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Augmented Natural Killer Activity in Ovarian Cancer Patients Treated with Cimetidine*

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Abstract—*The effect of cimetidine on natural killer (NK) activity of peripheral blood lymphocytes (PBL) was studied in 37 post-operative patients with ovarian cancer. There was no significant difference in the NK activities between normal healthy women and patients with no residual tumor after surgery, while those in patients with large residual tumor after surgery were suppressed to less than a half of those in normal healthy women. Cimetidine augmented the NK activities in patients with no residual tumor but not in normal healthy women. Particularly, the NK activities in patients with low baseline NK status before cimetidine treatment was markedly enhanced. Furthermore, the NK boosting by cimetidine was more effective in patients with large residual tumor than in patients with no residual tumor. The NK activity in PBL from such patients was also enhanced by* in vitro *exposure to cimetidine. These findings suggest that cimetidine may be used as an immunorestorative-therapy for ovarian cancer patients with depressed NK activity.*

INTRODUCTION

LYMPHOCYTES bearing histamine type-2 receptors may modulate lymphokine (for example, leukocyte migration inhibitory factor) production and to a degree, skin test reactivity [1]. Earlier work showed that histamine receptors modulate other leukocyte immunologic functions, such as antibody production [2] and lymphocyte-mediated cytotoxicity [3]. A growing body of evidence suggests that suppressor T cells carrying histamine type-2 receptors may play an important immunoregulatory role in the execution of the normal immune response. Abberations in this subpopulation of cells were shown to be associated with the development of pathologic conditions, including cancer [4–8]. The suppressor cell function seems to be mediated through the release of a soluble factor (induced by histamine), which can be abrogated by a histamine type-2 receptor antagonist may be used as therapeutic immunomodulators to inhibit suppressor T cells pharmacologically. It has been well known that cell-mediated immunity (CMI) in patients with different types of solid tumors was depressed and/or impaired [9]. Natural killer (NK) cells which represent natural CMI have been shown to be important in prevention of tumor metastasis [10] as well as in host defense mechanisms directed against tumor surveillance [11, 12]. In addition, NK activity has been demonstrated to be depressed under the response of malignant tumor [13].

Recently, cimetidine, a histamine type-2 receptor antagonist, has been shown to enhance the tumor host CMI by abrogating suppressor cell activity and subsequently to inhibit the tumor growth *in vivo* [14, 15]. In the present study, we now report stimulatory effect *in vivo* as well as *in vitro* of cimetidine on the NK activity.

MATERIALS AND METHODS

Subjects

The study comprised 33 normal healthy women (median age 40), 16 patients (median age 52) with no residual tumor after surgery and 21 patients (median age 48) with large residual tumor (more than 5 cm in average of the major and minor diameters of tumor) after surgery. To avoid the effect of surgery on the NK activity, measurement of NK activity was performed 3–6 months after surgery. No other intervening therapy during that period was given that might influence NK activity. The normal healthy women were randomly selected from laboratory, clinic or nursing personnel and secretarial staff and served as controls. The patients were given a 1-month course of oral cimet-

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idine (1000 mg daily in four divided doses). Blood from patients was taken before, on 14 and 28 days after initiation of treatment with this drug. No cytostatic therapy was given during this period.

Peripheral blood lymphocytes

Blood was collected by venipuncture into heparinized (10 U/ml of blood) tubes. Lymphocytes were separated by centrifugation on a Ficoll Hypaque gradient [16], washed three times with phosphate-buffered saline (0.15 M, pH 7.2), counted and resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco Laboratories, New York, NY, U.S.A.) and 2 mM glutamine.

In vitro *cytotoxicity assay*

Aliquots containing 10^6 target cells were labeled with 50 μCi of sodium chromate 51 solution (New England Nuclear, Boston, Massachusetts, U.S.A.) for 1 hr in 1 ml of medium (same medium as used for preparation of PBL). After three washings, 10^4 cells in 0.1 ml of medium were pipetted into flat-bottomed micro-titer plates (Limbro Scientific Inc., Hamden, CT, U.S.A.). Target cell used in this study was K 562 (a cell line derived from a patient with chronic leukemia). Various concentrations of effector cells in 0.1 ml of medium were added in triplicate to give effector cell : target ratios of 50 : 1, 25 : 1 and 12.5 : 1, respectively. After incubation for 18 hr at 37° C in a humidified atmosphere of 5% CO_2 in air, supernatants were harvested using a Titertek collection system (Flow Laboratories, Rockford, MD, U.S.A.) and counted in a gamma counter. Per cent specific ^{51}Cr release was calculated as follows:

$$\frac{\text{cpm test release—cpm spontaneous release}}{\text{cpm maximum release—cpm spontaneous release}} \times 100.$$

Spontaneous and maximum releases are cpm releases from target cells incubated in medium alone and in 1 N HCl-added medium, respectively. The range of spontaneous release from K 562 was 5–15% of the total isotope count. Maximum release determined by 1 N HCl was about 95%.

RESULTS

The immediate response of patients toward treatment with cimetidine was measured by NK cell activity in PBL. These experiments were designed after results previously obtained in nude mice, in which treatment with cimetidine led to an induction of cell population responsible for rejection of xenograftic tumor [17].

Table 1 shows comparison of NK activity in PBL from normal healthy women, patients with no residual tumor after surgery and/or large residual tumor. The NK activity in normal healthy women and patients with no residual tumor was 71.9 ± 17.8% and 58.8 ± 12.9%, respectively. The difference in these values was not statistically significant. On the other hand, the NK activity in patients with large residual tumor was about a half of that in patients with no residual tumor.

Table 2 shows the percent cytotoxicities of PBL of 10 normal healthy women before and after initiation of oral administration of cimetidine. The average cytotoxicities were 65.0, 64.1, and 64.5% before administration, on 14 and 28 days after administration, respectively. Thus, the level of NK activity in normal healthy women remained unchanged after administration of cimetidine.

In contrast, the average cytotoxicities were 50.7, 53.3, and 56.9% before administration, on 14 and 28 days after administration, respectively. The level of NK activity was increased after treatment with cimetidine and statistical analysis confirmed

Table 1. Comparison of NK activity in PBL from normal healthy women and ovarian cancer patients

Patients	*n*	Age* (Range)	NK activity†
Normal healthy women	33	40 (22–55)	71.9 ± 17.8
No residual tumor‡	16	52 (33–67)	58.8 ± 12.9
Large residual tumor§	21	48 (20–76)	31.6 ± 17.7‖

*Median age.
†Per cent cytotoxicity (mean ± S.D.). Effector : target cell ratio; 50 : 1.
‡Patients with no residual tumor after surgery and received no cytostatic drug.
§Patients with large residual tumor after surgery.
‖$P < 0.01$, compared to normal healthy women and patients with no residual tumor. Student's *t*-test.

Table 2. NK activity in normal healthy women before and after initiation of treatment with cimetidine

No. of patients	Per cent cytotoxicity*		
	Before	14 Day	28 Day
1	61.5	63.0	62.5
2	71.1	70.0	68.5
3	64.6	60.5	63.4
4	52.5	59.5	60.3
5	76.5	73.8	74.4
6	80.5	79.7	81.2
7	54.1	76.5	77.1
8	66.8	41.5	39.6
9	83.3	78.3	80.2
10	39.2	38.5	37.3
Mean ± S.D.	65.0 ± 13.7	61.1 ± 14.6	64.5 ± 15.6

*Per cent cytotoxicity before or 14 and 28 days after initiation of cimetidine oral administration. E : T ratio; 50 : 1.

that such enhancement is significant ($P < 0.01$, $P < 0.005$). The maximal level in NK activity was obtained on 28 days after treatment (Table 3).

From results of the day-to-day variation of NK activity (data not shown), the mean value obtained from differences in percent cytotoxicity between the two assays was 9.5 ± 5.7. On the basis of these results, an increase in percent cytoxicity of more than 20%, as compared to that before treatment, was considered to be significant in the following analysis.

To compare NK boosting effect by cimetidine, the 16 treated patients with no residual tumor were arbitrarily divided into two groups according to their baseline NK activity before treatment: patients with low (less than 50% cytotoxicity) and high (greater than 50%) NK activities. As shown in Table 4, five of seven patients (77.1%) in the low-NK group showed significantly enhanced activity after cimetidine administration, whereas in the high-NK group, only three of nine patients (33.3%) showed more than 20% enhancement in NK activity. Thus, as a whole, patients with low base-line NK activity had a sensitivity to the boosting effect higher than those with high baseline NK activity (Fig. 1).

Table 3. NK activity in patients with no residual tumor before and after initiation of treatment with cimetidine

No. of patients	Per cent cytotoxicity*		
	Before	14 Day	28 Day
1	46.9	55.8	60.5
2	32.1	34.1	35.8
3	50.3	56.7	62.5
4	59.7	61.1	60.9
5	65.1	63.2	64.7
6	43.1	51.9	58.6
7	25.6	29.3	43.4
8	52.2	55.5	60.3
9	61.8	58.7	42.3
10	53.3	57.4	61.2
11	40.5	51.6	60.7
12	45.9	48.3	53.8
13	59.4	50.7	55.8
14	71.9	60.3	66.2
15	48.4	59.9	65.7
16	55.3	58.1	57.2
Mean ± S.D.	50.7 ± 12.0	53.3 ± 9.4	56.9 ± 8.9
		$P < 0.01$†	$P < 0.005$

*Per cent cytotoxicity before or 14 and 28 days after initiation of cimetidine oral administration.
†Paired *t*-test.

Table 4. Comparison of NK activity in cimetidine-treated patients with no residual tumor according to their baseline NK status before treatment with cimetidine

	Total	No. of increase*	% increment
Low (< 50%)	7	5 (77.1%)	35.3 ± 19.7
High (> 50%)	9	3 (33.3%)	1.5 ± 10.7†

*Per cent increase on 28 days after treatment with cimetidine of more than 20%, as compared with that before treatment.
†$P < 0.001$, Student's *t*-test.

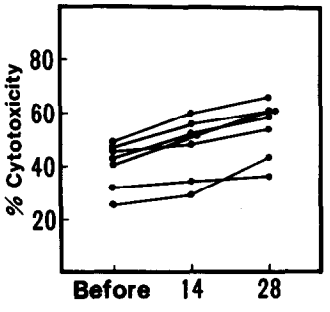

Fig. 1. NK boosting by cimetidine in no residual tumor patients with low baseline NK status before treatment.

As described in Table 1, patients with large residual tumor had low baseline NK activity. Accordingly, the NK boosting effect by cimetidine was compared to that of untreated patients with large residual tumor. As shown in Fig. 2, 10 of 11 cimetidine-treated patients with large residual tumor (90.9%) had more than 20% enhanced NK

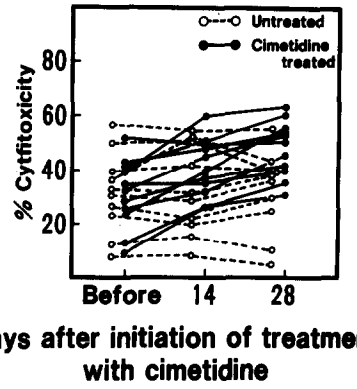

Fig. 2. NK boosting by cimetidine in patients with large residual tumor.

activity, while a patient with high baseline NK activity (greater than 50%) remained unchanged. *In vivo* NK boosting effects by cimetidine were summarized in Table 5. The average per cent increment of NK activity was greatest on 28 days (55.8%) and 14 days (31.9%) after treatment of patients with large residual tumor, followed by 16.3% on 28 days and 6.9% on 14 days after treatment of patients with no residual tumor. Thus, there seemed to be an inverse relation between the baseline NK activity and the boosting effect.

In addition, we attempted to determine whether such low baseline NK activities can be enhanced by *in vitro* exposure of PBL with cimetidine. As shown in Table 6, exposure of PBL to cimetidine stimulated the NK activity in a dose-dependent manner. The percent increment showed 23.7% at 0.01 mM and 63.4% at 0.1 mM of cimetidine, respectively.

DISCUSSION

The present study has demonstrated that the NK activity in patients with large residual tumor

Table 5. Comparison of NK boosting by cimetidine in patients with no or large residual tumor

Subjects	*n*	Days after initiation of treatment: Before	14	28
Normal healthy women	10	65.0 ± 13.7*	64.1 ± 14.6	64.5 ± 15.6
No residual tumor	16	50.7 ± 12.0	53.5 ± 9.4† (6.9%)§	56.9 ± 8.9‡ (16.3%)
Large residual tumor	11	31.0 ± 13.0	40.9 ± 11.0‖ (31.9%)	48.3 ± 10.3‖ (55.8%)
Untreated patients with large residual tumor¶	10	31.6 ± 15.2	32.4 ± 16.8	32.0 ± 15.2

*Mean ± S.D.
†$P < 0.01$, compared to values before treatment. Paired *t*-test.
‡$P < 0.005$, compared to values before treatment. Paired *t*-test.
§Average of % increment.
‖$P < 0.001$, compared to values before treatment. Paired *t*-test.
¶These patients were not treated with cimetidine.

Table 6. *NK boosting* in vitro *by cimetidine in patients with large residual tumor**

Cimetidine	NK activity (%lysis)	% increment†
None	27.9 ± 7.4	
0.01 mM	34.5 ± 5.1‡	23.7
0.1 mM	45.6 ± 3.9‖	63.4

*The duration of exposure of PBL to cimetidine was 18 hr. Results from six separate experiments (mean ± S.D.).
E : T ratio; 50 : 1.
†Average value.
‡$P < 0.01$, compared to non-treated PBL.
‖$P < 0.001$.

was significantly lower than that in patients with no residual tumor and normal healthy women (Table 1). Similarly, pronounced reduction of NK activity is documented in patients with advanced cancer [13, 18] but not in patients with regionally-locally controlled tumors [19]. Uchida *et al.* [20] reported that at 1–2 weeks after surgery, significant reduction of NK activity was observed in 16 of 21 patients with breast cancer and was not related to the stage of disease. Accordingly, to avoid the effect of surgery on the NK activity, measurement of the NK activity was performed after 3–6 months of surgery. During that period, no other therapy that might influence the NK activity was given. The NK activity in patients with large residual tumor remained unchanged during the experimental period (Table 5). Histamine produced by remote tissues under the influence of the growing tumor [21] or by host basophils attracted to the tumor site [22] may activate suppressor T cells carrying H_2 receptors on their surface. By effectively competing for these H_2 receptors, cimetidine may block the activation of suppressor T cells and relieve the cellular immune apparatus of the cancer patients of the histamine-induced paralysis. Recent data from experimental animal tumor models suggest that cimetidine indeed slows down tumor growth and prolongs survival, in conjunction with abrogating suppressor cell activity [14, 15]. Cimetidine treatment has been reported to manipulate favorably a certain parameter of the immune function in cancer patients, including cell-mediated cytotoxicity [23] and graft vs. host reaction [24]. Our experiments were designed to provide direct *in vivo* and *in vitro* evidence for enhanced NK activity in postoperative ovarian cancer patients treated with cimetidine. Although the expected augmented NK activity in the cimetidine-treated patients was observed, the enhancing effect was in general more marked in patients showing low levels of activity before treatment (Table 4). Similar observations have been reported by Lotzova *et al.* [25] in patients treated with interferon and Hovanessian *et al.* [26] in patients treated with polyadenylic;polyuridylic acid. The NK activity in patients with large residual tumor was significantly lower, compared to that in normal healthy women or patients with no residual tumor (Table 1). These results are consistent with previous reports [13, 19]. Although this depression of NK activity in peripheral blood of tumor-bearing individuals might be attributed to migration of these effector cells to the tumor site, this seems unlikely, because most studies have failed to detect NK cells within clinically detectable tumors [27]. In only one study did NK cells appear to be present within a tumor [28]. However, there have not been many systematic studies on the presence of NK cells within the tumor. It is becoming more apparent that excessive suppressor cell function in cancer patients is a significant factor in their defects in cellular and humoral immunity [29, 30]. The excessive suppressor cell function has been shown to be inhibited by cimetidine [26]. In the present study, we have demonstrated that the NK boosting in no residual tumor patients with low baseline NK activity (but not high) or large residual tumor patients with depressed NK activity was most effective, suggesting that cimetidine might abrogate cell functions responsible for inhibiting NK activity. *In vitro* exposure of PBL from patients with large residual tumor to cimetidine resulted in a significant enhancement of the NK activity, indicating a further evidence of existence of excessive suppressor cells in PBL from these patients. Based on our current knowledge of H_2 receptor antagonists and their inhibition of regulator-suppressor cells, cimetidine would be preferable as a selective immunostimulatory agent since levamisole is a nonspecific immunostimulator, augmenting lymphocyte effectors as well as suppressors [29].

In conclusion, the present study suggests that cimetidine may prove useful as a selective immunomodulator for treatment in ovarian cancer patients with depressed NK activity.

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