CHAPTER 5

Regulation of NK cell function by the dose of tumor transplanted in the peritoneum
5.1 INTRODUCTION

Natural killer cells are large granular lymphocytes distinguishable from T and B lymphocytes by their surface phenotype, cytokine profile and their ability to mediate spontaneous cytotoxicity against a broad range of targets including MHC class I negative tumor cells (Farrar et al., 1986). Necrosis and apoptosis have been the two proposed modes of CTL /NK mediated target cell death (Duke et al., 1989). Several cytokines such as IL-2, IFN-γ and IL-12 have been shown to augment NK cell proliferation and activation (Herberman et al., 1986). Activated NK cells produce a spectrum of cytokines such as TNF-α, IFN-γ, GM-CSF, lymphotoxin and IL-8 (Farrar et al., 1986). The production of IFN-γ by NK cells is enhanced by IL-12 (Cui et al., 1997), which favors a Th1 type cellular response. A lymphocyte subpopulation called NKT cells express both NK cell receptor (CD161) and a single invariant T cell receptor encoded by the Vα14 and Jα281 gene segments (Masuda et al., 1997). NKT cells mediate their tumor cell cytotoxicity by an NK-like effector mechanism after activation with IL-12 (Cui et al., 1997).

Stimulation of T cells via their antigen receptor can induce either proliferation or unresponsiveness / cell death (anergy); however, the precise mechanism by which the ‘choice’ is made is not well understood (Lenardo et al., 1991). It has been demonstrated that high dose of antigen can lead to a paradoxical suppression of immune response that is termed as high zone tolerance. While NK cells fail to rearrange TCR subunit, nonetheless they share a number of features with T cells, including expression of surface molecules and secretion of similar cytokines (Lanier et al., 1986 & Ortaldo et al., 1986). T and NK cell are also developmentally related and they also undergo functional anergy and apoptosis. NK cells become inactive, lose their cytotoxic function following cross-linking of CD16 receptor or incubation with target tumor
cells after overnight incubation (Jewett et al., 1997 & Jewett and Bonavida, 1996). In patients with advanced cancers, NK cell cytotoxic activity is usually suppressed (Oh et al., 1987). Inactivation by tumor cells, reduced number of NK cells, reduced responsiveness to IFN-γ or IL-2, ability to produce IFN-γ or IL-2, presence of suppressor cells (including monocyte/macrophages acting through release of prostaglandins), presence of inhibitory substances such as glycoproteins and glycolipids, and other mechanisms have been described as responsible for NK cell suppression in cancer patients. AK-5 is a highly immunogenic rat histiocytic tumor (Khar, 1986 & Khar, 1993) which regresses spontaneously in syngeneic animals when transplanted s.c. The AK-5 tumor follows a site-specific growth pattern when transplanted in syngeneic hosts. Upon s.c. transplantation (5 x 10^6 cells) the tumor grows up to day 15 (growth phase), after which it starts getting rejected during the regression phase followed by the healing phase when the animal is totally normal but immune to subsequent challenges of AK-5 tumor (Kausalya et al., 1994). Tumor regression in s.c. transplanted animals is mediated by CD8⁺, CD3⁻ NK cells through antibody dependent cellular cytotoxicity (ADCC) and apoptosis (Bright et al., 1995). However, the i.p. transplanted tumors (5 x 10^6 cells/animal) divide rapidly and form a peritoneal bulge by day 3 and the animals start dying by day 5. The death of 100% animals in i.p. injected tumors was attributed to the rapid growth in the peritoneum, not giving sufficient time to the immune system to mount a strong immune response and to the immune escape strategies adopted by the AK-5 cells (Khar et al., 1998).

Recent experiments *in vitro* have implicated antigen dose as an important factor in immune response and a major regulator of effector cell function. Previous studies from our laboratory have shown that low dose of antigen (fixed tumor cells) activated NK cell cytotoxic function whereas high dose of antigen suppressed NK cell cytotoxicity and induced anergy *in*
Fig. 5.1: Effect of i.p. transplantation of different number of tumor cells on the survival rate of animals. Animals (20 per group) were injected with $10^4$, $10^5$, $10^6$ and $10^7$ AK-5 cells per animal on day 0. Survival rate was monitored regularly after tumor transplantation.
Fig. 5.2: Effect of AK-5 tumor transplantation on the cytotoxic activity of NK cells. NK cells isolated from PEC on different days after i.p. tumor transplantation were tested for cytotoxicity against YAC-1 cells. Rejectors (- ■ -), non-rejectors (- △ -) and control animals (- ● -), NK cells from normal animals of the same age group were used as controls and the bars represent ± SD.
vitro (Das et al., 2001 and Das et al., 2000). However, a direct correlation between dose of tumor cell inoculation (i.p.) and NK cell cytotoxic function in vivo has not been demonstrated. The aim of our present study was to find out whether AK-5 tumor cells can modulate NK cell cytotoxic function in a dose dependent manner following injection of tumor cells intraperitoneally.

5.2 RESULTS

5.2.1 NK cell cytotoxicity after i.p. transplantation of AK-5 cells

Antigen concentration could be a major regulator of effector cell function. In order to analyze whether the concentration of antigen (live AK-5 cells) regulates NK cytotoxic function, Wister rats were injected i.p. with $10^4$, $10^5$, $10^6$ and $10^7$ AK-5 cells per animal.

5.2.1.1 Inoculation of $10^4$ AK-5 cells per animal

We investigated the effect of intraperitoneal injection of $10^4$ AK-5 tumor cells on syngeneic rats. When $10^4$ cells were injected per animal (20 per group) (Fig. 5.1), all animals survived upto day 25. Cytolytic activity of NK cells isolated on different days after tumor injection from the peritoneal cavity were checked against YAC-1 target cells. Three days after tumor transplantation, NK cells isolated from PEC demonstrated 20% cytotoxic activity, which increased steadily upto a maximum of 52% on day 7. Thereafter, there was a gradual decrease of cytotoxic activity of isolated NK cells to 20% on day 15, while NK cells isolated from the peritoneal cavity of PBS injected rats showed cytotoxic activity of 10% on day 0, reaching to a maximum of 18% on day 15 (Fig. 5.2).

Similarly, cytotoxic activity of NK cells isolated from splenocytes was checked against $^{51}$Cr labeled YAC-1 cells. Splenic NK cells from animals bearing 3 days tumor demonstrated 15% cytotoxic activity, which increased gradually to a maximum of 34% on day 7. Thereafter, there was a slight decrease in cytotoxic activity of NK cells to 28% on day 15, while NK cells
Fig.5.3: Effect of AK-5 tumor transplantation on the cytotoxic activity of splenic NK cells. NK cells isolated from the splenocytes on different days after i.p. tumor transplantation were tested for cytotoxicity against YAC-1 cells. Rejectors (-■-), non-rejectors (-▲-) and control animals (-○-), NK cells from normal rats of the same age group were used as controls and the bars represent ± SD.
isolated from splenocytes of PBS injected rats showed cytotoxic activity of 10% on day 3 reaching a maximum of 14% on day 15 (Fig. 5.3). These results demonstrate that i.p. injection of $10^4$ AK-5 cells per animal resulted in augmentation of cytotoxic activity in NK cells from both splenocytes and PEC as compared to controls and the cytotoxicity of NK cells isolated from the peritoneum was higher than the splenic NK cells.

5.2.1.2 Inoculation of $10^5$ AK-5 cells per animal

Similarly, 20 animals were injected i.p. with $10^5$ AK-5 cells per animal. After three days, animals were monitored for tumor growth. Based on the survival pattern, the animals were categorized into two groups. 80% animals (16 / 20) were free of tumor development beyond 25 days, which survived (rejectors), whereas 20% animals died within 15 days (non-rejectors) (Fig. 5.1). Non-rejectors showed a peritoneal bulge from day 4 onwards and died within 15 days. We isolated the NK cells from the PEC of non-rejectors on different days and assessed their cytotoxic activity against YAC-1 cells (Fig. 5.2). Four days after tumor transplantation, NK cells isolated from PEC showed 18% cytotoxic activity: which increased gradually to 28% on day 7. Thereafter, there was a steady decrease in cytotoxic activity of NK cells to 1% by day 12. NK cells isolated from PEC of control animals showed cytotoxic activity from 12% on day 0 to a maximum of 18% on day 15. The other set of animals, which did not develop tumors (rejectors) showed a higher number of TIM present in the peritoneal cavity upto day 15. Four days after tumor transplantation NK cells isolated from PEC of rejectors demonstrated 25% cytotoxic activity, which increased sharply to 70% on day 7. Thereafter, there was a gradual decrease of NK cytotoxicity to 25% observed on day 15.

We also assessed the cytotoxic activity of splenic NK cells of non- rejectors on different days against YAC-1 cells (Fig. 5.3). Four days after tumor transplantation, splenic NK cells from
non-rejectors showed 12% cytotoxic activity, which increased gradually to 24% on day 12. However, in case of rejectors four days after tumor transplantation, splenic NK cells showed 20% cytotoxic activity, which increased gradually to 39% on day 8. Thereafter, it maintained the same activity up to day 15. These results indicate that following i.p. inoculation of $10^5$ tumor cells, NK cells isolated from the splenocytes of both rejectors and non-rejectors possessed higher cytolytic activity than control; however, cytotoxic activity of NK cells isolated from rejectors was higher than those isolated from non-rejectors.

5.2.1.3  Inoculation of $10^6$ AK-5 cells per animal

We also studied the effect of intraperitoneal injection of $10^6$ tumor cells per animal. 50% of the animals did not develop the tumor in this group after 25 days and the other 50% animals died within 15 days (Fig. 5.1). Here again we categorized the animals into two groups, one set being the rejectors where the tumor did not develop and the other set as non-rejectors where the animals died within 15 days because they were unable to clear the tumor burden from the peritoneum. NK cell cytotoxicity was evaluated from non-rejectors. Three days after tumor injection, NK cells isolated from PEC of non-rejectors showed 15% cytotoxic activity which decreased gradually to 1% on day 10, whereas the NK cells from control animals showed cytotoxic activity from 10% on day 0, to a maximum of 16% on day 15. We also isolated the NK cells from the peritoneum of rejectors and checked their lytic activity. All the rejectors had higher number of TIM present in the peritoneal lavage up to day 18. Three days after tumor inoculation, NK cells isolated from PEC of rejectors showed 30% cytotoxic activity, which increased steadily to 85% on day 7. Thereafter, there was a gradual decrease of NK cell cytotoxic activity to 30% by day 15 (Fig. 5.2).
NK cells from the splenocytes of non-rejectors were assayed for their cytotoxic activity. Four days after tumor injection, splenic NK cells showed 14% cytotoxic activity, which increased gradually to 25% on day 12 (Fig. 5.3). However in case of rejectors, splenic NK cells isolated after 4 days demonstrated 18% cytotoxic activity which increased gradually to 42% on day 12. These observations showed that NK cells from both rejectors and non-rejectors possess higher cytotoxic activity than the controls when $10^6$ tumor cells are transplanted i.p.

5.2.1.4. **Inoculation of $10^7$ AK-5 cells per animal**

We also investigated the effect of intraperitoneal injection of $10^7$ tumor cells per animal. Injection of $10^7$ cells led to a rapid development of ascites and mortality within 5-8 days. The i.p transplanted tumor cells divided rapidly and formed a peritoneal bulge by day 3 and the animals started dying from day 5 (Fig. 5.1). All the rats developed the ascites and died. NK cells from ascites were evaluated for their cytotoxic activity against YAC-1 target cells. There was a drastic increase in AK-5 cell number from day 3 with simultaneous depletion of NK cell population in the ascites. Cytotoxic activity of NK cells isolated from PEC against YAC-1 was completely lost by day 3. After day 3, no cytotoxic activity was observed although NK cells isolated from ascites of control animals showed cytotoxic activity of 12% on day 0 and a maximum of 15% on day 7 (Fig. 5.2).

Cytotoxic activity of NK cells isolated from splenocytes of non-rejectors following i.p. transplantation of AK-5 tumor was also checked. NK cell cytotoxic activity increased gradually from 14% on day 0 to 22% on day 7, whereas control animals did not show any increase in their cytotoxic activity (Fig. 5.3). These results indicate that though NK cells isolated from ascitic fluid showed suppression of their cytotoxic activity, there was a slight enhancement in the lytic potential of NK cells isolated from the spleens.
5.2.2 NKT cell cytotoxicity after inoculation of AK-5 cells

PEC were collected 5 and 10 days after injection of different doses of AK-5 tumor cells. NKT cells were isolated from PEC by Dynal immunomagnetic beads and the cytotoxic activity was checked against YAC-1 target cells (Fig. 5.4). NKT cells also show dose dependent activation and inactivation following AK-5 tumor injection. NKT cells from rejectors showed higher cytotoxic activity than from controls whereas NKT cells from non rejectors were inactivated and showed lower or negligible cytotoxic activity depending on the tumor dose injected.

5.2.3 AK-5 tumor cell dose modulates the ratio of NK and NKT cells

In normal rats, in addition to T cells approximately the same number of NK cells (5.4 ± 1.1%, n=5) and NKT cells (4.2 ± 0.9%, n=5) are present in PEC. Six days after tumor inoculation (10⁴ to 10⁷), PEC from rejectors and non-rejectors were isolated and stained with anti CD3 and anti CD161 and the percentage of NK and NKT cells present in PEC were analysed (Fig. 5.5). In tumor rejectors, both NK cells and NKT cells increased significantly (p < 0.01). However, in non rejectors, no significant increase in the percentage of NK and NKT cells was observed following inoculation of 10⁵ tumor cells per animal. At higher tumor doses (10⁶ and 10⁷), the percentage of NK cell and NKT cells decreased significantly (p < 0.01). These results indicate that high doses of tumor reduced the survival time of animals and also depleted both NK and NKT cells from the peritoneal cavity.

5.2.4 Cytokine levels in serum and ascitic fluid of tumor rejectors and non-rejectors

When 10⁶ tumor cells were injected per animal, 50% animals survived and were free from tumor after 20 days and the other 50% died within 15 days. Based on the intraperitoneal tumor development we classified the animals as rejectors (survived after 20 days with no
Fig. 5.4: Effect of AK-5 tumor transplantation on the cytotoxic activity of NKT cells. NKT cells isolated from PEC on day 5 and 10 after AK-5 tumor transplantation (i.p.) were tested for cytotoxicity against YAC-1 cells. Open bars represent controls, closed bars represent rejectors and the shaded bars represent non rejector animals. *p < 0.001; control group versus experimental group.
**Fig. 5.5:** Number of peritoneal NK and NKT cells after injection of AK-5 tumor into the peritoneal cavity. AK-5 cells ($10^4$-$10^7$) were injected i.p. and the PEC from rejectors and non-rejectors were analyzed on day 6 after immunofluorescence staining. Open bars represent NK cells and the closed bars represent NKT cells. *p < 0.01; control group versus experimental group.
peritoneal bulge) and non-rejectors (died within 15 days). Figure 5.6 shows the serum cytokine profile in rejectors and non-rejectors estimated on different days. Levels of IL-2, IL-12, IFN-γ and TNF-α were higher in rejectors than in non-rejectors. We also analysed the presence of IL-2, IL-12, IFN-γ and TNF-α in the cell free ascitic fluid from rejectors and non-rejectors on different days as shown in Figure 5.7. Ascitic fluid also showed higher cytokine levels in rejectors than in non-rejectors. These results suggest the requirement of higher levels of cytokines and their role in the activation of effector cell function leading to the inhibition of tumor growth.

5.2.5 Anti-IFN-γ and anti IL-12 injections shortened the survival time of animals

To study the role of IFN-γ and IL-12 produced by PEC in suppressing the tumor growth, either anti-IFN-γ mAb (1 mg Ig/injection) or anti-IL-12 mAb (1 mg Ig/injection) were injected i.p. on day 0 (just before the tumor inoculation) \(10^5\) cells/animal) and on day 2. The results show that treatment with either antibody significantly reduced the survival time of animals. The effect of anti IFN-γ mAb was stronger than that of anti-IL-12 mAb. (Fig. 5.8). Anti IL-2 had no significant effect on the survival time of animals.

5.2.6 Expression of CD95-L by AK-5 cells

Following i.p. transplantation of \(10^7\) AK-5 cells per animal, the tumor cells were isolated from the peritoneum on different days and analysed for the expression of CD95-L after immunostaining with specific antibody. CD95-L expression by AK-5 cells is maximum on day 3 and 4 after transplantation and decreased from day 5 onwards (Fig. 5.9). These observations suggest that AK-5 cells are able to regulate CD95-L expression on their surface, which may have a role in antitumor immune response.
Fig. 5.6: Cytokine levels in the sera of tumor rejectors (---) and non-rejectors (---) on different days after i.p. tumor transplantation. The values represent mean ± SD of 3 animals. Cytokine levels in control animals on different days were very low (data not shown). The values were compared between rejectors and non-rejectors by Student’s “t” test and were significant (p < 0.01) from day 4 onwards.
Fig.5.7: Cytokine levels in ascitic fluid of tumor rejectors (–•–) and non-rejectors (–■–) on different days after i.p. tumor transplantation. The values represent mean ± SD of 3 animals. Cytokine levels in control animals on different days were very low (data not shown). The values were compared between rejectors and non-rejectors by Student’s “t” test and were significant (p < 0.01) from day 4 onwards.
Fig. 5.8: Anti IFN-γ or anti IL-12 antibody injections significantly shortened the survival time of rats transplanted i.p. with $10^5$ AK-5 cells. 1 mg Ig / injection / animal was injected on day 0 and day 2 (i.p.). Control rats received only $10^5$ AK-5 cells per animal. 10 animals were used in each group.

*p < 0.001; control group versus anti-IL-12 and anti IFN-γ treatment.
Fig.5.9: Differential expression of CD95-L by AK-5 cells (10^7 cells / animal) transplanted i.p. on different days. The values shown are mean ± SD of triplicates and are representative of two similar experiments.
5.2.7 Expression of CD95 and perforin by NK cells

After transplantation of AK-5 tumor cells i.p. (10^6 cells / animal), we isolated NK cells from ascitic fluid of non-rejectors on different days and analysed for the expression of CD95 and perforin after immunostaining with specific antibodies. Three days after tumor transplantations, NK cells isolated from ascites showed 2% CD95 positive cells, which increased sharply to 72% on day 10 (Fig. 5.10). On the other hand, 5% NK cells showed perforin expression on day which increased gradually to 15% on day 10.

Similarly, NK cells from ascitic fluid of tumor rejectors were analysed for the expression of CD95 and perforin on different days. Four days after tumor inoculation, 15% NK cells were perforin positive which increased steadily to 65% on day 12 (Fig. 5.10). However, only 4% NK cells were positive for CD95 expression on day 4, which increased gradually to 10% on day 8. These observations demonstrated that when the tumor burden was high, NK cells expressed more of CD95 and less of perforin, whereas when the tumor load is low, NK cells expressed high levels of perforin and low levels of CD95 on their cell surface, thereby suggesting CD95 and perforin as the markers of suppressed and activated NK cells respectively.

5.3 DISCUSSION

AK-5 is a rat histiocytoma which when transplanted s.c. grows as solid tumor and develops into ascites when injected i.p. upon transplantation of 5 x 10^6 cells. All the i.p. injected animals are killed by day 10, however, about 70% s.c. transplanted animals reject the tumor spontaneously (Kausalaya et al., 1995). We have demonstrated NK cell as the effector cells involved in tumor cell killing (Bright et al., 1995). In the present study, we have evaluated activation of effector cell function based on the dose of tumor cells transplanted i.p.
Fig. 5.10: Differential expression of CD95 (white bar) and perforin (black bar) by NK cells isolated from ascitic fluid of rejectors and non-rejectors on different days following i.p. transplantation of AK-5 cells (10^5 cells/animal). The values shown are mean ± SD of triplicate.
We have observed that a low dose of tumor cells mounts a high immune response which is manifested by increased number of NK and NKT cells in the peritoneum, higher cytotoxic activity and upregulation of perforin expression by NK cells followed by the rejection of AK-5 tumor from the peritoneal cavity. However at higher tumor doses, animals could not mount an effective immune response, which was reflected by the presence of low number of NK and NKT cells in the ascitic fluid, lower cytotoxic activity and upregulation of CD95 expression by NK cells. These observations indicate that the tumor cell load in the peritoneum is responsible for the regulation of NK cell activity and the surface phenotype on NK cells. Lower tumor dose also resulted in higher cytotoxic activity of splenic NK cells in comparison to control NK cells isolated from naive splenocytes. However, cytotoxic activity of NK cells isolated from PEC was higher than NK cells isolated from splenocytes when low dose of tumor cells was inoculated. NK cells in PEC are in direct contact with tumor cells, which might be responsible for the higher activity of NK cells present in ascites as compared to NK cells isolated from splenocytes, which had no direct contact with the AK-5 cells. Moreover, lower tumor cell dose also evoked an appropriate immune response followed by the secretion of several cytokines which are involved in the enhancement of cytotoxic activity of NK cells, whereas, in case of high tumor dose splenic NK cells showed higher cytotoxic activity than NK cells isolated from the peritoneal cavity. NK cells in the peritoneal cavity are exposed directly to the high tumor load and hence had undergone suppression of their cytotoxic activity. Since the NK cells in the spleen are not in direct contact with tumor cells, therefore they did not show any suppression of their cytotoxic function and the cytokines secreted by immune cells present in ascites following interaction with tumor cells were responsible for the activation of NK cells from the spleen. These observations demonstrate that when AK-5 tumor cells are in direct contact with NK cells, the tumor cell
could either activate NK cells at low dose, or suppress NK cell activity when the tumor load is high. Hence, this dose-dependent activation or inactivation of NK cell function determines the fate of the tumor growth and drives it either towards tumor rejection or tumor progression.

In the present study, we have detected IL-2, IL-12, IFN-γ and TNF-α in the sera as well as ascites from both rejectors and non-rejectors. IL-2 has been shown to trigger the activation and tumoricidal capacity of NK and LAK cells (Hara et al., 1995). Similarly, IFN-γ induces cytotoxic activity of CTLs, LAK and NK cells (Perussia et al., 1991). IL-12, a hetero-dimeric cytokine which was originally called natural killer cell stimulatory factor, augments spontaneous as well as antibody dependent cell mediated cytotoxicity of resting NK cells (Trinchieri and Perussia, 1985). Besides, contact between tumor cells and macrophages is known to release TNF-α without mediation of endotoxin (Carswell, 1975 and production of biologically active TNF-α has been demonstrated in malignancy (Zacherchule et al., 1983). Though we have detected all the above cytokines in both rejectors as well as non-rejectors, the levels of cytokines in the rejectors were higher than in non-rejectors. Therefore, high levels of TNF-α along with increased IL-2, IL-12 and IFN-γ levels in rejectors evokes a higher level of immune response which might be responsible for the rejection of AK-5 tumor. However, in case of non-rejectors, which secreted comparatively low levels of IL-2, IL-12, IFN-γ and TNF-α, an effective immune response could not be evolved against AK-5 tumor and hence could not cause rejection of the tumor in the peritoneum.

Tumor cells are known to have devised mechanisms of immune escape by masking or shedding of the antigens (Herlyn et al., 1987). In addition, a novel mechanism of immune evasion by tumor cells was demonstrated where the tumor cells expressed CD95-L, thereby keeping the activated immune cells which normally express CD95, away from the immune attack
(O’Connell et al., 1996 & Niehans et al., 1997). Tumor cells expressing CD95-L have been shown to deliver the death signal to CD95-expressing targets in vitro (O’Connell et al., 1996 & Niehans et al., 1997 & Strand et al., 1995). AK-5 cells express CD95-L on their surface. We have shown that NK cells isolated from ascites with a huge peritoneal bulge had higher expression of CD95 on their surface. Thus, it is possible that when the tumor burden is high, CD95-L positive AK-5 cells upon interaction with NK cells may cause upregulation of CD95 on NK cells. The CD95-CD95-L interaction between the effector cells and the target cells leads to the suppression of NK cell cytotoxicity and the depletion of NK cell population in the ascites. The present study suggests a direct interaction between NK cells and tumor cells in vivo, leading to the dose dependent activation or suppression of NK cell function that determines the fate of the tumor either towards progression or regression in syngeneic hosts.
Summary of the results

**In vitro studies**

The role of endogenously secreted TNF-α in mediating functional inactivation and apoptosis in NK cells when they are co-cultured with fixed AK-5 cells at 1:1 ratio.

A change in the phenotype from CD95\textsuperscript{dim} to CD95\textsuperscript{bright} was observed in NK cells co-cultured with target cells.

The up-regulation of CD95 expression on the NK cells after co-culture with fixed AK-5 cells may play an important role in signaling NK cells for functional anergy and apoptosis.

The induction of NK cell lytic function after their interaction with fixed tumor cells and the requirement for cell-secreted accessory signals for the augmentation of the lytic function.

AK-5 cell mediated augmentation of NK cell cytotoxicity in the presence of T cells is mediated by lymphokines (IL-2 and IFN-γ) and that NKT cells possess higher cytotoxic potential than NK cells.

We have also observed over-expression of CD95L and perforin in NK cells following exposure to fixed AK-5 cells.

**In vivo studies**

Inoculation of AK-5 tumor cells intraperitoneally modulate the cytotoxic function of NK and NKT cells present in the peritoneal exudates cells (PEC) in a dose dependent manner.

Low dose of tumor causes activation of NK and NKT cell cytotoxic function and enhanced NK and NKT cell population in PEC whereas high doses of tumor cause inactivation of NK and NKT cell cytotoxic function and depletion of the two sub-populations in the peritoneum.

Different doses of tumor inoculation in the peritoneal cavity did not suppress the cytotoxic function of NK cells from spleen suggesting that a direct interaction between NK cells and tumor cells is required for the suppression of NK cell cytotoxic function.