Cancer Immunol Immunother (1986) 21: 240–245

Cancer Immunology Immunotherapy

Effect of chemotherapy on NK function in the peripheral blood of cancer patients*

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Summary. The effects of cytotoxic chemotherapy on NK cell function and on glass adherent cell regulation of NK cell function was evaluated in the peripheral blood mononuclear cells of 20 previously untreated solid tumor patients. Most of the patients studied had lung cancer and received one of four combination chemotherapy treatment regimens. In addition, one patient with colon carcinoma and one patient with melanoma were studied, each of whom received treatment with a single agent. The results demonstrated that chemotherapy exerted a differential influence on NK activity which correlated with the pretreatment NK level of function in the individual patient. In patients with depressed NK levels prior to treatment, chemotherapy augmented NK function; in patients with normal levels prior to treatment, chemotherapy depressed NK function. The effects observed appeared to be associated with the capacity of chemotherapy to influence glass adherent cell regulation of NK activity. There was no apparent correlation between the effects of chemotherapy on numbers of NK effector cells, Leu11+ cells, or latex-ingesting cells. Also, there was no correlation between the effects seen and the type of drug treatment that was administered; rather, this was dependent on the pretreatment NK level of function which in turn was associated with glass adherent cell regulation of NK function.

Introduction

Several decades of experience with the cytotoxic drugs used for cancer chemotherapy have shown that these agents can act as immunomodulators [5, 15]. Their influence on the immune response is dependent on such conventional parameters as the type of drug used, its pharmacokinetics, the dose and schedule of drug administration, and the differential effects of single agent versus combination agent treatments [1, 13]. Recently, however, it has become clear that the effect of drug treatment on the immune response is influenced by the patient's immunocompetence at the time of drug administration. Thus, patients with normal immune function at the time of drug administration may be differentially affected compared to patients with abnormal immunocompetence at the time of drug administration [3, 11].

The potential for cytotoxic chemotherapy to augment the immune response in cancer patients has only recently come under systematic investigation. Much of this work is based on studies in animals which demonstrate that depending on the dose of cytotoxic chemotherapy administered, drug treatment may lead to potentiation of antitumor immunity rather than to its suppression [12, 16]. These studies parallel comparable investigations conducted in the early 1980s in cancer patients [10, 14], suggesting that some patients who receive chemotherapy experience potentiation of immunity following drug treatment. This occurred in association with clinical responsiveness of the tumor. Other patients who experienced immune depression or failed to experience immune potentiation also failed to demonstrate clinical tumor responsiveness.

We have demonstrated that potentiation of mitogen-induced lymphoblastogenesis in the peripheral blood of cancer patients who receive chemotherapy is associated with a reduction in monocyte-mediated suppressor function [3]. This is associated, in certain instances, with a reduction in monocyte percentages, but not with a reduction in lymphocyte T cell, OKT4 cell, or OKT8 cell percentages [4]. In the present investigation, we have extended these findings to natural killer (NK) function. The studies were based on reports suggesting that NK function is altered in patients with solid tumors [19] and may be under the control of peripheral blood monocytes [8]. The results obtained show that the effects of chemotherapy on NK activity are dependent, at least in part, on pretreatment NK levels of function; which are in turn, dependent on glass adherent cell regulation of NK activity.

Materials and methods

1. Patient population

The patient population studied consisted of 20 patients with untreated nonlymphoreticular solid tumors (see Table 1 for patient population and chemotherapy). No patient was studied within 10 days of a surgical procedure and no patient had received previous radiation therapy or chemotherapy prior to immunological assessment. In general, patients were evaluated at weekly intervals throughout their first cycle of chemotherapy.

* Supported in part by PHS Grant No. 27598

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Table 1. Patient population and chemotherapy

Patient group	Diagnosis	#	Chemotherapy
1	Non-small cell carcinoma of lung	2	Cytoxan 500 mg/m^2 days 1 and 8 Adriamycin 30 mg/m^2 days 1 and 8 Methotrexate 25 mg/m^2 days 1 and 8 Procarbazine 100 mg days 2–11 Every 21 days
2	Non-small cell carcinoma of lung	6	Cytoxan 500 mg/m^2 days 1 and 8 Adriamycin 40 mg/m^2 days 1 and 8 Methotrexate 30 mg/m^2 days 1 and 8 5-Fluorouracil 600 mg Every 21 days
3	Non-small cell carcinoma of lung	6	Mitomycin C 10 mg/m^2 Vinblastine 6 mg/m^2 Cisplatin 50 mg/m^2 Every 21 days
4	Small cell carcinoma of lung	4	Cytoxan 750 mg/m^2 day 1 Adriamycin 50 mg/m^2 day 1 VP-16 330 mg days 1–5 Every 21 days
5	Carcinoma of Colon	1	5-Fluorouracil 500 mg/m^2 days 1 and 8 Every 28 days
6	Malignant Melanoma	1	Methyl-CCNU 70 mg/m^2 Every 21 days

2. Isolation of peripheral blood mononuclear cells (PBMC)

Venous blood was aseptically drawn into sterile tubes containing preservative-free heparin, diluted with an equal volume of Hanks Balanced Salt Solution (HBSS) and layered over Lymphocyte Separation Medium (LSM, Bionetics, Kensington, Md.). After centrifugation, the mononuclear cell layer was recovered and washed three times in HBSS before further manipulation.

3. NK assay

The K562 cell line used as the target cell for NK activity was maintained in RPMI 1640 (Gibco, Grand Island, NY), medium containing 20% heat inactivated fetal calf serum (FCS), 100 units/ml penicillin and 100 μg/ml streptomycin, and was subcultured twice weekly. Target cells were prepared by incubation of $1.0–2.0 \times 10^6$ cells in 0.2 ml RPMI 1640 medium with 50 μCi of sodium chromate (^{51}Cr) for 1 h at 37 °C in an atmosphere of 5% CO_2 with frequent shaking. The labeled cells were washed three times with HBSS containing 1% bovine serum albumin (BSA) and suspended in RPMI 1640 medium with 10% FCS. The target cells were distributed into U-shaped microplate wells at a concentration of 10^4 cells/well. Various numbers of effector PBMC were added, yielding a total volume of 0.2 ml/well, and the plates were incubated for 4 h at 37 °C in 5% CO_2. After incubation, the cell-free supernatants were collected using the Titertek Supernatant collection system (Flow Laboratories) and counted in a gamma counter to determine ^{51}Cr-release. Spontaneous release was determined by incubation of target cells without addition of PBMC. Maximum ^{51}Cr-release was determined by lysis of target cells with 1% sodium dodecyl sulfate. Specific ^{51}Cr-release (cytotoxicity) was calculated using the following formula:

% cytotoxicity = ((Experimental ^{51}Cr-release − spontaneous release) ÷ (maximum-spontaneous release)) × 100.

All values were means of triplicate determinations.

Patients samples were tested for NK function on the day of blood sampling since it was determined that cryopreservation substantially altered both NK function and glass adherent regulation of NK function. For these reasons, it was necessary to control for the variability of NK activity using simultaneously tested normal PBMC from a pool of normal donors. This permitted us to define variation in NK function over time which at an effector : target ratio of 50/1 was determined to range from 8.4% to 14.9% (SD of NK activity determined weekly over a 4-week period in five normal donors [8]. In the present study, therefore, we considered changes in NK function which exceeded this normal range of variation to be significant.

4. Depletion of glass adherent cells

PBMC were resuspended in RPMI 1640 medium supplemented with 10% inactivated human AB serum 50 units/ml penicillin and 50 μg/ml streptomycin to a final concentration of 2×10^6/ml and 5 ml of cells were dispensed into sterile glass tissue culture flasks and incubated for 2 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. At the end of incubation, nonadherent cells were recovered by gentle pipette aspiration and the percentage of monocytes remaining was determined by staining for alpha naphthyl acetate esterase (ANAE, Sigma St. Louis, Mo.) and by latex ingestion. We have determined that this method reduces the percentage of ANAE positive, latex-ingesting cells in PBMC to 1%–5%. Also, we have determined that glass adherent cells were unable to lyse K562 targets in a 4-h NK assay.

5. Quantitation of latex-ingesting cells, K562-binding cells, and Leu11+ cells

a) Analysis of K562-binding cells. PBMC were incubated with K562 cells for 1 h at 37 °C, gently resuspended, and examined under phase contrast microscopy for enumeration of target binding cells. In the K562-binding assay we enumerated large granular lymphocytes that bound to the target as these cells have been identified as NK cells. We report the results as a percentage of the PBMC in the preparation used which bound to target cells.

b) Analysis of PBMC with monoclonal antibodies. PBMC were prepared for immunofluorescent analysis by suspension in 0.85% ammonium chloride in 0.17 M Tris buffer (pH 7.20) to lyse residual red blood cells. After incubation for 10 min at 37 °C, the cells were centrifuged at $900 \times g$ for 10 min. An aliquot of PBMC was treated with carbonyl iron by suspension in RPMI 1640 containing 30% heat-inactivated FCS (GIBCO) and 100 mg carbonyl iron. After incubation at 37 °C for 30 min with mixing, the carbonyl iron-containing cells were removed with a pencil magnet. Untreated PBMC and carbonyl iron-treated PBMC were suspended in HBSS containing 1% BSA. The carbonyl iron-treated cells (10^6 in 100 µl) were incubated with 5 µl of reconstituted OKT3, OKT4, or OKT8 monoclonal antibodies (ORTHO) and the Leu11+ monoclonal antibody (Becton-Dickenson). Cells were quantitated on an ORTHO III fluorescence activated cell sorter; Leu11+ cells were reported as a percentage of the peripheral blood mononuclear lymphocytes.

c) Analysis of latex-ingesting cells. An aliquot of 1.5×10^6 PBMC, was incubated with latex (Bacto, Difco Laboratories) for 45 min at 37 °C for monocyte identification. Cells were gently resuspended and at least 200 cells were counted on a Leitz-Wetzler microscope. Monocytes were defined as latex-ingesting cells; monocytes in the LSM-isolated preparation were expressed as a percentage of the total mononuclear cell fraction.

6. Statistical analysis

NK cell values for individual patients were considered significantly different from normal whenever a value fell >2 SD below the mean level of NK function which was determined at the identical effector:target ratio in the peripheral blood cells of normal individuals. Group comparisons in this study were by a nonparametric statistic, the Wilcoxon-Rank Sum test.

Results

1. Effect of chemotherapy on NK activity in the PBMC of solid tumor patients

The effect of chemotherapy on NK activity in the PBMC of cancer patients varied with pretreatment NK status. Patients with normal NK function prior to chemotherapy demonstrated a progressive decline in NK function throughout the posttreatment weekly assessment period. Of the 20 patients studied, 8 were found to have a level of NK function which was considered to be within the normal range immediately prior to treatment (Fig. 1). At the day 7 assessment point all patients had a reduced level of NK function; in 4/8 patients, this had dropped to a level which was considered significantly reduced compared to normal. This trend continued and by the end of the 3-week follow-up period, 8/8 patients had a level of NK function which was considered significantly depressed compared to normal.

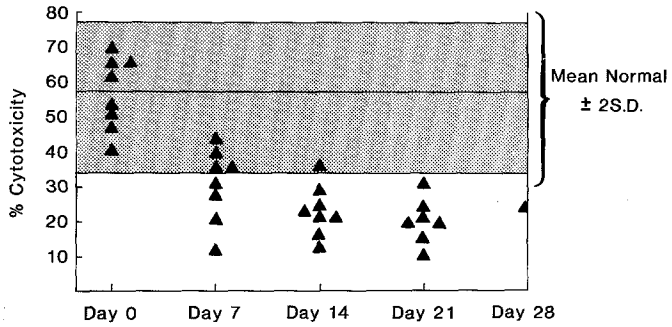

Fig. 1. Influence of cytotoxic chemotherapy on NK function in patients with normal pretreatment levels of NK activity. The shaded area represents the mean ±2 SD level of function for normal PBMC tested at an effector:target ratio of 50:1

In contrast, chemotherapy appeared to potentiate NK activity in the peripheral blood of those patients who had significantly depressed NK function prior to chemotherapy. Of the 20 patients studied, 12 patients were found to have a level of NK function which was considered significantly depressed compared to normal immediately prior to treatment (Fig. 2). Following therapy in this group of patients, NK function improved steadily and by the day 21 assessment period, 8/12 patients were found to have NK function which was considered to be within the normal range (Fig. 2). Significantly improved NK function was seen as early as 1 week posttreatment in 4 patients and by 2 weeks posttherapy in 2 additional patients.

The relationship between pretreatment NK levels and the subsequent effects of chemotherapy on NK function was consistent for effector cell:target cell ratios of 50:1, 25:1 and 12.5:1 (Table 2). There was no consistent effect of a particular chemotherapy treatment regimen on NK function in this study. For example, of the 6 non-small cell lung cancer patients who were treated with mitomycin C, vinblastine and cisplatin, 2 had normal NK function prior to chemotherapy which declined following treatment and 4 had reduced NK function prior to chemotherapy which improved following treatment (data not shown).

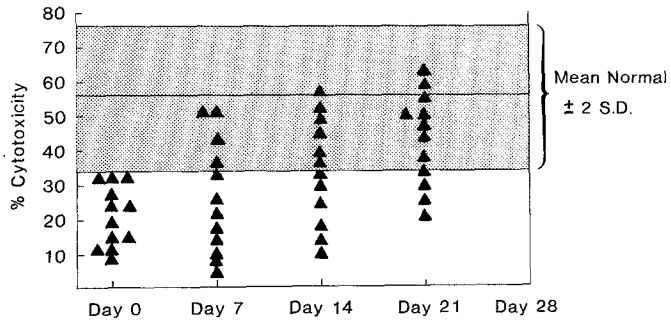

Fig. 2. Influence of cytotoxic chemotherapy on NK function in patients with depressed pretreatment levels of NK activity. The shaded area represents the mean ±2 SD level of function for normal PBMC tested at an effector: target ratio of 50:1

Table 2. Effect of chemotherapy on NK activity of whole PBMC and glass nonadherent PBMC (GNAD) from cancer patients

Group	Time of assessment		%^{51}Cr-Release at E/T[a]		
			50/1	25/1	12.5/1
Nomal NK pretreatment ($n = 8$)	Pretreatment[b]	(whole)	58.6 ±12	41.0 ± 8	31.4 ±11
		(GNAD)	61.6 ± 8	43.3 ±11	38.0 ±13
	Posttreatment[c]	(whole)	19.5 ± 6	13.6 ± 4	5.6 ± 5
		(GNAD)	44.6 ± 8[d]	35.6 ±11[d]	24.3 ± 5[d]
Depressed NK pretreatment ($n = 12$)	Pretreatment	(whole)	21.2 ± 9	13.0 ± 5	8.5 ± 5
		(GNAD)	47.5 ± 9[e]	42.0 ± 4[d]	22.8 ± 6[d]
	Posttreatment	(whole)	50.2 ±14	30.0 ± 7	17.8 ± 6
		(GNAD)	55.8 ± 7	43.7 ±14	22.3 ± 7
Normal subjects		(whole)	57.6 ±13	39.9 ±14	22.2 ±10
		(GNAD)	71.1 ±14	42.6 ±11	21.0 ±13

[a] E/T is effector cell to target cell ratio
[b] Pretreatment is the NK level with cells collected prior to administration of chemotherapy
[c] Posttreatment is the NK level following chemotherapy which was found to deviate by greatest amount from the pretreatment values
[d] $P < 0.01$
[e] $P < 0.05$

2. Effects of chemotherapy on the NK activity of glass nonadherent cells from solid tumor patients

Due to the reports that implicate glass adherent cells in the control of NK function in cancer patients [8] we investigated the effect of depleting glass adherent cells from the PBMC of the patients entered on this study. Prior to chemotherapy, glass adherent cell depletion did not significantly augment NK function in the group of patients with normal pretreatment NK levels (Fig. 3). Following chemotherapy, at the time of maximum NK depression, glass adherent cell depletion was found to significantly augment NK function (Fig. 3). The reverse situation was found in PBMC from patients that had depressed NK levels prior to treatment (Fig. 3). In that group of patients, depletion of glass adherent cells prior to therapy significantly augmented NK function (to within normal levels). Following chemotherapy, at the time of maximum NK augmentation, depletion of glass adherent cells did not significantly change the NK levels that were determined for the whole PBMC preparations (Fig. 3). As was the case for whole PBMC preparations, these relationships were maintained at effector cell : target cell ratios of 50 : 1, 25 : 1, and 12.5 : 1 (Table 2).

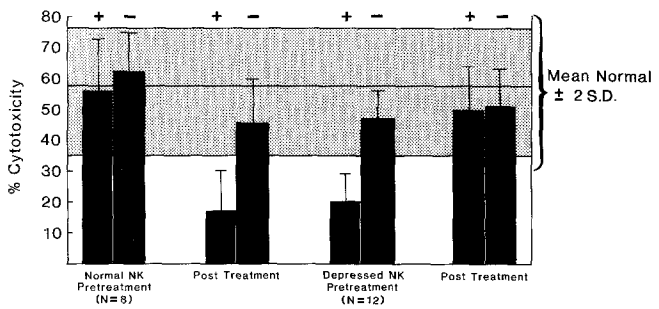

Fig. 3. Effect of chemotherapy on glass adherent cell regulation of NK function in patients with normal pretreatment levels of NK activity or depressed pretreatment levels of NK activity. The shaded area represents the mean ±2 SD level of NK function in normal PBMC tested at an effector: target ratio of 50 : 1. Plus (+) cultures are cultures with glass adherent cells present and minus (−) cultures are cultures in which glass adherent cells have been depleted

3. Effects of chemotherapy on K562-binding cells, Leu11+ cells, and latex-ingesting cells in the PBMC of solid tumor patients

The possibility that chemotherapy might influence NK function by altering the percentages of NK effector cells or NK regulatory cells in the peripheral blood of treated patients was also considered. However, this did not appear to be the case (Table 3). The group of patients with normal NK levels prior to therapy did not demonstrate a significant change in either K562-binding cells, Leu11+ cells, or latex-ingesting cells as a consequence of having received chemotherapy. In the patients with depressed NK function prior to treatment, some reduction in latex-ingesting cells was found as a consequence of chemotherapy, but this was not statistically significant. K562-binding cell percentages and Leu11+ cell percentages were also unchanged following chemotherapy in this group of patients. It was of interest to note, that the percentages of these cell subsets in the treated patient populations were not significantly different from the corresponding normal percentages determined in the control group either prior to chemotherapy or following chemotherapy.

Discussion

The results of this study demonstrate that chemotherapy can differentially influence NK activity in patients with malignant disease depending on the pretreatment NK level of function in the individual patient. In those patients with depressed NK levels prior to treatment, chemotherapy tended to augment NK function; in those patients with normal NK levels prior to treatment, chemotherapy

Table 3. Effect of chemotherapy on K562-binding cells, leu11+ cells, and latex-ingesting cells in the PBMC of solid tumour patients

Group	Pretreatment			Posttreatment		
	% Latex-Ingesting	% K562-Binding	% Leu 11 +	% Latex-Ingesting	% K562-Binding	% Leu 11 +
Normal	16.4 ±5	13.0 ±2	4.4 ±2.4	–	–	–
Depressed NK pretreatment ($n = 12$)	25.6 ±8	11.6 ±3	4.2 ±4.0	20.9 ±7	10.2 ±4	4.3 ±1.5
Normal NK pretreatment ($n = 8$)	20.1 ±3	12.0 ±2	3.9 ±3.6	21.6 ±4	10.9 ±3	4.0 ±2.2

tended to depress NK function. This effect appeared to be associated with the capacity of chemotherapy to influence glass adherent cell regulation of NK function in the peripheral blood of the treated cancer patients. In pretreatment situations where glass adherent cells appeared to depress NK function, chemotherapy seemed to modify this and permit NK augmentation. In pretreatment situations where glass adherent cells did not appear to depress NK function, chemotherapy depressed NK function. There did not appear to be a consistent relationship between the effects of chemotherapy on NK function or glass adherent cell regulation of NK function and modification of the relative numbers of latex-ingesting cells, K562-binding cells, or Leu11 + cells in the circulation of treated patients. Also, there was no consistent effect of specific chemotherapy treatment regimens on NK function in this study. Thus, patients who received a particular drug treatment regimen could experience either suppression of NK function or potentiation of NK function in the posttreatment interval depending on their pretreatment NK level; that, in turn, was related to the regulatory activity of their glass adherent cells.

Cytotoxic chemotherapy appeared to modify glass adherent cell regulation of NK function leading, in some patients, to a potentiation of NK activity. These results are consistent with other studies that demonstrate the potentiation of mitogen-induced lymphoproliferation and alloantigen-induced lymphoproliferation following chemotherapy due to modification of abnormal monocyte-mediated suppression [4, 7]. They are also consistent with the demonstration that glass adherent cells appear to regulate NK levels in the PBMC of cancer patients [8] and are responsible for much of the depressed NK activity that has been reported for such patients [19]. What is not clear from the present studies, however, is the means by which glass adherent cells act to regulate NK function in cancer patients and the means by which chemotherapy may modify this situation. One possibility is that glass adherent cells from cancer patients can depress NK function by a mechanism related to immunoregulatory prostaglandins [20]. That monocytes from cancer patients produce excessive amounts of certain immunoregulatory prostaglandins has become widely appreciated [6, 21]. We would tentatively discount that explanation, however, in the present study since we have been unable to potentiate NK function in the PBMC of cancer patients with the prostaglandin synthesis inhibitor, indomethacin [8]. Another possibility which might be considered is that glass adherent cells from cancer patients suppress interferon synthesis by large granular lymphocytes or T lymphocytes which may be required for the full expression of NK activity [22]. The results of the present study do not address that issue and further investigation in this area would seem warranted. We did consider the possibility that the NK activity in the PBMC of cancer patients and the differential effects of chemotherapy on that activity was related to relative numbers of K562-binding cells, Leu11+ cells, or latex-ingesting cells. That explanation does not seem to be correct judging from the results presented in this report. This is consistent with observations which have failed to demonstrate a correlation between numbers of large granular lymphocytes or K562-binding cells and NK cytotoxicity [8].

Studies concerned with the effects of chemotherapy on NK function in cancer patients have yielded variable results. For example, Saijo et al. [18] reported improvement in NK function in patients receiving chemotherapy for lung cancer, particularly in those patients who achieved disease stabilization as a consequence of therapy. In contrast, Forbes et al. [9] reported that patients with small cell carcinoma of the lung who were treated with Cytoxan, Adriamycin, and vincristine had reduced NK activity following drug treatment which persisted for extended periods of time and which was not associated with clinical treatment responses. In that study, however, most patients had received radiation therapy making it difficult to draw conclusions about the effects of chemotherapy alone on NK function. In the study by Bhoopalam et al. [2], the effects of chemotherapy with a combination treatment regimen on NK activity in patients with a variety of solid tumors was investigated. In most of the patients studied, normal NK function was found prior to treatment and depressed NK function was found following treatment. NK function was found to increase slightly in four patients despite disease progression and overall, any changes in NK activity following chemotherapy could not be correlated with tumor response. It is also possible that under some circumstances, chemotherapy may induce suppressor cells capable of reducing NK function. In one such study [17], the treatment of normal human peripheral blood cells in vitro with amphoteracin B led to the induction of cells which suppressed both NK and ADCC activities. In mixing experiments, it was suggested that amphoteracin B promoted the differentiation of precursors of suppressor cells for these forms of cellular cytotoxicity.

As can be appreciated from the results of this and similar studies, chemotherapy for cancer can be viewed in the

context of biological response modifier therapy. Ultimately, improved tumor responsiveness might be realized if drug treatments were designed to take full advantage of their immunomodulating potential. Decisions about scheduling of drug treatments and determinations of drug dosages might be influenced by the functional state of the immune system of the patient at the onset of therapy. Alternatively, regimens which combine cytotoxic chemotherapy with other biological response modifiers might be developed; these could, for example, be aimed at eliminating suppressor T cells or monocytes together with potentiating immune effector function. As knowledge of the cellular and biochemical events which control the immune response are applied to the design of cancer chemotherapy, greater capacity of favorably modify immunity in cancer patients should be realized.

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Received April 29, 1985/Accepted August 6, 1985