INTRODUCTION

INTRODUCTION

Nowadays, it is viewed that malignancy is often correlated with the breakdown of the immune surveillance mechanism. In innate immunity, the surveillance mechanism operates spontaneously as the first line of defence that keeps potential pathogens in check before they can establish an overt infection and contact with immunocompetent cells for adaptive responses.

Adaptive immunity is carried out by clonally expanded antigen specific B and T lymphocytes which can generate immunological memory (Sprent and Miller, 1976; Sprent and Tough, 1994). Until recently, T cells got much attention for their central role against malignancy. However, the abundance of NK cells in nude mice and the resistance of T cell deficient animals against some tumours (Kiessling *et al.*, 1976 and Warner *et al.*,1977) pointed towards the NK cells as the major effectors in immune surveillance against cancer.

Further, Roder and Duwe (1979) showed that impaired NK cell function in beige mutant mice causes less efficient rejection of tumour transplants and metastasis (Karre and coworkers, 1980). A loss of systemic NK activity in tumour bearing host has been reported in a variety of clinical and experimental tumour models (Ehrlich *et al.*,1980; Gerson *et al.*,1980; Stutman *et al.*,1980; Lala *et al.*,1985; Moy *et al.*,1985). Mice bearing advanced Lewis lung

carcinoma (LLC) were found to have significantly decreased NK cell activity in spleen as well as in blood (Kurosawa *et al.*,1993).

Although without much understanding, the NK cell activity was previously considered as 'undesired background' (Herberman *et al.*,1980). The potential of this spontaneously reactive cell type was realized when investigators became interested in a subpopulation of lymphoid cells which had the ability to eliminate tumour cells in *in vitro* cytotoxicity assays.(Kiessling *et al.*,1975, Herberman *et al.*, 1975), and soon this subpopulation of lymphoid cells were named Natural Killer (NK) cells and was taken seriously for playing an important role in surveillance of cancer *in vivo* (Kiessling and Haller,1978; Haller *et al.*,1977; Herberman and Holden,1978; Kiessling and Wigzell, 1979; Riccardi *et al.*,1980, Herberman, 1983).

Working with NK sensitive K 562 and NK resistant YAC cell lines, biologically significant roles of NK cells in host defence mechanism *in vivo* against the development of transplanted tumours (Kiessling *et al.*,1975; Sendo *et al.*,1975; Hanna and Burton,1981) and tumour metastasis (Talmadge *et al.*,1980 and Gorelik *et al.*,1982) were understood.

Initially it was difficult to categorize NK cells as because they have certain characteristics common with other lymphocytes. This could however be achieved with the discovery of selective markers of the NK cells. However, till today NK cells have been recognized as a functionally distinct subset of lymphocytes with large granular lymphocyte (LGL) morphology in humans (Saksela, 1979; Timonen *et al.*,1979a and b; Herberman, *et al* 1980;

Timonen and Saksela 1980; Timonen *et al.*,1981) rats (Reynolds *et al.*,1981) and mice (Luini *et al.*,1981). The characteristics are distinct from T and B cells, monocytes and macrophages. NK cell surface do not express the CD3 complex or any of the known T-cell receptor chains (TcR $\alpha,\beta,\gamma,\delta$) (Tutt *et al.*,1986 and Biron *et al.*,1987). The majority of NK cells express the CD16 (Fcγ RIII) (Perussia *et al.*,1989) and CD56 (N-CAM) antigens in humans (Lanier *et al.*,1986; Trinchieri,1989 and Whiteside *et al.*, 1990) and the NK 1.1 antigen in mice(Glimcher *et al.*,1977;Tutt *et al.*,1986; Karlhofer and Yokoyama 1991; Lanier and Philips,1992; Kung and Miller, 1995).

The NKR-P1 is found to be present in NK cells from different species (Raulet *et al.*,1995 and Yokoyama,1995).This marker on NK cells help in recognizing oligosaccharide moieties on target cells including abnormal and infected cells, in the process of lysis (Bezouska *et al.*,1994;Ryan *et al.*, 1995; Whiteside and Herberman,1995).

NK cells can lyse target cells with aberrant or without MHC Class I expression (Ljunggren and Karre, 1990; Liao, 1991; Chadwick *et al.*,1992; Karlhofer *et al.*,1992; Correa *et al.*,1994; Karre,1995; Yokoyama,1995; Lopez-Botet *et al.*, 1996.). NK cells are capable of killing virus infected cells expressing viral antigen (Welsh and Zinkernagel,1977; Santoli *et al.*,1978 a and b; Brooks *et al.*,1979; Reid *et al.*,1979;Herberman,1980).NK cells can also kill allogeneic haemopoietic cells (Bellone *et al.*,1993 ;Kiessling *et al.*, 1977; Sentman,1989; Karre, 1992; Lanier, 1995) and certain parasites (Scott and Trinchieri,1995).King and coworkers (1997)

have recently shown that NK cells may have a homeostatic role in reproduction and control of placentation.

In addition to its varied effector functions, NK cells also play a role in immune regulation (Nabel *et al.*,1982; Arai *et al.*,1983; Targan *et al.*,1985; Robles and Pottack,1986; Horwitz *et al.*,1997.). Morio and others (1989) have demonstrated the inhibition of immunoglobulin synthesis *in vitro* by NK cells.It has been shown that NK cells produce several cytokines that promote B cell proliferation (Scala *et al.*,1984; Procopio *et al.*,1985; Vyakarnam *et al.*,1985; Kimata *et al.*,1988; Wilder *et al.*,1996).

As spontaneous killing by NK cells itself is effective against malignancy much thought have been given for their potential in treatment of cancer especially whether activation could enhance their cytotoxic function was tested under different conditions. Further, NK cells were found to lyse most tumours and NK resistant cell lines after stimulation with immunoregulatory cytokines like Interleukin-2 (IL-2) (Henney *et al.*, 1981; Stutman, 1981; Trinchieri *et al.*,1984; Trinchieri, 1989), Interleukin-12 (IL-12), (Kobayashi *et al.*,1989; Tahara *et al.*,1994; Lanier and Phillips, 1992; Brunda *et al.*,1994; Nastala *et al.*,1994; Jordan *et al.*1996; Lamont *et al.*, 1996) and Interferon-α(Inf-α) (Trinchieri and Santoli, 1978; Djeu *et al.*,1979).

A number of natural products and synthetic immunopotentiators collectively termed as biological response modifiers (BRM) have been tried for stimulation of host antitumour immunity (Oldham 1983;Wiltrout *et al.*,1984 and Foon 1989).Natural BRMs include bacterial products such as

BCG (Mizutani *et al.* 1994). *Corynebacterium parvum* (Shu *et al.*,1989), fungal products (Vanky *et al.*,1992), Inf γ, hormones, cytokines, plant products and products of tumour origin. Synthetic BRMs include synthetic oligopeptide pyran copolymers and some cancer therapeutic components. Among these BRMs, systemic application of cytokines have been found to be more effective against malignancy.

Besides stimulating the cytolytic function, activation of NK cells induce production of interferon (INF)-γ, granulocyte-macrophage colony stimulating factors (GM-CSFs) (Perussia, 1991), tumour necrosis factor (TNF)-α (Paya *et al.*,1988), IL-8 and other cytokines (Trinchieri *et al.*, 1984; Trinchieri, 1989).Interferon and Tumour necrosis factor can directly affect the tumour cells and other cytokines produced by NK cells can boost their own function too.

Among the various cytokines, IL-2 is one of the best studied and found to play a central role in vertebrate immune response (Smith, 1988). Congenital absence of IL-2 production in humans causes a severe combined immune deficiency with life threatening infections (Pahwa *et al.*,1989; Weinberg and Parkman,1990).

IL-2 is produced by activated T lymphocytes which helps in the proliferation of mature T lymphocytes (Morgan *et al.*,1976; Gillis and Smith, 1977; Watson *et al.*,1980; Hefeneider *et al.*,1983; Smith ,1992). It also activates the NK cells (Biron *et al.*,1990; Voss *et al.*, 1990) and promotes the proliferation and differentiation of B cells (Mingari *et al.*,1984; Smith,1988) and monocytes (Malkovsky *et al.*,1987 and Espinoza Delgado *et al.*

1990). IL-2 has also been found to be an immunostimulant in cancer patients (Rosenberg *et al.*,1985, Rosenberg,1988; Lotze *et al.*,1985; Kasai *et al* 1990).

IL-2 therapy though promising (Lotze *et al.*,1985), has its own limitations. High dose IL-2 therapy is seen to cause many systemic side effects in humans. Such side effects are associated with life threatening toxicities like severe hypotension, pulmonary edema, renal failure, cardiac arrhythmias, neurologic dysfunction and vascular leak syndrome(Rosenstein *et al.*,1986; Belldegrun *et al.*,1987; Ognibene *et al.*,1988; Schwartzentruber *et al.*,1988; Denicoff *et al.*,1989;Lee *et al.*,1989;Margolin *et al.*, 1989; Kragel *et al.*,1990; Thijs *et al.*,1990; Siegel and Puri,1991).

Use of judious amount of IL-2 would rule out the toxic effects of high dose IL-2. Caliguiri *et al.*,(1991) showed that low dose IL-2 infusion in humans resulted in the selective expansion of NK cells *in vivo* upto 80% of all peripheral mononuclear cells; this treatment was extremely well tolerated without any sign of toxicity (Thews *et al.*,1993; Hladik *et al.*,1994).

IL-2 infusions *in vivo* leads to the generation of lymphokine activated killer (LAK) cells (Grimm *et al.*,1982; 1983 a,b; Rosenstein *et al.*,1984; Merluzzi *et al.*,1984; Forni *et al.*,1985; Rosenberg, 1985; Herberman *et al.*,1987; Gunji *et al.*, 1989).LAK cells which are clearly divergent from NK cells (Herberman *et al.*,1987) can lyse a wide variety of fresh, NK-resistant tumour cells (Lotze *et al.*,1981, Rosenstein *et al.*,1984) and freshly isolated autologous and allogeneic tumour cells.

LAK cell therapy also mediates the regression of established metastasis from a variety of immunogenic and non immunogenic tumours in tumour bearing animals (Mule *et al.*,1984; Lafreniere and Rosenberg, 1985; Mule *et al.*,1985; 1986;Ottow *et al.*,1986; Papa *et al*, 1986; Salup and Wiltrout, 1986) and man (Rosenberg,1986; 1988; Rosenberg *et al.*, 1985;1987;1993).

However, Ettinghausen *et al.*,(1985) have shown that LAK cells are exclusively dependent on IL-2 for maintenance of its antitumour activity and its continued proliferation *in vivo*. Immunotherapy utilizing LAK cells is highly dependent on the number of LAK cells infused (Ettinghausen and Rosenberg,1986) and the dose and duration of IL-2 injections, risking its toxic side effects.

On the other hand, it has been shown by Herberman and coworkers,(1987) and Herscend and Schmidt, (1988) that NK cells after activation are more effective than LAK cells obtained from spleen. In view of total dependence of LAK cells on IL-2 for their proliferation and cytotoxicity and risk of side effects of repeated IL-2 doses, NK cells are likely to be a better choice of cancer therapy.

Unlike LAK cells, NK cells normally possess high affinity IL-2 receptor heterodimers and thus require low doses of IL-2 for enhancement of its lytic activity (Caliguiri *et al.*,1993) and differ from T and B lymphocytes which express these high affinity IL-2 receptor heterodimers only after activation by antigens or mitogens(Wang and Smith, 1987; Smith, 1989). Caliguiri and his coworkers also demonstrated a gradual expansion of NK cell

number in patients with advanced cancer receiving low dose IL-2 therapy. This indicates that low dose of IL-2 can drive the NK cells to proliferate and differentiate all the way to fight malignancy more effectively and simultaneously reduce the side effects substantially.

In the present investigation, before employing the IL-2 activation for various activities of NK cells, certain other parameters of NK cells were studied.

To begin with, the number of NK cells in spleens of normal and tumour bearing mice were determined to find out whether it varies in tumour condition.

The degree of activation with IL-2 were studied in reference to blastogenic response and DNA synthesis by the NK cells from both normal and tumour bearing mice. Different doses of rIL-2 and cell culture generated IL-2 were employed.

Next, we measured the spontaneous cytotoxicity of NK cells from normal (NK^N) and tumour bearing mice (NK^T) with or without IL-2 activation in *in vitro* ^{51} Cr release assay.

NK cells were also found to eliminate malignant cells in Antibody Dependent Cell Mediated Cytotoxicity (ADCC)(Peter *et al.*, 1975;West *et al.*,1976;Kay *et al.*,1977;Lanier *et al*, 1988; Ravetch and Kinet, 1991). Fc γRIII receptors on NK cells bind to the Fc portions of immunoglobulins which are coated on the target cells (Herberman *et al.*,1977; Oehler *et al.*,1978).We also tried to find out the lytic potential of IL-2 activated NK cells

from both normal and tumour bearing mice in ADCC assay. The immunoglobulins were raised against TAAs.

The best measure to test the efficacy of cytotoxic cells against tumour target cells *in vivo* is to find out whether they can seed in at the tumour site. The radiolabelled IL-2 activated NK cells were injected in the tumour bearing mice intravenously and then followed their homing pattern following the established protocol in our Laboratory (Chakravarty and Jha, 1997).

Next, an attempt was made to use IL-2 activated NK cells for adoptive immunotherapy. Maitra(1986) and Maitra and Chakravarty (1990) have shown that polyclonally activated T lymphocytes adoptively transferred in tumour bearing mice was effective in curbing the growth of solid tumours *in situ*.

In a series of experiments, IL-2 activated NK cells were injected at tumour sites in two different doses, $0.5x10^6$ and 10^6 per animal. In some of these animals, cells were injected intravenously or at the site of the tumour; IL-2 was given to some at weekly intervals for five times for expansion and function of the already injected cells.

In another series of experiments, IL-2 and IL-2 activated NK^N cell therapy were tried after surgical removal of tumour mass, and low doses of weekly IL-2 injections were given for five times for long term maintenance of the adoptively transferred cells. The growth and survival of the host was noted as usual. Activated T cells were found to perform better in adoptive transfer experiment after surgical removal of tumour mass (Chakravarty and Jha, 1997).

Suppressor factors are often found to inhibit the immunocompetent cells favouring the growth of tumour. Certain factors released by tumour cells like gangliosides(Bergelson *et al.*, 1989) or transforming growth factor β was found to cause immunosuppression. Most of the suppressor factors are borne by serum so, we studied the immunosuppressor role in cytotoxicity assay.

In course of the study we tested whether the serum contains anti tumour associated antigen (TAA)antibodies. This might act as blocking factor and play a significant role in ADCC *in vivo*.

This study measures the activity of NK^N and NK^T cells, with or without IL-2 activation, against the tumour target cells in *in vitro* and *in vivo* situation and suggests a direction for balance use of IL-2 and NK cell therapy for malignancy.