. These NK cells appear to be endogenously activated and demonstrate enhanced cytolytic activity when freshly isolated. Although these activated NK effectors are seen after either autologous or allogeneic BMT, they are not detected after intensive combination chemotherapy alone. Clinical applications of the GVL activity of NK cells are discussed below.

NK CELL MALIGNANCIES AND THE GRANULAR LYMPHOCYTE PROLIFERATIVE DISORDERS

Human NK cell malignancies appear to be quite rare. Three children with ALL of probable NK cell lineage have been described. The leukemic blasts of these children expressed CD2 and Fcy receptors but not CD3 and mediated spontaneous killing of K562 target cells. A case of NK cell ALL has also been reported in an adult; the malignant blasts of this patient demonstrated a characteristic NK cell phenotype but no cytolytic activity. Three patients with clinically aggressive leukemia involving CD3—large granular lymphocytes have been described. The malignant lymphocytes of these patients expressed CD16 or CD56 and mediated NK cytolytic activity. Germline configurations of T-cell receptor and immunoglobulin genes were demonstrated in two of these cases, and a clonal cytogenetic abnormality was evident in the third. It should be noted that CD56 expression by malignant cells does not necessarily indicate an NK cell origin. CD56 has been detected on tumor cells from the majority of patients with small cell lung carcinoma and myeloma, as well as a subset of patients with myeloid leukemias.

Chronic proliferative disorders of granular lymphocytes are more frequently recognized than acute leukemias with NK cell characteristics; more than a hundred patients with the former have been described over the past 15 years. These patients typically present with a granular lymphocytosis, and a peripheral blood absolute granular lymphocyte count in excess of 2,000/μL for at least 3 months with no obvious cause is generally required for the diagnosis. Most patients also demonstrate lymphocytic infiltration of the bone marrow. Splenomegaly is present in about half and hepatomegaly in 20% to 30% of patients, but lymphadenopathy and skin involvement are distinctly unusual. Severe neutropenia is typical and recurrent bacterial infections may occur. Associated diseases, most commonly rheumatoid arthritis, hepatitis, or cancer, are present in 25% to 50% of patients. The clinical course of these patients is often relatively benign and many never require specific therapy. A variety of names have been given to these disorders, including large granular lymphocyte leukemia, LGL lymphocytosis, lymphoproliferative disease of granular lymphocytes, chronic...
granular lymphoproliferative disorders, chronic granulated T-lymphocytosis, and Tγ-lymphoproliferative disease.190-193 Although early phenotypic studies and the characteristic LGL morphology suggested that chronic granular lymphocytoses were indeed NK cell malignancies, subsequent work has demonstrated that CD3+ cells account for the expanded lymphocyte population in most patients. These granular lymphocytes also commonly express CD16 and CD57.191-193,197 Clonal TCR α/β or γ/δ gene rearrangements are usually detected if assayed, although this has been done in relatively few cases.191,192,198 Thus, monoclonal proliferation of T cells appears to underlie the granular lymphocytosis in most of these patients. Although fewer than 2% of T cells normally express CD16, a small subset of CD16+ T cells can be detected by flow cytometry in the peripheral blood of some healthy persons.91 These CD3+ CD16+ lymphocytes exhibit LGL morphology and mediate ADCC but not NK activity; most are both CD4 and CD8 negative, but some express low levels of CD8. These normal T cells closely resemble the malignant lymphocytes present in most cases of the granular lymphoproliferative disorders; whether they represent the normal counterpart of the latter is uncertain.

Although the CD3+ phenotype is by far the most common, in a minority (about 15% to 30%) of patients the granular lymphocytes are CD3− but CD16+ and thus appear to be NK cells; CD56 expression has not been examined in most series.191,193,197 Increased NK activity has been described in about 20% of all patients with granular lymphoproliferative disorders, although this does not necessarily correlate with the CD3− CD16+ phenotype. Because the CD3− cells do not demonstrate TCR gene rearrangements192,198 and chromosomal abnormalities are rarely found in these disorders, it has been difficult to assess the clonality of the CD3− granular lymphocytes. The NK cell expansion in some of these patients may be reactive rather than neoplastic.

**THERAPEUTIC APPLICATIONS OF NK CELLS**

**Activated NK cells and the cellular immunotherapy of cancer.** Experiments first performed more than a decade ago revealed that PBL cultured for several days in IL-2 could destroy fresh autologous and allogeneic tumor cells without MHC restriction; these lymphokine-activated killer (LAK) cells could also efficiently kill target cell lines resistant to lysis by unstimulated NK cells.130,199 Because bulk LAK cultures contain a heterogeneous mixture of cells, it was initially uncertain which cells mediated LAK activity. Early studies suggested that a novel cell lineage distinct from T cells or NK cells was responsible for the LAK phenomenon.200 However, substantial data now indicate that activated NK cells are the predominant LAK effectors.201-203 Though CD56− T cells, CD11b+ T cells, and γ/δ T cells may also mediate MHC-unrestricted cytoxicity after IL-2 stimulation, they appear to contribute relatively little to the cytolytic activity of standard LAK cultures. Animal studies demonstrated that infusions of LAK cells could result in resistance to metastasis as well as the regression of established tumors.204-206 Based on these observations, initial phase I clinical trials were conducted at the National Cancer Institute (NCI) to determine the potential therapeutic utility of LAK cells in humans.206-207 These trials used high doses* of IL-2 (~100,000 U/kg by intravenous (IV) bolus injection every 8 hours) combined with infusions of LAK cells generated in vitro. Ten of the first 25 patients so treated demonstrated objective partial responses, and one patient with melanoma experienced complete regression of all disease for greater than 10 months.207 All patients had advanced cancers for which no effective therapy had previously been available. These encouraging results prompted further clinical trials, performed at the NCI and elsewhere, using high-dose IL-2 alone or in combination with LAK cells. These studies confirmed the modest efficacy of IL-2/LAK therapy; objective responses were demonstrated in 16% to 35% of patients with renal cell carcinoma and 19% to 21% of patients with malignant melanoma.209-210 A small number of durable complete remissions have been described.206-210 Limited studies involving patients on these protocols have confirmed that activated NK cells, rather than T cells, appear to be responsible for the LAK activity generated in vivo by the high-dose IL-2 infusions as well as in the bulk in vitro cultures.203,211

Although the IL-2/LAK trials demonstrated that activated lymphocytes can cause the regression of advanced cancers in humans, they have also documented substantial systemic toxicity.208-210 In attempts to improve the response rate and limit toxicity, many investigators have undertaken modifications of the original Rosenberg protocol. Because standard LAK cultures contain large numbers of T cells that do not appear to be cytolytically active, adoptive immunotherapy using highly enriched NK effectors has been undertaken.243,244 Hercend et al241 have administered IL-2 and lymphokine-activated natural killer (LANAK) cells to 12 patients with metastatic renal cell cancer. In most cases more than 85% of such LANAK cells were CD56+, whereas T cells accounted for fewer than 5% of cells infused. Compared with unfraccionated LAK cells, LANAK cells exhibited approximately 100-fold greater killing of NK-resistant targets in standard cytotoxicity assays.243 Objective partial responses were seen in three of nine evaluable patients treated with IL-2 and LANAK cells. Purification and expansion of LANAK cells in vitro are, however, arduous procedures.243 Short-term incubation of PBL with IL-2 results in preferential adherence of NK cells to plastic culture flasks, and adherent LAK (A-LAK) cells have much more potent cytolytic activity than do unfractionated LAK cells.219,214-216 The Pittsburgh group has undertaken a phase I/II clinical trial of IL-2 and A-LAK cells in patients with melanoma and renal cell carcinoma.244

Although preclinical and initial clinical studies suggested that neither high-dose IL-2 alone nor LAK cell infusions alone had significant antitumor effect,206 subsequent work has shown that treatment with high-dose IL-2 results in

*Throughout this review, doses of IL-2 are given in International Units, which are the same as those used by the Biological Response Modifiers Program at the NCI. When investigators have reported their doses in "Cetus units," the latter have been converted to International Units for ease of comparison. One Cetus unit is approximately equivalent to 3 to 6 International Units.

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overall objective response rates comparable with those obtained with combined IL-2/LAK therapy.\textsuperscript{208,212} A large prospective randomized trial at the NCI demonstrated more complete responses in patients receiving both IL-2 and LAK cells compared with those receiving IL-2 alone, but no significant differences in overall responses or survival.\textsuperscript{212} It is presently unclear whether infusions of LAK cells add any therapeutic benefit over high-dose IL-2 alone. IL-2 itself possesses no known direct antineoplastic effect, and its efficacy when used alone probably reflects the killing of tumor cells by NK effectors expanded and activated in vivo. However, direct tumor cell lysis by activated NK cells may not be the only mechanism causing tumor regression. Other effector cells might be recruited in vivo, and cytokines released by several cell types in response to IL-2 may be important as well. Indeed, endogenous production of IFN-\(\gamma\) and TNF has been detected in patients receiving IL-2 therapy.\textsuperscript{213}

Several groups are using IL-2 alone in various doses and schedules to treat patients with advanced cancer. The Madison group has administered IL-2 by continuous infusion or IV bolus at doses of 1.0 or \(3.0 \times 10^6\) U/m\(^2\)/day for 4 consecutive days on each of 4 consecutive weeks.\textsuperscript{214,215} Toxicity was generally mild; patients were treated on medical oncology wards and no patient required transfer to an intensive care unit. Three of seventeen patients with advanced renal cell cancer demonstrated objective partial responses; no responses were seen in six patients with melanoma.\textsuperscript{214} The Loyola group has treated patients with advanced melanoma or colon cancer using repetitive once or twice weekly infusions of IL-2 at doses from \(1.0 \times 10^4\) to \(3.0 \times 10^7\) U/m\(^2\)/doses up to \(1.0 \times 10^8\) U/m\(^2\)/day were very well tolerated.\textsuperscript{216} Objective partial responses were seen in two of six patients with melanoma. Both the Madison and Loyola investigators reported very similar changes in the peripheral blood of patients receiving this "moderate dose" IL-2 therapy.\textsuperscript{211,215,217,218} IL-2 induced a prominent eosinophilia and an initial lymphopenia followed by a rebound lymphocytosis. Dramatic increases in peripheral blood NK cells were seen, with a preferential expansion of cells expressing the CD56\(^{bright}\)CD16\(^{-}\) phenotype.\textsuperscript{217,218} PBL from these patients exhibited enhanced killing of NK-sensitive targets and could lyse NK-resistant targets without any in vitro activation.\textsuperscript{215,216} Sorting and complement lysis experiments confirmed that virtually all of this LAK activity was mediated by NK cells and not by T cells.\textsuperscript{211,218} Furthermore, the CD56\(^{bright}\)CD16\(^{-}\) NK cells appeared to be more potent LAK effectors than the CD56\(^{dim}\)CD16\(^+\) cells.\textsuperscript{218}

Outpatient administration of biologically active doses of IL-2 has also been shown to be feasible. Ambulatory patients have tolerated several weeks of IV bolus or intramuscular IL-2 given 5 days weekly in doses from \(1.0\) to \(3.6 \times 10^6\) U/m\(^2\)/day.\textsuperscript{219,221} In one study, immunomodulatory effects similar to those described by the Loyola and Madison groups were reported.\textsuperscript{219} In an ongoing phase I trial at the Dana-Farber Cancer Institute, patients with advanced cancer receive continuous intravenous infusions of IL-2 for 90 consecutive days entirely on an outpatient basis. IL-2 so given has been well tolerated in doses as high as \(6.0 \times 10^5\) U/m\(^2\)/day. Even at this relatively low total daily dose, patients have demonstrated marked increases in circulating NK cells. As with the "moderate dose" inpatient regimens, CD56\(^{bright}\) NK cells are most prominently expanded. By contrast, no significant change has occurred in the absolute number of T cells. Thus the administration of lower doses of IL-2 can selectively activate NK effectors in vivo without the severe toxicities of high-dose bolus IL-2. Further clinical studies will be required to determine the optimal means of exploiting these activated NK cells.

### Manipulation of the graft-versus-leukemia activity of NK cells

The IL-2/LAK cell trials have involved patients with advanced cancer; cellular immunotherapy might be more effective in patients with a smaller tumor burden. Indeed, adjuvant IL-2 therapy has been given to a small number of patients with resected stage C colon cancer.\textsuperscript{211} Most adults with acute leukemia in complete remission also have minimal residual disease that might be responsive to immune effector cells. The presence of endogenously activated NK cells after allogeneic and autologous BMT makes these particularly attractive settings in which to use the GVL activity of NK cells therapeutically.\textsuperscript{183,185} However, reluctance to accentuate the known toxicities of BMT and fear of aggravating GVHD after allogeneic BMT have hindered the use of IL-2 posttransplant. Indeed, a child given IL-2 after receiving a histocompatible allograft for metastatic neuroblastoma experienced reactivation of acute GVHD.\textsuperscript{222} One group has described nine patients treated with IL-2 after autologous BMT for hematologic malignancy.\textsuperscript{223,224} These patients were given IL-2 in doses of approximately \(1.0\) to \(2.0 \times 10^6\) U/m\(^2\)/day for 3 to 5 days. Toxicity was similar to that experienced by nontransplant patients given comparable IL-2 therapy.\textsuperscript{223} As expected, increased NK cell numbers and enhanced LAK activity were evident in the peripheral blood of these patients after IL-2 administration.\textsuperscript{224} In contrast to most other IL-2 trials, however, both activated NK cells and activated T cells appeared to contribute prominently to LAK activity.\textsuperscript{224}

Our experience with prolonged continuous infusions of IL-2 in patients with advanced solid tumors suggested that similar treatment might be tolerable to patients after BMT. Furthermore, GVHD has been effectively abrogated in our allograft patients by donor marrow treatment with anti-CD6 MoAbs and complement, without the high incidence of graft rejection reported after some methods of T-cell depletion.\textsuperscript{223} As in other transplant series, our patients demonstrate early engraftment of NK cells,\textsuperscript{186} and the latter appear to be relatively activated in vivo. We have begun a clinical trial of IL-2 in selected patients at high risk for relapse after allogeneic or autologous BMT; these patients receive prolonged continuous infusions of IL-2 in relatively low doses after demonstrating stable neutrophil engraftment. Others are also exploring IL-2 therapy as an adjunct to BMT.\textsuperscript{223,226,229} A minority of patients have an appropriate HLA-matched sibling donor for allogeneic BMT, and the rigor of the procedure precludes even autologous BMT for many patients. Thus there is considerable interest in manipulating the antileukemic activity of NK cells in clinical contexts other than BMT. Several investigators have demonstrated that LAK activity can be generated from the peripheral blood of...
patients with acute leukemia in remission\textsuperscript{180,227,228} or CML in stable phase.\textsuperscript{229} Phase I trials have confirmed the feasibility of IL-2 therapy for patients with acute leukemia.\textsuperscript{223} Further clinical studies of IL-2 in the treatment of leukemia are in progress.\textsuperscript{223}

CONCLUSIONS

Less than 15 years ago NK activity was little more than an immunologic curiosity, even considered an experimental artifact by some; today NK cells are the subject of intensive investigation by a number of laboratories. Initially defined only by their spontaneous cytolytic activity, NK cells can now be unequivocally identified as a discrete subset of lymphocytes with characteristic morphologic, immunophenotypic, and functional attributes. The availability of monoclonal antibodies with well-defined reactivity and the use of homogeneous clonal populations of NK cells have been largely responsible for recent advances in our understanding of these unique effector cells. Although precise knowledge regarding the role of NK cells in normal physiology remains elusive, a growing body of evidence suggests these lymphocytes are involved in the defenses against viral infections, the regulation of hematopoiesis, and the destruction of malignant tumor cells. It is also apparent that the antitumor activity of NK cells can be used therapeutically. The near future is likely to bring important new insights into fundamental NK cell biology as well as improved clinical applications of these effector cells.

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Biology and clinical relevance of human natural killer cells

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