1. The basic aim of the present work was to study the influence of tumor cells on NK cell system. Since normal tumor cells grow and overwhelm the culture, we examined the effect of irradiated tumor cells which cannot proliferate.

2. Addition of irradiated tumor cells resulted in a progressive decline in the recovery of viable cells from IL-2 activated spleen cell cultures. The decline was dependent on the number of irradiated tumor cells added.

3. Irradiated YAC as well as EL4 tumor cells could inhibit the IL-2 induced NK activation and the inhibition could be demonstrated by using any of the two tumor cells as targets.

4. Presence of irradiated tumor cells (YAC or EL4) during IL-2 activation also inhibited proliferative activity of spleen cells. The inhibition was proportional to the tumor/spleen cell ratios used.

5. Culture supernatants of irradiated as well as control YAC cells could induce a marked inhibition of NK cell activation. Cell recovery was also poor in the presence of tumor supernatants.

6. Time kinetics of the effect of YAC supernatant showed that the inhibitory effect on NK activation required three days of culture, as no inhibitory effect was observed at earlier time points. Dose response showed that tumor cell supernatants were effective in inhibiting NK activation and cell proliferation at very low (less than 1% v/v) doses.
7. The supernatant from YAC cells had an anti-proliferative effect which was not specific for IL-2 induced spleen cell proliferation. Proliferation in response to LPS and Con A was also inhibited by tumor cell supernatants.

8. Generation of suppressor activity was not restricted to YAC tumor cells alone since EL4 and SP2O cell supernatants also had similar effect.

9. Heating the YAC supernatant to 90 C for 45 minutes did not abrogate the suppressor activity, though it resulted in improved cell recoveries. The anti proliferative effect of tumor supernatants was also heat stable. The suppressor activity of YAC supernatant, did not pass through 3K and 10K Amicon filters indicating that the molecular weight of the suppressor factor may be higher than 10 kDa.

10. Paraformaldehyde fixed tumor cells were used to study the effect of tumor cells in absence of suppressor factors. At all tumor to spleen cell ratios, fixed YAC cells, boosted NK cell activation in response to IL-2. In the absence of IL-2, NK activity was not boosted by fixed tumor cells.

11. Enrichment of NWC passed spleen cells for T-cells and total depletion of T-cells by anti Thy-1 + C treatment did not abrogate the enhanced cytolytic activity induced in presence of fixed tumor cells and IL-2.
12. Increased cytolytic activity was observed against YAC and EL4 target cells when the effector cells are activated in the presence of either of the fixed tumor cells. However, the anti-YAC cytolytic activity generated is relatively higher when fixed YAC cells are added during the activation phase as compared to cultures generated in the presence of fixed EL4 cells. Similarly, relatively higher anti-EL4 activity is observed when fixed EL4 are added during the activation phase. Thus, the addition of fixed tumor cells caused a generalized boosting of NK activation, yet the boosting was relatively greater against the tumor cells used for boosting the NK activity.

13. The addition of fixed syngeneic or allogeneic spleen cells did not affect the anti-YAC or anti-EL4 activity generated in response to IL-2, indicating that the difference in MHC haplotypes did not play a role in the activation induced by fixed tumor cells.

14. Mouse spleen cells were activated by IFN-γ either alone or in the presence of fixed tumor cells. Activation induced by IFN-γ at different doses was relatively poor as compared to IL-2, yet fixed YAC cells could significantly boost the cytolytic activity generated in response to IFN-γ. In addition, some target specific boosting of NK activation with fixed tumor cells was also suggested when a shorter activation period (24 hours) was used.
15. The activation of spleen and bone marrow cells in the presence of fixed YAC or EL4 tumor cells resulted in an increase in the percentage of cells expressing the Ly 49A receptor. A time kinetics of the Ly 49A receptor expression on bone marrow cells activated with IL-2 either alone or in the presence of fixed YAC tumor cells showed that the induction of Ly 49A expression, in response to fixed tumor cells was maximum on day 4 and day 5.

16. Spleen cells cultured in the presence of fixed tumor cells showed a high expression of Ly 49A and Ly 49C in both the TCR positive and NK1.1 positive populations. Similarly, activated bone marrow cells showed a significant increase in the expression of Ly 49A and Ly 49C on the NK1.1 and TCR positive cells.

17. Bone marrow cells activated in the presence of fixed YAC cells showed significantly higher levels of Ly 49A and Ly 49C expression, as compared to the cells activated in the presence of fixed EL4 cells.

18. To ascertain the influence of MHC molecules on the development/activation of NK cells, the effect of masking MHC molecules during the activation of NK by using a polyclonal anti H-2b antisera, was studied. The addition of anti H-2b alloantiserum during the IL-2 activation of bone marrow cells, resulted in greater activation of NK cells.

19. Activatory effect of anti H-2b alloantiserum was dose dependent and best effect of the alloantiserum was obtained on day 4. Augmentation of NK activation of spleen cells by alloantiserum was observed against both EL4 and YAC tumors, indicating a lack of target specificity in NK activation.
20. To test the effect of Ig coated cells on IL-2 activation, rabbit anti mouse IgG antiserum was added during the IL-2 induced NK cell activation of bone marrow cells. NK activation was significantly greater in the presence of the anti mouse IgG antiserum also, indicating that the boosting of NK activation by anti MHC antibodies might be mediated by antibody coated cells generated in the culture.

21. To avoid the interference caused by antibody (Fc intact) coated cells, the effect of F(ab)$_2$ fragment of anti H-2$^b$ alloantibodies on NK activation. was studied. Addition of F(ab)$_2$ fragment derived from the anti H-2$^b$ alloantiserum inhibited, in a dose dependent manner, the NK cell activation in response to IL-2. These results suggest that MHC I antigen may play an important role in NK activation process.