INDUCTION OF LYMPHOKINE-ACTIVATED KILLER-LIKE CELLS BY CANCER CHEMOTHERAPY

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The mechanism of action of the cancer chemotherapeutic agents (alkylating agents, antimetabolites, antibiotics, plant alkaloids, enzymes, and hormones) is thought to be the blocking of cancer growth and cell division, with the exception of low-dose cyclophosphamide, which seems to inhibit suppression of immune responses against cancer cells (1, 2). In general, these anticancer drugs inhibit immune functions, including natural cell-mediated cytotoxicity (3). On the other hand, combined cancer chemotherapeutic agents instead of a single agent are frequently used and they are usually administered in several cycles to cancer-bearing patients. The mechanism of action of this combined chemotherapy is also thought to be the blocking of cancer cell growth and cell division. The effect of combined cancer chemotherapeutic agents on natural killing has not been examined extensively.

From the experiments of examining NK activity against K562 human myelogenous leukemia cells and killer activity against NK-resistant B cell lymphoma Raji cells during MVAC (methotrexate, vinblastin, adriamycin, and cis-platin) combined chemotherapy (4) in bladder cancer patients, we found the appearance of very strong lytic activity against NK-resistant Raji cells in the peripheral blood after ~1 mo of each cycle of therapy. The target spectrum of this killing was wide. mAbs and complement treatment revealed that the effector cells express CD8 and SL1 phenotype weakly, but do not express CD4, CD5, CD16, Leu-7, or OKM1, suggesting that these effector cells are lymphokine activated killer (LAK)-like cells. Examinations are now under way with other protocols of cancer chemotherapy, but up to now, MVAC for bladder cancer or ureter cancer, PEB (cis-platin, etoposide, and bleomycin) (5) for testicular cancer, and MA (methotrexate, adriamycin) for testicular cancer, which are all we have examined, induced this strong LAK-like activity in the peripheral blood.

Materials and Methods

Patients. Among the six bladder tumor patients and two testicular tumor patients whose cytotoxic activities during chemotherapy we have monitored, four bladder tumor patients and one testicular tumor patient were chosen to show in Fig. 1.

Treatment. From day 7 to 11, bladder tumor patients got 2,000 rad preoperative irradiation. On day 14, total cystectomy was performed. Two or three cycles of MVAC chemotherapy were performed with 1-mo intervals starting on day 27. Each MVAC cycle consisted of 2 d of intravenous injection (1 mg/kg of methotrexate on day 1 and 0.1 mg/kg of vinblastin, 1 mg/kg of adriamycin, and 2 mg/kg of cis-platin on day 2). For testicular tumor patients, two
cycles of PEB chemotherapy without prior radiation were administered. Each PEB cycle consisted of an intravenous injection of 1 mg/kg of cis-platin, 2 mg/kg of etoposide, and 0.2 mg/kg of bleomycin per day for 3 d.

**Effector Cells.** PBL were isolated as effector cells from the carbonyl iron--treated blood of these patients by separation on Ficoll-Hypaque gradients. Adherent cells were removed by a 1-h, 37°C incubation on plastic dishes precoated with autologous plasma.

**Target Cells.** The cell lines used in this study included human B cell lymphoma Raji cells, erythroleukemia K562 cells, bladder tumor T-24 cells, prostatic cancer PC-3 cells, murine lymphoma YAC cells, UV-induced fibrosarcoma UV female 1, and mammary carcinoma MM2.

**Cytotoxicity Assay.** A cytotoxicity assay was performed by a standard 3Cr-release assay as described (6). LU were calculated from cytotoxic titration curves; 1 LU was defined as the number of effector cells required to cause 20% lysis of $5 \times 10^3$ targets. The data are presented as LU/10^7 effector cells.

**Negative Selection of Lymphocyte Populations.** Monoclonal anti-Leu-7 (HNK-i), anti-Leu-11B (CD16), anti-Leu-3A (CD4), and anti-Leu-1 (CD5) antibodies were obtained from Becton Dickinson & Co. (Mountain View, CA). Monoclonal anti-T8 (CD8) antibody was purchased from Coulter Electronics, Inc. (Hialeah, FL). Monoclonal SL-1 antibody is an anti-human pan T cell antibody and was obtained from Ortho Diagnostic Systems Inc. (Tokyo, Japan). This antibody can stain 100% of peripheral T cells and <5% of peripheral B cells and bone marrow cells (7). Effector cells were treated with mAbs for 30 min at 4°C and after centrifugation the supernatant was discarded and the young rabbit complement obtained from Pel-Freeze Biologicals (Rogers, AR) was added and incubated for 40 min at 37°C. After washing three times, antibody-treated effector cells were assayed for cytotoxicity and aliquots were used for assuring phenotypes by FACS staining.

**Results**

We first planned experiments for assaying NK and killer activity against NK-resistant targets in order to determine the optimal timing of adding biological response modifiers (BRM) to usual chemotherapy. Hence, we assayed these two types of killer activity once a week during all the courses of treatment. As shown in Fig. 1A, before irradiation, NK activity was comparatively high but killer activity against NK-resistant Raji cells was as minimal and negligible as that of normal persons (20 ± 4 LU from 25 normal volunteers and <1 LU from 10 bladder tumor-bearing patients before therapy) and a first peak of killer activity against NK-resistant Raji cells appeared after radiation. After the first course of MVAC and PEB therapy (as before radiation), we could see no killer activity against Raji cells until the burst appearance of this activity after ~30 d. Without further chemotherapy no more peaks were seen (data not shown). And ~30 d after the second or third chemotherapy, we could always (all of the bladder and testicular tumor patients we examined) see the second or third peak of this killer activity. These results show that appearance of these potent killing activities against NK-resistant target cells was induced by MVAC or PEB combination chemotherapy.

To know what component of combined chemotherapeutic agents is responsible for this killer induction in peripheral blood, we further assayed this killer activity of testicular tumor-bearing patients who were treated with different combined chemotherapeutic agents MA. MA also induced this killer activity after ~1 mo of chemotherapy (data not shown). From these results, no special chemotherapeutic agents, but some kinds of changes (e.g., bone marrow suppression and regeneration) common to these combinations, were thought to induce this killer activity.

NK activity during MVAC chemotherapy had the same tendency as this killer
activity against NK-resistant target cells. To characterize this killer activity, first, we used various tumor cells as target cells (Table I). These killer cells could lyse not only NK-resistant Raji cells and NK-sensitive K562 cells but also a broad spectrum of target cells, including human bladder tumor T-24 cells, human prostatic cancer PC-3 cells, and murine fibrosarcoma UV female 1 cells. This shows that these killer cells can recognize and lyse without restriction. Target specificity of MVAC-induced killer activity, PEB-induced killer activity, and LAK cells induced in vitro were quite similar. Phenotypes of these chemotherapy-induced killer cells were examined using mAbs and complement treatment (negative selection). As shown in Table II, HNK-1 or CD16 treatment did not influence this killer activity at all, though HNK-1⁺ or CD16⁺ cells in the treated preparation were only 2% or 3% of original

![Table I](image)

**Target Spectrum of MVAC-induced Killer Cells, PEB-induced Killer Cells, NK Cells, and LAK Cells**

| Killer cells       | Raji | Daudi | YAC  | T-24 | PC-3 | K562 | UV 1 | MM2 |
|-------------------|------|-------|------|------|------|------|------|-----|
| MVAC-induced killer | 460* | 640   | 73   | 160  | 222  | 492  | 221  | 0   |
| PEB-induced killer  | 446  | 800   | 400  | 320  | 337  | 1280 | 356  | 0   |
| NK                 | 0    | 0     | 0    | 0    | 0    | 376  | 0    | 0   |
| LAK\(^1\)        | 100  | ND    | 246  | 397  | ND   | 376  | ND   | 0   |

* Results are expressed as LU calculated from cytotoxic titration curves of an 18-h ⁵¹Cr-release assay.

\(^1\) PBL from a normal volunteer were cultured in 10 U/ml of human rIL-2 for 5 d and the cytotoxic activity was assayed by an 18-h ⁵¹Cr-release assay.
population by FACS (data not shown). Among T cell markers, only SL-1 and CD8 were thought to be weakly present on these chemotherapy-induced killer cells. Hence, the effector cells were thought to be CD4⁻, CD5⁻, CD16⁻, Leu-7⁻, OKM1⁻, and CD8 weakly positive SL-1 weakly positive LAK-like cells.

**Discussion**

We found LAK-like cell induction in the peripheral blood after administration of anticancer drugs. Perhaps this is the first report of the presence of in vivo activated LAK cells without any administration of IL-2.

From the results that MVAC, as well as the PEB or MA combination, equally induced LAK-like activity at ~1 mo after administration, we think that this LAK-like cell induction is not due to specific drug actions but due to some common phenomenon, for example, regeneration from myelosuppression among these chemotherapeutic agents. Indeed, we usually could see mild or severe lymphocytopenia 2 wk after chemotherapy and recovery from it ~1-2 wk later. But, how it regenerates is one of the important questions to be solved in the future. As it is reported with IL-2 and IL-3, hematopoietic progenitor cell colonies could be directly differentiated into large granular lymphocytes (LGL) (8), which are known to be activated into LAK cells (9, 10), it seems possible that IL-2 or IL-3 may also play some roles to generate this chemotherapy-induced LAK-like cell activity, though these effectors are T cells instead of being LGL. In the murine system, it is reported that after a sublethal dose of cyclophosphamide administration, all of the killer cell activities (LAK, NK, CTL) were abolished at once. And recovery was in the order of allogeneic CTL, NK, LAK (11-13). In these reports, however, a sublethal dose of cyclophosphamide did not induce LAK activity but the precursors of LAK cells reappeared after regeneration. These precursors could be activated after incubation with IL-2 in vitro. The situation is the same in the case of murine bone marrow chimeras (14). The difference is that in our experiments LAK-like effector cells, instead of precursor cells, were induced after chemotherapy. And this is the reason we could not perform positive selection for surface phenotype determination.

Kinetics of killing revealed that from 4 h, we could see cytolysis, and in 18-24 h,
it reaches a plateau (data not shown). The magnitude of killing is very high (at least >500 LU and sometimes >1,000 LU) and the duration is comparatively long (~2 wk). From this potent lytic activity, we think that these LAK-like cells may play some role in destroying tumor cells resistant to chemotherapy, because some kinds of chemoresistant murine or human tumor cells are reported to be more sensitive to LAK cells (15). But the real role of these chemotherapy-induced LAK-like cells is still obscure and experiments are now under way to clarify it.

Summary

Natural cell-mediated cytotoxicity against NK-resistant target tumor cells was found in the peripheral blood of tumor-bearing patients ~1 mo after combined chemotherapy. The recognition specificity of these effector cells was broad and had no restriction. From the experiments of negative selection with mAbs and complements, these newly developed killer cells after chemotherapy were thought to be LAK-like cells. Contribution of these LAK-like cells to the mechanism of action of anticancer drugs remains to be clarified.

Received for publication 6 July 1988 and in revised form 10 August 1988.

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