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Induction of Killer Activity in Peripheral Blood Mononuclear Cells after Chemotherapy with Methotrexate, Vinblastine, Adriamycine and Cis-platin (M-VAC)

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SUMMARY: We have studied NK activity against K562 cells and killer activity against NK-resistant Raji cells after administration of Methotrexate, Vinblastine, Adriamycine and Cis-platin (M-VAC) in patients with transitional cell carcinoma and found the appearance of strong lytic activity against K562 cells and Raji cells (lymphokine activated killer(LAK)-like activity) appeared 24.3 ± 6.5 days after the administration of first cycle of M-VAC and the lytic unit (LU) was 418.4 ± 177.4 (LU of pre-chemotherapy: 88.6 ± 61.2). NK activity against K562 cells showed the same course as lymphokine activated killer-like activity. There was a significant difference in activity of NK cells and LAK-like cells between pre-chemotherapy and maximum. NK cells and LAK-like cells activities after second or third cycle of M-VAC showed the same course as those of first cycle of M-VAC. Lymphocyte count decreased temporarily and increased during maximal activities of NK cells and LAK-like cells in each cycle of M-VAC therapy. However, maximal peak of lymphocyte count delayed in comparison with that of maximal activities of NK cells and LAK-like cells. Lymphokines, such as IL-2 may play a role to generate these enhanced NK activity and LAK-like activity. The data presented in this report suggests that the nature, dose, and timing of chemotherapeutic agent should be considered from the point of immunological mechanism.

INTRODUCTION

Most anticancer drugs have immunosuppressive capabilities, and the immunosuppressive action can be detected in various experimental systems. However, there are not so many investigations of cytotoxicity in peripheral blood lymphocytes (PBL) after cancer chemotherapy. We have studied the natural cell-mediated cytotoxicity of patients with carcinoma after cancer chemotherapy and found the strong natural cell-mediated cytotoxicity against NK-resistant target cell after administration of Methotrexate, Vinblastine, Adriamycin and

Cis-platin (M-VAC)⁴. We have further investigated the natural cell-mediated cytotoxicity in patients with urinary bladder carcinoma or pyeloureteral carcinoma, and the relation between lymphokine activated killer (LAK)-like activity and natural killer (NK) activity or these cytotoxicities and lymphocyte count.

MATERIAL AND METHOD

Patients: Between January 1986 and July 1990, 10 patients with transitional cell carcinoma of the bladder (6) and pyeloureter (4) were subjected to this study.

Chemotherapy: Our chemotherapy consisted

of 30 mg/m² methotrexate on day 1, and 3 mg/m² vinblastine, 30 mg/m² adriamycine and 70 mg/m² cisplatin administered on day 2, with the cycle repeating every 28 days²⁾. Cis-platin was administered after appropriate hydration as 1-hour infusion followed by routine post-hydration fluid. Methotrexate and vinblastin were not administered on days 15 and 22 in all 10 patients as the white blood cell count was less than 2000 per mm³. Seven patients received 3 cycles of therapy.

Effector cells: PBL were isolated as effector cells from the heparin-treated blood of patients by separation on Ficoll-Hypaque gradients. Adherent cells were removed by 1 hour, 37°C incubation on plastic dishes precoated with autologous plasma.

Target cells: The cell line used in this study included human B cell lymphoma Raji cells and erythroleukemia K562 cells. Those cell lines were maintained in RPMI 1640, supplemented with 10% fetal calf serum. These cell lines were also monitored regularly for contamination and were found to be mycoplasma-free.

Cytotoxic Assay: A cytotoxicity assay was performed by 18 hours ⁵¹Cr-release assay. Percent specific lysis was determined by using the formula.

$$\% \text{ lysis} = \frac{\text{cpm test} - \text{cpm medium}}{\text{cpm max} - \text{cpm medium}} \times 100$$

Counts per minute (cpm) max was determined by counting an aliquot of resuspended target cells, cpm medium was determined in wells containing targets only with no effectors added. All experiments were done in triplicate in V-shaped 96 well microplate (Nunc, Denmark)

Lytic units were calculated from cytotoxic titration curves. One LU was defined as the number of effector cells required to cause 20% lysis of 5×10³ target cells. The data were presented as LU/10⁷ effector cells.

Statistical analysis: Statistical analysis was done by Wilcoxon signed-rank test.

RESULT

1) Characteristics of patients

The characteristics of evaluable patients are shown on **Table 1**. Among 10 patients, 9 were men and one was a woman, between 38 and 67 years of age (mean age 59.3 years). With regard to performance status there were 7 patients with grade 0 and 3 with grade 2.

2) NK and LAK-like activity

NK activity and LAK-like activity temporarily decreased after the administration of M-VAC in every cycle. The minimal activities of NK cells and LAK-like cells after the first cycle of M-VAC therapy were 264.1 ± 419.9 LU (pre-chemotherapy: 613.6 ± 432.9 LU) and 21.9 ± 27.8 LU (per-chemotherapy: 88.6 ± 61.2 LU) respectively. These activities increased gradually from the nadir and maximal activities of NK cells and LAK-like cells after the administration in first cycle of M-VAC therapy were 970.6 ± 567.4 LU and 418.4 ± 177.4 LU respectively. Maximal activities of NK cells and LAK-like cells after chemotherapy express much higher activities than that of pre-chemotherapy level (**Fig. 1, 2**). There was a significant difference in activities of NK cells and LAK-like cells between pre-chemotherapy and maximum. Activities of NK cells and LAK-like cells after second or third

Table 1. Patient characteristics

No.	Case	Sex	Age	P. S.	Primary lesion	Stage	Grade
1	T. M.	M	63	0	Bladder	pT3b	3
2	S. K.	M	65	2	Bladder	pT4	3
3	M. N.	M	67	0	Bladder	pT2	3
4	H. F.	F	38	0	Renal Pelvis	pT2	2
5	K. E.	M	67	0	Bladder	pT2	3
6	H. M.	M	46	0	Bladder	T4	2
7	N. S.	M	65	0	Ureter	pT2	3
8	S. K.	M	58	2	Ureter	pT3b	2
9	M. H.	M	63	2	Renal Pelvis	pT3b	2
10	S. T.	M	61	0	Bladder	pT3a	3

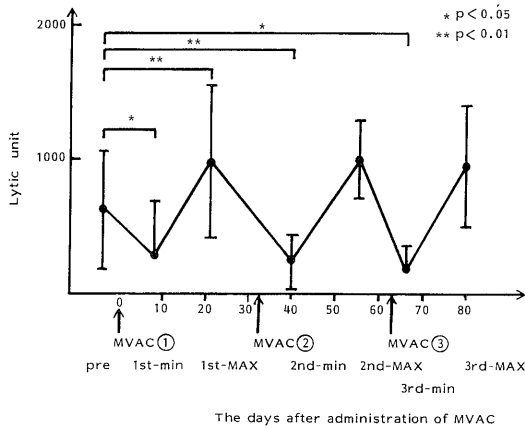


Fig. 1. NK cell activity against K562 cells after administration of M-VAC. Cytotoxic activity was assayed by 18 hour ⁵¹Cr-release assay. LU was calculated from cytotoxic titration curves of ⁵¹Cr-release assay.

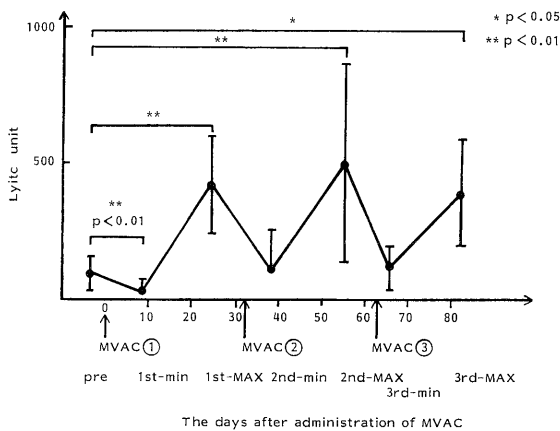


Fig. 2. LAK-like cell activity against NK-resistant Raji cells after administration of M-VAC. Cytotoxic activity was assayed by 18 hour ⁵¹Cr-release assay.

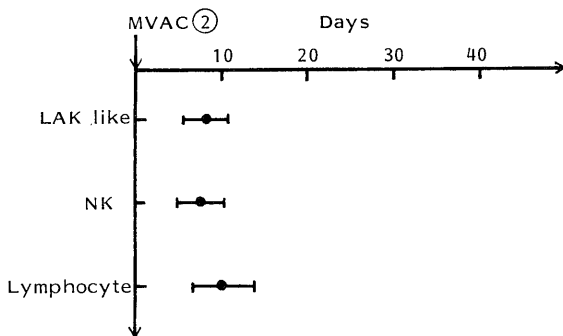


Fig. 3. The day of minimum NK cell activity, LAK-like cell activity and lymphocyte count after administration of M-VAC.

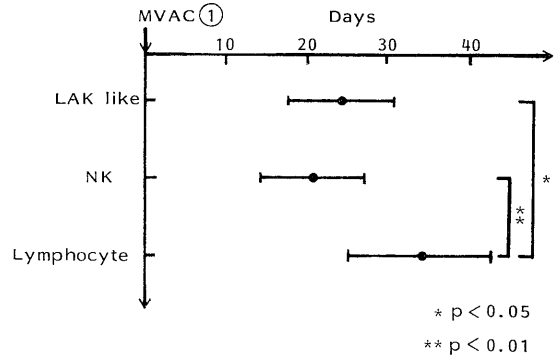


Fig. 4. The day of maximum NK Cell activity, LAK-like cell activity and lymphocyte count after administration of M-VAC.

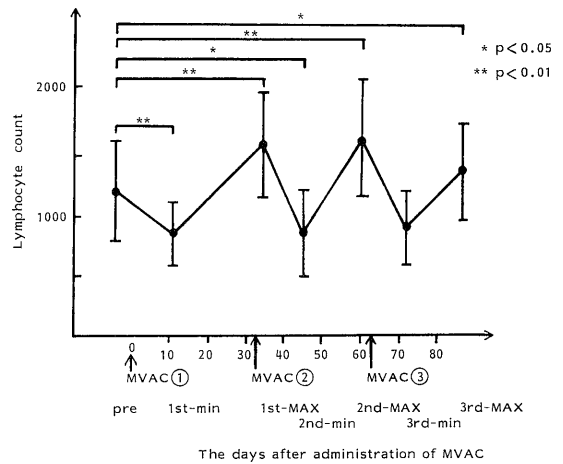


Fig. 5. Lymphocyte count after administration of M-VAC

cycle of M-VAC therapy showed the same course as those after the first cycle of M-VAC therapy.

Minimal activities of NK cells and LAK-like cells developed 7.7 ± 2.9 days and 8.4 ± 2.8 days respectively after the administration of first cycle of M-VAC therapy (Fig. 3). Maximal activities of NK and LAK-like cells appeared 21.5 ± 6.5 days and 24.3 ± 6.5 days respectively after the administration of first cycle of M-VAC therapy. There were no significant difference in the day of appearance of minimal and maximal activities between NK and LAK-like activity, although the response to recover of NK activity was slightly earlier than LAK-like activity in the mean time. (Fig. 4)

3) Lymphocyte count during chemotherapy of

M-VAC

Fig. 5 shows lymphocyte count during chemotherapy of M-VAC. After M-VAC therapy lymphocyte count decreased significantly during minimal activity of NK cells or LAK-like cells, but later it increased significantly during maximal activity of NK cells or LAK-like cells in each cycle of M-VAC therapy than that of pre-chemotherapy level. However, the maximal peak of lymphocyte count delayed in comparison with that of maximal activities of NK cells or LAK-like cells.

DISCUSSION

It is well known that normal PBL can exhibit a number of killer cell functions including NK cell activity, cytotoxic T lymphocytes and LAK cell activity. NI cells can lyse certain cancer cells without prior sensitization and they are considered to play a role in the defense against cancer development. LAK cells are generated by culture of normal lymphocytes with interleukin 2 (IL-2), without any additional antigenic stimulation. LAK cells lyse a wide spectrum of allogenic and syngenic tumor cells^{3, 4}.

We found an appearance of very strong killer activity against NK-resistant target cells in PBL after administration of M-VAC^{1±}. The target spectrum of this killer cell was wide, and monoclonal antibody and complement treatment revealed that effector cells express CD8 and SL-1 phenotypes weakly, but do not express CD4, 00CD5, CD16, Leu-7, OKM1. These results suggest that effector cells are LAK-like. This strong killing activity against NK-resistant target cells was also induced by a combination chemotherapy with Cis-platinum, Etoposide and Bleomycin for testicular tumor patients. We think that this LAK-like cell induction is not due to specific drug actions but due to some common phenomenon such as a regeneration of myelosuppression among these chemotherapeutic agents. Indeed, our result showed that initially lymphocyte count decreased significantly during minimal activity of NK cells or LAK-like cells after M-VAC therapy, but later it increased significantly during maximal activity of NK cells or LAK-like cells than that

of pre-chemotherapy level. The peak of lymphocyte count delayed in comparison with that of maximal activity of NK cells or LAK-like cells. That is, maximal activity of NK cells or LAK-like cells appeared during the rapid recovery of the number of lymphocytes. Hematopoietic progenitor cell colonies are known to be activated into LAK cells^{5, 6}, and lymphokine such as IL-2 may play a role to generate enhanced NK activity and LAK-like activity. It is reported that after administration of a sublethal dose of cyclophosphamide in the murine system, LAK cell, NK cell and CTL were abolished at once and the recovery was in the order of allogenic CTL, NK cell, LAK cell^{7, 8}. However, a sublethal dose of cyclophosphamide did not induce LAK activity. In our results there was found no significant difference of recovery time between NK activity and LAK-like activity, although the response to recover of NK activity was slightly earlier than LAK-like activity in mean time.

The optimal drugs in patient with transitional cell carcinoma remain unknown. LAK-like activity seems to be higher in clinical responders than in clinical non-responders. This fact shows that LAK-like activity may be related to the tumor reduction. The present data suggest that the nature, dose and timing of chemotherapeutic agents should be considered from the view point of immunological mechanism. The data also indicate usefulness of good timing of the additional chemotherapy or immunotherapy. If this killer activity could increase by administration of any biological response modifier, M-VAC therapy would be more effective.

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