Kinetics of NK cell activity during tumor development in a mouse model.

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ABSTRACT - We have previously reported the profiles of natural killer cell mediated cytotoxicity (NKC/MC) in the spleen cells of tumor bearing Balb/c mice during the development of Dalton's lymphoma ascites (DLA) tumor, a murine T cell lymphoma, In the present paper we have used two different methods of assessing NK cytotoxicity *in vitro* to dissect the relative importance of various steps involved in attaining the overall result, The ^{51}Cr release assay has been combined with a recently established single cell conjugate assay using poly-L-lysine coated coverslips to allow better quantitative analysis. With this double procedure we could estimate the percentage of active killer cells, V_{max} and recycling capacity of the effector cells at different intervals of tumor progression. A large granular lymphocyte (LGL) enriched population obtained by discontinuous density gradient centrifugation of Percoll was used as effector cells against the murine NK susceptible target YAC-1 in both assays. Also, the ability of the effector cells to release natural killer cytotoxic factor (NKCF), on stimulation with YAC-1 cells was estimated in a micro-supernatant assay of 48 h, A significant enhancement in the number of active killer cells coupled with increased NK:CF production was observed on day 7 and day 12 after tumor inoculation with respect to normal control. A decline in NKCWC observed at the advanced stage of tumor growth (day 19) was found to be associated with diminished NKCF production as well as a reduced number of active killer cells among the effect or cell population. Estimated maximal recycling capacity showed minimal differences at different phases of tumor growth. Pretreatment of day-19 effector cells with 200 IU/ml of human recombinant IL-2 resulted in augmentation of NKCMC, V_{max}, percentage active killer cells, and NKCF production.

Key words - Natural Killer Cytotoxic factor, Large granular lymphocytes, Interleukin-2.

INTRODUCTION

NATURAL KILLER (NK) cells, a morphological subpopulation of large granular lymphocytes are able to kill a wide spectrum of target cells including certain tumor cells and virus infected cells (Herberman and Ortaldo 1981) without prior sensitizatlon (Nunn *et al.*. 1975). NK cells have been reported to express receptors for the Fc portion of IgG (Kay *et al.* 1977, Herberman *et al.* 1977). According to Wright and Bonavida (1983), murine spleen cells that release NKCF during co-culture with NK sensative target cells share the following characteristics with NK effector cells : 1) They are plastic non-adherent, thy 1- 2- and asialo-GM1 positive; 2) enrichment of NK cells by Percoll gradient centrifugation results in enhancement of cells that release NKCF. The first step in NK cell-mediated cytotoxicity is that the effector cell recognizes and binds to the target cell.

Next the target cell stimulates the effector cell to activate the mechanism for release of NKCF and finally the effector cell releases NKCF which binds to the target cell membrane and mediate target cell lysis. We have previously reported that profiles of NK cell activity show a definite pattern during tumorogenesis in Balb/c mice (Suresh *et al.* 1989). The rationale of the present investigation is to assess the enhancement in killing potential of large granular lymphocytes purified on Percoll density gradients at different intervals of tumor progression, using single cell conjugate assay and ^{51}Cr release assay.

Maximal recycling capacity of the effector cells and ability to release NKCF were also studied. These experiments could explain the observed NK cell profiles seen during tumor development. The large granular lymphocytes (LGL) obtained from animals in the late phase of tumor growth were activated *in vitro* with human recombinant IL-2 and the effect of IL-2 pretreatment was also studied.

MATERIALS AND METHODS

Tumor model - Inbred Balb/c mice, maintained in our animal house were provided standard mouse feed and water *ad libitum*. Dalton's lymphoma ascites tumor (DLA) obtained from Chittaranjan National Cancer Research Centre, Calcutta, was maintained in ascites form by intraperitioneal passage. Balb/c mice, 6-8 weeks old, were given DLA cells (10^7) i.p. Tumor bearing mice were sacrificed at various intervals from day 0 to death, their spleens removed asceptically and processed at 0°C. Spleen cells, after washing in cold RPMI-1640 supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated newborn calf serum (NBS) (Hysel, India), were gently squeezed through a wire mesh with a plastic plunger of a syringe into a Petri dish containing medium. The cells were washed three times in medium and adherent cells were removed by passage through a nylon wool column (Julius *et al.* 1973).

Isolation of LGL of Percoll density gradient (Timonen et al. 1982) - Percoll (Sigma Chemicals) was adjusted to 285 mOsmol/kg, a seven-step density gradient, ranging from 38.6% to 70%, was prepared by diluting the Percoll with plain medium. Each fraction (1.5 ml) was layered careful-

ly into a 15-ml centrifuge tube starting from 70% fraction at the bottom. Nylon wool-purified cells, 50 x 106 in 1-ml were carefully layered on the top, avoiding mixing. The tubes were centrifuged at 150 x g for 30 min at room temperature. After centrifugation, fractlons 2 and 3 were collected, washed in phosphate-buffered saline (PBS) three times, resuspended in RPMI-1640 medium and checked for viability. The cells were morphologically identified as LGL by Giemsa staining.

Target cells - A murine NK cell sensitive cell line, YAC-1 (obtained from National Tissue Culture Facility, Pune, India) was used. The cells were propagated as stationary cultures in RPMI-1640 plus 10% NBS and subcultured twice weekly. Prior to the labeling, viability was assessed by trypan blue exclusion.

^{51}Cr release assay - The chromium release assay was done as described previously (Suresh *et al.* 1990). Approximately 2 x 10^6 target cells in 0.3 ml of medium were incubated with 100 µCi of ^{51}Cr for 2 h at 37°C. The cells were washed three times in RPMI-1640 and resuspended at a concentration 10^5 cells/ml. LGL and YAC-1 (target) cells at effector target ratios of 5:1, 10:1, 20:1, 40:1 were incubated at 37°C for 4 h in RPMI-1640 with 10% NBS. All the tests were done in triplicate. After incubation, supernatant was removed and radioactivity was measured in a gamma ray spectrometer. Percent specific cytolysis was calculated as:
(Mean experimental release - mean spontaneous release)/(Mean total release - mean spontaneous release) x 100.

Spontaneous release was determined by incubating labeled target cells in the absence of effector cells. Total release was obtained by adding 1N HCI in the labeled target cells.

Single cell conjugate assay on poly-L-lysine coated cover slips - This was done as described by Vargas-Cortes *et al.* (1983). 22mm x 22mm cover slips were thoroughly washed with 70% ethanol, placed on plastic Petri dishes and covered with 0-5 ml of (2 µg/ml) of poly-L-lysine hydrobromide (PLL, type 1B, M.W 70,000; Sigma Chemical Company) in PBS. The coverslips were incubated at room temperature for 45 min and were washed with PBS to discard unbound PLL. 100 µl of the effector cell suspension was mixed with an equal amount of target cells (1 x 10^5 cells) and pelleted by low-speed centrifugation in conical plastic tubes for 4 min. The cell pellet was incubated for 15 min at 37°C in a 5% CO_2 atmosphere. After incubation most of the supernatant was aspirated and the pellet resuspended in serum-free medium (RPMI-1640, 0.5 ml/tube). The mixture was plated on the PLL-coated coverslips and incubated at room temperature for 20 min. The slides were then transferred to a 37°C incubator for 4 h. Control slides containing only target cells were prepared in the same way. After incubation, the control and experimental slides were washed by drop wise addition of medium. The medium was decanted, the slides were stained for 3 min with trypan blue (0.1% in PBS) and washed. They were then fixed with 1% formaldehyde in PBS. After washing, the cover slips were inverted to microscope slides to be scored under the light microscope.

Calculations - The number of target binding lymphocytes and their cytotoxicity was calculated as previously described by others (Ullberg and Jondal, 1981). Spontaneously dead target cells were determined in control slides, by scoring the percentage of dead cells assessed by trypan blue staining. The proportion of lymphocytes forming conjugates with target cells (TBC) was determined by counting the frequency of lymphocytes binding to YAC-1 cells. At least 200 lymphocytes were counted. The percentage of target cell binding lymphocytes that were cytotoxic was determined by the equation A= B-(B x C) where B is the percentage of conjugates containing dead target cells (100 conjugates counted) and C is the fraction of spontaneously dead target. The percentage active killer cells present in the LGL population (A x D) 100 where D is the percentage of total lymphocytes binding to target cells (TBC). According the Ullberg and Jondal (1981), it is possible to get a rough estimation of Vmax for effector cells by using a E:T ratio 5:1 (5 x 10^4 lymphocytes and 10^4 target cells in triplicate) in the ^{51}Cr release assay and then using the formula V_{max}= 1.4 x 10^3 + 4.2 x 10^2 x (percentage ^{51}Cr release at E:T 5:1 ratio).

Estimation of maximal recycling capacity (MRC)- MRC was calculated by combining data from the ^{51}Cr release assay and the conjugate assay. The V_{max} value was divided by the absolute number of killer cells, i.e., the percentage active killer cells multiplied with number of effector cells in the V_{max} assay (5 x 10^4 cells). MRC is an estimation of the average number of target cells that an active NK cell can kill in 4 under optimal conditions.

Production of cell-free supernatants containing NKCF (Wright and Bonagida, 1983) - 5 x 10^4 LGL and 10^4 YAC-1 stimulator cells were co-cultured in 2ml RPMI-1640 containing 1% bovine serum albumin, at 37°C in 5% CO_2 for 48 h. After incubation, the cells were spun down, the supernatant was filtered through a 0.45 µm filter (Millex) and stored at -20°C until assayed for cytotoxicity. Control supernatant from 10^4 YAC-1 cells incubated alone were used to ensure that the cytotoxic effect of NKCF supemata-tants were not merely due to build-up of toxic waste products. Control supernatants of 5 x 10^4 LGL incubated alone were used to measure spontaneous NKCF release.

Microsupernatant assay - Cell free supernatants were assayed for cytotoxicity as previously described by Wright and Bonavida (1983). 10^4 YAC-1 target cells in 50 µl culture medium were incubated with 150 µl of test supernatants in 90-well sterile microtitre plates (Nunc). Cultures were set up in triplicate; control cultures contained 150 µl medium instead of test supernatant or 48 h culture supernatant of YAC-1 cells alone. After a 48 h incubation at 37°C in 5% CO_2, cell viability was determined by trypan blue exclusion. Viability of YAC-1 in control cultures was always greater than 90%. The percentage cytotoxicity was calculated as follows:
% cytotoxicity = % viability of control - % viability of test)/% viability of control x 100

Activation of LGL with human rIL-2 - LGL separated by a discontinuous density gradient of Percoll were incubated in medium containing different dilutions (50, 100, 200,

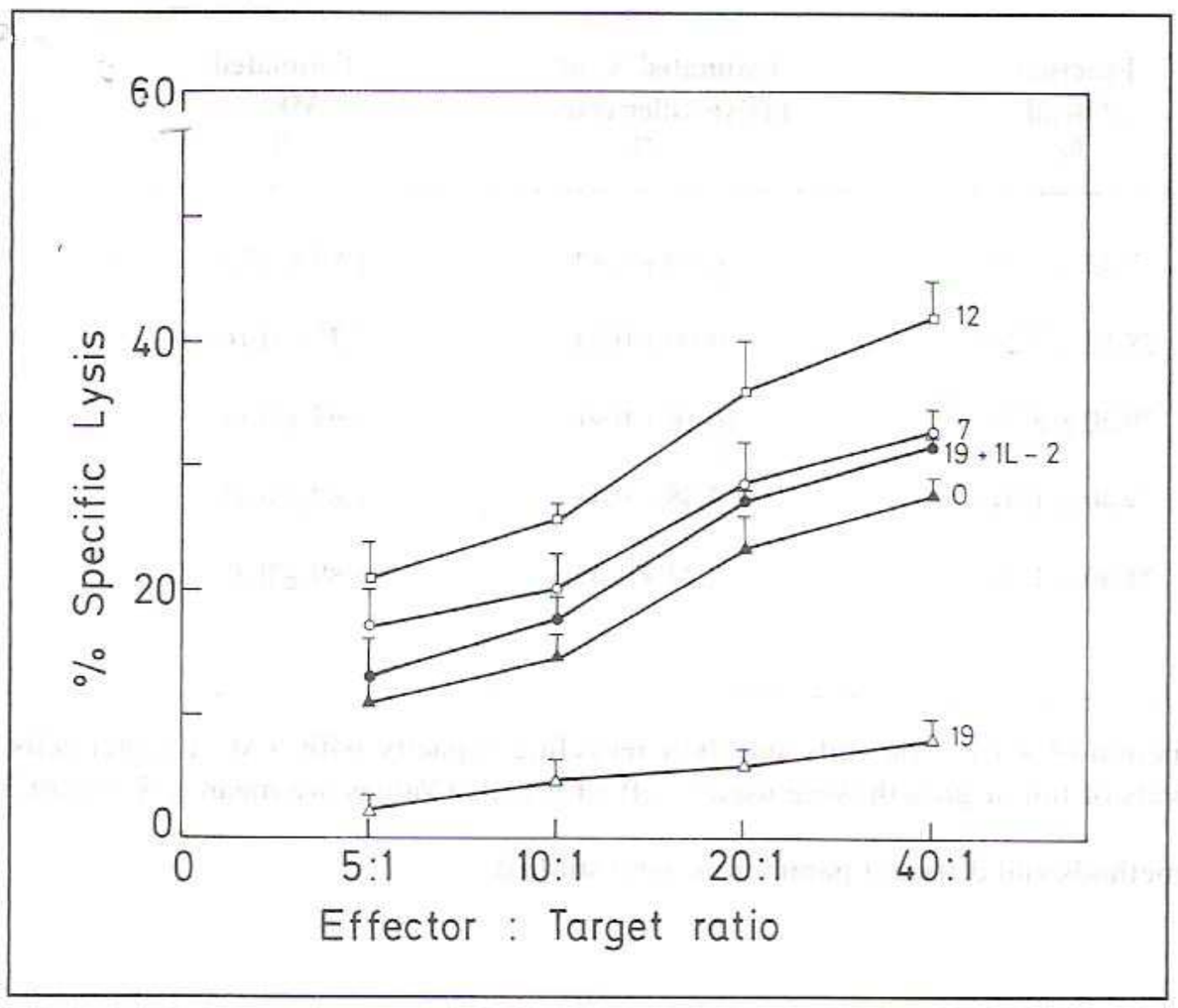

Fig. 1. Natural Killer Cell (LGL) mediated cytolysis of radiolabeled YAC-1 target cells at different time points after tumor inoculation. (▲ day 0, ○, day-7, ❑ day 12, Δ day 19, ✻ day, 19+IL-2) at various effector: target ratios. Numbers indicate the days after tumor inoculation values are mean ± SD from 3 different experiments in each group.

400 IU/ml) of human recombinant interleukin-2 (IL-2) (kindly donated by Dr. J. Rossio, National Cancer Institute, U.S.A) for 72 h at 37°C in 5% CO_2 atmosphere.

Statistical analysis - The above experiments were repeated three times and the mean and SD of the values were used to calculate significance based on Student's t test.

RESULTS

^{51}Cr release cytotoxicity assay - NK cell activity assessed using a single-cell suspension of spleen cells at different intervals of tumor growth showed a gradual increase in specific lysis to a peak on day 12 after tumor inoculation (59.67% ± 0.4) at an effector:target ratio of 100:1. NK cell activity was not detectable on day 19 (Suresh *et al.* 1989). The augmentation of NK activity in the spleen coincided with slight increase in the number of lymphoid cells with characteristics of LGL that could be isolated by density gradient centrifugation on Percoll. (Table I). When the LGL-enriched fraction was used as effector cells against

Days after tumor inoculation	Absolute number of cells per spleen x 10^7	% LGL recovered	% specific cytolysis
0 (normal)	8.23 ± 0.38	4.5 ± 0.59	10.90 ± 1.27
7	8.50 ± 0.30	5.5 ± 0.89	17.10 ± 2.40
12	9.10 ± 0.36	7.4 ± 1.40	20.53 ± 2.15*
19	7.70 ± 0.40	3.5 ± 0.50	2.26 ± 0.76**
+*19	-	-	12.96 ± 2.9

Table I. Absolute number of spleen cells, frequency of LGL in the spleen and NK cell (LGL) mediated cytolysis of radiolabeled YAC-1 cells on different days after tumor inoculation at effector: target ratio of 5:1 50 x 10^7, non adherent, nylon wool purified spleen cells (in put) were fractionated on Percoll discontinous density gradient. Fractions 2 and 3 were collected. Cell preparations were evaluated for morphology by microscopic analysis of Giemsa stained cytocentrifuge preparations. Values are mean ± SD from 3 different experiments in each group.
+*= day 19 LGL were pretreated with human rIL-2 as described in materials and method and 51Cr release assay was performed.
*= P< 0.01
**= P< 0.001.

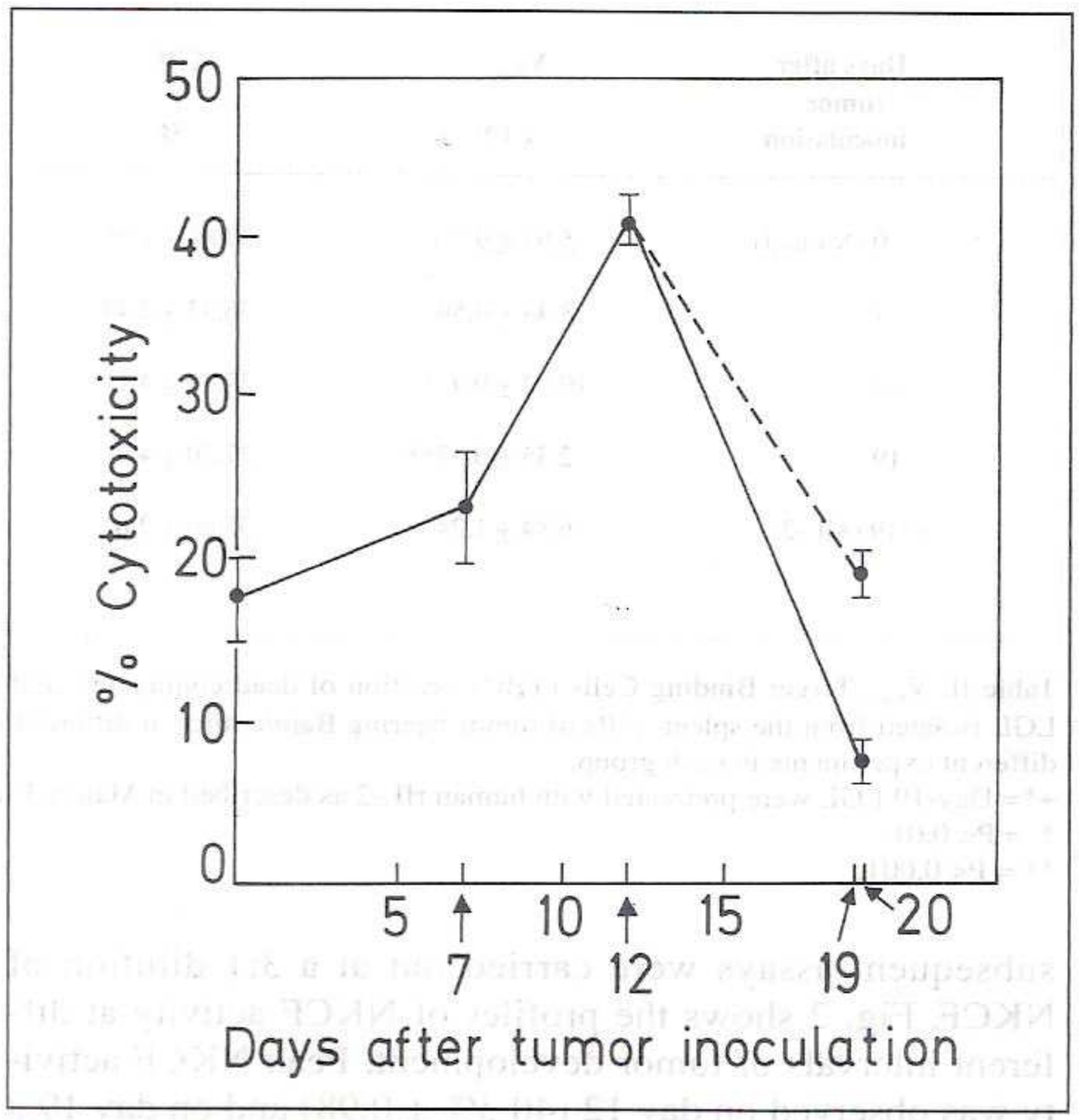

Fig. 2. Production of NKCF by LGL isolated from spleen cells of tumor bearing Balb/c mice at different intervals of tumor development assessed in 48 h. Microsupernatant assay using YAC-1 target cells. Doted lines represent NKCF activity by IL-2 pretreated LGL on day-19 tumor inoculation. Values are mean ± SD from 3 different experiments in each group.

radiolabeled YAC-1 targets in 4 h assay, an appreciable amount of specific lysis was observed even at a low effector : target ratio of 5:1 (10.9% ± 1.3), which increased gradually to a peak on day 12 after tumor inoculation (20.5% ± 2.1). The spleen-derived LGL efficiently lysed YAC-1 cells in a dose-dependent manner (Fig. 1). The activity was found to be reduced significantly on day 19 (2.26% ± 0.7), when the animals were at the advanced stage of tumor growth (Table I). Pretreatment of effector cells on day 19 with 200 IU/ml of human rIL-2 *in vitro* resulted in enhancement of the killing potential of LGL (2.26% ± 0.7 to 12.96 ± 2.9) (Table I).

Single cell conjugate assay - Among a purified population of LGL 30-40% could form conjugates with YAC-1 cells indiscriminately, iof the day after tumor inoculation. However, the percentage of conjugates containing dead targets increased gradually from day 0 to day 12 after tumor inoculation and was found to be slightly reduced on day 19 as compared to other days (Table II)- The percentage of active killer cells was found to be increased on day 7 (9.6% ± 0.9) and day 12 (10-2% ± 0-9) with respect to control (6-3% ± 0.8) (Table II).

The estimated maximal recycling capacity of the effector cells, however, did not show much variation when compared to normal controls (Table II).

NKCF Assay - Culture supernatants of effector cells with YAC-1 were tested for NKCF activity against YAC-1 cells in a microsupernatant assay as described in Materials and Methods. Optimum NKCF activity was observed at an NKCF dilution of 3:1 in an initial standardisation assay (Suresh and Vasudevan, unpublished observation) Hence,

Days after tumor inoculation	V_{max} x 10^3	TBC %	Fraction of dead %	Estimated N° of active killer cells %	Estimated MRC %
0 (Normal)	5.97 ± 9?54	37.83 ± 1.55	17.45 ± 1.78	6.30 ± 0.82	1.89 ± 0.13
7	8.48 ± 0.59	36.33 ± 2.49	28.12 ± 2.29*	9.60 ± 0.93	1.77 ± 0.16
12	10.02 ± 0.91*	37.33 ± 4.11	29.20 ± 5.38	10.16 ± 0.91	1.97 ± 0.14
19	2.35 ± 0.32**	27.20 ± 4.9	10.40 ± 1.04*	2.58 ± 0.48	1.82 ± 0.21
+*19 (+IL-2)	6.84 ± 1.24	35.60 ± 2.6	21.30 ± 1.85	7.24 ± 1.47	1.89 ± 0.27

Table II. V_{max}, Target Binding Cells (TBC), fraction of dead conjugates, and estimation of active NK cells and their recycling capacity with YAC-1 target cells. LGL isolated from the spleen cells of tumor bearing Balb/c mice at different intervals of tumor growth were used as effector cells (Values are mean ± SD from 3 different experiments in each group.
+*= Day-19 LGL were pretreated with human rIL-2 as described in Materials and methods and different parameters were studied.
* = P< 0.01.
** = P< 0.001.

subsequent assays were carried out at a 3:1 dilution of NKCF. Fig. 2 shows the profiles of NKCF activity at different intervals of tumor development. Peak NKCF activity was observed on day 12 (40.3% ± 0.98) and on day 19 a significant fall in activity was noticed (7.4% ± 1.4) with respect to control (17-6% ± 2.6; Fig. 2).

IL-2 dose response for activation of LGL- Initial dose response studies were performed to determine the optimal amount of IL-2 required to activate LGL isolated from spleen cells.

As shown in Fig. 3, incubation of LGL with 200 IU/ml of rIL-2 produced an appreciable amount of cytolysis against YAC-1 cells. Almost similar results were obtained with higher doses of IL-2 (400 IU/ml). Therefore 200 units were used in subsequent experiments. Incubation of day-19 effector cells with 200 IU/ml of human rIL-2 for 72 h at 37°C in 5% $C0_2$ resulted in a significant increase in NKCMC (2.26% ± 0.7 to 12.9% ± 2.9) and in the number of active killer cells (2.58% ± 0.5 to 7.2% ± 1.5). Maximal recycling capacity of the effector cells, however, did not show any variation (1.82% ± 0.2 to 1.89% ± 0.3) (Tables I and II). Moreover, NKCF activity was found to be augmented after pretreatment of the effector cells with rIL-2 (7.4 ± 1.4 to 18.4 ± 1.27; Fig. 2).

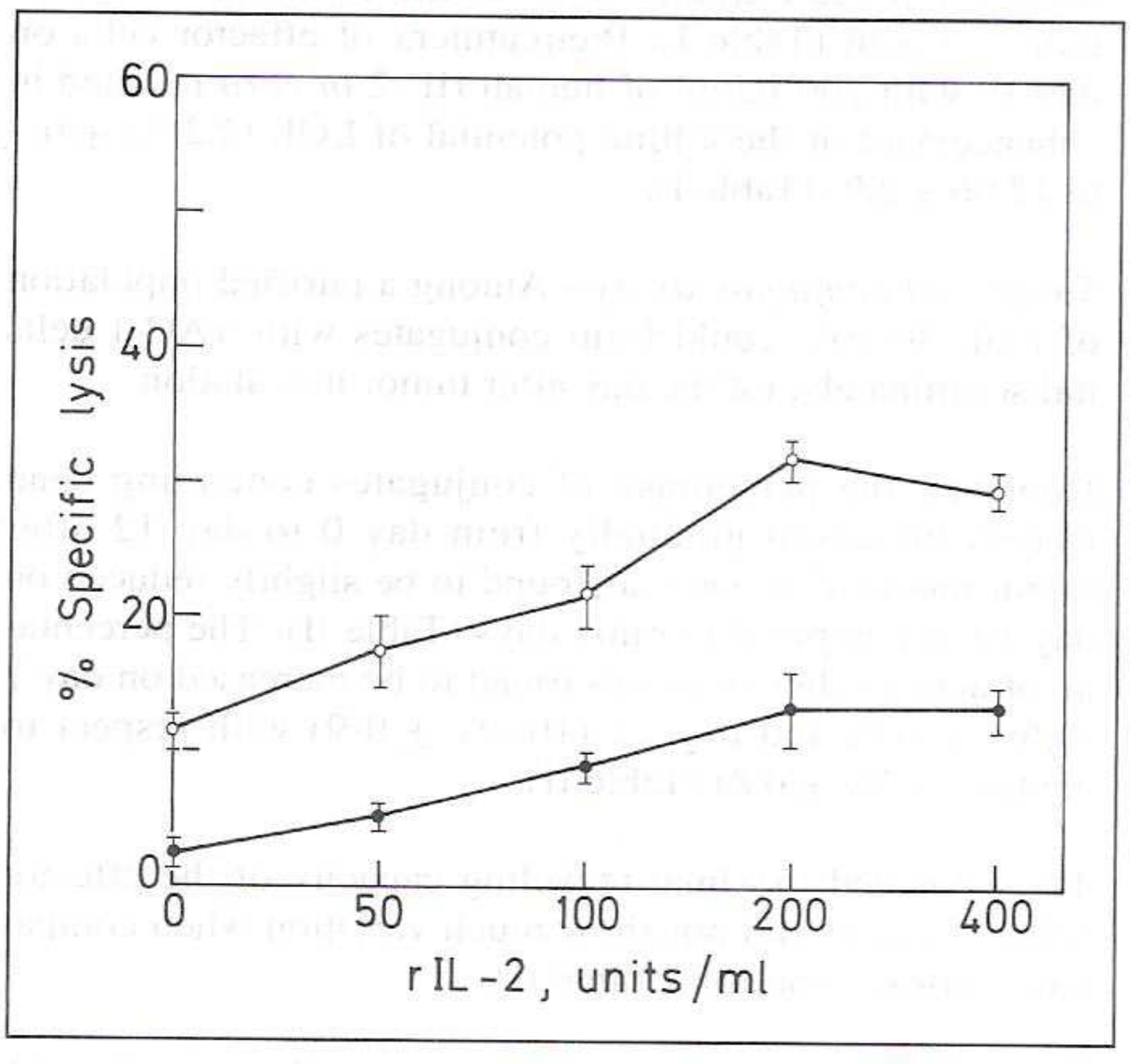

Fig. 3. IL-2 dose response for activation of LGL. LGL isolated from spleen cells of ○ normal, ● day-19 animals were incubated in medium containing different dilutions of IL-2 (50, 100, 200, 400 IU/ml) for 72 h at 37°C in 5% CO_2. After incubation cultures were harvested and cytotoxicity assessed against radiolabeled YAC-1 cells at effector: target ratio of 5:1. Values are mean ± SD from 3 different experiments in each group.

DISCUSSION

In our previous studies we have reported the profiles of NK cell-mediated cytotoxicity during tumor development in Balb/c mice (Suresh *et al.* 1989) and demonstrated that a functional NK (asialo-GM1+) cell population is essential for biological response modifiers to exert their inhibitory effect on DLA tumor growth (Suresh and Vasudevan 1990). It has been shown that spleen cells from DLA tumor bearing animals at different intervals of tumor growth display NK cell mediated cytotoxicity in a dose dependent manner (Suresh *et al.* 1989). The present investigation is intended to explain the NK cell profiles observed in our earlier studies. Since the increase in the number of spleen cells in tumor-bearing mice correlated well with augmented NK activity (Table I), further enrichment of LGL by fractionation on a Percoll density gradient was performed. This resulted in even higher levels of NK cell activity against YAC-1 cells even at a low effector : target ratio of 5:1 (Table I). Purified LGL from tumor-bearing mice at various time points of tumor growth lysed YAC-1 cells in a dose-dependent manner (Fig. 1). An enhancement in NK cell mediated cytotoxicity was observed in the initial phase of tumor development (first 12 days), which peaked on day 12, and then gradually declined to the basal level. Several authors have demonstrated that NK cell activity in the mouse could be augmented by inoculation of tumor cells bearing a relevant antigen (Wolfe *et al.* 1977, Tracey *et al.* 1977). By using anti-interferon antibodies and fluorescence microscopy it has been shown that approximately 40% of the LGL can produce interferon when they come into contact with tumor cells (Timonen *et al.* 1982). Therefore the augmentation of NK cytotoxicity may be partially due to the generation of soluble factors

such as interferon in the early phase of tumor development. Previously, it has been demonstrated that both NK and ADCC activities, spontaneous and interferon-inducible, are confined to the LGL enriched fraction (Wright and Bonavida 1983). We have already shown that antigen-specific T cells generated in MTLC display negligible cytotoxicity against YAC-1 cells as well as DLA cells at the peak of the response in this tumor model (Suresh *et al.* 1989, Suresh *et al.* 1990) Thus the contribution of antigen specific T cells towards the augmentation of NK cytotoxicity has been ruled out. Enumeration of LGL forming conjugates with target cell provides a direct method for determining the number of cells capable of recognizing target cells and potential lysis. The percentage active killer cells and maximal recycling capacity of effector cells, estimated at dlfferent phases of tumor progression, correlated well with our previous findings on NK cell profiles. NKCF production by LGL has been found to be closely associated with the lytic potential of the effector cell at different phases of tumor growth. Substantial levels of NKCF detectable in a 48-h ^{111}In-release cytotoxicity assay of K.562 target cells has been reported in the supernatant of LGL cocultured with K.562 target cells (Herberman *et al.* 1984). Since no impairment of conjugate formation was observed in the LGL of tumor-bearing animals, even at the advanced stage of tumor growth, when NKCF production was significantly diminished, it was evident that post binding events in the cytotoxic process were mainly affected in the later stages. These include lymphocytes bound to dead targets, the percentage of active killer cells, the killing potential and NKCF production However, estimated MRC showed minimal differences at different intervals of tumor progression. A limited production or release of NKCF may be responsible for the observed depression in NKCMC on day 19 (Fig. 2). Patients with advanced malignant tumors have been reported to exhibit low natural cytotoxic status (Pross *et al.* 1984). Defective functioning of the lytic event which occurs after the formation of an effector-target conjugate could be the reason for the decline in NKCMC in animals at a later stage of tumor growth.

In our dose response studies 200 IU/ml of rIL-2 was found to give an optimal response (Fig. 3). This is more or less consistent with earlier reports on IL-2 dose response for activation of LAK cells (Merluzzi *et al.* 1985, Froelich and Guiffant 1987).

Pretreatment of day-19 effector cells with human rIL-2 resulted in appreciable enhancement in NKCLC and NKCF activity. The finding that the IL-2 induced augmentation of NKMC in the ^{51}Cr release assay correlates well with IL-2 induced augmentation of NKCF activity, supports the hypothesis that NKCF could be the lytic mediator in NKCMC. Interferon or polycytidilic acid pretreatment of effector cells have been reported to increase NKCF activity and NK activity in the ^{51}Cr release assay (Wright and Bonavida 1983). Thus defective IL-2 production, which in turn may interfere with NKCF production or release by LGL, could be one of the potential mechanisms of NK cell immunodeficiency in tumor-bearing animals. It has been reported that defective NK cells from chronic myeloid leukemia patients could be restored to normal cytotoxic activity on exposure to recombinant IL-2 (Fujiyama *et al.* 1987, Chang *et al.* 1989). The ability of IL-2 to stimulate continuous activity of NK cells has been well demonstrated in animal tumor models (Hereneider *et al.* 1982, Majumdar and Rosenberg 1984). IL-2 enhances the depressed NK cell activity from patients with acquired immuno-deficiency syndrome. IL-2 can either directly activate LGL to enhance NK activity in the absence of adherent cells or induce the synthesis of gamma interferon by NK cells which can also be augmented independently (Rooks *et al.* 1985). We have clearly demonstrated that depressed NK cell activity displayed by day-19 LGL could be restored to normal by treatment with exogenous IL-2. Thus IL-2 induced augmentation of NK activity could be accounted for by an increase in synthesis of release of NKCF.■

REFERENCES

1. Chang LWC, Fujiyama Y, Casteel N, Pattengale P. Natural Killer cell immunodeficiency in patients with chronic myelogenous leukemia- III-Defective interleukin-2 production by T-Helper and natural killer cells. Int J Cancer 43, 591-597, 1989.

2. Domzig W, Stadler BM, Herberman AB. Interleuk1n-2 dependance of Natural Killer Activity. J Immunol 139, i970-i973, 1983.

3. Froelich C and Guiffant S. Lysis of Human T cell Leukemia virus infected T and B lymphoid cells by interleukin-2 activated killer cells. J Immunol 139, 3637-3643, 1987.

4. Fujiyama Y, Chang WJC, Bakke A, Horwitz D and Pattengale PK. Natural killer (NK) cell immunodeficiency in patients with chronic myelogenous leukemia. II. Successful cloning and amplification of natural killer cells. Cancer Immunol Immunother 24, 213-220, 1987.

5. Hefeneider SH, Henney CS, Gillis S. In vivo interleukin-2 induced augmentation of Natural Killer activity. In: Werberman RB, Eds- "NK cells and other natural effector cells". New York, Academic Press 421-420, 1982.

6. Herberman AB, Batram S, Haskill JS, Nunn ME, Wolden WT, West WH. Fc receptors on mouse effector cell, mediating natural cytotoxicity against tumor cells. J Immunol 119, 322-328, 1977.

7. Herberman AB, Orataldo JA. Natural Killer Cells their role in defense against desease. Science 24, 214-219, 1981.

8. Herberman AB, Allavena P, Scala G *et al.* Cytokine production by human large granular lymphocyte (LGL). In: Hoshino T, Koren HS, Uchida A, eds- Natural killer activity and its regulation. Amsterdam. Excerpta Medica 409-413, 1984.

9. Julius MH, Simpson E, Herzen Berg LA. Rapid method for isolation of functional T lymphocytes. Eur J Immunol 3, 645-649, 1973.

10. Kay Ho, Bonnard GD, West LWH, Herberman AB. A functional comparison of human Fc-recepter bearing lymphocytes. J Immunol 118, 2058-2066, 1977.

11. Mazumder A, Rosenberg SA. Successful immunotherapy of Natural Killer resistant established pulmonary melanoma metastases by intravenous adoptive transfer of syngeneic lymphocytes activated *in vitro* by interleukin-2. J Exp Med 159, 495-507, 1984.

12. Meruluzzi VJ, Welte K, Last Barney K, Mertlemann K, Souza L, Boon T, Savage DM, Quinn D, Oreilly JO. Production and response to Interleukin-2 *in vitro* and *in vivo* after bone marrow transplantion in mice.J Immunol 134, 2426-2431, 1985.

13. Nunn ME, Herberman AB, Holden HT. Natural cell mediated cytotoxicity in mice against non-lymphoid tumor cells and some normal cells. Int J Cancer 16, 230-236, 1975.

14. Pross HF, Aubin P, Baines GM. The assessment of Natural Killer activity in cancer patients. In: Herberman AB eds- NK cells and other natural effector cells. New York, Academic Press, 1175-1187, 1982.

15. Rooks AH, Hooks J, Quinnan GV, Lane H, Manischewit FA, Djeu JY. Interleukin-2 enhances the natural killer cell activity of immunodeficiency syndrome patients through gamma interferon induction mechanism. J Immunol 134, 1503-1506, 1985.

16. Suresh K, Nirmala K, Kezia Kuruvilla, Vasudevan DM. Assessment of immunological parameters during tumor development in a murine model. Indian J Exp Blology 27, 497-501, 1989.

17. Suresh K, Nirmala K, Vasudevan DM. Adoptive immunotherapy using human interleukin-2 activated specific cytotoxic T lymphocytes. J Exp Clin Cancer Research 9, 155-160, 1990.

18. Suresh K and Vasudevan DM. Augmentation of murine NK cell and ADCC activity by Phyllanthus emblica, a new immuno modulator. Int J Immunopharmacol (in press).

19. Timonen T, Reynolds CWJ, Ortaldo JA, Herberman AB. isolation of human and rat natural killer cells. J Immunol Methods 51, 269-277, 1982.

20. Tracey DE, Wolfe SA, Durdik JM, Henney CS. BCG induced murine effector cells. I Cytolytic activity in peritoneal exsudates. An early response to BCG J Immunol 119, 1145-1152, 1977.

21. UllBerg M, Jondal M. Recycling and target binding capacity of human natural killer cells. J Exp Med 153, 615-628, 1981.

22. Vargas-Cortes M, Hellstrom V, Perlmann P. Surface markers of human natural killer cells as analyzed in a modified single cell cytotoxicity assay on poly-L-lysine coated coverslips- J Immunol Methods 62, 87-99, 1983.

23. Wolf SA, Tracey DE and Henney CS. BCG induced murine effector cells. II. Characterisation of natural killer cells in peritoneal exsudates. J Immunol 119, 1152-1158, 1977.

24. Wright SC, Bonavida B. Studies on mechanism of natural killer cytotoxicity-III. J Immunol 130, 2960-2965, 1983.

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Cinétique de l'activité des cellules NK pendant la croissance d'une tumeur de souris.

RESUME - Nous avons précédemment rapporté les modes de cytotoxicité à médiation cellulaire par les cellules "natural killer" (NKCMC) dans la rate de souris Balb/c pendant la croissance du lymphome ascitique de Dalton (DLA), lymphome T murin. Dans le présent travail, nous avons utilisé deux méthodes différentes d'évaluation de la cytotoxicité NK *in vitro* pour analyser l'importance relative des différentes étapes nécessaires pour atteindre le résultat global. La méthode de relargage du ^{51}Cr a été combinée avec la méthode récente de conjugaison à une seule cellule utilisant des lamelles couverte de poly-L-lysine ce qui permet une meilleure étude quantitative. Grâce à ce double procédé nous pouvons estimer le pourcentage de cellules NK active, le V_{max} et la capacité de rentabilisation des cellules effectrices à différents moment de la progression tumorale. Une population enrichie en lymphocytes à gros granules, obtenue par centrifugation en Percoll avec un gradient de densité discontinu fut utilisée dans les deux tests comme source de cellules effectrices contre des cellules cibles murines YAC-1 sensibles aux NK. La capacité des cellules effectrices de relarguer le facteur cytotoxique NK (NKCF), sous l'effet d'une stimulation avec les cellules YAC-1 fut estimée à l'aide d'un essai dans le microsurnageant de 48 heures. Une augmentation significative du nombre de cellules NK actives couplée à l'accroissement de production du facteur cytotoxique NK fut observée les jours 7 et 12 après l'inoculation tumorale, par rapport aux témoins normaux. La baisse de la NKCMC à un stade plus avancé de la croissance tumorale (jour 19) fut observée, en association avec la baisse de production du NKCF, ainsi que la réduction de l'activité NK dans la population de cellules effectrices. La capacité de réutilisation maximale estimée montrait des différences minimes aux différentes phases de la croissance tumorale. Le prétraitement des cellules effectrices du jour 19 avec 200 IU/ml d' IL-2 humain recombinant provoqua une augmentation de la NKCMC, de la V_{max}, du pourcentage de cellules NK activées et de la production de NKCF.

Evolución de la actividad NK celular durant el desarrollo de un tumor, en un modelo murino.

RESUMEN - En trabajos previos informamos sobre los perfiles de la citotoxicidad mediada por células asesinas naturales ("natural killer", NKCMC) en células de bazo de ratones Balb/c con ascitis por inducción de un linfoma de Dalton (DLA), linfoma murino de tipo T. En este trabajo, hemos usado dos métodos de valoración de la citotoxidad NK *in vitro* para esclarecer la importancia relativa de las distintas etapas implicadas en el desarrollo de la enfermedad tumoral. La prueba del ^{51}Cr liberado se combinó con otra prueba recientemente etablecida y realizada sobre células aisladas, usando cubreobjetos revestidos de poli-L-lisina, que facilita el análisis cuantitativo. Con este doble procedimiento, pudimos estimar el porcentaje de células asesinas activas, la V_{mas} y la capacidad de renovación de células efectoras, a intervalos diferentes de la progresión tumoral. Usamos una población enriquecida de Linfocitos Granulares Grandes (LGL) obtenida por centrifugación en gradiente de densidad discontinua de Percoll, como células efectoras contra celulas YAC-1 como diana de celulas NK murinas. Medimos tambien, en micro-sobrenadantes de 48 h, la capacidad de las células efectoras para liberar el factor citotóxico de las células asesinas naturales (NKCF), en la estimulación con celulas YAC-1. En los dias 7 y 12 tras la inoculación de las celulas tumorales, observamos una amplificación significativa en el número de células asesinas activas, unido a un incremento de la producción de NKCF, con respecto al control normal. En estadíos avanzados del crecimiento del tumor (dia 19) observamos una caida de la actividad NKCMC asociada a la disminución de la producción de NKCF y la reducción del número de células asesinas activadas entre la población de células efectoras. La capacidad máxima estimada de renovación mostró diferencias mínimas en las distintas fases del crecimiento tumoral. El tratamiento previo de las celulas efectoras del dia 19 con 200 IU/ml de IL-2 humana recombinante, provocó el aumento de la MKCMC, la V_{max}, el porcentaje de células asesinas activas y la producción de NKCF.