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Changes in Natural Killer Cell Activity (NK Activity) by Surgical Stress

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ABSTRACT : NK activity in relation to proliferation of cancer cells was clinically evaluated in the postoperative course in patients with carcinomas of the stomach and colon as compared with that in patients with benign diseases.

1) Preoperative NK activities in patients with advanced cancers were inhibited as compared with those in patients with benign diseases and those of normal individual. In particular, it was significant in patients with poor PS of grade 2.

2) NK activities enhanced until two hours after surgery and gradually fell down to the minimum at 12 hours, thereafter resumed the activities.

3) Intraoperative decrease in NK activity was minimal in patients with favorable PS. In contrast, in patients with poor PS and advanced cancers, a decrease and recovery of NK activity was aggravated.

4) NK activity also related to the operation time and the amount of blood transfusion.

5) Main causes of depressed NK activity in the postoperative course were prolonged operation time, with or without blood transfusion, and malnutrition.

INTRODUCTION

It is well known that NK activity may acts as a natural inhibitor against carcinogenesis[1] and also may be inhibited by surgical stress for considerably long period following surgery[2]. Depressed NK activity in a tumor-bearing host is unfavorable state for enhancing metastasis and proliferation of cancer cells[3].

The purpose of this study is to clarify changes in NK activity with elapse of postoperative time and to analyze influential factors on depression of NK activity in terms of the intensity of surgical stress.

MATERIALS AND METHODS

1. Subject

Fifty-seven gastric cancer, 22 colon cancer, and 20 benign disease patients were eligible for this study in comparison with 57 normal individual. They were operated upon at the First Department of Surgery, Nagasaki University School of Medicine during the period from December 1984 to April 1985. They included 23 in stage I, eight in stage II, eight in stage III and 18 in stage IV according to the general rules of Japanese Gastric Cancer Study and five in stage I, six in stage II, four in stage III, four in stage IV and three in stage V according to the general rules of Japanese Colon Cancer Study.

The mean ages in patients with gastric cancer were 58.7±10.1 years old. The sex distribution was 35 to 22 of men to women. Meanwhile, in patients with colon cnacer, the ages averaged 62.0±11.4 and the men to women was nine to 13. On the other hand, in 20 patients with benign

diseases, 10 in cholelithiasis, four in gastric and duodenal ulcers, two in colon diverticulum, two in blind pouch syndrome, one in gastric adenoma, one in scar hernia of the abdominal wall. Their mean age was 56.1±12.8 and sex distribution was 14 men to six women.

The ages in normal individuals averaged 44.7±13.6 years old, with 56.7±7.5 years old on the average, which were composed of 14 men and 14 women were compared as being controls.

2. Estimation of skin reaction to PPD

Cellular immunity was estimated by means of skin reaction to PPD.

3. Immunology

(1) Separation of lymphocytes from peripheral blood

Approximately 20ml of peripheral blood was collected in heparinized tubes from each patient at between 8 and 9 am. Each blood sample was diluted with a two fold volume of Hank's balanced saline solution and mononuclear cells were isolated by the Ficoll-Conray gradient centrifugation. Mononuclear cells were collected and washed three times with HBSS, and resuspended in 10ml of RPMI-1640 tissue culture medium, supplemented with 10% fetal calf serum (GIBCO) and 2mM N-2 hydroxyethylpiperazine-N7-2 ethanesulfonic acid buffer, collectively referred to as complete medium.

(2) ^{51}Cr release assay

Cytotoxicity assays were routinely performed immediately following collection. K562 cells were washed twice with HBSS, then resuspended in complete medium and counted. Five $\times 10^5$ K562 target cells, in 0.9ml of complete medium, were incubated with 100μCi of ^{51}Cr ($Na_2\ ^{51}CrO_4$) for one hour at 37°C and 5% CO_2 in a humidified incubator. The ^{51}Cr labelled target cells were washed three times with cold medium and resuspended in complete medium as a concentration of 1×10^5 cells/ml. Labelled target cells (1×10^4) in 0.1ml of medium, were added to each well of replicate 96 well-rounded bottomed microtest plates. Effector cells, in 0.1ml of complete medium, were added to the wells at an effector: taraget cell ration of 50: 1. For the determination of spontaneous or maximal ^{51}Cr release, 0.1ml of complete medium, alone or with detergent respectively was added to the labelled taraget cells. After six hours, plates were centrifugated at 1650g for five minutes. The radioactivity in 0.1ml of the supernatant was counted with a gamma counter. The percentage of specific ^{51}Cr released from the target cells was calculated using the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{experimental CPM} - \text{spontaneous CPM}}{\text{maximal CPM} - \text{spontaneous CPM}} \times 100$$

as follows:

$$\text{corrected \% cytotoxicity} = \text{experimental \% cytotoxicity} \times \frac{\text{average control \% cytotoxicity}}{\text{control \% cytotoxicity}}$$

The average control % cytotoxicity was the average of experimental results, using normal individual. Control % cytotoxicity was the result obtained from a single experiment.

4. Statistical analysis

Student's t-test was used to determine the significance of the differences between the means of the two groups.

RESULTS

[1] Basic evaluation of NK activity measurement

1) Changes in NK activity by a ratio of E to T (Fig. 1)

NK activities in accordance with alteration of a ratio of E to T from 10:1 to 70:1 were measured. As a result, NK activities were increased according to an increase in a ratio of E to T.

Therefore, a ratio of E to T was set up to

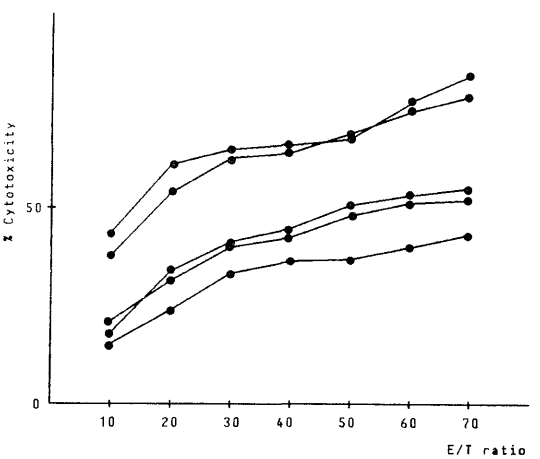

Fig. 1. Changes in NK activity by E/T ratio

50 : 1 in this series.

2) Changes in NK activity by ^{51}Cr volume used for labelling (Fig. 2 and 3)

NK activities were measured by changing in ^{51}Cr volume from 25 to 300μCi/ml.

When adding 25μCi/ml ^{51}Cr, the maximum free counts of ^{51}Cr were a low value. In contrast, when using 300μCi/ml ^{51}Cr, ^{51}Cr counts were lower than when adding 200μCi/ml ^{51}Cr. These implied an unadequate condition for measurement of NK activities.

Therefore, it is adjusted in this series, considering a half life time of Na_2CrO_4 was attempted to be 100μCi/ml ^{51}Cr.

As a result, it is certified that the use of a range of 50 to 200μCi/ml showed in lineal proportion to free ^{51}Cr counts and % cytotoxicity values remained stable.

3) Measurement error of NK activities

1) Measurement errors

NK activities in this series were measured triplicate and the variation of these values ranged within 3% as being % cytotoxicity.

2) Daily changes in NK activity (Fig. 4)

Daily NK activity ranged from 43.0 to 85.6 with

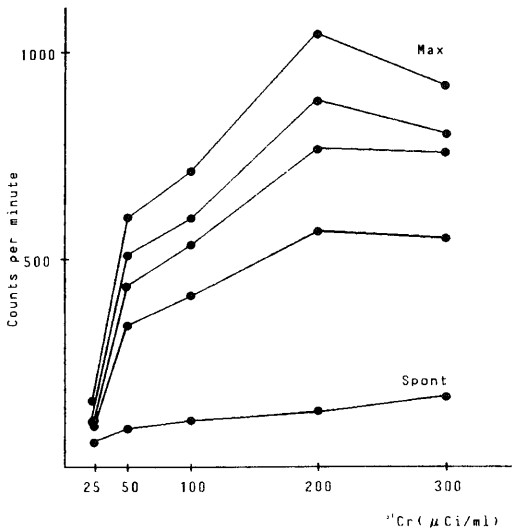

Fig. 2. Changes in radiation counts per minute followed by quantity of ^{51}Cr for labelling K562 cells

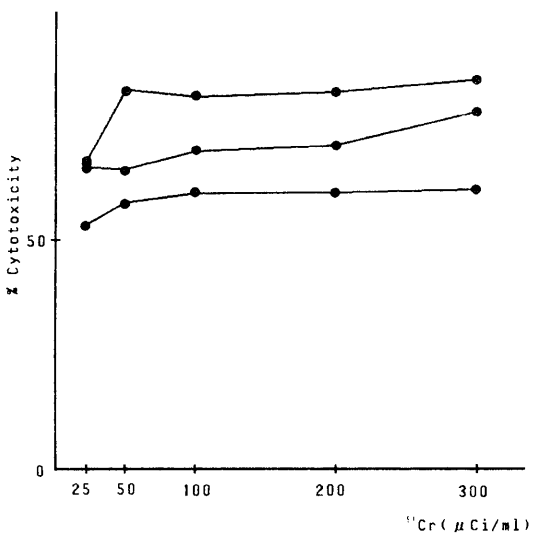

Fig. 3. Changes in NK activity by ^{51}Cr for labelling K562 cells

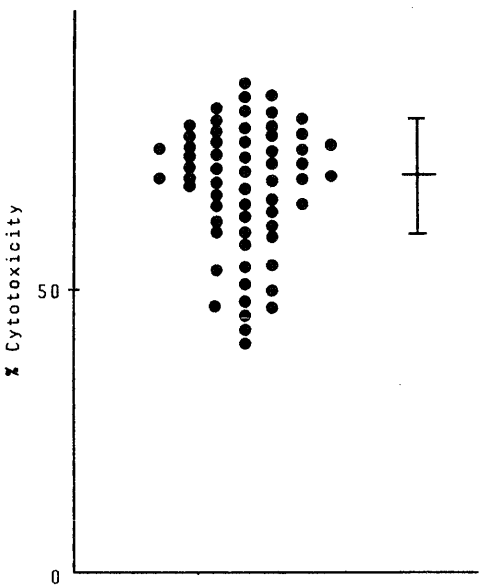

Fig. 4. Variation of NK activity obtained from identical subject

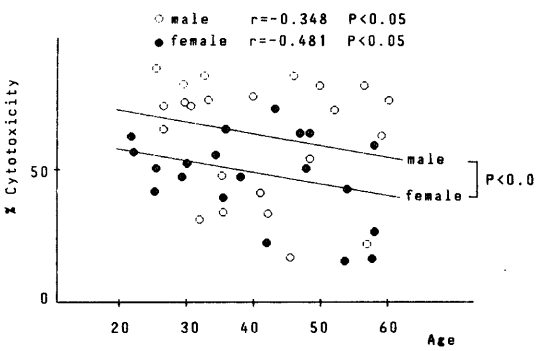

Fig. 5. Correlation among NK activity, sex and age under 60 years old of normal individual

an average of 68.3±10.1% in identical subject. The values of less than 10% in the control were discarded as errors concerning the condition of K562 cells, the intensity of labelling and ill effects of RPMI 1640 culture.

[2] NK activity in healthy controls.

NK activity decreased with ages in healthy controls under 60 years old ($p<0.05$). NK activities in men remained higher rather than those in women ($p<0.01$) as shown in Fig. 5. However, as far as cancer ages might be over 45 years old, there was not significantly different between NK activity and age or sex (Fig. 6).

[3] Preoperative NK activity

1) benign disease

Ages in 20 patients with benign disease averaged 56.1±12.8 and sex distribution was 14 to six of men to women. There was no close relation between NK activity and age or sex. The average of NK activity was 51.7±16.9% without significant difference as compared with the control (Fig. 7).

NK activity in patients with grade 0-1 of PS was significantly higher ($p<0.05$) than that in patients with grade 2 of PS (Fig. 8).

The serum albumin levels averaged 4.07± 0.40g/dl, showing almost over 3.5g/dl. There was not significantly different between NK activity and serum albumin level (Fig. 9).

2) NK activity in patients with gastric cancer and colon cancer.

Seventy-nine patients without any immunochemotherapy were subject to this study. The mean age was 59.6±10.6 years old. There was not significant correlation with sex and age (Fig. 10).

NK activities in patients with cancers were retrospectively evaluated in accordance with operation findings.

(1) It is a tendency toward further decrease in NK activities in patients with histologic stage II to IV than in patients with stage I without

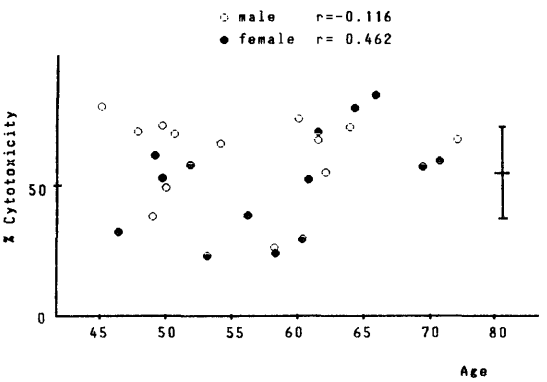

Fig. 6. Correlation among NK activity, sex and ages over 45 years old in normal individual (mean ± SD)

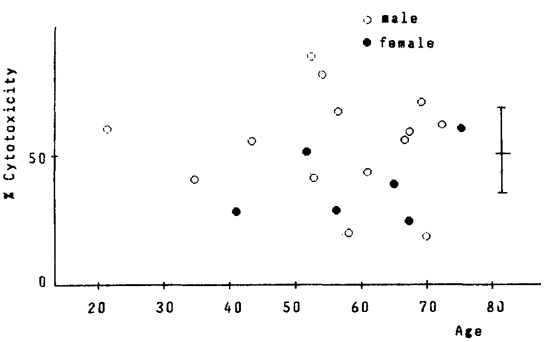

Fig. 7. Comparison between NK activity and age in patients with benign disease (mean ± SD)

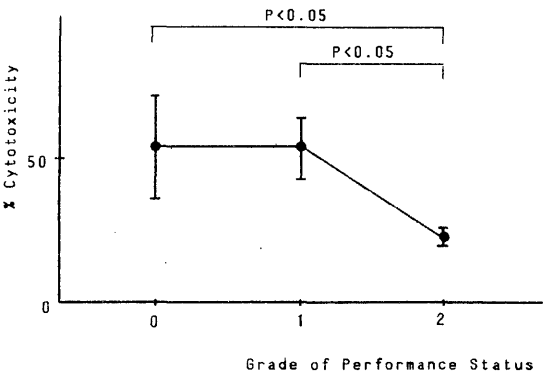

Fig. 8. NK activity and the grade of performance status in benign diseases

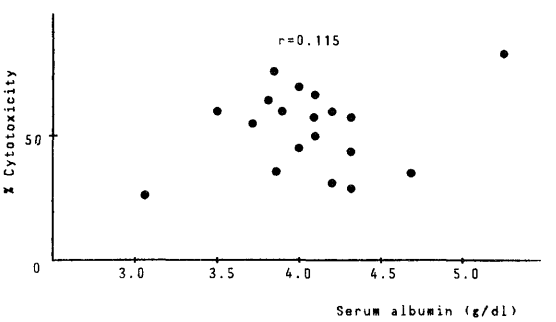

Fig. 9. NK activity and serum albumin in benign diseases

significant difference as compared with those of the control and benign diseases (Fig. 11).

(2) According to gross finding, type II carcinoma tended to reduce NK activities without significant difference (Fig. 12).

(3) According to serosal invasion (S-factor)

NK activites in patients with negative serosal invasion remained higher than those with positive serosal invasion (Fig. 13).

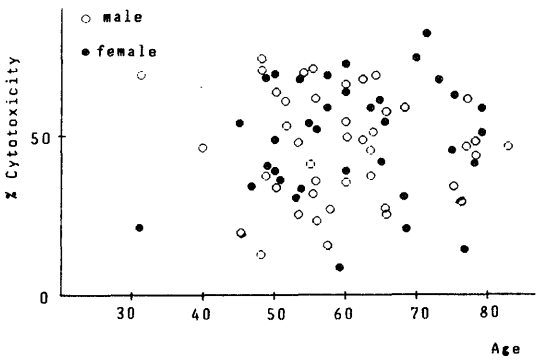

Fig. 10. NK activity and patient's ages

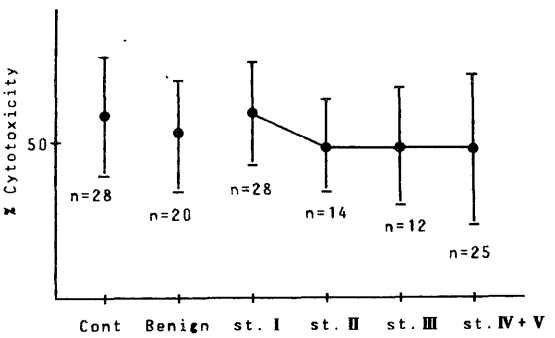

Fig. 11. Mean NK activity±SD in comparison with control, benign diseases and cancer stages

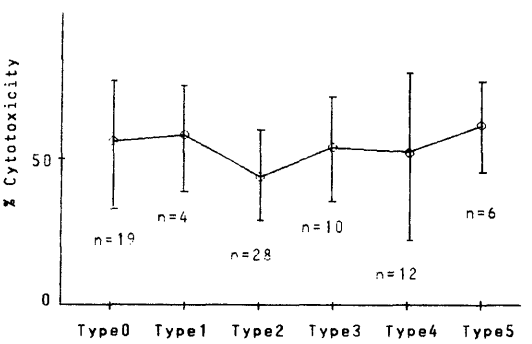

Fig. 12. NK activity and gross findings of cancer (mean ± SD)

(4) As for peritoneal dissemination (P-factor)

There was not significant difference between patients with positive and negative peritoneal dissemination (Fig. 14).

(5) According to hepatic metastasis (H-factor)

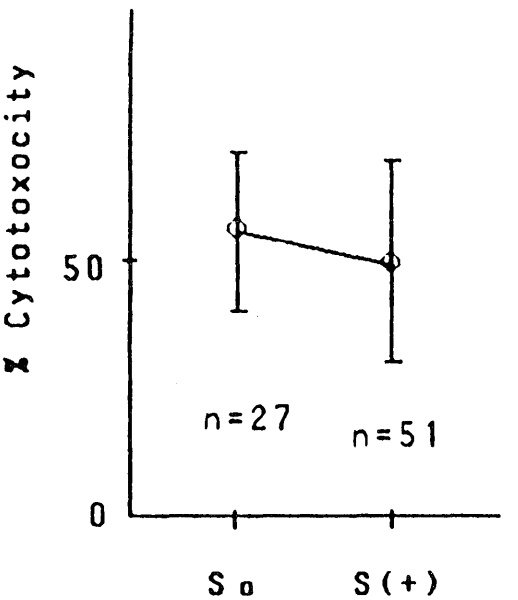

Fig. 13. NK activities and S-factor

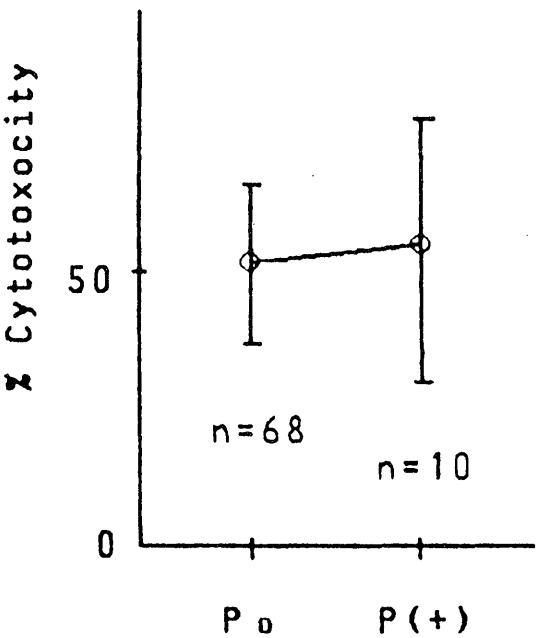

Fig. 14. NK activies and P-factor

NK activities in patients with hepatic metastasis were significantly reduced ($p<0.05$) as compared with those in patients without hepatic metastasis (Fig. 15).

(6) According to histologic types, there was not significantly different among histologic types (Fig. 16).

(7) According to the modes of infiltrative growth, there was not significant difference among INF α, β and γ factors (Fig. 17).

(8) According to the depth of cancer infiltration, NK activities were gradually decreased but there was not significantly different (Fig. 18).

(9) There was not significant difference in NK activities regarding vascular invasion of lymphatics (Fig. 19).

(10) According to vascular invasion of vessels, there was not significant difference in NK activities (Fig. 20).

(11) There was a decrease in NK activities in

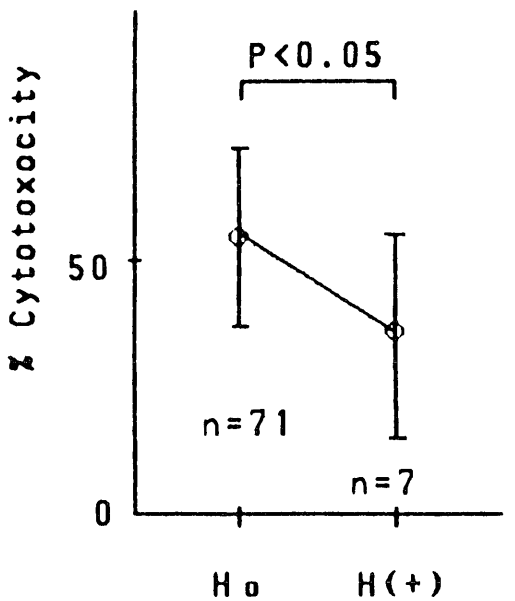

Fig. 15. NK activies and H-factor

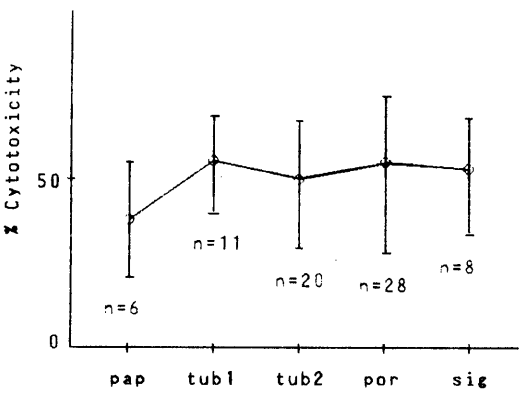

Fig. 16. Mean NK activity ± SD and histologic type of cancer

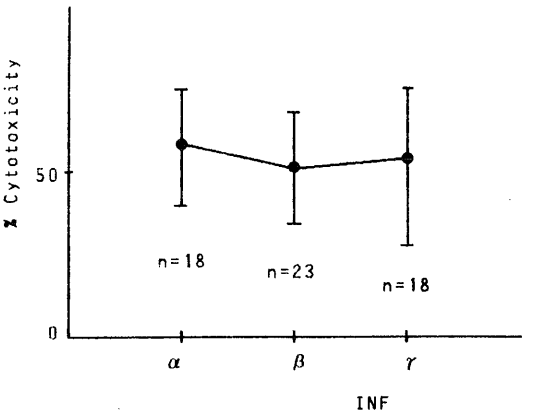

Fig. 17. Mean NK activity ± SD and infiltrative growth (INF)

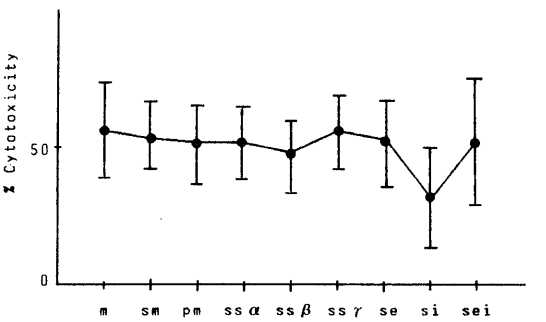

Fig. 18. NK activities and depth of infiltration

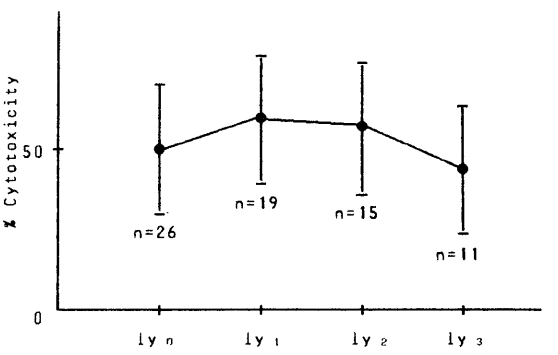

Fig. 19. Mean NK activity ± SD and lymphvessel infiltration

patients with node involvement $n_2(+)$ as compared with the other groups (Fig. 21).

(12) As shown in Fig. 22, there was no close correlation with NK activities and tumor size (Fig. 22).

(13) According to performance status classified by Oyama-Saito group study, NK activities in patients with grade 2-4 of PS were significantly decreased ($p<0.01$) as compared with the other grade of PS despite no correlation between grade 0 and 1 (Fig. 23). Most patients with over grade 2 of PS complained of advanced cancer of stage IV and V as shown in Table 1.

(14) According to the intensity of the response to PPD

NK activities with positive response to PPD were significantly higher rather than those with negative or suspicious responses ($p<0.02$) as shown in Fig. 24. Patients with negative response to PPD had the diseases of stage III or more advanced cancers (Table 2).

(15) As for the levels of tumor markers such as

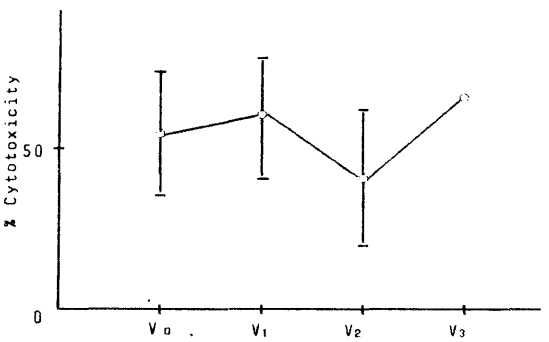

Fig. 20. Mean Nk activity ± SD and vascular infiltration

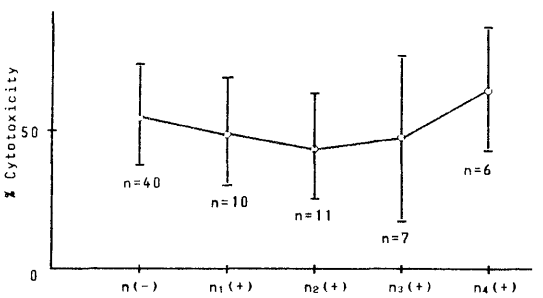

Fig. 21. Mean NK activity and lymphnode metastasis

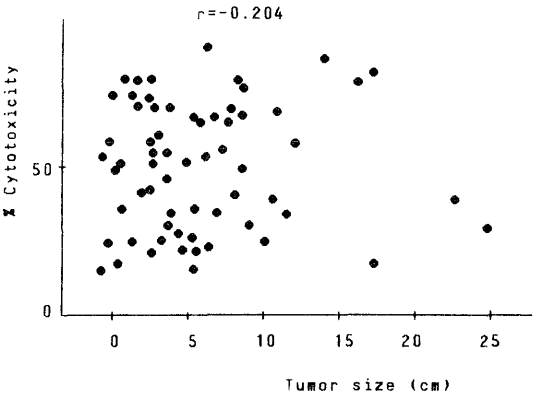

Fig. 22. Correlation between tumor size and NK activity

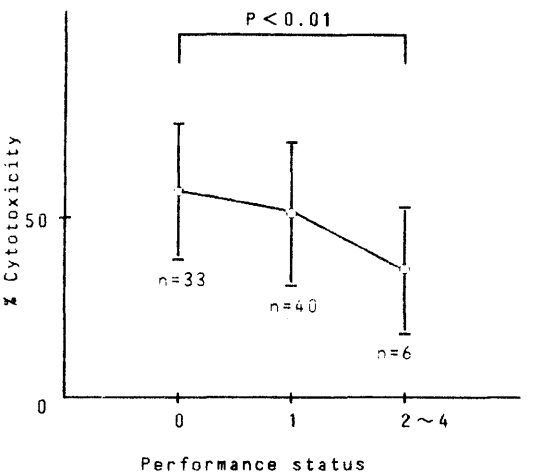

Fig. 23. Mean NK activity ± SD and preoperative performance status in cancer patients

Table 1. Cases of cancer, performance status over Grade 2

Grade	Organ	stage	Operative procedure	Curability
2	Colon	II	right hemicolectomy	absolute curative
2	Gastric	IV	exploratory laparotomy	absolute non curative
2	Gastric	IV	total gastrectomy	absolute non curative
2	Colon	IV	sigmoidectomy	relative curative
3	Colon	V	Hartmann's operation	absolute non curative
4	Gastric	IV	total gastrectomy	absolute non curative

Table 2. Nagative cases of PPD reaction in cancer patients

Organ	stage	Operative procedure	Curability
Gastric	III	total gastrectomy	absolute curative
Colon	III	right hemicolectomy	absolute curative
Gastric	IV	gastrectomy	absolute non curative
Gastric	IV	subtotal gastrectomy	absolute non curative
Colon	V	Hartmann's operation	absolute non curative

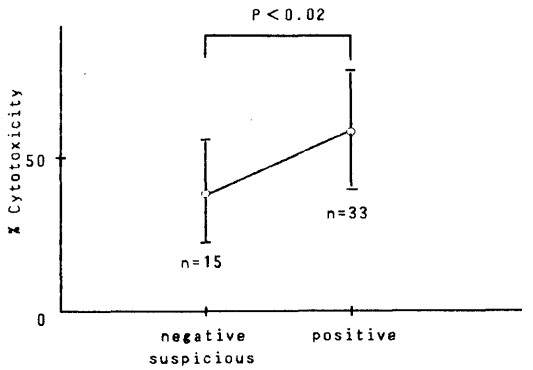

Fig. 24. NK activity and PPD reaction

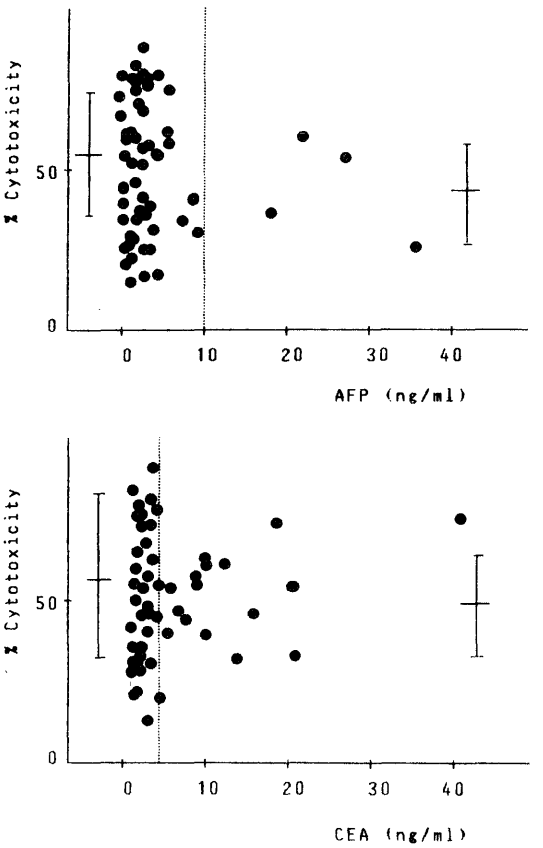

Fig. 25. Relation between tumormarker (AEP, CEA) and NK activity. Broken lines mean each upper normal range. For each range, the bars represent the mean NK activity ± SD.

AFP and CEA, NK activities in patients with abnormal levels were lower than those with normal levels without significant difference (Fig. 25).

(16) There was no close correlation with serum albumin and NK activities (Fig. 26).

On the basis of a result concerning pre-operative NK activities, NK activities were markedly reduced in patients with advanced cancers such as clinical manifestation such as over grade 2 of PS, negative or suspecious positive PPD skin response, positive hepatic metastasis and also showed a tendency toward a decrease in NK activities in patients with serosal invasion, peritoneal dissemination and abnormal serum AFP and CEA values.

Progression of cancer also correlated well with general factors such as PS ($p<0.01$) and serum albumin ($p<0.01$). Fig. 27 showed that a decrease in serum albumin levels was more likely to be seen in patients with over grade 2 of PS.

[4] Changes in NK activities during and postoperation

Table 3 showed changes in NK activities during the period of surgery in patients with gastric and colon cancers. The measurements were carried out before operation, 30 min after anesthesia, two hours after the start of operation, one hour after the end of operation, 12 hours and 24 hours after operation, respectively.

The average operation time was 4.56±1.16 hours and the amount of blood transfusion was 5.29±6.16 units on the average.

NK activity was increased at the time of the

Table 3. 7 Cases of cancer studied throughout operation

Organ	stage	Operative procedure	Op. time	NK activity					
				Pre	Anes	Intr	POlh	PO12h	POld
Gastric	I	gastrectomy	4.3	60.7	69.4	74.1	65.2	36.3	54.1
Colon	III	sigmoidectomy	3.0	38.8	48.6	52.7	16.3	2.0	14.9
Gastric	III	total gastrectomy	4.5	36.3	46.2	29.0	36.8	15.2	10.8
Gastric	III	gastrectomy	4.0	62.9	71.5	61.9	54.9	10.4	30.6
Rectal	IV	Miles' operation	5.0	35.8	23.3	51.4	15.7	4.6	7.2
Gastric	IV	total gastrectomy	6.8	71.3	69.7	72.5	55.1	44.5	40.3
Gastric	IV	gastrectomy	4.3	59.9	67.6	71.9	75.2	37.6	59.4

start of anesthesia and two hours after the start of operation ($p<0.05$), thereafter gradually decreased and reached at the lowest ($p<0.01$) and then recovered slightly from the minimum, remaining still lower as compared with preoperative levels ($p<0.01$) (Fig. 28). The variation rates of NK activities at 12 hours after the end of operation were calculated by an equation (NK activity − preoperative NK activity)/preoperative NK activity × 100 and analysed a correlation with the operation time and the amount of blood transfusion, but there were no significance (Fig. 29).

[5] Changes in NK activity on days after surgery

The surgical procedures were distal gastrectomy in 23, subtotal gastrectomy in 13, total

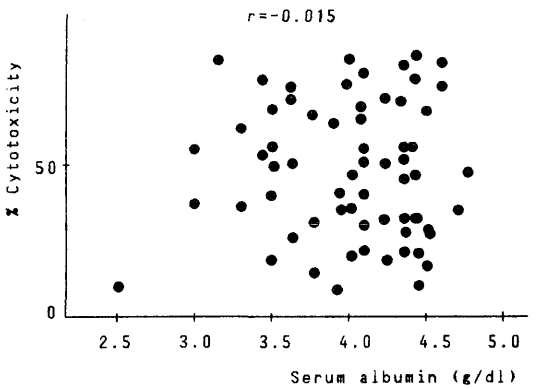

Fig. 26. Correlation between the value of serum albumin and NK activity

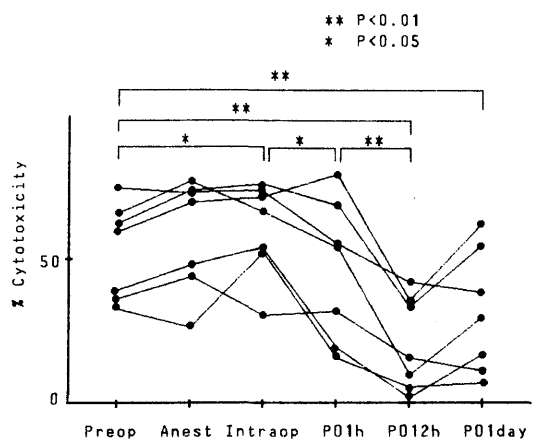

Fig. 28. Changes in NK activities during and postoperation

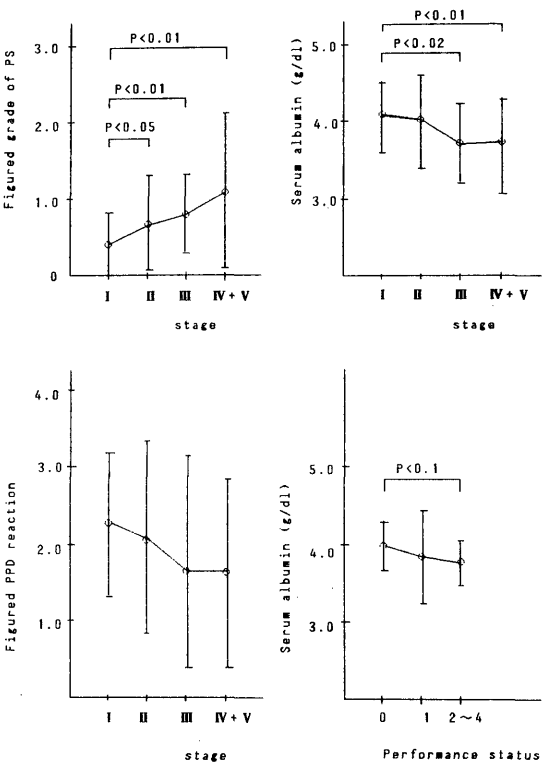

Fig. 27. Evaluation of genaral condition using figured performance status. PPD reaction and serum albumin in relation to the progress of cancer

gastectomy in 10, exploratory laparotomy in three for gastric cancers and colon resection in 16, Miles' operation in two, Hartmann's operation in two for colon and rectal cancers (Table 4).

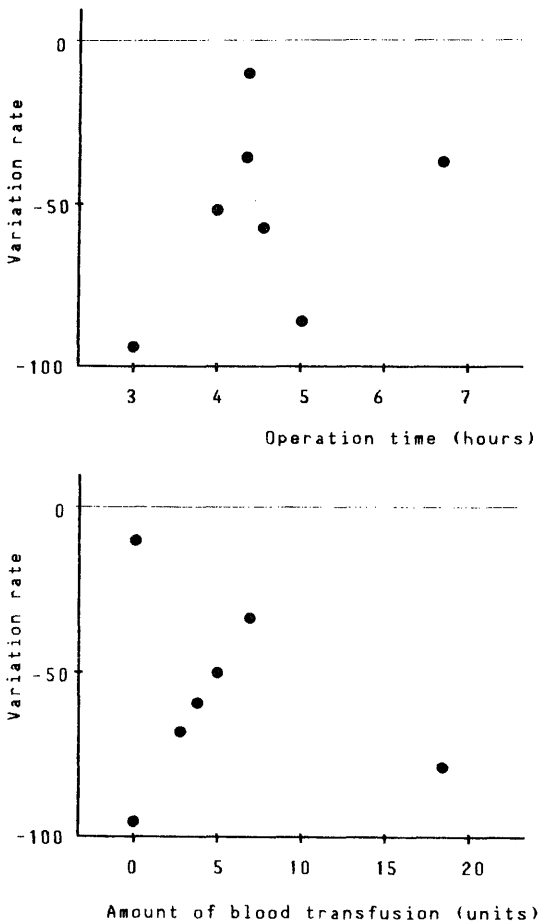

Fig. 29. Variation rate of NK activity at 12 hours after the end of operation in relation to operation time or the amount of blood transfusion

Table 4. Operative procedures in 49 cases of gastric cancer and 20 cases of colon and rectal cancer

Operative procedure	number
distal gastrectomy	23
subtotal gastrectomy	13
total gastrectomy	10
exploratory laparotomy	3
colon resection	16
Miles' operation	2
Hartmann's operation	2

The mean operation time was 3.8±1.2 hours and the amount of blood transfusion averaged 4.4±6.0 units.

NK activity was significantly decreased on day 1 ($p<0.01$) and still remained lower on day 7 ($p<0.01$) and on day 14 ($p<0.05$) and returned to the normal at four weeks after surgery (Fig. 30).

On the other hand, NK activity in patients with benign diseases was significantly reduced on day 7 ($p<0.01$) and reverted to the normal at four weeks in reflection of not so grave operation insult with 2.5±1.2 hours of the operation time and 0.5±1.9 unit of blood transfusion, without significant decrease on day 14 respectively, thereafter significantly increased at two months after surgery ($p<0.05$) and returned to the normal at three months as did those in patients with cancers show (Fig. 31).

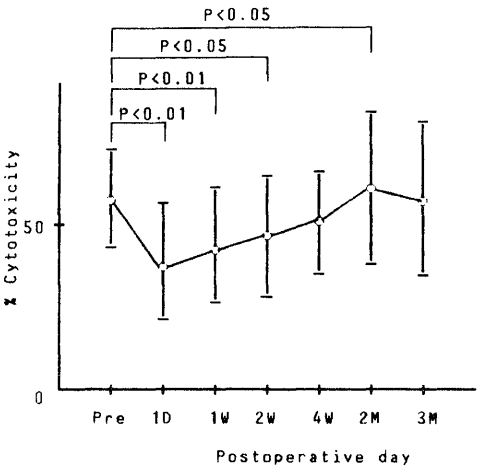

Fig. 30. NK activity in postoperative day

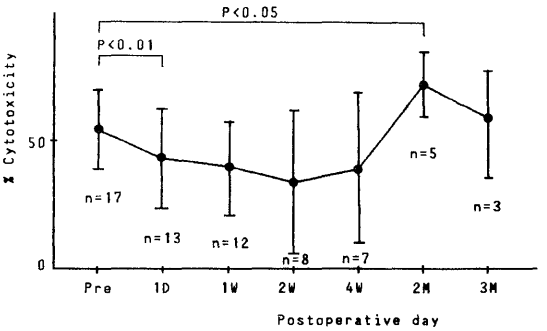

Fig. 31. Postoperative NK activity in benign diseases

Subsequently, the variation rates in NK activities in patients with cancers were evaluated in analysis of pre-and postoperative factors.

1) Stage

According to disease stages, NK activities in stage I patients were higher ($p<0.01$) than those in stage II - V and also less decreased than those in stage II, III and IV, V and patients in stage I prompted NK activity to resume the normal level. On the contrary, marked decrease in NK activity was seen in patients with stage II, III and IV, V on day 1 and 7, and also recovery was retarded.

Furthermore, NK activity in patients with stage I was sigfinicantly enhanced ($p<0.05$) at two months after surgery in contrast with nothing to enhance at the same period as seen in others (Fig. 32).

2) Performance status (PS)

NK activities in patients with grade 2 of PS prior to surgery was less ($p<0.01$) and further decreased on day 1 ($p<0.01$), thereafter gradually reverted ($p<0.05$).

In general, NK activities with preoperative grade 0 of PS remained higher throughout the observation than those with grade 1 ($p<0.01$) and slight decrease was seen on day 1 and 7.

In contrast, significant decrease in NK activity on day 1 and 7 ($p<0.01$) and delay in recovery were marked in patients with grade 1 of PS (Fig. 33).

According to postoperative recovery from PS, the more smooth recovery of PS the higher NK activity was observed ($p<0.01$) until two weeks following surgery. However, there was not significant difference at four weeks after surgery (Fig. 34).

3) Serum albumin level

NK activities before surgery were not in

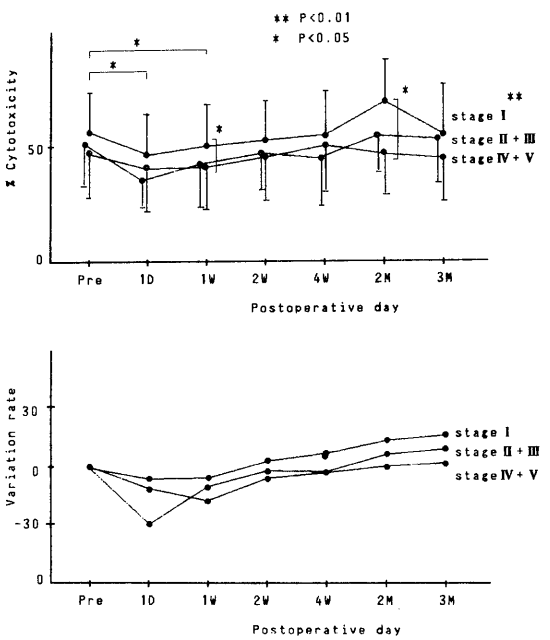

Fig. 32. Postoperative NK activity in relation to stage of cancer

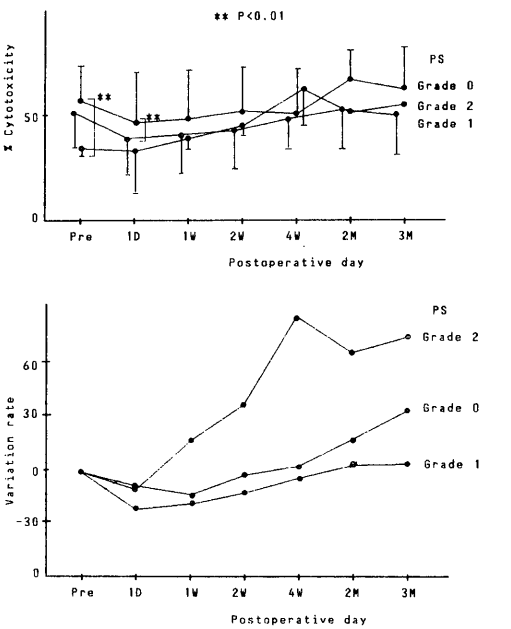

Fig. 33. Postoperative NK activity in relation to preoperative grade of performance status

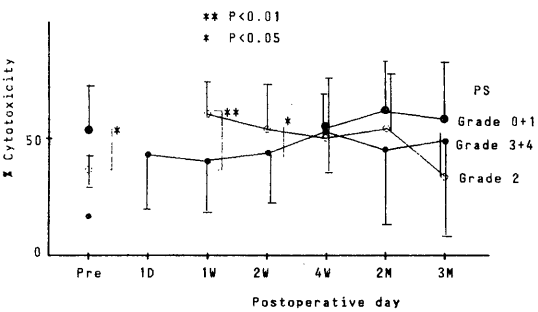

Fig. 34. Postoperative NK activity ± SD related with performance status at each period

proportion to the serum albumin levels and those after surgery with serum albumin of less than 3.5g/dl were significantly low ($p<0.01$) (Fig. 35).

4) Skin response to PPD

NK activities in patients with negative and/or suspicious skin response to PPD were significantly reduced ($p<0.05$) throughout the observation (Fig. 36).

5) Tumor marker

NK activities in patients with abnormal AFP and CEA values were significantly low ($p<0.01$) and postoperative recovery were retarded at two and three months ($p<0.01$) as shown in Fig. 37.

6) Surgical curability

Recovery of NK activities from postoperative decrease was prompt in patients with curable surgery (Fig. 38).

7) Operation time

In this series, the operation time ranged from two hours to six hours. However there was not significant difference between NK activity and operation time.

The variation rates of NK activities in patients with a two to four hour operation time were less than those with a four to six hour operation time and recovery in the former was seen in two weeks and that in the latter was delayed (Fig.

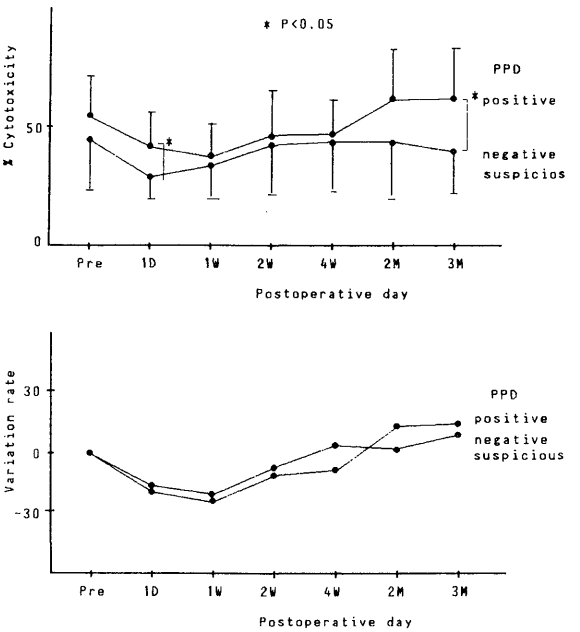

Fig. 36. NK activity in relation to preoperative PPD reaction

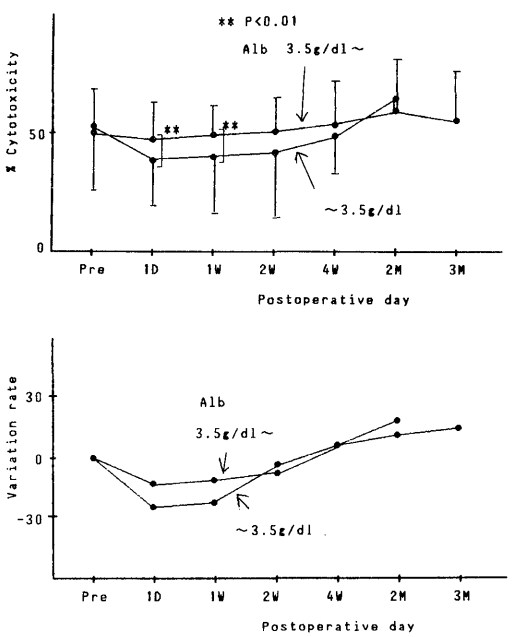

Fig. 35. Postoperative NK activity in relation to serum albumin levels above 3.5g/dl or below 3.5g/dl

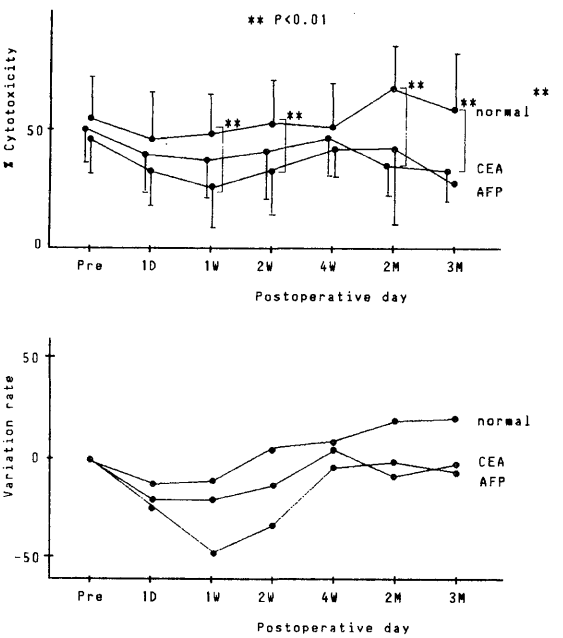

Fig. 37. Postoperative NK activity in relation to tumor marker (AEP, CEA), AEP>10ng/ml and CEA>4ng/ml

39). In contrast, NK activities in patients with benign diseases had become the lowest at two weeks and then reverted to the normal (Fig. 40).

8) Blood transfusion

NK activities in patients with 1 to 5 units blood transfusion were significantly lower for

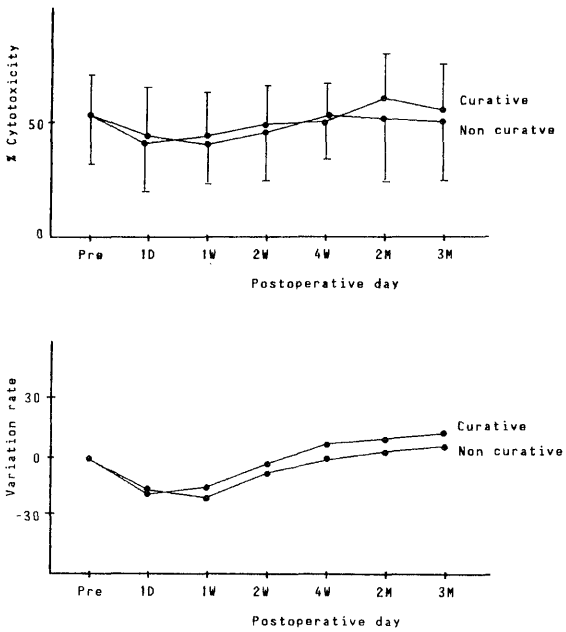

Fig. 38. Postoperative NK activity in relation to curability

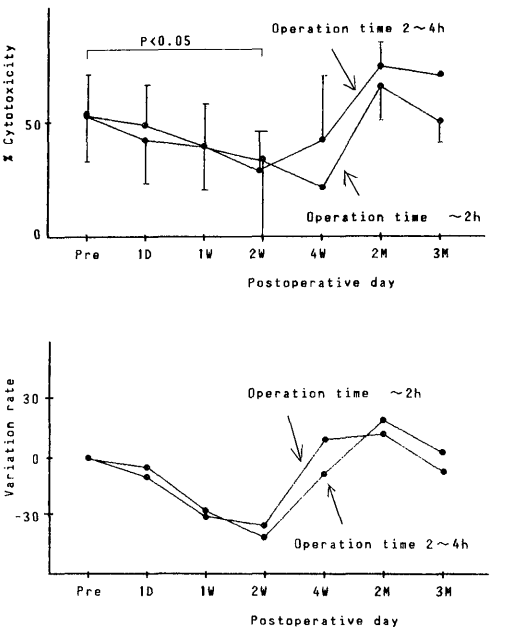

Fig. 40. Postoperative NK activity in relation to operation time in benign diseases

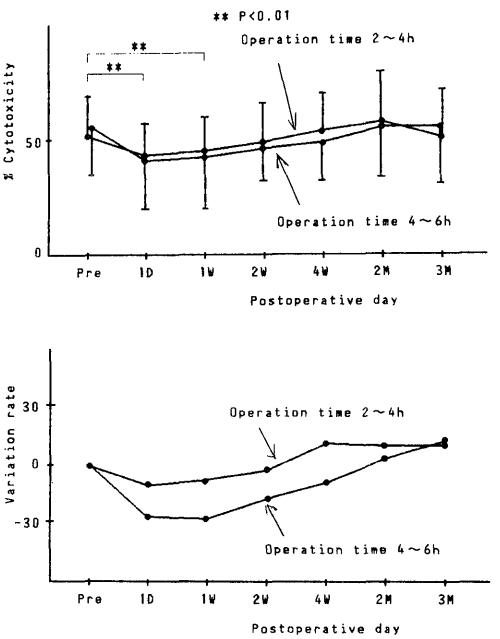

Fig. 39. Postoperative NK activity in relation to operation time in cancer patients

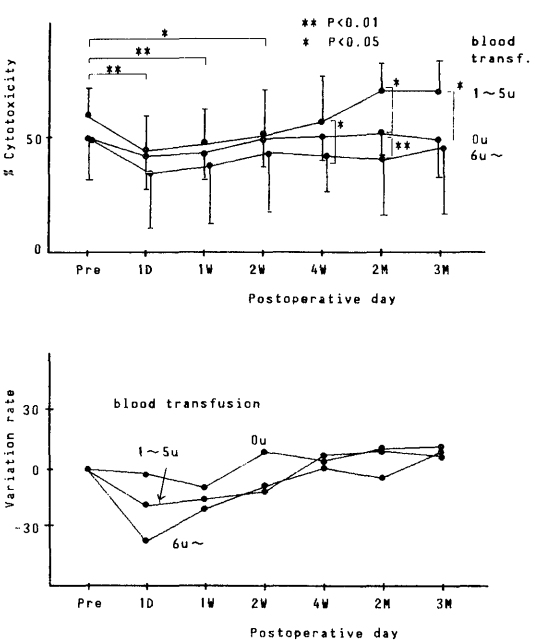

Fig. 41. Postoperative NK activity in relation to the amount of blood transfusion

two weeks ($p<0.01$-$p<0.05$) than those without blood transfusion. When the amount of blood transfution increased to 6 units, postoperative decrease in NK activities had become manifest (Fig. 41).

DISCUSSION

NK activity has been recognized as being maintained immunodefensive mechanism prior to development of acqcuired immunity[1]. NK activity plays a key role in immunosurveillance[8] on the basis of the fact that it is proven that NK activity is kept high in nude mice which are devoid of T-Cells[4][5] and the incidence of cancer generation is also low[6], in addition, the incidence of metastasis is frequent in beige mice which are characteristic of low NK activity[7].

On the other hand, immunocapacity in tumor-bearing hosts is inhibited[9][10] and NK activity is reduced with progression of the tumor[11]. It is due to no specific immunosuppressant[12], activity of suppressor T-cells[13][14] in association with tumor mass and nutritional states as reported by Ohtani[15].

In addition to an operation insult, it is certain that NK activity may as well be depressed[16][17]. As a result, unfavorable condition for the host is imperative that metastasis and proliferation of cancer cells are facilitated as reported by Tanaka[2]. The principle of surgery for cancers is that complete resection is main and reduction of the tumor mass aims at enhancement of immune response.

However, surgical stress has become grave because of prolonged operation time over two hours in addition to undernutrition and depressed cellular immunity[18].

This fact means that metastasis may be facilitated and tumor growth may also be enhanced during the period of postoperatively impaired immunoresponse.

In this study, the factors concerning depressed immunity were analysed in the postoperative course. As a rule, many investigators explained that NK activity was inhibited with ages[19]-[22], much more significant in women. In this series, there were varying variety in NK activities without certain tendency. NK activities correlated well with performance state, and disease stages.

On the other hand, changes in NK activities during the postoperative course reached the lowest at 12 hours, thereafter gradually recovered and reverted to the normal at four weeks. Decrease in NK activities was significant in patients with malnutrition and weak skin response to PPD and long-time recovery was required. Patients with smooth and quick recovery from surgical stress showed early resumed NK activities although patients with retarded recovery from surgery had an opposite course. With respect to surgery it closely related to operative curability, the operation time and the amount of blood transfusion.

It is well known that malnutrition is in association with impaired cellular immunity[23] decrease in T-cell population[24] and weak response to PPD[25]. It also is suggestion that NK activity closely relates to nutrition[15][23]. Copeland[26] and Sakamoto[27] reported that hyperalimentation facilitated skin response to PPD in patients with carcinomas. It is needed for improved nutritional state in a tumor-bearing host to enhance NK activity.

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