

Special Article

The Biology of the Human Natural Killer Cell

JOHN C. RODER¹ and HUGH F. PROSS^{1,2}

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Natural killer (NK) cells in the human are a population of large granular lymphocytes (LGL) with at least one unique surface antigen not expressed on cells of other lineages. NK-target-cell interaction appears to involve carbohydrate recognition and, following binding, the NK cells are induced to generate O_2^- , transmethylate membrane phospholipids, and activate phospholipase A_2 . Some or all of these activities trigger a cascade of events which ultimately leads to the secretion of a substance toxic to the target cell. A variety of genes controls various steps in this cytolytic pathway. There is a good deal of evidence in the mouse, and some in the human, that NK cells play a role in host surveillance against tumor development, resistance to viral infections, and, possibly, hematopoietic regulation.

KEY WORDS: Natural killer cells; immune surveillance; viral resistance; hematopoietic regulation.

INTRODUCTION

Natural killer (NK) cells are lymphocytes from normal nonimmune donors with the capacity to rapidly lyse (within hours) tumor cells, virally infected cells, or certain undifferentiated normal cell types both *in vivo* and *in vitro*. This "spontaneous" cytotoxicity was first recognized as the function of a discrete lymphoid subpopulation in 1975 (1-3) and several excellent reviews on early developments in the field have been written (4-6). In this article we attempt to review some recent advances in our understanding of endogenous human NK cells.

CHARACTERISTICS

NK cells originate in the fetal liver of man as early as 9 weeks of gestation (7) and are present in significant levels at birth in the cord blood (7, 8). Fetal liver also harbors NK progenitors in the mouse (9). Following birth there is a gradual increase in NK levels to high levels in adulthood, with no decrease in activity late in life (10-16). Most NK activity in the adult is found in the peripheral blood and spleen, with low levels in bone marrow, lymph node, and thoracic duct and no activity in thymus (14, 16, 17).

Human NK cells have been described morphologically as large granular lymphocytes (LGL) with a high cytoplasm:nucleus ratio, pale cytoplasm, and slightly eccentric reniform nucleus with 6 to 12 azurophilic granules in their cytoplasm (18). It has not been possible to separate LGL from cytolytic function by adherence to target cells (18) or sedimentation in Percoll density gradients (19) and the number of LGL binding to K562 tumor cells correlated with the distribution of cytotoxicity in normal individuals (18). Therefore most, if not all, NK cells are LGL.

Most LGL bear receptors for the Fc portion of IgG and express low-affinity receptors for sheep erythrocytes (20), and two new monoclonal antibodies have been shown to bind selectively to LGL. Almost all (>92%) HNK-1⁺ cells separated on the fluorescence-activated cell-sorter (FACS) exhibited the LGL morphology and cytolytic function, whereas HNK-1⁺ cells lacked both characteristics (21). No other cells in the blood or a panel of tumor cell lines bound to HNK-1 with the exception of HSB-2, the line used to immunize the mice for

¹Department of Microbiology & Immunology,
²Department of Radiation Oncology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

hybridoma production. In double marker studies no HNK-1⁺ cells expressed surface immunoglobulin (sIg) or phagocytosed latex beads. However, some HNK-1⁺ cells stained with OKT3 or leu4, which are pan T reagents, but all of the cytolytic activity was confined to a OKT3⁺, HLA-DR⁺, OKM1⁺ subpopulation comprising approximately 60% of the HNK-1⁺ pool (Abo and Balch, personal communication). Since NK cells originate in the bone marrow (9, 22) and since all of the HNK-1⁺ cells in the bone marrow were OKT3⁺, then one could speculate that the cytolytically active HNK-1⁺, OKT3⁺, NK subpopulation differentiates from an HNK-1⁺, OKT3⁺ precursor (pre-NK) which is not yet cytolytic (Abo and Balch, personal communication). Another monoclonal antibody, NK-8, from mice immunized with human LGL, bound to a subpopulation of LGL (33%) but no other cell types in peripheral blood or a panel of cell lines representing the T, B, myeloid, or erythroid lineage (23). Seventy percent of the LGL forming conjugates with K562 tumor cells were NK-8⁺. Pretreatment of effectors with anti-NK-8 antibody in the absence of complement blocked cytotoxicity against isotope-labeled K562 cells and also blocked interferon release by LGL, although target-effector conjugate formation was normal. The mechanism of blocking is unknown but it is interesting to note that NK-8 did not block cytotoxicity against fetal fibroblast targets. In summary it would appear that HNK-1 marks all LGL, whereas NK-8 is specific only for those LGL capable of binding to target cells. It should be kept in mind that the number of cytolytically active NK cells accounts for only a few percent of peripheral blood lymphocytes.

Human NK cells share a plethora of additional surface antigens in common with other cell lineages. OKM1 is found on NK cells, monocytes, and granulocytes (24-26), whereas MAC-1 is present on monocytes and NK cells (27). It is unlikely that NK cells belong to the monocyte lineage, however, since they lack the monocyte lineage-specific marker Mo2 detected on all human monocytes but no other cell types (28). In our hands the majority of nonadherent, mononuclear cells enriched for NK cells on Percoll gradients was HNK-1⁺ and exhibited LGL morphology, whereas <1% stained with Mo2 or phagocytosed antibody-coated erythrocytes (29). Furthermore, LGL are positive for α -naphthyl acetate esterase (ANAE) in the presence of sodium fluoride, which is known to inhibit the monocyte ANAE reaction (30).

Human LGL also share a number of antigens with T cells. OKT10 is expressed on 62% of LGL (31), most immature thymocytes, and some (10%) myeloid cells in the bone marrow (32), whereas markers restricted exclusively to mature T cells (OKT3, leu4) are absent from LGL. 3A1 and 5A12 are expressed on both T cells and LGL (31, 33).

These findings do not allow the placement of NK cells on a known lineage, and until proven otherwise we assume that NK cells diverge very early in lymphopoiesis on a lineage of their own. The existence of NK-specific markers will greatly facilitate work in the field.

MECHANISM

The development of a single-cell assay for dissociating NK-mediated binding and lysis in the mouse (34, 35) led to the development of a stimulus secretion model for NK-mediated cytotoxicity (35, 36). The model has some similarities to, but also distinct differences from, earlier work in the cytolytic T-cell (CTL) field (37, 38) as discussed previously (39). Under this model specific target-cell contact results in a change in the cyclic nucleotide balance, or some other second messenger, which triggers secretion of the lethal substance (39). These results have been confirmed and extended in the human (40) following the development of conjugate assays in this species (41-43).

One of the earliest events (seconds) that can be detected in the human NK cell, subsequent to target-cell contact, is the rapid burst in the generation of the superoxide anion, $O_2^{\cdot-}$, as measured by chemiluminescence or cytochrome reduction (44, 45). Intact cells or plasma membrane vesicles from NK-sensitive, but not NK-insensitive, targets induced the $O_2^{\cdot-}$ response which was necessary for cytotoxicity to proceed since superoxide dismutase, but not catalase, specifically blocked both $O_2^{\cdot-}$ generation and cytotoxicity. Although $O_2^{\cdot-}$ was necessary for NK-mediated cytotoxicity, it was not sufficient since Chediak-Higashi patients produced normal amounts in the absence of cytotoxicity. We do not believe that $O_2^{\cdot-}$ is the lytic agent but postulate its role as a cofactor or activator for some subsequent step in the cascade of events leading to lysis.

The triggering mechanism in NK cells subsequent to target-effector binding is unknown, but indirect evidence implicates the cyclic nucleotides. In both the mouse (46) and the human (47) the addition of

cAMP, or inducers thereof, to highly enriched NK cells caused an inhibition of cytotoxicity, whereas cGMP or inducers, including interferon (48), caused augmentation. Although specific antagonists blocked these effects, specific measurements of intracellular cAMP and cGMP in the NK cell, before and after target-cell contact, have not yet been made. The initial finding that cGMP or inducers could reverse the defective NK response in Chediak-Higashi (CH) patients (49) has not been confirmed in a study of four additional patients (50) or in beige mice (36), the animal model of the CH syndrome.

A later event in the cytolytic pathway, measurable directly at 1 hr postbinding, involves the methylation of phospholipids within the NK cell (51). Inhibitors of transmethylation (3-deazaadenosine) blocked cytotoxicity, and it is conceivable that transmethylation may enhance signal transduction across the plasma membrane of NK cells as occurs in many other systems (52). A concurrent increase in phospholipase A₂ activity was also measured directly and could be blocked by several specific inhibitors of this enzyme including DL-(2,3-distearoyloxy propyldimethyl-2-hydroxy)ethylammonium acetate. These results have been confirmed in the mouse (53) but it remains to be shown if phospholipase A₂ or its detergent-like product, lysophosphatidylcholine, is the lethal substance.

The evidence that secretion may be important in the cytolytic pathway of human NK cells can be summarized as follows: (i) pretreatment of LGL with Sr²⁺ led to degranulation, and following washing, there was a concomitant loss of cytotoxic activity (54); (ii) cytotoxicity was inhibited by monensin, a carboxylic ionophore, which blocked cellular secretion in LGL (40); (iii) agents which inhibit cytotoxicity (cAMP, PGE₁, theophylline, histamine) (46) also block lysosome secretion in other cells (46, 55) at the same concentrations, and conversely, agents which increase lysosomal discharge (cGMP, carbamylcholine) augment NK cytotoxicity (46); (iv) chloroquine, a selective inhibitor of lysosomes, simultaneously decreased NK cytotoxicity and *N*-acetyl- β -glucosaminidase levels in mouse spleen (36) and human peripheral blood lymphocytes (PBL) (56); (v) secretion is energy dependent, and DNP and CCCP, uncouplers of oxidative phosphorylation, block both NK cytotoxicity (35, 40) and secretion; and (vi) CH patients and beige mice carry a gene mutation which selectively impairs both the lethal-hit stage of NK-mediated cytotoxicity (39) and

the secretion of lysosomal enzymes in the kidney and into the phagolysosome of leukocytes (57). Since HNK-1⁺ LGL from CH patients have a single giant granule rather than many small granules (58), it is likely that lack of secretion may also explain this genetic NK insufficiency. Neither monocytes nor T cells from these patients exhibited giant granules, and both effector-cell types had normal cytotoxic functions (59, 60). It seems likely, therefore, that the giant granules in NK cells may be linked to the functional defect in CH patients.

Two hypotheses have been put forward for the nature of the lethal-hit phase of NK-mediated cytotoxicity. First, it was suggested that a lysosomal protease was the lethal substance since secretion of lysosomal or granule contents may be involved (above) and since inhibitors of various esterases (PMSF, DIFP) blocked killing but not binding to the target (35, 39). Subsequent work using a larger panel of more specific inhibitors has revealed that a chymotrypsin-like activity, as opposed to a trypsin activity, may be involved in early activation events rather than the lethal hit itself (55). The second hypothesis suggests that soluble toxic molecules are released by the NK cell and bind to specific receptor sites on the target-cell membrane (59). Human PBL stimulated by lectins release soluble factors which are toxic to NK-sensitive, but not NK-resistant, targets (61). It remains to be shown if these toxins are released from NK cells or other cells in the mixture. The existence of toxin receptor sites on the target-cell membrane has been inferred from data showing that NK-resistant mutants of YAC bound normally to the NK cell but resisted lysis (62). Cytotoxicity by alloimmune CTL or antibody and complement was normal, thereby suggesting the existence of a specific target-cell determinant important for lysis but not involved in target-effector binding. It is conceivable that the toxin contains a hexose phosphate residue which binds to the receptor sites since mannose-6-phosphate and stereochemically similar fructose-6-phosphate inhibit NK-mediated cytotoxicity at the target-cell level, and gelonin conjugated to a mannose-6-phosphate was highly toxic to NK-sensitive, but not NK-resistant, targets (56). In a similar manner various carbohydrates competed with toxins derived from PBL in another system (60). The inability of NK cells to kill NK-insensitive targets (noncancer bystanders) after triggering with NK-sensitive targets does not exclude the existence of soluble toxins. The insensitive target may lack receptor sites for

the toxin or there may be a specialized mode of delivery that prevents leakage.

SPECIFICITY

NK cells bind to their targets using one of two alternative modes. The first mode involves a receptor-ligand interaction between a target structure (TS) on the plasma membrane of the target cells and a recognition receptor (RR) on the surface of the NK cell. In the second mode the target cell is coated with antibody specific for a target-cell antigen and the NK cell then binds the Fc portion of the antibody to complete the necessary bridge. Both of these binding events appear to activate the same cytolytic pathway within the NK effector cell.

The nature of the RR is not known, but in the mouse (35) and man (unpublished observations) pretreatment of NK cells with proteolytic enzymes (trypsin, papain, pronase) eliminated both target-cell binding and cytotoxicity. These two functions regenerated in parallel over a 4-hr time course and could be blocked by cycloheximide. Treatment of nontrypsinized cells for 18 hr with the minimum concentrations of puromycin or cycloheximide required to inhibit protein synthesis by 90% also decreased both target-cell binding and lysis. It is likely, therefore, that the NK RR is a *de novo* synthesized protein with a turnover time similar to that of other membrane proteins. The ability of simple sugars to inhibit target-effector binding in the mouse (63) suggests that lectin-like receptors are involved, although the sugar specificities in the human are clearly different (56; Werkmeister, personal communication). In view of the lack of significant segregation of target specificities in cloned NK lines in the mouse (64), it is unlikely that NK RR are clonally distributed. It is possible, however, that the RR is widely distributed among non-NK leukocytes since nonlytic thymocytes and peritoneal macrophages preferentially bind NK-sensitive target cells (65).

The target structure (TS) has been more extensively analyzed (66, 67). Preincubation of human PBL with solubilized glycoproteins from NK-sensitive, but not NK-resistant, target cells inhibited binding to the homologous intact target cells by approximately 50% (66). However, these molecules were not related to any known surface proteins including viral and MHC products, and the quantities recovered were too low to inhibit cytotoxicity. In retrospect it became clear that only a minority of

the conjugates detected in this assay consisted of lytic NK cells since extensive purifications were not employed (68). Other investigators have used absorption techniques whereby NK cells were depleted on target-cell monolayers and then analyzed for cytotoxicity against a panel of targets (69). Absorption of PBL from some donors with certain cell lines removed cytolytic activity against the homologous cell line and some third-party lines which were presumed to share an antigen. Reactivity against some third-party lines was not removed, and this analysis concluded that up to seven TS were involved. Some were unique to a given group of targets, whereas others were shared among all the cell lines. Similar evidence for multiple specificities was obtained by others using "interaction analysis" (70), monolayer absorptions (71), or competitive inhibition assays with unlabeled targets (72, 73) or purified membrane glycoproteins (66).

In some target cells the TS may represent early antigens that are lost upon differentiation or maturation. Hence immature thymocytes from neonates, but not mature thymocytes or T cells, were sensitive to NK-mediated cytotoxicity in the mouse (74, 75). Human thymocytes were also NK sensitive (76) and there was an inverse correlation between the sensitivity of fetal fibroblasts and the age of the donor fetus (41). Adult fibroblasts were usually resistant to NK cells. Undifferentiated embryonal carcinoma cells were highly NK susceptible in the mouse, whereas their more differentiated endodermal progeny were NK resistant (77). In the human there was a good correlation between controlled differentiation in K562, U-937, or GM-86 tumor cell lines and increased resistance to NK-mediated cytotoxicity (78). These results have been confirmed and extended in differentiated K562 and HL-60 lines, and Werkmeister *et al.* (79) have shown that the NK resistance is due to a loss of TS and is selective since cytotoxicity mediated by monocytes or T cells was normal. One could hypothesize that NK cells recognize "fetal-like" antigens which are lost upon differentiation of undifferentiated tumor cells or normal cells. In the case of the more differentiated tumors, these antigens may be derepressed during the malignant transformation process. However, exceptions exist, and in theophylline-induced melanoma lines (79) NK sensitivity was increased rather than decreased following differentiation. An intriguing alternative hypothesis has been developed by Karre which suggests that NK cells are triggered by a lack of self-MHC antigens of the class I type, and

It is known that undifferentiated lines express less TS (80).

The changes that occur in the TS during differentiation are not yet known. However, analysis of several TS loss variants in the mouse (81-83) has revealed a negative correlation between NK sensitivity and the amount of cell-surface sialic acid (83), whereas a positive correlation between asialo-GM₂ and NK sensitivity was found in two independent studies (83, 84). These results suggest that sialic acid may mask the TS but further work is needed to implicate GM₂ or other gangliosides. It has recently been proposed that the mechanism of effector-target interaction involves the binding of effector-borne transferrin to target-borne transferrin receptors (85), and therefore one would predict a correlation between the rate of proliferation in a given target cell and NK sensitivity since the quantity of transferrin receptor expression is related directly to the proliferation rate (86, 87). Alternatively, the transferrin receptor itself may be the TS.

REGULATION

Human NK function is markedly augmented by human interferon of either the alpha or the beta family (88, 89). As previously observed in the mouse (35), interferon does not increase the number of conjugate-forming NK cells in the human (43, 90) but may recruit nonlytic pre-NK cells (FeR⁻) to become cytolytic NK cells (FeR⁺) by a process requiring mRNA and protein synthesis (91), which is also required for the induction of the antiviral state (92). Similar findings have been made in the mouse (93, 94). It is of interest that the totally deficient NK activity in patients with defective immune interferon production (DIIP) (95) can be completely restored to normal levels following *in vivo* injection of interferon (96). During human trials it should be kept in mind that interferon can oppose the augmented NK activity by simultaneously protecting the malignant cells from lysis as shown in the mouse both *in vitro* and *in vivo* (97).

A great many other agents including BCG (98) have also been shown to augment NK activity but most likely operate as inducers (reviewed in Ref. 39). The human NK cell itself can be induced by tumor cells to synthesize large amounts of interferon within a short time (18 hr) after stimulation, whereas antigen-stimulated T cells require several days (99). A variety of agents including β -estradiol at nonphysiological concentrations (100), prosta-

glandins (101), and corticosteroids (102) has been shown to inhibit NK function (39). Some of these agents seem to act through suppressor macrophages in mice (39, 103). Tumor growth itself can often inhibit NK activity by unknown mechanisms (39). On the other hand interleukin-2, a T-cell product, can also augment NK cytolytic function (104). NK cells therefore are enmeshed in a cellular network of regulatory influences within the immune system.

GENETIC AND CLINICAL FACTORS

Target-cell recognition in the mouse is under the control of autosomal dominant, H-2-linked genes on chromosome 17 (34), whereas the postbinding cytolytic pathway is blocked by mutations in the *bg* gene on chromosome 13 (105) and possibly other mutations on other chromosomes (106; reviewed in Ref. 39).

In the human, males have slightly higher NK activity than females (10, 12, 107), and an association between NK activity and HLA A3, B7 (low NK) (107, 108), and Rh negativity (high NK) (109) has been reported. Not all investigators have obtained similar results (10, 110). It is well established, however, that the level of endogenous NK activity in healthy donors is a stable characteristic of the donor, even when tested over many years (10), and it is possible to categorize healthy people as "high" or "low" NK responders. Even cord blood lymphocytes show distinct patterns of high and low NK activity (14), as well as the slight male/female difference seen in adulthood (10).

Differences between high and low individuals are due largely to differences in the triggering of active NK cells, as opposed to differences in recycling capacity (68). The frequency of HNK-1⁺ cells and target-binding cells does not differ between the two groups, suggesting that low NK responders have a "defect" in the postbinding cytolytic pathway. Chemiluminescence and O₂⁻ production upon target-cell contact was deficient, which suggests an impaired triggering mechanism in low NK donors (111). Studies on the *in vitro* BCG reactivity of low normal donors (112) indicate that these individuals are less reactive than those with high or normal NK activity. Others have found that the responsiveness of low normal adults (Pross unpublished) or neonates (14) to interferon is normal.

It is presumed that NK activity is genetically determined and analogous to the strain differences in NK activity seen in inbred mice (39). Family

studies have suggested that similar patterns of reactivity exist among family members (110) but the results are not clear-cut, both because of difficulties in NK quantitation (113, 114) and because marriages between donors at the upper and lower 10th percentile extremes of NK activity are infrequent (2/100). We have studied two pairs of monozygous twins, and the NK activity of each member of the pair was identical to that of the sibling. One of the pairs was studied 20 times over 4 years to establish that they both have similar, high NK activity. Since their NK activity was higher than that of all except 1 of 105 healthy females who were repeatedly tested, it is unlikely that this observation is due to chance. Identical NK activity was confirmed in these twins in a single-cell cytotoxicity assay, by HNK-1 staining, and using kinetic analysis methods (Rubin, Callewaert, and Pross, unpublished).

Several genetic diseases are also associated with an NK deficiency. The Chediak-Higashi (CH) syndrome caused a profound (59) and selective impairment in NK cytolytic activity (60, 115), whereas the frequency of HNK-1⁺ cells (24) and their ability to bind target cells (60) were normal. These results have been confirmed by two independent studies (96, 115). Patients with X-linked lymphoproliferative syndrome (XLP) also had impaired NK activity compared to phenotypically normal XLP carriers or normals (117). Patients with either XLP or CH usually develop fatal lymphoproliferative malignancies, which are clearly EBV associated in the case of XLP. Of the classical immunodeficiencies, only severe combined immunodeficiency (SCID) had markedly impaired NK function (118, 119). In general, patients with X-linked agammaglobulinemia, common variable immunodeficiency, hyper-IgM immunodeficiency, hypogammaglobulinemia, and immunodeficiency thymoma were relatively normal in NK function (120). In other disorders such as IgA deficiency, ataxia telangiectasia, and Wiscott-Aldrich syndrome some patients were low and others were normal in NK function depending on the investigation and the methods employed (119, 121).

THE EFFECT OF DISEASE

Low NK activity has been described in association with malignant disease (122-128), leukemia (22, 129-132), aplastic anemia (22), chronic ulcerative herpes simplex infection (133), chronic renal failure (134, 135), fibrocystic breast disease (128), and

diseases such as rheumatoid arthritis (136), systemic lupus erythematosus (136, 137), Hashimoto's thyroiditis (138), and multiple sclerosis (139, 140). In none of these disorders is there an invariable correlation between low NK function and disease, and some investigators have been unable to find any abnormalities in NK function in patients with multiple sclerosis (140) or rheumatoid arthritis (141). The existence of NK depression due to therapy has been reported in association with radiation therapy (142), extensive surgery (143), cytotoxic chemotherapy (131), prednisone (102), and diethylstilbestrol (144). Removal of these agents results in recovery of normal NK function in most patients, and it has also been reported by Guillou *et al.* (143) that NK function may gradually recover in spite of long-term immunosuppression with steroids and azathioprine. The mechanisms by which various diseases bring about NK depression are extremely complex and may involve one or more components of the NK system and modulating substances such as interferon and prostaglandins (145-147).

Decreased NK in MS patients was accompanied by a decrease in the ability to make or respond to interferon (139) (McGarry, personal communication). A similar defect was inferred by Hersey *et al.* in Fanconi's syndrome (148) and by Kadish *et al.* in a study of low NK donors with breast cancer (127). Low NK activity in most cancer patients with solid tumors and leukemia can be accounted for by a low frequency of cytolytic NK cells as detected in a single-cell assay, an observation similar to that made in low NK responder normals (Pross, unpublished observation). Immunosuppressive therapy may also result in reduced NK frequency as well as defective recycling capacity (68). In other studies suppression of NK in PBL, in lymph nodes, and *in situ* in cancer patients has been attributed to suppressor cells (126, 149-151), a conclusion based on the inhibitory effect of tumor-associated lymphocytes on NK cells *in vitro*. NK does not correlate with serum immune complexes in malignant disease (Dorval, Pross, and Baines, unpublished), and in most instances, the mechanisms underlying low NK activity have not been elucidated.

Elevated NK activity *in vivo* has usually been attributed to activation by interferon. A number of inflammatory disorders such as atopic dermatitis (152), chronic hepatitis (153, 154), incipient renal transplant rejection (143), and graft vs host disease (155, 156) have been correlated with increased NK activity, although it is unknown whether interferon

is the activating agent in all cases. As in animal models, viral (156, 157) and parasitic (158) infections lead to an elevation in NK activity which may precede or coexist with specific effector cells. The NK response of normal donors to viral infection was recently determined in a double blind trial in which influenza virus or placebo was administered intranasally to volunteers. NK-mediated lysis of K562 tumor cells and MRC-5 fibroblasts increased in parallel with interferon induction, peaking on the third day and correlating with clinical evidence of viral infection (157).

ANTITUMOR SURVEILLANCE IN MICE

As reviewed in detail elsewhere (39, 159, 160), there is a large body of evidence which suggests that NK cells play a role in host defense against transplantable tumors. More recently it has been shown that selectively NK-deficient (105) homozygous *bg/bg* mice are more susceptible to the growth and metastasis of transplantable, syngeneic tumors than their normal *+/bg* littermate controls (161–163), whereas no differences were found using NK-resistant tumors or regressing MSV-induced tumors (80), which are killed by a T cell-dependent mechanism rather than NK cells (164). In preliminary studies by Haliotis *et al.* beige mice have a higher incidence of spontaneous tumors (usually lymphomas) late in life, compared to a lower incidence in a group of age-matched littermate controls (165). As expected, treatment with NK-suppressive doses of carcinogens or radiation did not show any differences in the incidence of primary tumors in these two groups of mice (165, 166). In chickens NK cells may prevent the growth of tumors induced by Marek's disease virus (167). These data are compatible with the suggestion that NK cells play a role in host defense against primary oncogenesis, at least in the lymphoid system.

If NK cells are important in antitumor surveillance, then one would predict a positive correlation between NK sensitivity and tumorigenic potential in transformed cells. In one study (168) a cloned line of normal fetal fibroblasts from Balb/c mice was found to resist NK lysis *in vitro* and these cells did not grow in agarose and were nontumorigenic in normal mice or NK-deficient mice (type N cells). Clones of chemically transformed variants grew in agarose and formed tumors in NK-deprived mice (NK⁻) but not normal mice with high levels of NK

cells (NK⁺). These type I cells were highly NK sensitive *in vitro*. Cell lines established from type I cells growing as tumors in NK⁻ mice were NK resistant and grew as tumors in normal mice (NK⁺) (type C cells). The N-1 mutation leading to anchorage independence was linked to NK sensitivity, whereas a second mutation was required to escape surveillance and grow as a tumor in normal NK⁺ mice. In an independent study (170) it was found that a permanent cell line of L cells (presumably type I) was highly sensitive to NK cells, whereas several malignant sublines (type C) were NK resistant. There was no difference between malignant and nonmalignant sublines in terms of susceptibility to lysis by alloimmune CTL, macrophages, or antibody and complement. These findings in mice therefore explain experiments in the human whereby spontaneous tumors are often only poorly lysed by NK cells, if at all.

ANTITUMOR SURVEILLANCE IN HUMANS

Animal studies have shown that if tumor cells are NK sensitive *in vitro*, then animals with high NK activity are more efficient than low-NK animals at resisting the growth and metastatic spread of these tumor cells *in vivo* (39, 170, 171). It is also certain that this resistance is due to NK cells (171a). Conversely, patients with NK deficiencies such as CH or XLP have an increased incidence of malignancy (117, 172). These observations suggest that NK cells play a fundamental role in antitumor surveillance. Such a theory must take into account several seemingly paradoxical observations: (i) the vast majority of cancer patients in the early stages of disease has normal levels of NK activity (122–128, 151, 173), (ii) fresh tumors are relatively insensitive to lysis by NK cells (174, 175), (iii) lymphocytes isolated from tumor biopsies lack NK activity, (151, 176–178) (iv) interferon is relatively ineffective at inducing NK activity against autologous biopsy-derived target cells (179), and (v) the level of NK activity in irradiated leukemia patients after successful bone marrow transplantation does not correlate with the prevention of relapse (120).

All of these observations are compatible with the hypothesis which states that NK cells are circumvented by tumor variant formation (62, 81, 82, 180). As shown in the mouse (62, 81, 82, 181), early neoplastic cells may be highly NK sensitive, whereas variants can be selected which resist NK lysis

and grow up as an observable tumor. This mechanism may also be operative in metastasis formation: it was shown by Brooks *et al.* (182) that mammary adenocarcinoma cells isolated from some metastatic deposits in the rat were less NK sensitive than the primary tumor. Conversely, Gorelik *et al.* (181) selected an NK-resistant subline of Lewis lung carcinoma by *in vivo* passage and found that these cells had an increased potential for spontaneous metastasis formation. In the human, few studies have been underway long enough to relate individual NK activity in early-stage disease with ultimate survival. Hersey *et al.* (125) found that the postsurgical cell-mediated cytotoxicity of patients with melanoma correlated inversely with recurrence. Mukherji and Dayal (183) found no correlation between the development of new primary melanomas and NK activity in six patients. This "model" resembles one of surveillance more than studies on tumor recurrence. On the other hand, Morales (personal communication) has noted high NK activity in patients receiving intravesical BCG, a treatment which reduces the frequency of new transitional cell carcinomas in patients with unstable bladder epithelium (184, 185). Other investigators have also reported the stimulation of human NK activity *in vivo* with agents such as BCG (186), interferon (187, 188), poly(IC) (189), and Streptococcal Preparation OK-432 (190). A cause and effect relationship between NK function and apparent therapeutic benefit can only be inferred from these studies, however.

Escape from NK surveillance may also result from the local suppression of NK activity by tumor products [e.g., prostaglandins (147), tumor surface "antigens" (66, 191)] or even carcinogens and tumor promoters, such as has been shown with urethane (192) and phorbol esters (193). It is unlikely that NK cells function against allogeneic targets only, *in vitro*, since early work established that fresh, Fc receptor-positive lymphocytes from normal donors can lyse syngeneic EBV-transformed cells (4).

Based on the data from animal models, the peripheral blood and splenic distribution of NK-cell activity, the NK resistance of established tumors, and the lymphoproliferative nature of malignancies in NK-deficient patients, we support the hypothesis that NK cells are responsible for early surveillance against malignancies of the hematopoietic system and, possibly, blood-borne metastases from solid tumors.

OTHER FUNCTIONS

A detailed discussion of other functions which have been attributed to human NK cells is beyond the scope of this brief review. As mentioned above, NK cells may also be important in host reactivity against virally infected cells. Human cell lines which are NK resistant (Hela) become markedly NK sensitive *in vivo* in nude mice upon infection with a variety of persistent viruses (194). Infected cell lines are also sensitive to human NK cells (195, 196) and NK-deficient beige mice have a much lower LD₅₀ for murine cytomegalovirus than normal littermate controls (197), an effect which is due to impaired NK resistance rather than impaired viral replication. Since human NK cells lysed virus-producing cells at an early stage of the EBV cycle before virus particles were assembled, it was suggested that they may restrict the spread of virus from latently infected cells (198).

It is also possible that NK cells serve primarily as regulator cells of hematopoiesis. The evidence for this is derived mainly from studies on hybrid resistance by Cudkiewicz and colleagues (103) which show a high degree of parallelism between the characteristics of the parental murine spleen cells which are the effectors in F₁ bone marrow rejection and those of NK cells. In man, it has been suggested that bone marrow transplant-recipient NK cells (as defined by HSV-1-infected fibroblast lysis) interact with HLA A, B, C, and D matched donor hematopoietic cells. The result of this interaction was stimulation of the donor cells to produce graft vs host disease. In transplant-surviving donors with no NK (HSV-1) activity prior to transplant, no GVH disease was observed. Aside from the prognostic value of these NK results, it is possible that NK cell-hematopoietic cell interaction is the result of the NK cells attempting to carry out a normal regulatory function. Other evidence suggesting that NK cells may have a regulatory function stems from studies on patients with NK proliferation disorders. Although several patients have been described with T_H or K-cell lymphocytosis (199-201; reviewed in Ref. 201), only two have been described in which NK cells are functionally active and increased in number (200, 201). In these patients, leukopenia and recurrent infections were the principal presenting problems, suggesting that NK cells actively suppress leukopoiesis. Research into the effects of purified human NK cells on hematopoiesis *in vitro* will hopefully provide definitive answers as to the regulatory role of these cells.

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