

Natural Killer Cells in Relation to Disease and Treatment

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Key words: bone marrow transplantation; cancer; natural killer cells; virus.

Accepted for publication 5 May 1985

Natural Killer (NK) cells were discovered about 10 years ago (48, 82) and were for some years considered as ill-defined, lymphoid cells, mediating nonspecific spontaneous cytotoxicity. Although heterogeneity within NK cells exists (67) NK cells are today far from ill-defined. Their role *in vivo*, however, has still to be clarified.

Characteristics of NK cells

NK cells are mainly functionally identified by their ability to lyse a variety of target cells. NK cell activity is measured in a 51-chromium release assay. Recently, it has been possible to quantitate the cells by surface markers. In addition, the quality of NK cells can be detected by their ability to be boosted by interferon. NK cytotoxicity occurs as a two-step process. The first step involves recognition and binding of the NK cells to target cells, the second step is the lytic reaction which leads to the destruction of target cells. The ability of NK cells to bind the target cells can be detected in a single cell agarose assay.

NK cells are found within the same subpopulation of lymphoid cells, morphologically identified as large granular lymphocytes (LGL), that comprise about 5% of peripheral blood lymphocytes (for a recent review on the characteristics of NK cells, see ref. 67). LGL can be identified morphologically in Giemsa stained cytocentrifuge preparations of low-density cells separable by Percoll density gradient cen-

trifugation. LGL are characteristically large lymphoid cells with slightly indented nuclei; they possess distinct azurophilic cytoplasmic granules and range from 16-20 μ m in diameter. LGL are non-phagocytic non-adherent cells, that lack surface receptors for the third component of complement but contain low affinity receptors for sheep erythrocytes and Fc-receptors for IgG. The Fc-receptors allow the cells to bind antibody-coated target cells and mediate antibody-dependent cellular cytotoxicity (ADCC), a function previously attributed to the killer (K) cells. Today it seems established that the same cells, NK/K cells, are able to mediate both NK cell activity and ADCC.

The following monoclonal antibodies: Leu 11, B73, 3G8, OK 10 and OKM 1, react with most human LGL. Treatment of mononuclear cells with these antibodies plus complement results in a depletion of most or all detectable NK activity. NK cells are most numerous in spleen and blood and develop from stem cells in the bone marrow (27, 32); they develop in the absence of the thymus (32) and do not belong to conventional B or T lymphocytes or the macrophage-monocytic lineage.

In addition to mediation of NK cell activity, LGL are secretory cells, which in response to various stimuli release a variety of cytokines including interferon (IF), interleukin 1 (IL-1), interleukin 2 (IL-2), and B cell growth factor (BCGF). The ability of LGL to mediate NK cell activity and ADCC is dependent on an

intact secretory system, as these functions are inhibited if the secretory system is blocked (11, 74). The ability of NK cells to produce cytokines has also raised the possibility that NK cells are important regulatory cells, particularly in a variety of immune responses that are affected by, e.g., IF and interleukins. That NK cells rapidly produce IF and IL-2 following stimulation, and that NK cell activity is augmented by these cytokines, provides a mechanism for positive self-regulation.

NK cells lyse a wide range of targets including neoplastic cells, several types of normal cells, subpopulations of bone marrow and thymus cells, cells infected with viruses or cells infected with other microbial agents (30, 67).

Because of their spontaneous levels of reactivity and/or their ability to be rapidly activated, NK cells together with macrophages and polymorphonuclear cells seem well suited to play an important role in the first line of defense. It is possible that this broader range and readily available defense system controls tumor cells and virus infected cells until the more potent and specific immune system begins to respond adequately. If so, it would be understandable that a defect in the natural killer cell system could be the cause of many different diseases. In this review the focus will mainly be on NK cells in relation to disease and treatment.

Possible role of NK cells in immune surveillance

The concept of immune surveillance against cancer is primarily based on the considerable evidence that various forms of immunodepression have been associated with an increased incidence of tumors. Initially, it was suggested that T cells play an important role in immune surveillance against cancer. The importance of T cells seems, however, limited to tumors with strong tumor-associated transplantation antigens, particularly virus-induced tumors, and today it is clear that T cell-mediated immunity alone cannot account for resistance against development of tumors. For example, athymic nude or neonatally thymectomized mice do not have a particularly high incidence of various

types of spontaneous or carcinogen-induced tumors (33). However, this and other observations that argue against a central role of T cells in immune surveillance against cancer did not lead to total rejection of the basic hypothesis of immune surveillance, but to a consideration of the possible involvement of other effector cells, including NK cells. There are several lines of evidence that are consistent with a role of NK cells in immune surveillance against cancer, as summarized in Table 1.

It has been observed that NK cells have a rapidly activated ability to lyse a variety of established tumor cell lines and that NK cells accumulate at sites of inflammation and in small primary as well as transplanted tumors (21). In several murine models, a good correlation exists between high *in vitro* NK activity and *in vivo* resistance against neoplasia (26, 27, 83). It has also been reported that injections of NK-enriched lymphocytic populations delay tumor growth *in vivo* (43).

NK cells seem also able to eliminate metastatic tumor cells and thereby resist tumor spread. It was shown that cells from the lung metastases of a transplantable tumor in mice were more

Table 1
Evidence for a role of NK cells in immune surveillance against tumors

1. NK cells seem able to eliminate metastatic tumor cells and thereby counteract tumor spread.
2. NK cells accumulate at sites of inflammation and in primary as well as transplanted tumors.
3. NK cells lyse a variety of tumor cell lines.
4. Increased tumor incidence is found in individuals with depressed NK cell activity: (e.g. patients with Chediak-Higashi syndrome, X-linked lymphoproliferative disease and kidney allograft patients).
5. Some carcinogens (urethane, γ - or X-irradiation, and dimethylbenzanthracene) have been shown to cause depression of NK cell activity.
6. Low NK cell activity has been demonstrated in many cancer patients and patients suffering from premalignant diseases (e.g. carcinoma *in situ*, preleukemic syndrome and Sjögren's syndrome).

resistant to NK activity than locally growing tumor cells (22). Suppression or augmentation of NK cell activity of mice was associated with parallel alterations in resistance to artificial metastases produced by intravenous inoculation of tumor cells (23, 38). In mice it was also shown that the depression of NK activity induced by antibodies to NK cells, or chemotherapy, resulted in the promotion of metastatic diseases (86). From these studies it might be concluded that NK cells have a key role in the control of metastases.

In the murine system some carcinogens (urethane, gamma- or X-irradiation and dimethylbenzanthracene) have been shown to cause early depression of NK cell activity, while NK activity has been shown to be substantially augmented by retinoic acid, which has been reported to retard the development of some primary tumors (31).

In the beige mice, that have a high incidence of lymphomas, it has been described that a point mutation on chromosome 13 is responsible for a defect in NK activity (57).

While many experimental studies (*in vitro* and in animal models) have thus emphasized the role of NK cells in anti-tumor surveillance, in humans the role of NK cells is still uncertain.

NK cell activity in cancer patients

An increased tumor incidence has been found in individuals with depressed NK cell activity. Patients with the genetically determined Chediak-Higashi syndrome (CHS) have a high risk of development of lymphoproliferative disease. They have a selective deficit in NK and K cell activities, but a normal number of NK cells and normal target cell binding capacity. In addition, it has been shown that the direct addition of cyclic GMP to mononuclear cells of CHS patients corrects NK cytotoxicity in a dose-dependent fashion (44). Another genetic abnormality of X-linked lymphoproliferative disease has been associated with a defect in the ability to control proliferation of B cells infected with EBV, and it has been shown that the patients have very low NK cell activity (101). Patients receiving immunosuppressive therapy,

e.g. recipients of renal allotransplants, have a high risk of developing tumors and a very low NK cell activity (52).

Reduced NK cell activity has been reported in several groups of patients with malignant disease, especially lymphoid malignancies. In untreated patients affected with malignant lymphoma of Hodgkin's as well as non-Hodgkin's type, NK activity was depressed to the same degree in stages I-IV (29, 104). Other workers, however, found normal cytotoxic activity in the blood of patients with Hodgkin's disease in all but the most advanced stages (28, 29), while increased NK activity of cells from spleens and lymph nodes in patients with Hodgkin's disease has been reported (1, 85).

Patients with chronic lymphocytic leukemia (CLL) have a high incidence of secondary malignancies, and several workers have found severely impaired NK cell activity in the blood of CLL patients (47, 106, 112). The impaired NK cell activity in CLL patients is associated with cells that are phenotypically and morphologically NK cells, but which lack azurophilic granules that are thought to play a role in NK-mediated lysis (47). In patients suffering from acute myeloid leukemia (3, 100), or acute lymphoblastic leukemia (51), low NK cell activity corresponded to clinically active disease, while patients in remission exhibited normal cytotoxicity. Furthermore, depressed NK activity has been described in patients with hairy cell leukemia (84), aplastic anemia (53), and lymphohistiocytosis syndrome (77).

Regarding the NK cell activity of patients with non-lymphoid solid malignancies, both normal and deficient NK cell activity has been found (20, 95, 97, 104).

Untreated women with malignant breast disease had low NK activity and low numbers of LGL compared to women with benign breast disease, or healthy controls. Depressed NK activity correlated to the tumor spread, but not to estrogen or progesterone receptor states (19, 25, 109). Patients with ovarian carcinoma had lower blood NK cell activity than normal individuals. Moreover virtually no NK cell activity was detected in ascitic fluids of these patients. The decreased blood NK cell activity

of ovarian cancer patients was significantly augmented after intradermal injections of a virus-modified tumor cell extract, and the NK cell activity in ascitic fluids was similarly augmented after intraperitoneal administration of the same extract (56, 60).

In addition, low NK activity has been found in a wide variety of cancer patients including patients with cervical cancer (79), lung carcinoma (87, 90), melanoma (87), hepatocellular carcinoma (93), adenocarcinoma of the pancreas (18), oral cancer (38) and colon cancer (5). Generally, there is normal or minimally depressed NK activity in patients with primary localized tumors of a variety of types, but a marked diminution in a proportion of patients with disseminated or metastatic disease. Advanced cancer patients with low NK activity show no decrease in blood concentration of LGL, but the function of the NK cells was defective. The defective function included reduced binding and recycling capacity as well as decreased killing activity (97, 107).

NK cell activity in patients at risk for cancer

The demonstration of significantly defective NK cell activity in many studies of patients with advanced malignancy, suggests that there is a role for NK cells in resistance to tumor growth and spread. The question raised by these results is, however, whether the impaired immune function precedes or is a consequence of the tumor. If deficient NK activity contributes to the development of cancer, one might expect patients at risk for cancer to have a deficiency in natural cytotoxicity.

In 79 normal individuals NK activity correlated with the incidence of cancer in each individual's family. NK cytotoxicity was significantly lower in individuals with a high family incidence of cancer compared to those with a low incidence of cancer (99). Moreover, NK activity correlated inversely with the number of family members with cancer. In another study low NK activity was found in patients with familial malignant melanoma and their close relatives (34).

Of great importance is the analysis of the NK function in patients who suffer from diseases considered to be premalignant. Patients with primary acquired preleukemic syndrome frequently develop AML, and recently a defective NK function has been found in patients with preleukemic syndrome (50, 102). Among untreated patients with cervical precancerous lesions about one third develops cervical carcinoma. An impaired NK activity was found in 26 % of patients with carcinoma *in situ* (89), but it is not known if the subset of women with diminished NK cytotoxicity are more likely to develop invasive cancer. Patients with liver cirrhosis represent a group of subjects at high risk of developing hepatocellular carcinoma. NK activity was significantly reduced in patients with non-alcoholic liver cirrhosis, but no association existed between HBsAg positivity and NK activity (64).

As certain autoimmune disease may be considered premalignant it is of interest that a defective NK activity has been described in this group of diseases as well (Table 2).

NK cell activity in autoimmune diseases

It is possible that, in addition to genetic predisposition, viral infections may play a role in the pathogenesis of immune inflammatory diseases, and it has been proposed that a defective antiviral immunity could explain such viral infections. In order to investigate this possibility NK cell activity has been studied in patients with certain autoimmune disease (Table 2).

Significantly reduced NK cell activity has been found in a number of studies in patients

Table 2	
Autoimmune diseases associated with low NK activity	
	Systemic lupus erythematosus
	Sjögren's syndrome
	Multiple sclerosis
	Graves' disease
	Crohn's disease
	Primary cardiomyopathy

with systemic lupus erythematosus (SLE) (15, 35, 42, 45, 46, 59, 65, 68, 91, 98), Sjögren's syndrome (SS) (59, 62, 63, 75) and multiple sclerosis (MS) (110, 111).

Concerning the NK cell activity of rheumatoid arthritis (RA) conflicting results have been obtained, as both unchanged (12, 65, 71), reduced (42), as well as slightly enhanced NK cell activity in the blood (59) has been reported. Reduced NK cytotoxicity was found (12) in lymphocytes from the synovial tissue of RA patients.

Some of these studies include RA patients receiving drugs which are known to influence NK cell activity (42, 58, 65). A comparison between 45 patients who had not received remission-inducing drugs and 45 controls did not show any significant difference in baseline and interferon-enhanced NK cell activities (71). In SLE and SS impaired NK cell activity as well as IF-enhanced NK cell activity, but normal target cell binding capacity, has been found, and it has been suggested that the presence of circulating immune complexes in these diseases may inhibit the NK cell function (62, 63, 76, 91). The recent finding of a defect in NK cell function of patients with primary cardiomyopathy (2) supports the hypothesis of an autoimmune pathogenesis in this disease. A reduced number of LGL have been found in Graves' disease (37), while there have been conflicting results regarding the NK cell activity in Crohn's disease (4, 9).

In most autoimmune disease states, in which a natural killer defect has been found, a defect in suppressor cells has been identified. It has been suggested that suppression of NK cell activity might be the result of a loss of normal surveillance mechanisms predisposing toward autoimmunity through the failure to eliminate autoreactive B lymphocyte clones. But whether this loss of activity is a primary or secondary phenomenon is not known. SS is known for its high incidence of lymphoid neoplasms, and in MS an increased incidence of pulmonary tumors and perhaps malignant tumors in general malignancy has been reported. The cancer diathesis of SLE patients has been discussed, while there is no increased incidence of malignancy in RA. As NK activity may reflect resistance against malignancy the finding of low NK activity in SS, MS and SLE, but normal NK activity in RA is consistent with the immune surveillance theory.

Possible roles of NK cells in resistance against microbial infections

There is increasing evidence for a role of NK cells in resistance against some microbial infections (Table 3). Human NK cells show high cytolytic reactivity against cells infected with a wide variety of viruses, including herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein Barr virus (EBV), mumps, influenza, Sendai and measles viruses. Generally, cells persistently infected with virus are more susceptible to NK lysis than acutely infected cells (80). In several animal studies resistance to infections by some viruses is correlated with high levels of NK activity. NK cells seem to play an important role in natural resistance to another herpes virus, Marek's disease virus in chickens, may be mediated by NK cells. NK activity may also be involved in resistance against the protozoan parasite *Babesia microti* and the fungus *Cryptococcus neoformans* in mice (33).

In humans the best studied system in which NK cell activity appears to correlate with resistance to infection is that of herpes simplex. During the acute episode of herpes the individuals show elevated levels of NK activity. A heightened cytotoxicity persists for several

Table 3

Evidence for a role of NK cells in resistance against microbial infections

1. NK cells show high cytolytic reactivity against virus-infected cells.
2. Role of NK cells in resistance to several infections in animals, including CMV, HSV, Marek's disease virus, *Babesia microti* and *Cryptococcus neoformans*.
3. Drop in NK activity before reactivation of latent herpes simplex virus infections in humans.

weeks during prospective follow-up studies of the donors, but NK cell activity falls to low levels during the last week before the next episode of herpes labialis (81). The finding of low NK activity against CMV infected targets in renal transplant recipients receiving immunosuppressive therapy may be responsible for the severe CMV infections in these patients (94). Influenza virus infections increased the level of natural killer cell activity together with induction of interferon (17).

NK activity and IF levels have been measured in the blood of children acutely ill with *Plasmodium falciparum* infection. Malaria patients expressed an increased level of NK activity, which was positively correlated to the degree of parasitemia (66). Patients suffering from chronic schistosomiasis had normal NK activity (6). NK activity was significantly suppressed in a group of patients with lepromatous leprosy who were undergoing an episode of erythema nodosum leprosum (36).

Few studies have been done on the relation of NK cells to bacterial infections. It was, however, recently shown that during the primary syphilitic infection NK cell activity increases, but is significantly reduced during the progression of the disease (40).

NK activity in relation to other diseases

NK cells show high cytolytic reactivity both *in vivo* and *in vitro* against a subpopulation of normal bone marrow cells, including colony-forming stem cells, and it is now generally accepted that NK cells are the main effector cells in resistance to bone marrow transplants (31, 33). It is of interest that a relation has also been observed between NK cell activity and graft versus host (GVH) disease. GVH disease is more likely to occur in recipients who have high pretransplant levels of NK activity against virus-infected target cells. It is also reported that the occurrence of acute GVH disease was associated with an early reappearance of NK activity, within the first 4 weeks of grafting. In contrast, in patients not developing acute GVH disease, NK activity returned a few weeks later (13, 55). It is thus possible that high reactivity

of either donor or host NK cells against normal tissues of the recipient may contribute to the manifestations of GVH disease.

Asthma is related to high NK activity as it has recently been shown that asthmatic patients have significantly higher NK activity compared to controls (103). Patients suffering from atopic dermatitis are unusually susceptible to skin infections. Most of the patients have abnormal humoral and cellular immunity, which probably accounts for their decreased resistance to infection by microorganisms. Reduced NK activity was recently found in these patients (39). The acquired immunodeficiency syndrome (AIDS) is associated with severe opportunistic infections and in some cases with Kaposi sarcoma. The patients have abnormally low numbers of T helper cells and, in addition, deficient NK cell activity (54, 92).

NK activity in relation to therapy

Recently, reports on the influence of medical treatment on NK cell activity have appeared (Table 4). Concerning treatment of cancer patients, several authors have reported that various forms of cytotoxic drugs, radiation therapy and even surgery, may inhibit the NK cell activity (8, 61, 95, 108). On the other hand, treatment of cancer patients with interferon and other immunostimulants including BCG, OK 432 (a streptococcal preparation), azimexon and a virus-modified tumor cell extract has augmenting effect on NK cells (16, 69, 78).

Many drugs used in the treatment of immunoinflammatory diseases, e.g. RA and SLE,

Table 4
Modulation of NK activity by therapy

Augmentation	Inhibition
Interferon	Cytotoxic drugs
BCG	Radiotherapy
Azimexon	Oestrogens
Virus-modified cell extract	Corticosteroids
	Azathioprine
	Cyclophosphamide
Gold salts	Theophylline
Adrenaline	Terbutaline

may influence NK cell activity. Corticosteroids have profound effects on the human immune system, including NK cell activity. It has been stated that corticosteroids induce suppression of NK cell activity in SLE (68) and healthy volunteers (69). Other studies did not find any relationship between NK cell activity and prednisone treatment in SLE (15, 46). High-dose intravenous methylprednisolone produced a considerable fall in NK cell activity in RA patients, and 3 months later the NK cell activity was still less than half of that before methylprednisolone pulse therapy (72). Single-drug treatment of RA patients with azathioprine induced complete suppression of baseline- and interferon-enhanced NK cell activity (70, 73). That the inhibitory effect on NK cell function during azathioprine treatment occurs gradually with total inhibition being reached only after 5–8 months, that the suppressed NK cells cannot be boosted by IF, and that azathioprine and its metabolite 6-mercaptopurine do not have any inhibitory effects *in vitro* are compatible with the hypothesis that azathioprine or its metabolites act on precursor NK cells. This again would imply that the lifetime of NK cells is 5–8 months. The NK activity of renal transplant recipients treated with both prednisone and azathioprine was similarly suppressed (52). Treatment of MS patients with cyclophosphamide causes further suppression of their defect NK function (7). Both suppressed and enhanced NK cell activity in patients receiving gold therapy for RA has been described (42, 59), while penicillamine does not seem to influence NK cell activity (71). Prostaglandin (PGE)-mediated inhibition of NK cell activity as well as enhancement of NK cell activity by prostaglandin inhibition *in vitro* has been described (10, 14). Addition of indomethacin (1 µg/ml) to mononuclear cells from patients with Sjögren's syndrome and controls augmented the NK cell activity (Pedersen, B. K., et al., unpublished results), while it is unknown if treatment of patients with indomethacin or other prostaglandin inhibitors influences NK activity in humans.

In vitro experiments showed that inducers of cyclic AMP decreased NK activity, while inducers of cyclic GMP increased NK activity (45). Recently, it was shown that injection of

theophylline or terbutaline to asthmatic patients reduced the NK activity (96). On the other hand, administration of adrenaline to healthy human subjects caused an increased NK activity (105).

Experimental work in the mouse has suggested that oestrogens may influence NK levels and it was shown that mouse oestradiol, which is itself non-toxic for NK cells, may suppress NK activity, perhaps via its effect on the bone marrow, though doses of oestradiol 9 times the physiological level are required for 4–6 weeks before a decline in activity is seen. Neonatal administration of diethyl stilboestrol to female mice also suppresses NK activity (41, 88). There is no clear evidence linking oestrogens and progesterone with NK activity in humans, but, interestingly, it was found that healthy women donating blood during the first half of their menstrual cycle showed significantly reduced NK activity in comparison to those in the second half (19).

Conclusion

NK cells seem to be of importance in immune surveillance against cancer and microbial infections, and evidence for a role of NK cells in bone marrow transplantation and autoimmune diseases is forthcoming. Recent data have shown that several kinds of therapy influences NK cell activity, which is of potential clinical importance from two different standpoints. On the one hand, various forms of conventional therapy inhibit NK activity and might thereby have potential effects on resistance against tumor growth and infections. On the other hand, some treatments augment NK activity and such an effect might contribute to the therapeutic efficacy of the agent.

In future investigations of NK cell activity in patients with various diseases, the possible influence of medication should be taken into consideration.

ACKNOWLEDGEMENTS

I am grateful to Drs. V. Andersen and G. Bendixen for valuable discussions. The work was supported by the Danish National Association against Rheumatic Diseases.

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