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Study of Effect of Surgical Stress on Immunity in Patients with Gastrointestinal Cancer

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Abstract

We investigated the effect of surgical stress on immunity in patients with gastrointestinal cancer by three color flow cytometry centering on lymphocyte subsets. The control group consisted of patients with cholelithiasis as a benign disease and the cancer groups consisted of patients with gastric cancer and those with colorectal cancer. Total lymphocyte in peripheral blood, lymphocyte subsets by monoclonal antibody and NK cell activity were measured before and after operation in the target patients.

The cell ratio of $CD4^+CD45R^+Leu8^+$ (suppressor inducer T), $CD8^+CD11b^+$ (suppressor T) were significantly higher in the gastrointestinal cancer group when lymphocyte subsets were investigated after operation. Further, the cell ratio of $CD3^+CD16^-CD56^+$ (T-LAK) was significantly lower. These findings suggest that decrease in immunity as a result of surgical stress is greater in patients with gastrointestinal cancer than in those with a benign disease.

Introduction

It is known that the immunomechanism of the living body is much affected by surgical stress. Normally the immunomechanism is already in a lowered state even before operation in many cancer patients. Therefore, it is anticipated that the immunity is further lowered after opertion and metastasis and proliferation of cancer after operation are greatly affected when surgical stress is added in the cancer-patients.

In the present study we investigated the lymphocyte subsets by three color flow cytometry and immunological changes after operation in patients with gastric cancer or colorectal patients.

Materials and Methods

The subjects were 63 patients; 25 with gastric cancer (A group), 20 with colorectal cancer (B group) and 18 with cholelithiasis (C group) who had undergone surgery in our hospital from January 1989 to August 1990. The background factors of the subjects are shown in Table 1.

After consent was obtained from the 63 patients, peripheral blood was collected before opera-

Key words: Gastrointestinal cancer, Surgical stress, Immune activity, Lymphocyte subsets, Three color flow cytometry

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tion, and on the 1st, 5th, 10th, 20th and 30th day after operation. Lympnocyte subsets was determined by three color analysis using total lymphocyte count and monoclonal antibodies CD3, CD4, CD8, CD11b, CD45R, CD56, CD57 and Leu8. In addition, NK cell activity was measured. Anticancer drugs and biological response modifiers (BRM) were not given to these 63 patients before or after operation.

1) Measurement of lymphocyte subsets

Monoclonal antibodies CD3 (Leu4), CD4 (Leu3a), CD8 (Leu15), CD45R (Leu18), CD56 (Leu19), CD57 (Leu7) and Leu8 (Becton Dickinson) were used in the measurement of lymphocyte subsets. Ten μl of the optimally diluted monoclonal antibody liquid was added to 100 μl of the blood, incubated at 4°C for 30 min, centrifuged after hemolysis and resuspended in phosphate buffer saline (PBS). The cells in the suspension were analyzed by flow cytometry. For three color analysis, multihistograms were prepared by two color analysis and cell analysis was made with a computer to find the ratio of positive cells (%) to all lymphocytes.

As regard to lymphocyte subsets for labeling, pan T cell was labeled for CD3, suppressor T cell for $CD8^+CD11b^+$ as the two color analysis, cytotoxic T cell[1,2] for $CD8^+CD11b^-$, helper T cell[3] for $CD4^+CD45R^-Leu8^-$ as the three color analysis. T-LAK cell for $CD3^+CD16^+CD56^-$ and NK-LAK cell[4] for $CD3^-$ $CD16^+CD56^+$

2) Measurement of NK cell activity

Seven to 8 ml of peripheral blood was collected in heparinized tubes and diluted twice with phosphate buffered saline (PBS). Monocytes were separated from the solution by Ficoll-Lropaque specific gravity centrifugation and used as effector cells. Furthermore, ^{51}Cr-labeled human chronic myelocytic leukemia strain K562 was used as the target strain. After these were mixed at a constant rate (E/T=20) and incubated at 37°C in 5%CO2 for 4 hours, released ^{51}Cr was mesured with an auto well gamma system ARC-30 to determine the cytoparthy to strain K562 of NK cell activity,

Table 1 Subject

	A group (n=25)	B group (n=20)	C group (n=18)
Age	56～78 (68.2±16.4)	44～76 (63.9±13.8)	36～73 (58.4±21.9)
Sex (♂:♀)	14 : 11	13 : 7	11 : 7
Operative procedure	Total gastrectomy 5 Subtotal gastrectomy 20	Colectomy 12 Lower resection 3 Hartmann 1 Miles 4	Cholecystectomy 15 Cholecystectomy + Choledocholithotomy 3
Operating time (min)	128～276 (189±48.2)	118～334 (204±121.3)	68～248 (104±43.2)
Blood loss (mℓ)	139～530 (189±48.2)	118～334 (204±121.3)	86～386 (254±104.0)
Post operative hospital stay (days)	21～63 (32.4±16.3)	20～68 (34.2±23.1)	12～36 (21.3±8.3)

(mean±SD)

i.e., %lysis, by the following formula.

$$\frac{\text{Experimantal CPM—Control CPM}}{\text{Maximum CPM—Control CPM}} \times 100(\%)$$

Results

1) Changes in total lymphocyte counts

Total lymphocyte count after operation was low in A group, and was significantly lower on the 5th day after operation in A group than the 5th day after operation in A group than in C group (Fig. 1).

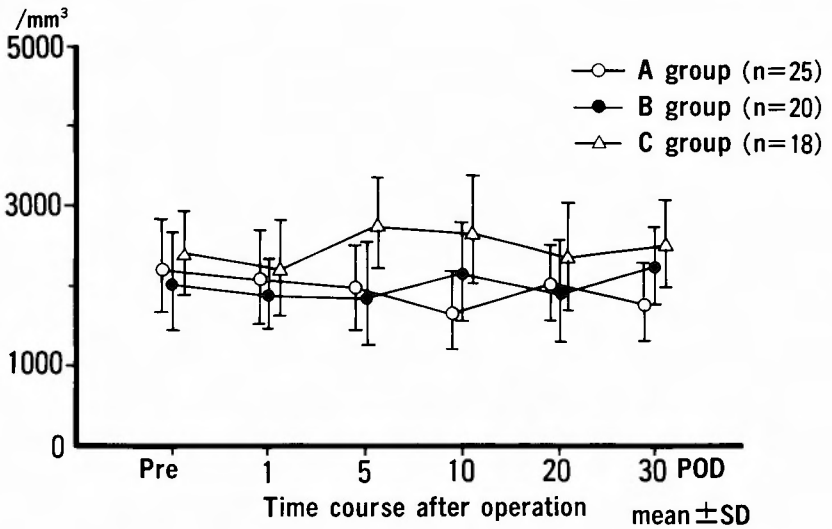

Fig. 1 Daily changes of Total Lymphocyte Counts

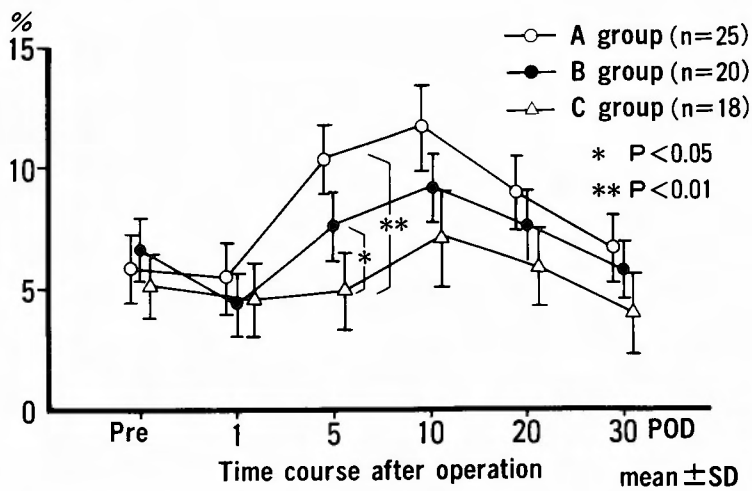

Fig. 2 Daily changes of $CD4^+CD45R^+Leu8^+$ (suppressor inducer T) cell subpopulation

2) Changes in lymphocyte subsets

There were no significant differences among A, B and C group in changes in CD3 cells, $CD4^+$ $CD45R^-Leu8^-$ (helper T), $CD4^+CD45R^-Leu8^+$ (helper inducer T), or $CD3^-CD16^+CD56^+$ (NK-LAK) cells before or after operation. On the other hand, the cell ratio of $CD4^+CD45R^+Leu$ 8^+ (suppressor inducer T) after operation was low in A group, and significantly lower on the 5th day in A group than B and C groups (Fig. 2).

The cell ratio of $CD8^+CD11b^+$ (suppressor T) after operation was low in C group than in A group than in A and B group (Fig. 3).

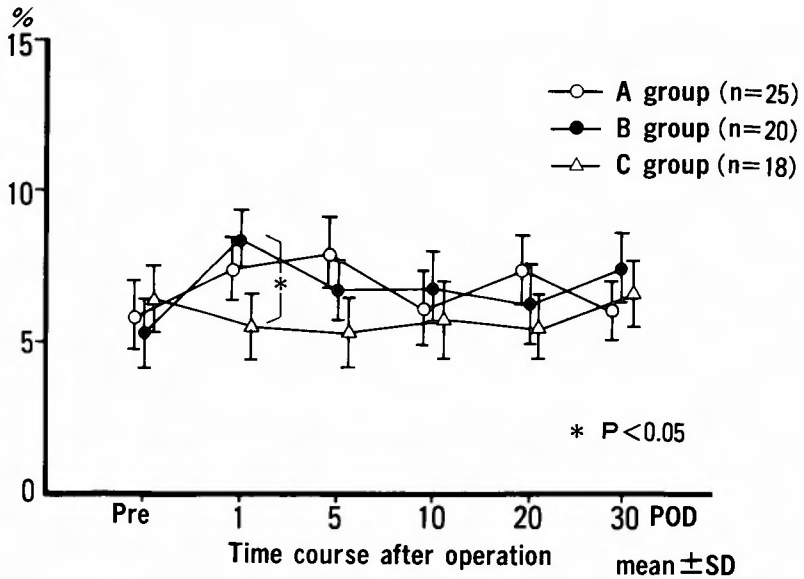

Fig. 3 Daily changes of $CD8^+CD11b^+$ (suppressor T) cell subpopulation

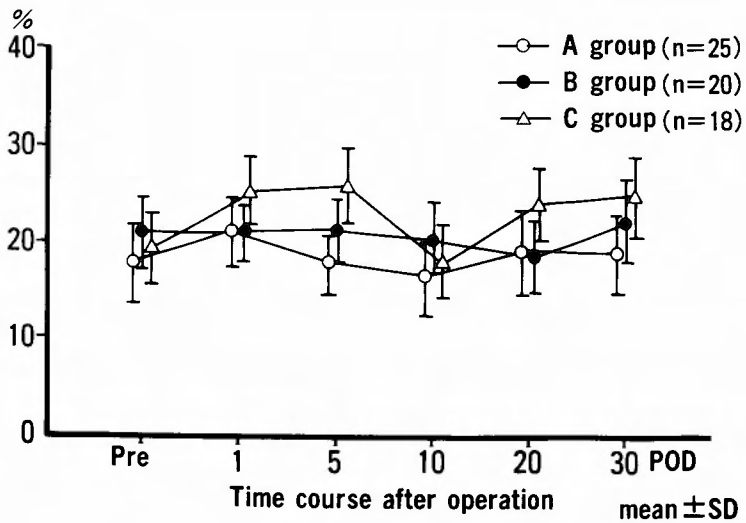

Fig. 4 Daily changes of $CD8^+CD11b^-$ (cytotoxic T) cell subpopulation

The cell ratio of $CD8^+CD11b^-$ (cytotoxic T) after operation was constant in A and B group but increased from the 20th day in C group (Fig. 4).

The cell ratio of $CD3^+CD16^-CD56^+$ (T-LAK) was higher in C group than in A and B group, and significantly higher on the 1st day in C group than in A group. Moreover it was significantly higher in B group than A group.

3) Changes in NK cell activity

NK cell activity immediately after operation was markedly low in A group and was significantly higher on the 1st day in C group than in A group (Fig. 6).

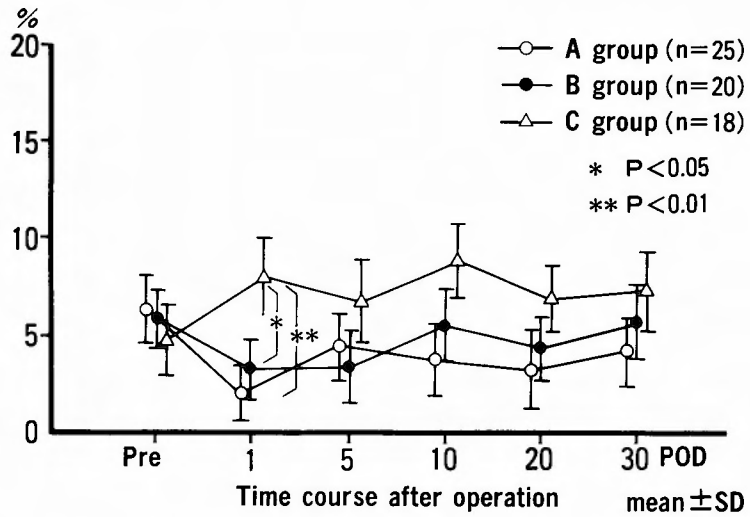

Fig. 5 Daily changes of $CD3^+CD16^-CD56^+$ (T-LAK) cell subpopulation

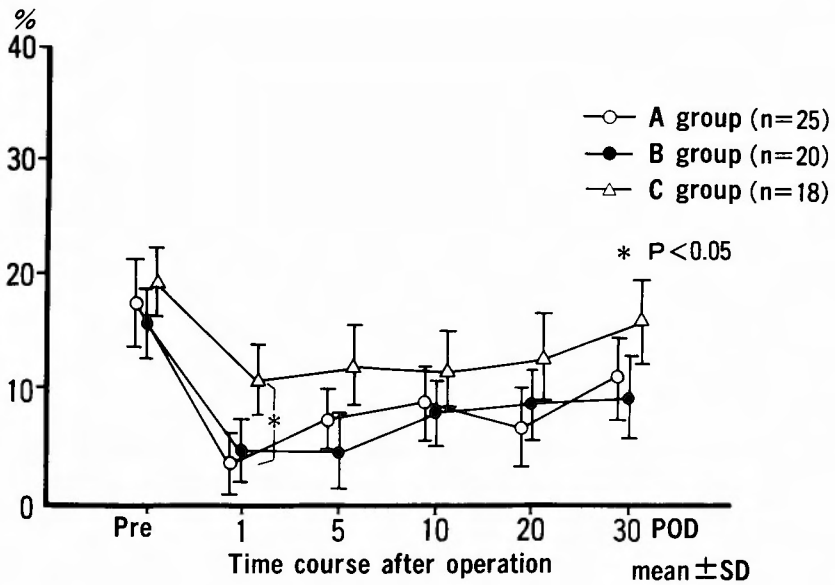

Fig. 6 Daily changes of NK cell activitiy

Discussion

It is reported[5] that the surgical stress has an inhibitory effect on immunological responsiveness in the living body. In paticular, abnormalities are often observed before operation in the immune system of the cancer-patients[6]. Since the immunity further decreases and the proliferation of the remaining cancer cells is facilitated when surgical stress is added, it is feared that the prognosis is affected by the proliferation[7].

Changes in T lymphocytes which are direct immunologically competent cells are important for evaluating the immunity of a cancer-patients. However, in recent years monoclonal anibodies to lymphocyte surface antigen have been successfully produced using the cell fusion method, and lymphocyte subpopulation can be easily analyzed by the whole blood method using flow cytometry. An attempt has been made to evaluat the immunity of the cancer body from living body from he aspect of lympnocyte fraction[8]. In the present study, an immunological investigation was made at the time of surgical stress in cancer patients from the aspect of lymphocyte subpopulation using three color flow cytometry.

Changes in T lymphocytes at the time of surgical stress tended to be lower after operation in patients with gastrointestinal cancer than in patients with benign diease, although no significant difference was observed. There are many reports[9] that the cell ratio of T lymphocytes does not decrease before operation in patients considered to be observed between patients with gastrointestinal cancer, and that no difference is cosidered to be observed between patients with gstrointestinal cancer and those with a benign disease in the cell ratio after operation.

In contrast, for fraction of T lymphocytes $CD4^+CD45R^+Leu8^+$ (suppressor inducer T) increased in A and B group, but was suppressed in patients with a benign disease after operation. $CD8^+$ $CD11b^+$ (suppressor T) cells hardly showed any change after operation in C group but increased after operation in A and B group. Tohge et al[10], started that suppressor inducer T and suppressor T cells were increased by the surgical stress, and the greater the surgical stress was, the clearer the increase of these cells was.

The immunological system is in a suppressed state even before operation in the cancer-patients. However, it was anticipated that since suppressor inducer T cells are markedly activated by surgical stress, they increase to result in a decrease in immunity in the living body. The changes in the suppressed cells for $CD8^+CD11b^+$ (cytotoxic T) cells were consistant after opertation in A and B groups, but tended to increase after operation in C group. However, as significant difference was observed.

In the present study, the patients with cholelithiasis served as controls. There were some differences between the treated groups and control group in terms of degree of the surgical stress, such as operation time and transfusion. The differences observed between the treated and control groups in immunomechanism after operation suggest that abnormalities in immunity were already present before operation in the living body, and that the abnormalities were markedly enhanced by the surgical stress. Like in our present study, Tohge et al.[10] pointed out that immunity decreased more markedly in operation of malignant diseases such as gastric and epigastric cancers than in that of benign diseases.

Moreover, immunologically competent cells such as NK cells and LAK cells are presumed to be closely related to the growth of cancer. In this study, NK cell activity decreased markedly on the 1st day in A and B groups and thereafter the recovery rates wre lower in malignant disease than in

benign diseases. Dieu et al.[11] repeated that NK cell activity in cancer-patients was reduced significantly by activation of suppressor T cells due to surgical stress.

On the other hand, LAK cell, one of the immunologically competent cells, was investigated in the present study. T-LAK derived from T cells were lower immediatedly after operation in patients with gastrointestinal cancer than in those with a benign disease. Nichiden et al.[13] reported that LAK cell activity decreases markedly in cancer-patients, and the greater the surgical stress is, the stronger the tendency is, and the recovery is delayed.

In our study we investigated the effect of surgical stress on immunity in patients with gastrointestinal cancer from the viewpoint of changes in lymphocyte subpopulation and NK cell activity. As the function and activity of these cells were not evidenced by the results of lymphocyte subpopulation of the present flow cytometry, there are also limitations in clinical studies. However, our findings suggested that the immunity becomes lower in patients with gastrointestinal cancer than in those with benign diseases when surgical stress is added. Therefore, in consideration of the decrease in immunity in cancer-patients, one must be careful when selecting the method and period of operation.

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和文抄録

外科侵襲が消化器癌患者の免疫能にあたえる影響に関する検討

木沢記念病院外科
田辺 博

消化器癌患者において外科侵襲が免疫能にあたえる影響について three color flow cytometry によるリンパ球サブセットを用いて検討した．悪性疾患は胃癌と大腸癌とし，良性疾患は胆石症を対象症例とした．対象症例に対し術前，術後第 1, 5, 10, 20, 30 病日に末梢血から総リンパ球数，モノクローナル抗体によるリンパ球サブセット，NK 細胞活性を測定した．

術後のリンパ球サブセットの検討では $CD4^+$ $CD45R^+Leu8^+$ (suppressor inducer T), $CD8^+CD11b^+$ (suppressor T) 細胞比率が悪性疾患群で有意に高値であった．また $CD3^+CD16^-CD56^+$ (T-LAK) 細胞比率の低下を示した．以上の結果より悪性疾患群では良性疾患群に比べ，外科侵襲による免疫能の低下がうかがわれた．