

Understand the Role of Natural Killer

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Abstract: Problem statement: Immunosuppression is a common approach for pathogens or cancer to escape from immune system of the infected host. Some of the antimicrobe or anticancer drugs may also weaken the body immune system. Among various types of immune cell, natural killer cells are the key component of the innate immunity which plays an important role in first line defense against cancer and microbe infections. Thus, understand the role of Natural killer cell can help to design a successful natural killer cell base therapy in cancer and infectious disease therapy. **Approach:** Natural killer cell unique activation/inhibition ligand, cytolytic granules and proinflammatory cytokines offered a specific killing mechanism toward cancer or infected cell target. These specific activation can be achieved in the presence of immunomodulator such as cytokines (IL-2 and IL-12) or mitogens (plant extract such as *Rhaphidophora korthalsii* methanol extract). **Results:** Key role of Natural killer cell base immunotherapy is to maintain enough activated Natural killer cell number in the infected host. **Conclusion/Recommendations:** Future study should expand the clinical use of *in vivo* or *ex-vivo* Natural killer cell base therapy especially in cancer treatment.

Key words: Peripheral blood mononuclear cell, Natural Killer (NK), Caspase-Activated DNase (CAD), immunological memory, Lymphokine Activated Killer (LAK), Cytotoxic T Cells (CTL), condition may happen

INTRODUCTION

Immune system is a network of cells, tissues and organs which work closely to defend the body from diseases through identifying and removing pathogens and tumour cells. Thus, human health is always related to immune responses (National Institutes of Health, 2003). The immune system can be grouped based on their functions. The classes of immune system are innate (or natural) immunity and acquired immunity (adaptive immunity) (Yeap *et al.*, 2007). Innate immunity is the non-specific first line body defense which provides immediate response to infection

penetrating the body physical barriers (Litman *et al.*, 2005). On the other hand, pathogen specific acquired immunity is a third layer of protection activated by the innate immunity. It improves the recognition and elimination of pathogen through discrimination of self- and non-self antigens and also development of immunological memory. Acquired immunity can be separated into two components which are cell-mediated immunity or humoral immunity. Cell mediated immunity is an immune response which involves the activation of lymphocytes such as macrophages, Natural Killer (NK) cells and T cells but do not involve the antibodies or complements which are mainly

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produced by the B cells. Since immunosuppression is a common phenomena during progression of certain diseases such as cancer, *ex-vivo* cell base adoptive tumour immunotherapy using Lymphokine Activated Killer (LAK) cell as the effector cells to maintain patients' immune system besides targeting the cancer cell have been proposed as the potential cancer treatment (Suck, 2006). LAK cells is a mixture population of killer cells including natural killer (NK) (CD3⁻/CD56⁺), cytolytic NK-T (CNK-T) (CD8⁺/CD56⁺), Cytokine-Induced Killer (CIK) (CD3⁺/CD56⁺), and Cytotoxic T Lymphocytes (CTL) (CD3⁺/CD8⁺) which could lyse a broad range of fresh and cultured tumour cells (Ozdemir and Savasan, 2005). It is generated by activating the Peripheral Blood Mononuclear Cells (PBMC) isolated from the cancer microenvironment (commonly known as tumor infiltrated lymphocytes) with the cytokine [especially interleukin 2 (IL-2)]. Generally, PBMC are critical to fight infections or any invaders. Many studies have shown that human PBMC functions can be stimulated or inhibited by immunomodulatory agents. Huang *et al.* (1995) described that PBMC pre-treated with immunomodulators, such as cytokines, either *in vitro* or *in vivo* manner, might increase anti-viral activity. Besides, influenza A virus induced PBMC displayed strong cytotoxicity against melanoma cells, if compared to the non-treated cells (Sturlan *et al.*, 2009). This cytotoxic effect depends on the cellular contact between PBMC and target cells, as well as the cytokines secreted, such as TNF, IL-6 and IFN. This adoptive immunotherapy is a passive immunization involving the transplantation of immune effector cells to kill tumour cells. However, low efficiency and toxic side effects was often couple with this type of treatment. The low success rate of adoptive immunotherapy may be due to low cytolytic activity of the patients' NK cells and the small population of NK cells (approximately 10%) in the activated NK cells (Suck, 2006). Thus, activating this adoptive immunotherapy.

Natural killer cell: Natural Killer (NK) cell is a class of large granular lymphocyte that is crucial for innate immune system and involved in a variety of biological processes, including antitumor activity and defense against viral, bacterial, and fungal infections, without any antigenic specificity (Biron *et al.*, 1999; Garcia-Penarrubia *et al.*, 1989; Arase *et al.*, 1995; Auernhammer and Melmed, 2000). NK cell function abnormalities are associated with viral infections (Ojo-Amaize *et al.*, 1994). NK cells are called "natural"

because they do not need to recognize any specific antigen to respond and take actions. And, they do not require activation in order to kill target cells that lack of the MHC class I antigens, like tumor cells and virus infected cells. Human NK cells comprise of about 5-20% of peripheral blood lymphocytes (Cooper *et al.*, 2001a).

In fact, NK cells are derived from human primitive progenitors. Differentiation of NK cells from progenitors requires interaction of primitive-acting ligands (c-kit ligands and Flt3 ligands), as well as cytokines (IL-2, IL-3, IL-7, IL-10, IL-15 and others) (Cooper *et al.*, 2001b; Miller and McCullar, 2001). All human NK cells express CD56 on the surface. The density of surface expression of CD56 can be used to classify functionally and developmentally distinct NK cell subsets. Minority (about 10%) of human NK cells are CD56^{bright} CD16^{dim} NK cells, which mediate low or no cytotoxicity, but produce high levels of inflammatory cytokines; while majority are CD56^{dim} CD16^{bright} cells that are potent mediators of cytotoxicity (Cooper *et al.*, 2001a; Caligiuri, 2008) as shown in Fig. 1.

Nowadays, numbers of NK cell lines are available that have eased the maintenance and expansion in culture. For example, stable IL-2 producing derivatives of NK-92 cell lines and KHYG-1 cell lines. In the absent of IL-2, NK cell lines maintain their cytotoxicity, as the cells manage to activate immune cells and eliminate disease effectively (Yan *et al.*, 1998). *In vitro* and *in vivo* studies demonstrated that NK-92 cells and KHYG-1 can kill half of newly diagnosed and relapsed acute myelogenous leukemias, T cell adult lymphocytic leukemias, blinlage acute lymphocytic leukemias, and chronic myelogenous leukemias (Yan *et al.*, 1998; Suck *et al.*, 2005). Preliminary study showed that intravenous administration of NK-92 cells is safe and the cells are not rejected by the immune system (Reid *et al.*, 2002).

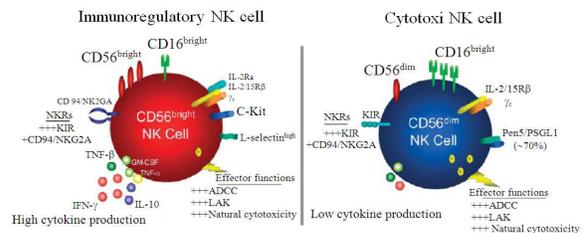


Fig. 1: Human NK cell subsets. The CD56^{bright}CD16^{dim} NK cells are immunoregulatory cells that secrete high levels of cytokines with low natural cytotoxicity. The CD56^{dim}CD16^{bright} NK cells are cytotoxic cells which produce low levels of cytokines (Farg and Caligiuri, 2006)

Natural killer cells reacted in different pathways:

Human Natural Killer (NK) cell activities are tightly regulated. NK cells possess two types of surface receptors, which are activating receptors and inhibitory receptors. The inhibitory and activating function is determined by the length of associated cytoplasmic tail, either Long (L) or Short (S), respectively (Uhrberg *et al.*, 1997). Balance between inhibitory and activating signals may affect immune responses. Inhibitory receptors recognize MHC class I molecules that are normally expressed on the surface of normal cells to protect from autoimmune disease. Therefore, NK cells are able to kill target cells possessing low or absent of MHC class I molecules. NK cells response to kill the targets in two different mechanisms, either by cytotoxic granule exocytosis or by induction of death receptor-mediated apoptosis.

Granule mediated cytolytic pathway: Granule mediated cytolytic mechanism involved the granules presented in CD4⁺ Cytolytic T Lymphocytes (CTL), CD8⁺ CTL, T cells and NK cells. Upon exocytosis of the granules, perforin changes its conformation, polymerizes into tube-like structure (polyperforins) and inserts into the membrane, in the presence of calcium ions (Masson and Tschopp, 1985). Transmembrane channels are generated; as a result, the target cells lose membrane integrity and appear to die by osmotic lysis (Liu *et al.*, 1995). Besides, granzymes diffuse to the cytosol by endosomal pathway, further activates cysteine proteases, which in turn cleave structural and regulatory proteins, and leads to apoptosis. Granzyme B caused DNA fragmentation in target cells through activating a nuclease called as Caspase-Activated Dnase (Cad) (Fan and Zhang, 2005). Recent reports showed that granzyme B deliver to target cells through electrostatic interactions, further undergo electrostatic exchange from serglycin to target cells (Raja *et al.*, 2005; Shi *et al.*, 2005). Generally, both perforin and granzyme B are acting synergistically. Nevertheless, without granzyme B, exposure of perforin is effectively lysing certain target cells, but cannot induce DNA fragmentation (Duke *et al.*, 1989). In contrast, in the absent of perforin, granzyme B could induce apoptosis in target cells via endocytosis (Spaeny-Dekking *et al.*, 1998). This suggested that granzyme B played more important role in mediating granule-mediated cytotoxicity.

Fas ligand and Trail pathway: Natural Killer (NK) cells demonstrated another cytolytic mechanism through the induction of death receptor-mediated apoptosis (Smyth *et al.*, 2002). These death ligands belong to the Tumor Necrosis Factor (TNF) family of

ligands, comprise of a large family, included TNF- α , Fas ligands (FasL), CD40L, lymphotoxin α (LT α) and LT β , Receptor Activator of Nuclear Factor-Kappa B Ligands (RANKL) as well as TNF-Related Apoptosis-Inducing Ligands (TRAIL) (Mirandola *et al.*, 2004).

Activation of NK cells have been linked to the expression of several death receptors, such as FasL (CD95) and TRAIL. Binding of Fas ligands of target cells with Fas receptors on NK cells induces cytotoxicity (1995). Another type of death receptor, TRAIL can bind to the death receptors DR4 (TRAIL-RI) and DR5 (TRAIL-RII) and induces apoptosis of target cells. Takeda *et al.* (2001) reported that TRAIL sensitive tumor cells proliferated, in the presence of neutralizing anti-TRAIL antibody against TRAIL transfected effector cells; but this not happens to TRAIL resistance tumor cells. Administration of anti-TRAIL antibody to NK cell depleted, interferon-deficient, TRAIL-sensitive tumor injected mice, also resulted in greater metastasis (Takeda *et al.*, 2001). However, this cell death pathway must happen in cell to cell direct contact manner.

Besides the death ligands, a large family of secreted cytokines of TNF family, that are pleiotropic, such as Nitride Oxide (NO), also mediate apoptosis (Cifone *et al.*, 1999; Kwak *et al.*, 2000).

Cytokines involved in activation of immune system:

Cytokines are small proteins secreted by specific cells of the immune system that characterized by considerable redundancy and pleiotropism. They acted as immunomodulator to regulate immune system functions, such as proliferation, cell survival or death, differentiation and various gene expressions (Street *et al.*, 2001). They also involved in development processes during embryogenesis. Some cytokines circulate in picomolar (10^{-12} M) concentrations, but able to increase up to 1000 fold, upon stimulation. Natural Killer (NK) cells secrete a lot of functional cytokines, such as IFN- γ , TNF, GM-CSF, IL-5, IL-10, IL-13 and more (Cooper *et al.*, 2001b; Smyth *et al.*, 2002).

Interferon-gamma (IFN- γ) is the hallmark cytokine of T_H1 cells. In fact, CD56^{bright} CD16^{dim} NK cells are the primary source of IFN- γ when the cells are being stimulated (Cooper *et al.*, 2001b). CD8⁺ Cytotoxic T Cells (CTL) and Natural Killer T (NKT) cells also produce IFN- γ . IFN- γ is well known with its immunoregulatory and anti-tumor properties (Street *et al.*, 2001). It triggers of pro-inflammatory cascade that causes increasing of antigen presentation, promotes adhesion and binding required for leukocyte migration, as well as

activation of T cell, NK cell and the macrophages lysosome activity (Nathan *et al.*, 1983; Winkler *et al.*, 2006). In addition, IFN- γ can up-regulate expression of MHC class I molecules and thus, increase the sensitivity of tumor cells to Fas ligands (FasL) mediated cytotoxicity.

Interleukin-2 (IL-2) is a multifunctional inflammatory cytokine which activates the immune system by cells proliferation, cytokines production and Lymphokine Activated Killer (LAK) cell generation (Hughes, 1998; Zhang, *et al.*, 2008). Instead, IL-2 is identified as a T-cell growth factor that regulates T cell proliferation and maturation (Conrad, 2003). Besides, IL-2 can augment cytotoxic activity by enhancing the target immunogenicity (Bradley *et al.*, 1998). Nowadays, IL-2 is approved by the U.S. Food and Drug Administration (FDA) for the treatment of metastatic renal cell carcinoma and melanoma. High dosage of IL-2 was commonly used to treat metastasis (Dutcher, 2002). However, severe toxicities were developed in most patients, such as hypotension, lack of appetite, and fever. Phase II trial of subcutaneous injection of recombinant IL-2 in advanced cutaneous T cell lymphoma resulted in expansion of T cells, but caused toxic side effects and less efficiency. Majority patients suffered with grade 1 or 2 in severity; and one patient developed grade 4 lymphopenia. To limit the toxicities, lower administration dosage or combination of IL-2 with other targeted agents may be the solutions for cancer patients (Querfeld *et al.*, 2007).

Interleukin 12 (IL-12) is a T cell stimulating factor, originally produced by dendritic cells, macrophages and B-lymphoblastoid cells (NC-37) in response to antigenic stimulation. IL-12 augments cytotoxic activity of NK cells and CD8⁺ CTL by stimulating the production of IFN- γ , further enhances T_H1 immune responses, includes cellular immunity and production of antibodies (Fehniger *et al.*, 1999; Tripp *et al.*, 1993). Thus, IL-12 possesses anti-inflammation and anti-angiogenesis activities, associated with IFN- γ production (Stone *et al.*, 2003). Brunda *et al.* (1993) reported *in vivo* antitumor activity was displayed by IL-12, without any gross toxicity. However, in phase I trial on patients, the clinical testing abruptly curtailed due to unexpected severe toxicities, although antitumor effect was obtained (Atkins *et al.*, 1997).

Cytotoxic and cytolysis: Cytotoxic is referring to the substances that are toxic to cells or caused cells death. Since 1950s, many scientists have focused on the application of cytotoxic agents in anticancer study and encouraging results had been obtained (Kennedy, 1991).

In contrast, cytolysis is relating to an important immune function of cell dissolution or destruction. Cytolysis process involved cytolytic cells that are able to lyses a broad range of cells, especially tumor cells and virus-induced cells. The cytolysis effect towards the target cells can be augmented when an immunomodulatory agent was administered to the immune cells (Yeap *et al.*, 2007; Lafleur *et al.*, 2001; Yeap *et al.*, 2011).

Antimicrobial property of NK cell: Natural Killer (NK) cells function as a key factor in immediate immune responses to virus infection and intracellular infections of mycobacterial and bacterial (Orange, 2002; Salazar-Mather *et al.*, 1998; Unanue, 1997). A patient with complete lacking of NK cells would sustain severe virus and other fungal infection. NK cells are populations of lymphocytes that can be activated to mediate significant levels of cytotoxic activity and produce high levels of certain cytokines, such as IFN- γ . NK cell-derived IFN- γ leads to T_H1-type immunity and activates macrophages, resulting in production of IFN- α , IL-12 and IL-18 (Biron *et al.*, 1999). IFN- γ limits transynaptic transmission, prevent viral reactivation and inhibit virus-induced apoptosis (Cantin *et al.*, 1999; Geiger *et al.*, 1995; Mikloska and Cunningham, 2001). In addition, MHC class I expression of viruses-infected cells are often being suppressed; hence, the infected cells become more susceptible to killing by NK cells. Activation of NK cell-mediated cytotoxicity would occur during viral infections, including arenaviruses, herpesviruses, Herpes Simplex Virus (HSV), influenza virus, Coxsackie virus and more (Biron *et al.*, 1999). Salazar-Mather (1998) reported that IFN- γ production was enhanced upon stimulation by influenza virus and murine cytomegalovirus (MCMV). NK cells also exhibit antiviral activity through antibody dependent cell-mediated cytotoxicity (ADCC) mechanism.

Antitumor property of NK cell: Natural Killer (NK) cells are cytotoxic cells and capable to mediate spontaneous cytotoxicity against tumor cells and blood-borne metastasis (Vujanovic *et al.*, 1996; Whiteside and Herberman, 1995) In fact, most of the cancer cells have reduced or no MHC class I expression; hence, become susceptible to killing by NK cells (Ljunggren and Karre, 1985). Zhang *et al.* (2008) showed that a number of cytokines produced, such as IL-2, IL-15 and IL-12, can enhance cytotoxic activity of NK cells. Moreover, production of interferon may augment NK cytotoxic activity. NK cell-mediated cytolysis is largely mediated by perforin and granzyme B as well as the death ligands of TNF family.

Unfortunately, NK cells therapy has limited efficacy, even though it may induce tumor regression in some cases. Therefore, intratumoral injection of tumor vaccine in combination of adjuvant, such as IL-2, may help in NK cells activation, and trigger tumor regression response (Ishikawa *et al.*, 2004). Added to this, not all types of cancer are suitable with the magic bullet IL-2 plus NK cell. Kim *et al.* (2004) has reported that hepatoblastoma cell was only susceptible to IL-2 activated NK cell granule-dependent necrotic death. Thus, this combination that induced necrosis which may further promote the cancer cell metastasis are not suitable for this hepatoblastoma immunotherapy. Due to this disadvantage of using cytokine to activate NK cell for tumor immunotherapy, attempt to search for NK cell immunomodulator from natural source is one of the current focus for the field of immunology. Yeap *et al.* (2011; 2007) have proved that *Rhaphidophora korthalsii* methanol extract was able to target hepatoblastoma and myelogenous leukaemia cell death via apoptosis. Effort to understand the mechanism of NK cell activation and the cytolytic process induced by the extract activated NK cell against the cancer target are still going on.

CONCLUSION

Although LAK cell generated from PBMC using recombinant IL-2 as a immunomodulator demonstrated the potential as effective killer cell in cancer immunotherapy, isolating the NK cell which was the most important player in LAK cell may be able to enhance the specificity and efficiency of that treatment. Furthermore, NK cell in the present of IL-2 cannot be treated as the magic bullet against all type of immunosuppressive disease since it may induce necrosis rather than apoptosis against the specific target cell. Thus, continuous effort on searching potential immunomodulators from various natural sources (Yeap *et al.*, 2011; 2007) and combination of cytokines (Capitini *et al.*, 2009) to activate NK cell cytolytic activity for different purpose may be a safe and warrant solution for different type of diseases. Caution needs to be work out to monitor the sensitivity of the patients against this NK cell base treatment since autoimmunity which complicate the patients' condition may happen.

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