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REVIEW

The Role of Natural Killer Cells in Viral Infections

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Natural killer (NK) cells are important effectors for the lysis of both neoplastic and virus-infected cells. Lectin-like receptors on human NK cells, such as NKR-PIA and CD94, bind to target cell carbohydrate ligands and initiate the lytic process. In addition, P58 and P70 bind to major histocompatibility class I antigens on targets and mediate negative signals. Models using NK cell-deficient mice have proven useful in elaborating the role of NK cells in the immune defence against multiple viral agents. In addition, studies in humans have suggested a vital role of NK cells in the host defence against human immunodeficiency virus, herpesviruses, hepatitis B and C and other viruses. Several genetic disorders, chronic illnesses and infections have been associated with decreased NK function.

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INTRODUCTION

Natural killer (NK) cells are large granular lymphocytes that lyse tumour or virus-infected cells. Mounting evidence suggests that these cells are important in the early response of the immune system to many viral infections [1]. Although acquired deficiency in NK function has been described in the chronic fatigue syndrome (CFS) [2], human immunodeficiency virus (HIV) infection [3], metastatic carcinoma [4], autoimmune disorders [5], depression [6] and exposure to occupational chemicals [7]; the clinical significance of the reduced NK function in these disorders has not been established. The Chediak–Higachi syndrome [8] and leucocyte adhesion deficiency [9] are genetic disorders characterized by profiles of depressed immune response, each of which includes a depression of NK-cell function. Patients with these disorders suffer from severe, recurrent infections, haematological malignancies and early mortality. In the present review, the basic immunology of NK cells, the importance of NK function as derived from viral infections in animals and the role of NK cells in specific viral infections in humans are discussed. Other human conditions associated with diminished NK function are considered.

BASIC IMMUNOLOGY OF NK CELLS

Immune mechanisms are mediated by multiple cell types. An anatomic classification can identify granulocytes, monocytes and lymphocytes. A functional classification of lymphocytes can further distinguish B cells, T cells and NK cells, utilizing the expression of specific cell surface glycoproteins and/or the specific mechanism by which the cell protects the host from foreign antigens. NK cells are larger than most B or T cells, ranging in diameter from 12 to 15 μm. They originate and differentiate in the bone marrow, and they ultimately represent 5–10% of the total mononuclear cells in the blood. Characteristics of these cells include a kidney-shaped nucleus and abundant cytoplasm containing numerous azurophilic granules. They are non-adherent cells. Most express the surface glycoprotein markers CD2 (sheep red blood cell receptor), CD16 (Fc receptor), CD45 (leucocyte common antigen), CD56 (NCAM), CD57, CD11a/CD18 (lymphokine function-associated antigen-1 LFA-1), CD11b/CD18 (Mac-1) and CD11c/CD18 (p150,95) [10, 11]. Some also express the T-cell markers CD7, CD8 and CD38. NK cells are often distinguished from other mononuclear cell populations by the presence of CD16 and CD56 molecules in the absence of CD3.

NK cells secrete several cytokines including interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-3 and granulocyte–monocyte colony-stimulating factor (GM-CSF) [12]. Foreign antigens, ligand-binding to the Fc receptor (CD16), treatment with IL-2 or binding of NK cells to preplasma cells can induce transcription of cytokine genes and secretion of the biologically active proteins [13]. NK cells, unlike T cells, are rapid and efficient producers of cytokines when stimulated even

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in a non-preactivated state, providing the potential for an important role in immune system regulation at an early stage of immune response [14].

Although NK cells can lyse certain normal stem cells as well as a variety of infected or transformed cells, cells vary significantly in their sensitivity to NK-mediated lysis [15]. It has been demonstrated that the binding of NK cells is controlled to some degree by the expression of major histocompatibility complex (MHC) class I molecules on target cells. There appears to be an inverse correlation between NK-cell sensitivity and expression of MHC class I antigens. Cells that lack surface expression of MHC class I molecules are susceptible to NK cell-mediated lysis and transection of these target cells with class I MHC genes makes them resistant [16]. Human NK cells express the surface receptors P58 and P70, which bind to target MHC class I molecules and inhibit cytolysis [17]. Receptors of the Ly-49A family play a similar role in rodents [18]. However, P50 receptors appear to activate cytolysis after binding to target MHC-I antigens [19]. CD2 and CD11a molecules on the NK surface may be important in NK cell binding to targets since monoclonal antibodies directed against these glycoproteins inhibit NK cell-mediated cytolysis [20, 21]. The respective ligands for these molecules, LFA-3 and intracellular adhesion molecule-1 (ICAM-1), are therefore candidate target cell ligands and CD2–LFA-3 interaction has been shown to trigger NK-cell cytotoxicity [22]. Another potential receptor is a protein expressed on the surface of NK cells which is closely related to the intracellular protein vimentin [23]. Monoclonal antibodies directed against the latter heterodimer block NK-mediated lysis and stimulate signal transduction in the cell. This molecule appears to be highly conserved in NK cells from many species. The transferrin receptor is also a candidate binding site on target cells [24].

However, recent work has focused on a superfamily of calcium-dependent lectin-like NK receptors encoded in a single genetic region called the NK complex [25]. These receptors include NKR-P1 and NKR-PIA families in rodents and humans, respectively, [26, 27]; Ly-49 (in mice and rats); NKG2 (humans and rats) and CD94 (humans). NKR-P1 and NKR-PIA bind to target cell carbohydrate ligands and trigger effector functions such as cytolysis and the release of cytokines such as IFN-γ [28]. Ly-49 and NKG2 inhibit lysis through activation of a cytoplasmic receptor (immunoreceptor tyrosine-based inhibitory motif; ITIM). CD94 usually mediates positive effector functions [29] but can cause inhibition via heterodimer formation with NKG2 [25].

When NK cells alternatively lyse cells through antibody-dependent cellular cytotoxicity (ADCC), another bond between target cell and NK cell can be identified. During the latter process target cells are coated with specifically bound immunoglobulin molecules which also bind to the low affinity Fc receptors (CD16) on the NK-cell surface.

The major physiological function of NK cells so far identified is the lysis of cells with foreign antigens, i.e. tumour cells or cells infected with intracellular pathogens. The NK response that occurs after initial contact of foreign antigens with the host immune system can be divided into the following phases: activation of resting NK cells to an enhanced state of cytotoxicity; proliferation; local accumulation; and lysis of target cells. With regard to *activation* of resting NK cells, several cytokines have been shown to enhance the cytotoxic capacity of NK cells both *in vitro* and *in vivo*. The cytokines are released primarily by T helper cells when they encounter foreign antigens. These include IL-2 [14], IL-7 [30], IL-12 [31] and all three classes of interferon (α, β and γ) [32]. IL-4 and IL-12 may be synergistic in their activity [33]. Morphological changes are induced by these activators, including a significant increase in the number of cytoplasmic granules. The expression of CD2 and CD16 molecules is enhanced. Corticosteroids [34], hepatitis B surface antigen [35] and ligand-binding to the IgM-Fc receptor site [36] suppress NK activation. The inhibitory effect of corticosteroids can be blocked by beta-endorphins and adrenocorticotrophic hormone (ACTH) [34]; vasoactive intestinal peptide (VIP) is capable of reversing the suppressive effect of hepatitis B surface antigen [37].

The importance of *proliferation* can be inferred from the studies that demonstrate a correlation between the number of NK cells and their ability to kill target cells [6]. The NK-cell proliferation is part of the response to activating cytokines produced by other immune cells and an expansion of NK cells can be demonstrated *in vivo* both in the periphery and in the bone marrow [6].

Accumulation of circulating NK cells at the site of target cells increases the local efficiency of target cell lysis. Local cytokine production plays an important role in concentrating the NK cells at the site of the target cells [6]. The rate of accumulation may also depend upon the location and poorly defined intrinsic characteristics of the target cells. When NK cells bind to target cell mechanisms of signal transduction are induced via the formation of inositol phosphates and an increase in intracellular Ca^{2+} [38–41]. There is evidence suggesting that phospholipase A2 activation and arachidonic acid generation are important in NK-cell activation [42].

Lysis of target cells occurs after cytoplasmic granules secrete their contents into the area of contact with the target cell in a manner similar to cytotoxic T cells [43]. The granules contain several proteases and nucleases as well as perforin, a molecule which polymerizes to form pores in the membrane of the target cell [44, 45]. The pores act as ion-permeable channels, which permit accumulation of ions in the target cell, increasing the osmotic pressure and inhibiting the escape of water and other ions. Consequently, the target cells swell and eventually lyse. Other mechanisms such as proteolytic degradation and endonuclease-induced fragmentation of nuclear DNA are also occurring.

In summary, NK cells are large, granular lymphocytes that lyse tumour or virus-infected cells.

ANIMAL MODELS OF NK FUNCTION

Murine models have been utilized to demonstrate the role of NK

cells in the immune response to many viral pathogens and tumours. Since YAC-1 cells are sensitive to murine NK cell-mediated cytolysis *in vivo*, they provide a convenient system for evaluation of NK function [46]. It is possible to demonstrate the enhancement of splenic NK-cell function in mice infected with specific micro-organisms in response to interferon induced by the infection. The degree of NK stimulation induced by infection has been correlated with microbial replication in target tissues. Furthermore, murine NK cells express a glycolipid, ganglio-*n*-tetraosylceramide (asialo GM1), on their cell surface. Some strains also express the NK1 and NK2 antigens. NK cells have been depleted *in vivo* by the administration of monoclonal antibodies directed against these antigens. The role of NK cells in limiting infection with specific viruses or the spread of tumour cells has then been assessed in the NK-depleted mice. Finally, beige mice homozygous for the *Bg* gene have profound depression in NK cytolytic functions, and have served as a useful model in the evaluation of NK-sensitive tumours and microbes.

Using these methods, studies in mice suggest that NK cells are important with enteroviruses and several other viruses. NK cells from the spleens of mice infected with a pancreatropic strain of coxsackievirus B4 (CVB4) manifest increased activity 4 days postinoculation compared to those from uninfected controls [47]. Furthermore, pathological lesions in the pancreas were least extensive in those strains of mice with the highest NK activity. In mice depleted of NK cells with anti-asialo GM1 antibody prior to infection, resistance to infection of the pancreas was significantly diminished when compared to mice with intact NK-cell populations. Such target tissue protection from acute virus replication was reported in similar studies with two other enteroviruses, CVB3 [48] and encephalomyocarditis virus D variant [49] as well as human herpes simplex virus type 1 [50].

There has been a demonstration that the significance of NK cell-mediated resistance to infection is not uniform for infection with all viruses. Groups of normal mice were first depleted of NK cell by the administration of anti-asialo GM1. Mice then infected with murine cytomegalovirus (MCMV), vaccinia virus or mouse hepatitis virus had significantly higher titres of virus in their livers and spleens and more extensive histopathological abnormalities in the liver as compared to NK-competent controls [51]. On the other hand, NK-cell depletion had no effect on virus titres in acute or persistent lymphocytic choriomeningitis virus-infected mice. These studies have been confirmed [46, 52].

Several studies have demonstrated the importance of NK cells in protection against pulmonary infection with myxoviruses. C57BL/6 mice are susceptible to lethal pneumonia after challenge with Sendai virus, a murine paramyxovirus [53]. Animals treated with antibodies against the NK 1.1 and asialo GM1 markers had increased severity of infection. On the other hand, treatment with low doses of anti-CD3 antibody protected animals from acute infection. The protective mechanism was due to a strong NK response via production of stimulatory cytokines.

There are data which suggest that pulmonary NK cells are important in controlling local replication in mice challenged with influenza A virus by the intratracheal route [54]. Mice first depleted of either pulmonary or systemic NK cells by intratracheal or intravenous anti-asialo GM1 antibody, respectively, had diminished survival after viral challenge compared to immune competent controls. Rats treated with 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) has decreased NK-cell activity and increased virus titre compared to controls after pulmonary challenge with influenza A virus [55]. Nude mice perish from influenza A unless they are also treated with IL-12. However, the protective effect of IL-12 is eliminated if the animals are also given anti-asialo GM1.

There is evidence that NK-cell responses to infection in terms of antiviral properties, activation and proliferation occur independently of T or B cells or their products. Mice with severe combined immunodeficiency (SCID) lack functional T or B cells, but NK-cell function and number is retained. In SCID mice infected with MCMV, NK cells proliferated and increased in number comparable to normal, virus-infected mice. In virus-infected SCID mice previously depleted of NK cells by anti-asialo GM1, the replication of MCMV was greatly enhanced and survival diminished [52]. Furthermore, MCMV infection in SCID mice induced the production of IL-12, which had direct antiviral effects but also conferred resistance to infection by stimulating NK cells to produce IFN-γ [56]. Finally, mice homozygous for the Ikaros null mutation lack all lymphocyte populations and had very little resistance to multiple viral infections [57].

Additional work with NK-depleted mice has demonstrated that virus could be isolated from the eyes of 50% of NK-depleted mice inoculated intraperitoneally with MCMV compared to none of the infected, normal mice [58]. In addition, after intravitreous challenge with MCMV, viral titres and histopathological changes in the eyes were significantly greater in NK-depleted mice compared to normals.

A study using NK-deficient C57BL/6 mice suggested that NK cells inhibit MCMV infections in spleens and livers by two different mechanisms [59]. In the spleen, the NK cells caused lysis in a cytotoxic, perforin-dependent manner while in the liver production of IFN-γ by activated NK cells appeared to be the predominant mechanism.

Thus, NK-deficient mice serve as a useful model for studies designed to establish the role of NK cells in the host response to infections with viruses. The accumulating evidence suggests that NK cells are important in immune defence to several viral agents including orthomyxovirus (influenza), herpesviruses, enteroviruses and one paramyxovirus.

ROLE OF NK CELLS IN VIRAL INFECTIONS IN HUMANS

A number of studies in human cells and/or in humans have demonstrated the importance of human NK cells in combating certain viral infections. In addition, treatment of humans with biologically active agents toward NK function, such as IFN-α, IL-2 and IL-12 has demonstrated efficacy in disorders associated

with depressed NK function. Considerable evidence has implicated NK cells as mediators of host defence against infections in humans with varicella zoster (VZV), herpes simplex (HSV), cytomegalovirus (CMV), Epstein–Bar (EBV), hepatitis B (HBV) and C (HCV), and human immunodeficiency (HIV) viruses.

Several investigations have suggested a role for NK cells in limiting infection with members of the herpesvirus group. NK function of normal children with natural varicella is significantly higher against VZV-infected and uninfected targets than NK function of uninfected children [60]. Immunosuppressed children with cutaneous herpes zoster showed significantly reduced NK activity within 3 days of the onset of lesions [61]. Their NK activity rose to normal levels during the healing phase. Another study reported depressed NK function early during the process of reactivation of VZV [62] with gradual improvement during the recovery stage.

It has been demonstrated that activated human NK cells lyse HSV-1-infected target cells *in vitro* [63, 64] and progression of HSV infection *in vitro* is limited by the presence of human NK cells. Similar results have also been demonstrated *in vitro* for human CMV-infected cells [65, 66]. It was also demonstrated that CMV early gene products in CMV-infected cells induced the expression of the transferrin receptor, a candidate molecule for NK-cell target binding [67]. In another study, high NK activity was demonstrated in patients with EBV-induced mononucleosis [68]. Lymphokine-activated killer (LAK) cells from the peripheral blood lymphocyte population in these patients demonstrated high activity against EBV-infected cells. The LAK cell population was shown to be composed predominantly of activated NK cells [69].

A significant role for NK cells in limiting replication of HBV and HCV has been suggested by several studies. One study demonstrated high NK-cell activity from 17 hepatitis B virus-infected patients compared to uninfected controls early in the course of acute infection [70]. HBV DNA-positive patients showed increased activity compared to HBV DNA-negative patients. In the recovery phase of the illness, NK activity returned to normal. In another study, NK function in patients with chronic active HBV infection was reduced compared to normal controls [71]. Finally, NK-cell function was reduced *in vitro* by HBV surface antigen [37].

Less compelling evidence of the role of NK activity in hepatitis B can be inferred from the multiple studies which have revealed efficacy of IFN-α therapy in patients with chronic HBV infections [72–74]. Response rates after 3–6 months of therapy ranged from 40% to 60%. Several parameters of the therapeutic response were assessed including decrease in serum alanine aminotransferase (ALT) levels, improvement in liver histopathological abnormalities, and loss of Hepatitis B e antigen (HBeAg), HBV DNA and HBV surface antigen in the serum. The mechanism of response to therapy remains controversial. Although NK function was enhanced by therapy, most investigators have felt that direct anti-viral effects of the IFN on virus-infected hepatocytes were primarily responsible for clinical response [75].

Histological examination of liver tissue harvested from patients with chronic active HCV infection has revealed the presence of NK cells [76]. Indirect evidence for the importance of NK cells in limiting chronic HCV infection was demonstrated by two studies of infected patients treated with IFN-α [77, 78]. Patients that responded clinically to therapy also demonstrated significantly greater enhancement of NK activity than controls after the initial dose of IFN-α. NK activity was not affected in treatment non-responders.

NK cells are capable of lysing HIV-infected cells [79]. Several investigators have demonstrated that NK-cell activity is decreased in HIV-infected patients [80] and attempts have been made to identify the nature of the defect in NK function. One study showed that HIV infection altered NK-cell activity by several mechanisms including a decrease in the number of NK cells; an alteration in the target cell recognition mechanism; and a post-binding inhibition of signal-transduction pathways [81]. The HIV-specific ADCC mechanism also declines with disease progression due to both a defect in the ability to generate anti-HIV antibodies required for ADCC as well as diminished lytic capacity of the NK cells [82, 83]. Several studies have demonstrated that cytotoxicity of NK cells from HIV-infected patients can be increased *in vitro* by co-administration of several cytokines including IFN-α [80], IL-2 [84], and IL-12 [31]. Therapy with IFN-α in HIV-positive patients increased several parameters of immune function, including NK-cell activity.

However, significant clinical response was not documented [85]. HIV-infected patients treated with IL-2 manifested a favourable clinical response [86]. Infection with human papillomavirus [87], HIV, HSV-1, or human herpesvirus 6 (HHV-6) inhibits NK function, presumably as a means of evading immune surveillance. The mechanism for this suppression of NK function is not completely understood. HHV-6 both infects, and is capable of lysing NK cells [88]. The function of NK cells is diminished after contact with HSV-1-infected target cells. This decreased activity is dependent on both close target-NK-cell interaction and viral entry into the NK cells [89].

Thus, studies in human cells and/or in humans have suggested a role for NK cells in the immune response to several viral pathogens including members of the herpesvirus group, HBV, HCV and HIV.

Genetic deficiencies of NK function have been described. Chediak–Higachi syndrome is a rare autosomal recessive disorder characterized by defective granulocyte and NK-cell function [90]. Patients present with a variety of clinical and laboratory abnormalities including occulocutaneous albinism, neurological changes; hepatosplenomegaly, diffuse lymphadenopathy, anaemia and thrombocytopenia. They suffer from recurrent bacterial, fungal and viral infections, and many develop lymphoid malignancies. Peripheral blood smears reveal giant blue-grey, peroxidase-positive granules in the cytoplasm of granulocytes. Chemotaxis and intracellular microbicidal activity are reduced in neutrophils. Natural killer cells demonstrate delayed killing kinetics. Most affected patients die from complications of infection or malignancy at an early age.

Heterozygous carriers can be identified by the presence of leucocyte granulation abnormalities on peripheral blood smears.

When peripheral blood mononuclear cells isolated from patients with Chediak–Higachi syndrome were stimulated *in vitro* with IL-2 [8], NK cytotoxicity was significantly enhanced compared to untreated cells.

Leucocyte adhesion deficiency is a rare genetic disorder characterized by abnormality of the surface glycoproteins that mediate the adherence-related functions of all leucocytes [6]. The syndrome is inherited in an autosomal recessive manner. Affected patients suffer from severe, widespread and life-threatening bacterial and fungal infections. Blood neutrophil counts are characteristically markedly elevated. All cytolytic leucocyte functions are inhibited, including NK-mediated lysis.

CFS is a heterogeneous disorder of unknown aetiology characterized by variable immune dysfunction and a variety of constitutional symptoms, including persistent fatigue. The aetiology of the immune system abnormalities has not been determined. Studies have reported a variety of manifestations including diminished NK-cell function in some patients [2].

One group of investigators described a clinical syndrome referred to as 'low natural killer syndrome' (LNKS) [91]. The illness was characterized by low NK-cell activity in association with symptoms of fatigue, low-grade fevers and depression. Other parameters of immune function were normal. Circulating numbers of NK cells were within normal limits. Patients with this syndrome responded well to the immunopotentiator Lentinan. Clinical symptoms were reduced and NK function was enhanced by administration of the drug.

Benzidine and β-naphthylamine have been implicated as triggers for transitional cell metaplasia predisposing to the development of bladder cancer. One study of workers exposed to these chemicals reported increased circulating NK cells, but decreased function, in these subjects compared to normal controls [7]. Other conditions associated with diminished NK function include pregnancy [92], psychiatric depression [6], thermal injuries [93], strenuous exercise [94] and malignancy [95–97]. A single case of a patient with absent NK cells has been reported [98]. The subject was an adolescent female who suffered from chronic leukopenia, bouts of varicella and CMV pneumonia, and disseminated cutaneous herpes simplex type I infection.

Thus, a deficiency in NK-cell number and/or function has been demonstrated in several genetic and acquired immune disorders.

CONCLUSION

NK cells are large lymphocytes with a granular cytoplasm, distinguishable from T and B cells by the presence of the surface markers CD16 and CD56 and the lack of CD3 and surface immunoglobulin. They have the capacity to lyse tumour and virus-infected cells without prior presentation of foreign proteins by antigen-presenting cells. NK cells bind to carbohydrate ligands on target cells via surface receptors of the NKR-P1A and CD94 families in humans, and NKR-P1 molecules in rodents and secrete various proteases, nucleases and perforin, which results in the lysis of the target. In addition to protection of the host from intracellular pathogens and tumorigenesis, NK cells secrete a large array of cytokines and are important in immune system regulation. *In vitro* experiments as well as studies in humans and mice have revealed the importance of NK cells in the early, non-specific immune response to several viral pathogens. Current evidence suggests a role for these cells in protecting the host from infection with herpes-viruses, coxsackieviruses, paramyxovirus infections, influenza A virus, hepatitis viruses B and C and HIV. The spectrum of viruses sensitive to NK cell-mediated destruction is still to be determined, however. Compromised NK function has been reported as part of a variety of chronic illnesses including genetic immunodeficiency syndromes, chronic fatigue syndrome, depression, and AIDS.

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