RESULTS

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NUMBER OF NK CELLS IN NORMAL AND TUMOUR BEARING MICE

The increase in size of the spleen in mice with developing tumours made it necessary to count the number of NK cells in the spleen of normal and tumour bearing mice. Spleens from normal mice contained about $3x10^6$ NK cells whereas spleens from tumour bearing mice contained about four times more NK cells than spleens from normal mice. (Fig.1).

BLASTOGENIC RESPONSE OF NK^N CELLS AFTER ACTIVATION WITH IL-2

For blastogenesis, NK cells from normal mice were activated with varying doses such as 10U, 50U, 100U and 200U of rIL-2 and 50 μl of generated IL-2 (g IL-2) for 24 hours and 48 hours. 100U rIL-2 is optimal for activating NK cells for blastogenesis *in vitro* with the peak at 24 hours(Fig.2). This value is nearly equivalent to the percentage of blastogenesis of NK cells activated with 50 μl of gIL-2. For all these doses, the response declines to some extent at 48 hours(Fig.3), still the 100U rIL-2 is the best. The results with 50 μl of gIL-2 at 48 hours is not exactly equivalent to that with 100U rIL-2 as it was at 24 hours.

DNA SYNTHESIS OF NK^N CELLS AFTER ACTIVATION WITH IL-2, MEASURED BY INCORPORATION OF ^3H-THYMIDINE

The *in vitro* proliferative response of NK^N cells after activation with IL-2 was also measured by incorporation of radioactive thymidine (^3H-TdR) in DNA (Fig.4). The maximum incorporation of ^3H-TdR in NK cells were with 100U of rIL-2 activation for 24 hours, almost equivalent result was obtained with 50 μl of gIL-2. Other doses of rIL-2 did not improve the thymidine incorporation in NK^N cells. At 48 hours of IL-2 activation, the thymidine incorporation came down to control values.

CYTOTOXICITY OF NK AND IL-2 ACTIVATED CELLS FROM SPLEEN OF NORMAL AND TUMOUR BEARING MICE

The difference in cytotoxicity of NK^N cells and NK^T cells, before and after IL-2 activation was tested in a ^{51}Cr release assay. Even without activation, NK^N cells exhibited minimal cytotoxic response as spontaneous cytotoxicity against fibrosarcoma cells (Table 2,Fig.5). This minimal cytotoxic value of NK^N cells however increased four times after 24 hours activation with IL-2. Activation with IL-2 upto 48 hours did not improve the results beyond minimal values. Interestingly, NK^T cells as such were found to be twice as cytotoxic than NK^N cells; this increased marginally by IL-2 treatment for 24 hours. At 48 hours of activation with IL-2, the cytotoxic value came down to background level as found in NK^N cells (Table 5).

ANTIBODY DEPENDENT CELL MEDIATED CYTOTOXICITY (ADCC) OF NK CELLS FROM SPLEEN OF NORMAL AND TUMOUR BEARING MICE

. NK^N cells were highly cytotoxic in ADCC assay where anti TAA antibody raised in rabbit was added (Table 3). This however, could not be improved by further activation with IL-2. NK^T cells showed lower index in ADCC and IL-2 treatment improved the results to some extent which is statistically significant (Table 4). At 48 hours of IL-2 activation (Table 5), ADCC of both NK^N cells and NK^T cells came down to the level of the response of NK^T cells without IL-2 activation.

CYTOTOXICITY OF ANTI TAA SERUM RAISED IN RABBIT AGAINST MURINE FIBROSARCOMA CELLS

Heat inactivated anti TAA serum was always used in ADCC assay to avoid participation of complement. In this experiment, the cytotoxic ability of heat inactivated anti TAA serum with or without complement has been tested. The percentage of cytotoxicity with heat inactivated anti TAA serum was in the range of 3.2 to 6.66 whereas in presence of complement the cytotoxicity increased enormously (Table 6). The specific activity of anti TAA serum in presence of complement was tested by performing the control experiment with normal rabbit serum.

CYTOTOXIC ACTIVITY OF NK^N CELLS IN PRESENCE OF SERUM FROM NORMAL AND TUMOUR BEARING MICE

As the NK^N cells perform better in ADCC assay (Table 3) in presence of anti TAA serum, we guessed that the serum of tumour bearing mice might have anti TAA antibodies and thus help in ADCC assay. NK^N cells perform better in presence of serum from tumour bearing mice than in control experiment where serum is added from normal mice. The indices with serum from tumour bearing mice fall in absence of complement i.e. serum inactivated at 56^0C for 30 mins. This is possibly indicative of NK cell activity plus activity of the anti serum as such.The response is restored by adding guineapig complement.

HOMING OF RADIOACTIVELY LABELLED IL-2 ACTIVATED SYNGENEIC NK^N CELLS AT TUMOUR SITE

IL-2 activated NK^N cells were radiolabelled with ^3H-thymidine for eight hours and injected intravenously, then the pattern of homing of these cells at different anatomical sites were followed by counting the radioactivity in the tissues.

Interestingly, the highest count was obtained from the centre of the tumour at 12 hours after adoptive transfer (Fig.6). This value was considered as maximum and all other values were expressed relative to this number for the comparison of the results. This homing index from centre of tumour stayed relatively high upto 48 hours. The periphery of tumour showed the second highest homing index of 77.4% after 12 hours of adoptive transfer. Decline in homing of NK^N cells at these two sites occured from 24 hours onward. Liver was the next site of

preference for homing of the activated NK^N cells with 69.6% infiltration. Infiltration by the NK^N cells was slightly lower but reasonably good at other sites, being the lowest at skin. Out of all the results, highest count at the tumour site definitely shows as preferential site for homing of radiolabelled IL-2 activated NK^N cells.

TUMOUR GROWTH AND SURVIVAL OF ANIMALS AFTER *in vivo* INFUSIONS OF IL-2

As IL-2 activated NK^N cells were found to be more cytotoxic towards the tumour target cells (Table 2), *in vivo* infusions of IL-2 was carried out to test whether low dose IL-2 infusions can activate the resident NK cells in tumour bearing hosts. Two routes, intravenous and at tumour site were chosen to deliver the IL-2. For each schedule, a dose of 50 μl of gIL-2 was injected per mouse five times at weekly intervals. The growth of the tumour was regularly plotted for 16 weeks(Fig.7). From the slope of the curve, it transpires that the intravenously injected IL-2 was found to be better in curbing the growth of the tumour than the IL-2 injected at the tumour site.

The growth of the tumour was faster in the control group of animals injected only with normal saline. Furthermore, the reflection of the tumour growth was also seen in the survival of the tumour bearing host where 20% of the mice receiving IL-2 recovered completely (Fig.8). When all control animals died at the end of ten weeks, some of the animals injected with IL-2 at the tumour site, could continue to live for two more weeks.

ADOPTIVE TRANSFER OF NK^N CELLS TO THE TUMOUR BEARING HOSTS

Normal NK cells were found to be cytotoxic in *in vitro* cytotoxicity assay (Table2, Fig.5). $0.5x10^6$ syngeneic NK^N cells adoptively transferred to the tumour site, were found to be effective in curbing the tumour growth, in comparison to control animals where only normal saline was injected. The slower rate of growth of the tumour also cause slower mortality rate of the tumour bearing mice (Fig.9). Some experimental animals survived upto thirteen weeks after tumour inoculation compared to ten weeks of survivality of control animals(Fig.10).

ADOPTIVE TRANSFER OF NK^N CELLS AFTER IL-2 ACTIVATION

It has been observed that spontaneous cytotoxicity of NK^N cells is further augmented significantly by 24 hours IL-2 treatment (Table 2). Thus, in this present set of experiments NK^N cells were cultured *in vitro* for 24 hours in presence of 50 μl of gIL-2 before adoptive transfer. Then the animals were divided into three groups. In the first group of animals, IL-2 activated NK^N cells were injected at tumour site. In second set of animals, IL-2 activated NK^N cells were injected intravenously. 50 μl of gIL-2 was administered in the first and second animal groups for five times at weekly intervals at the tumour site and intravenously, respectively. The third group of animals were kept as control which received only normal saline. The best result for restriction of tumour growth was observed in the first group of animals. The survival of the animals were also longer in this group. Comparing, the

slope of the line of tumour growth was 0.25 for this group of animals and 0.36 for the second group indicating the protocol of the injection of NK^N cells at tumour site followed by IL-2 injections at tumour site is the best among the three for curbing the growth of the tumour and increasing the life of the animals (Figs.11 & 12).

Increasing the inoculum of IL-2 activated NK^N cells to double (10^6 NK^N cells) at tumour site produced much better result with a slope of 0.09 and simultaneously increased the life expectancy of 30% of host animals to more than 16 weeks. (Figs.13 &14).

ADOPTIVE TRANSFER OF IL-2 ACTIVATED NK^T CELLS

Just as the prior experiment another experiment was performed taking the NK^T cells instead of NK^N cells. Here also tumour growth in the group of animals injected with the cells at tumour site were more restricted than when cells were injected intravenously. As in other cases, reflections of this experiment was seen in the survivality of these animals where 10% animals recovered completely (Figs.15 & 16).

ADOPTIVE TRANSFER OF IL-2 ACTIVATED NK^N CELLS AFTER SURGICAL REMOVAL OF TUMOUR

A dose of 50 μl gIL-2 was injected for five times at weekly intervals in one group of animals with surgically removed tumours and also in another group receiving the IL-2 activated NK^N cells after surgery (Figs.17 & 18). The tumours were surgically removed in control animals but they did not receive any NK^N cells or IL-2 injections.

Recurrence of tumour did not occur in 92.3% of the animals receiving IL-2 activated NK^N cells and weekly IL-2 injections, whereas recurrence of tumour occurred in 40% of the animals receiving IL-2 injections only. In contrast recurrence was in 75% control animals which did not receive any treatment after removal of tumour bulk. The difference in the results of both the experimental groups with control was found to be statistically significant ($p < 0.001$).