

Bull Yamaguchi Med Sch 40(1-2) : 29-35, 1993

Changes of Lymphocyte Subsets Following Various Surgical Stress

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(Received February 18, revised May 28, 1993)

Abstract This study evaluated any changes in the lymphocyte subsets using monoclonal antibodies and flow cytometry after surgery in forty-two patients who underwent surgery (malignant:36, benign:6). The lymphocyte subsets were compared prior to surgery, on day 0 and on days 3 and 7 following surgery.

The percentages of CD4+ cells (helper/inducer T-cells) increased, while two color analysis showed that the percentages of helper T-cells did not change and the percentages of suppressor inducer T-cells increased. Thus, the increase in the CD4+ cells is due to an increase in the suppressor inducer T-cells. The percentages of CD8+ cells (suppressor/cytotoxic T-cells) decreased, while two color analysis showed that the percentages of suppressor T-cells did not change and the percentages of cytotoxic T-cells decreased. Thus, the decrease in the CD8+ cells is due to a decrease in the cytotoxic T-cells. The percentages of CD56 cells (natural killer (NK) cells) decreased after surgery. These changes suggested some postoperative immunosuppression. However, the proportion of interleukin-2 (IL-2) receptor positive cells increased.

Thus, for the prevention of postoperative immunosuppression, augmentation of cytotoxic T-cells and NK cells and inhibition of suppressor inducer T-cells may be required. Because of the increase in the IL-2 receptor positive cells, IL-2 administration may be one strategy to prevent postoperative immunosuppression.

Key Words : Surgical stress, Lymphocyte subset, Flow cytometric analysis

Introduction

Despite many reports demonstrating immune suppression after surgery, it remains controversial whether surgical stress suppresses the immune system in patients. The evaluation of this immunosuppression was done chiefly by functional analysis (1-4). Furthermore, the mechanism of such immune suppression has yet to be established. Immune

competence in surgical patients is of vital importance because it influences an individual's susceptibility to bacterial, viral, and mycotic infections (5,6). In a cancer patient, immunocompetence can influence tumor dissemination after surgery (7).

In immune surveillance, lymphocytes play an important role in mediating the overall host defense response to microbial organisms and cancer. As a consequence of technologic

developments, various monoclonal antibodies to lymphocyte subsets and flow cytometry systems are available. Furthermore, specific T-cell subsets such as helper, cytotoxic, suppressor inducer, and suppressor T-cell can be detected by two color analysis. Therefore, analysis of lymphocyte subsets may yield more precise information on postoperative immune status. For example, if cytotoxic T-cells decrease after surgery, increasing their numbers postoperatively might reverse the immune suppression. However, there are a few reports on the analysis of lymphocyte subsets in postoperative patients (8-12).

The aim of this study was to identify the lymphocyte subsets which are impaired after surgery using various monoclonal antibodies to lymphocytes.

Patients and method

Patients

Forty-two patients who underwent elective surgery under general anesthesia in the Department of Surgery II, Yamaguchi University School of Medicine were used in this study. The patients consisted of 27 males and 15 females, ranging in age from 24 to 86 years. The diagnoses of the patients are listed in Table 1. Thirty-six patients had malignant disease, and 6 had benign disease. None of the patients with malignant disease received any chemotherapeutic agents prior to or after the

operation. Surgical blood loss ranged from 70 mL to 32,000 mL (mean: 1905 mL), and the duration of the surgical procedure ranged from 120 minutes to 685 minutes (mean: 386 minutes). Thirty-six of 42 patients received blood transfusion (600 ml to 30,000 ml). The informed consent was obtained from all patients.

Blood sampling

Peripheral blood samples were collected aseptically from patients on day 0 (prior to surgery), and on days 3 and 7 after surgery. Lithium heparin or EDTA anticoagulants were used. Specimens were held at ambient temperature in anticoagulant and prepared within 36 hours of collection.

Peripheral blood preparation method and flow cytometry analysis

For lysed whole blood preparations, 100 to 200 μ L of whole blood was mixed with 5 μ L of antibody reagent (see below) and incubated on ice for 30 minutes in conical-bottom 12 \times 75 mm polypropylene tubes. After staining, the erythrocytes were lysed by Ortho-mune (Ortho Diagnostic System Inc., Raritan, NJ). After the lysis was complete, the leukocytes were washed three times at 4°C with phosphate-buffer saline (PBS). Two hundred μ L of PBS was added and the samples were analysed on EPICS flow cytometers (Coulter Electronics, Inc., Hialeah, FL), using a fluorescence excitation of 200 to 500 mW at 488 nm. For each sample, 5,000 lymphocytes were analyzed. Two color analysis was performed using two different antibodies.

Antibody reagents (Table 2.)

All antibodies were purchased from Coulter Immunology (Hialeah, FL). Fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (T3), anti-CD4 (T4), anti-CD20 (B1), anti-CD25 (IL-2R1), anti-CD56 (NKH-1), anti-HLA-DR (I2), anti-CD11b (MO1) were used. Phycoerythrin (PE)-conjugated anti-CD8 (T8) and TQ1 (cluster unknown) were also used. Two color analysis was performed by a combination of TQ1/CD4 and CD8/CD11b.

Statistical analysis

Statistical analysis was performed using

Table 1. List of Diseases

Diseases	No of patients
Malignant disease	
Hepatocellular carcinoma	10
Colorectal cancer	9
Gastric cancer	8
Esophageal cancer	4
Pancreatic cancer	2
Carcinoma of the biliary tract	2
Metastatic lung cancer	1
Benign disease	
Chronic pancreatitis	2
Intrahepatic gall stones	2
Esophageal varices	1
Intestinal fistel	1
Total	42

Table 2. List of Lymphocyte Markers

Cluster	Monoclonal Antibody	Cell type
Single color analysis		
CD3	T3	pan T-cell
CD4	T4	helper/inducer T-cell
CD8	T8	suppressor/cytotoxic T-cel 1
CD4/CD8	T4/T8	ratio of CD4 and CD8 positive lymphocyte
CD20	B1	B-cell
CD25	IL-2R1	IL-2 receptor positive cell
CD56	NKH-1	NK cell
HLA-DR	I2	activated T cell, monocyte, or B-cell
Two color analysis		
CD? ⁺ CD4 ⁺	TQ1+T4 ⁺	suppressor inducer T-cell
CD? ⁻ CD4 ⁺	TQ1-T4 ⁺	helper T cell
CD8+CD11b ⁺	T8+MO1 ⁺	suppressor T-cell
CD8+CD11b ⁻	T8+MO1 ⁻	cytotoxic T-cell

All monoclonal antibodies were purchased from Coulter Immunology.

CD? : The CD cluster for the TQ1 monoclonal antibody remains unknown.

Table 3. Changes in the Lymphocyte Subsets after Surgical Stress

Lymphocyte subset	day 0	day 3	day 7
CD3	63.4±1.8(%)	63.5±1.8	66.4±1.6
CD4	43.9±1.6	49.6±1.6*	50.9±1.4*
CD8	27.6±1.1	22.5±1.2*	23.0±1.2*
CD4/CD8	1.75±0.12	2.57±0.19*	2.48±0.15*
CD20	8.8±0.5	11.7±0.9*	9.6±0.6
CD25	5.2±0.4	7.5±0.5*	8.2±0.5*
CD56	25.3±1.6	17.6±1.3*	18.2±1.1*
HLA-DR	21.8±1.6	24.9±1.5	29.6±1.6*
TQ1+T4 ⁺	29.5±1.6	33.8±1.7*	34.7±1.5*
TQ1-T4 ⁺	10.1±0.5	10.0±0.6	10.5±0.6
T8+MO1 ⁺	2.8±0.3	2.7±0.3	2.9±0.3
T8+MO1 ⁻	28.1±1.3	22.1±1.4*	23.0±1.2*

values (%) are mean ± S.E. (standard error). *: Preoperative values (day 0) vs. postoperative values (day 3 or day 7); p<0.002.

Student's t test for paired means. Values of p<0.05 were considered significant.

Results (Table 1 and Fig. 1-3)

The percentages of CD4(+) cells (helper/inducer T-cells, CD25(+) cells (interleukin-2 (IL-2) receptor positive cells), TQ1+T4+ cells (suppressor inducer T-cells), and

CD4(+)/CD8(+) ratio increased significantly on days 3 and 7 after surgery (p<0.002). The percentages of CD20(+) cells (B-cells) increased on days 3, and HLA-DR(+) cells (activated T-cells, monocytes, or B-cells) on days 7 (p<0.002).

The percentages of CD8(+) cells (suppressor/cytotoxic T-cells), CD56(+) cells (natural

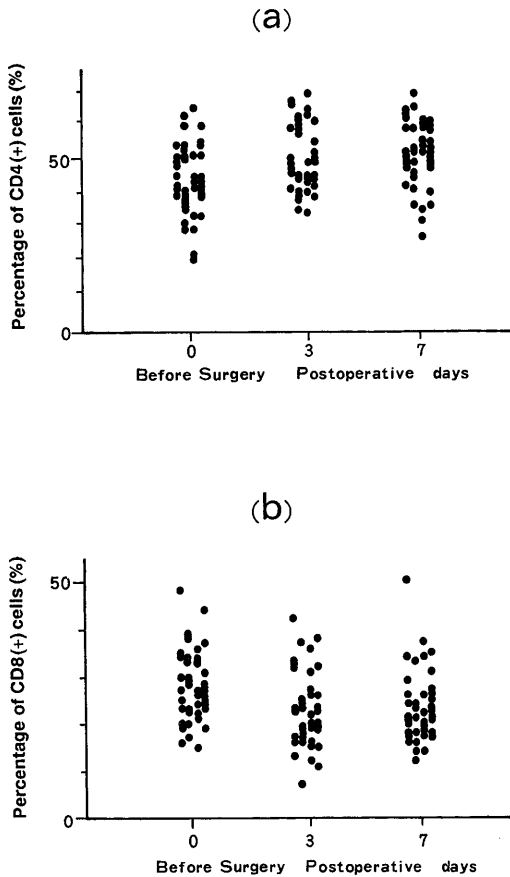


Fig. 1 (a) Postoperative changes in the percentages of CD4(+) cells in the peripheral blood. The percentage of CD4(+) cells increased significantly after surgery (day 0 vs. days 3 and 7, $p < 0.002$). (b) Postoperative changes in the percentages of CD8(+) cells in the peripheral blood. The percentage of CD8(+) cells decreased significantly after surgery (day 0 vs. days 3 and 7, $p < 0.002$).

killer (NK) cells) and T8+MO1-cells (cytotoxic T-cells) decreased significantly on days 3 and 7 after surgery ($p < 0.002$).

The percentages of CD3(+) cells (pan T-cells), TQ1-T4+ cells (helper T-cells), and T8+CD11b+ cells (suppressor T-cells) did not change following surgery.

Figure 1-3 demonstrates the values for individual patients. Figure 1 shows the percentage of CD4(+) cells and CD8(+) cells. The

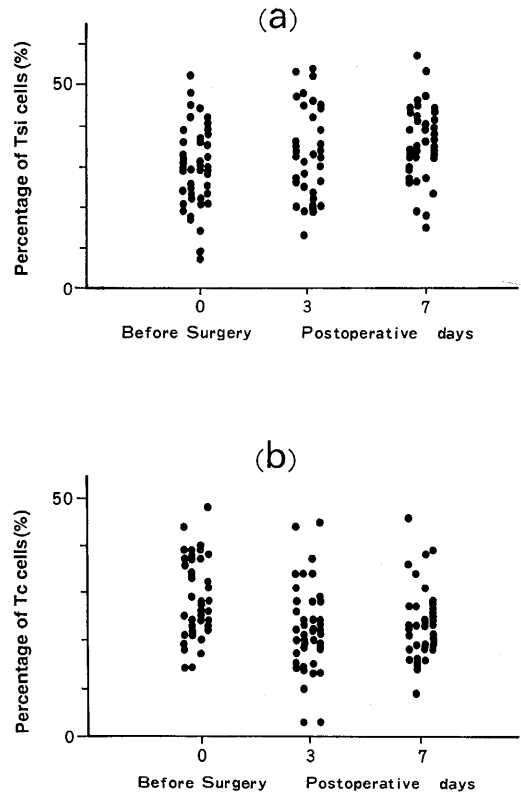


Fig. 2 (a) Postoperative changes in the percentage of CD25(+) cells in the peripheral blood. The percentage of CD4(+) cells increased significantly after surgery (day 0 vs. days 3 and 7, $p < 0.002$). (b) Postoperative changes in the percentage of CD56(+) cells in the peripheral blood. The percentage of CD8(+) cells decreased significantly after surgery (day 0 vs. days 3 and 7, $p < 0.002$).

changes in these two cells were opposite: the percentage of CD4(+) cells increased, while that of CD8(+) decreased. Two color analysis revealed that there was an increase in the suppressor inducer T-cells and a decrease in the cytotoxic T-cells (Fig. 2). Thus, the increase in the CD4(+) cells implies an increase in the suppressor inducer T cells, while a decrease in the CD8(+) cells means a decrease in the cytotoxic T-cells. A decrease of in the NK cells and an increase in the IL-2 receptor positive cells was noted (Fig. 3).

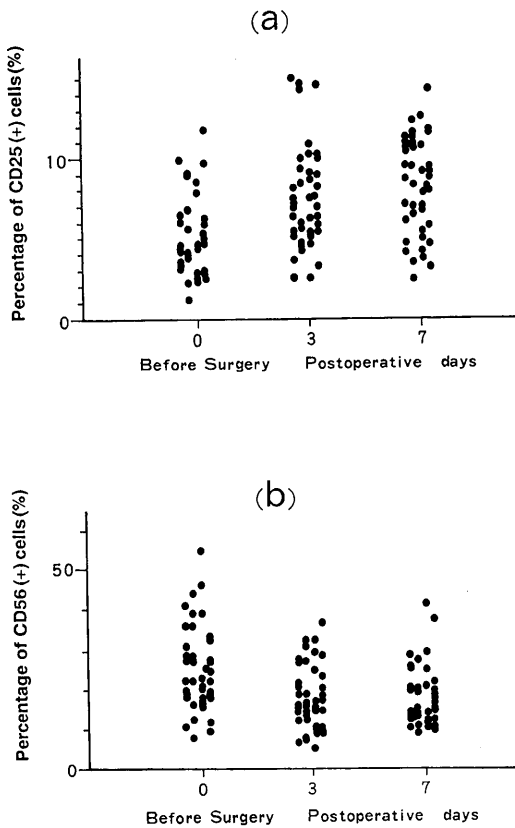


Fig. 3 (a) Postoperative changes in the percentage of suppressor inducer T-cells (Tsi) in the peripheral blood. The percentages of Tsi cells increased significantly after surgery (day 0 vs. days 3 and 7, $p < 0.002$). (b) Postoperative changes in the percentage of cytotoxic T-cells (Tc) in the peripheral blood. The percentage of Tc decreased significantly after surgery (day 0 vs. days 3 and 7, $p < 0.002$).

Discussion

Many investigators support the belief that surgical stress suppresses immunity: however, the mechanism of this immunosuppression following surgery remains controversial. New techniques, such as lymphocyte phenotypic analysis using monoclonal antibodies, allows one to probe the immune status of surgical patients (13). Direct measurement of the lymphocyte subpopulations, util-

izing staining and quantitation by antibodies specific for different cell types, may prove to be a more accurate and dependable assessment of cellular immune competence than in vitro stimulation assays. Detecting changes in the lymphocyte subsets postoperatively may not only elucidate the mechanism of immunosuppression but also suggest strategies to prevent or correct it. Several previous reports have found changes in the lymphocyte subsets following surgery. Hansbrough et al. (8) reported that the helper to suppressor ratios decreased on the first postoperative day, but returned to within normal limits on subsequent days. Grzelak et al. (9) observed a decrease in the percentage of CD4(+) and CD8(+) cells and an increase in the ratio of CD4(+) to CD8(+) cells. Hamid et al. (10) found no changes in the percentage of Leu 2a (suppressor T-cell) and Leu 3a (helper T-cell) positive cells. Lennard et al. (11) found a decrease in the Leu 4 (pan T-cell), Leu 2 (suppressor/cytotoxic T-cell), Leu 3 (helper/inducer T-cell), and Leu 11 (NK-cell) positive cells following major surgery. Platt et al. (12) found decreases in the CD3(+) and CD4(+) cells and increases in the CD8(+) cells. These reports suggested that there are significant changes in the lymphocyte subsets following surgery. However, since all analyses were done with single monoclonal antibodies, they were not definitive.

While absolute number of lymphocytes may decrease postoperatively (14), the immune status might be more accurately evaluated by changes in the more useful number or percentage of each lymphocyte phenotype. The cell to cell interaction of various lymphocytes is well known. Therefore, alternation in the lymphocyte subset populations may have a critical effect on the host's immune status. For example, if the ratio of cytotoxic T-cells to suppressor T-cells becomes altered toward excessive suppression, this can result in immunoincompetence and increased tumor growth. Accordingly, we evaluated changes in the percentage of each subset.

Our data indicated that: 1) the overall percentage of helper/inducer T-cells increases. Since two color analysis revealed that the percentage of helper T-cells did not

change and that the percentage of suppressor inducer T-cells increased, the increase in the helper/inducer T-cells is due to an increase in the suppressor inducer T-cells. These data are consistent with postoperative immunosuppression. 2) the percentage of suppressor/cytotoxic T-cells decreases. Since two color analysis showed that the percentage of suppressor T-cells did not change and that the percentage of cytotoxic T-cells decreased, the decrease in the suppressor/cytotoxic T-cells is due to a decrease in the cytotoxic T-cells. These data also are consistent with the postoperative immunosuppression. 3) the percentage of CD56 (NK cells) decreased after operation. Taken together, our data suggest that the prevention of postoperative immunosuppression may require both the augment of cytotoxic T-cells and NK cells and inhibition of the suppressor inducer T-cells.

However, our data also showed that the proportion of IL-2 receptor positive cells increased. IL-2, originally reported as a T-cell growth factor (15), is a multi-functional lymphokine regulating intercellular responses in the immune system. For example, IL-2 augments NK activity (16), LAK activity (17), and cytotoxic T-cell activity (18). For IL-2 to have these effects, it must bind to the IL-2 receptor. Therefore, IL-2 receptor positive cells may be important and can respond to IL-2 administration. Thus, the peri-operative administration of IL-2 may be effective in preventing postoperative immunosuppression. Sedoman et al. (19) administered low-dose interferon peri-operatively in surgical patients and reported that it prevented the fall in natural killer cell cytotoxicity, but failed to prevent the postoperative impairment of IL-2 production or lymphokine activated killer cytotoxicity. This report suggests that cytokine administration may modulate the immune system postoperatively. We currently are planning to administer low-dose IL-2 to surgical patients peri-operatively to assess its effects on the immune system.

Finally, the factors responsible for postoperative immunosuppression remain to be determined. The length and complexity of surgery, the associated blood loss and

hypotension, and the duration and type of anesthesia are factors which must be examined. In this study, six of 42 patients had a benign disease and six patients did not receive blood transfusion. Therefore, we could not observe the influence of malignancy or blood loss against lymphocyte subsets.

References

- 1) Park, S.K., Brody, J. I., Wallace, H.A. and Blakemore, W.S.: Immunosuppressive effect of surgery. *Lancet*, **1**: 53-55, 1971.
- 2) Cochran, A.J., Spilg, W.G.S. and Thomas, C. E.: Postoperative depression of tumor-direct cell-mediated immunity in patients with malignant disease. *Br. Med. J.*, **14**: 67-70, 1972.
- 3) Roth, J.A., Golub, S.H., Grimm, E.A., Eilber, F.R. and Morton, D.L.: Effects of operation on immune response in cancer patients: Sequential evaluation of in vitro lymphocyte function. *Surgery*, **79**: 46-51, 1976.
- 4) Slade, M.S., Simmons, R.L., Yunis, E. and Greeberg, L.J.: Immunodepression after major surgery in normal patients. *Surgery*, **78**: 363-372, 1975.
- 5) Polk, H.C., Wellhausen, S.R. and Regan, M. P.: A systemic study of host defense processes in badly injured patients. *Ann. Surg.*, **204**: 282-299, 1986.
- 6) Abraham E.: Immunologic mechanisms underlying sepsis in the critically ill surgical patients. *Surg. Clin. North. Am.*, **65**: 991-1003, 1985.
- 7) Tanemura, H., Sakata, K., Kunieda, T., Saji, S., Yamamoto, S. and Takekoshi, T.: Influences of operative stress cell-mediated immunity and tumor metastasis and their prevention by nonspecific immunotherapy: Experimental studies in rats. *J. Surg. Oncol.*, **21**: 189-195, 1982.
- 8) Hansbrough, J.F., Bender, E.M., Zapato-Sirvent, R. and Anderson, J.: Altered helper and suppressor lymphocyte populations in surgical patients. *Am. J. Surg.*, **148**: 303-307, 1984.
- 9) Grzelak, I., Olszewski, W.L. and Engeset, A.: Influence of operative trauma on circulating blood mononuclear cells: Analysis using monoclonal antibodies. *Eur. Surg. Res.*, **16**: 105-112, 1984.
- 10) Hamid, J.M., Bancewicz, J., Brown, R., Ward, C., Irving, M.H. and Ford, W.L.: The

- significance of changes in blood lymphocyte population following surgical operations. *Clin. Exp. Immunol.*, **56** : 49-57, 1984.
- 11) Lennard, T.W., Shenton, B.K. and Borzotta, A.: The influence of surgical operations on components of the human immune system. *Br. J. Surg.*, **72** : 771-776, 1985.
 - 12) Platt, M.P.W., Lovat, P., Watoson, J.G. and Aynsely-Green, A.: The effects of anesthesia and surgery on lymphocyte population and function in infants and children. *J. Pediatr. Surg.*, **24** : 884-887, 1989.
 - 13) Thronthwaite, J.T., Seckinger, D., Rosenthal, P. and Vazquez, A.: Characteristics of monoclonal antibody measurements in human peripheral blood. *Ann. New York Acad. Sci.*, **468** : 144-159, 1986.
 - 14) Oka, M.: Immunological studies on esophageal cancer -Cellular immunocompetence and histological responses in main tumor and regional lymph nodes in esophageal cancer patients. *Arch. Jpn. Chir.* **50** : 29-44, 1981.
 - 15) Morgan, D.A., Ruscetti, F.W. and Gallo, R.: Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science*, **193** : 1007-1008, 1976.
 - 16) Grim, E.A., Mazumder, A., Zhang, M.Z. and Rosenberg, S.A.: Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J. Exp. Med.*, **155** : 1823-1841, 1982.
 - 17) Suzuki, R., Handa, K., Itoh, K. and Kumagai, K.: Natural killer (NK) cells as a responder to interleukin 2 (IL 2). 1. Proliferative response and establishment of cloned cells. *J. Immunol.*, **130** : 981-987, 1983.
 - 18) Palladino, M.A., Obata, Y., Stockert, E. and Oettgen, H.F.: Characterization of interleukin 2-dependent cytotoxic T-cell clones: Specificity, cell surface phenotype, and susceptibility to blocking by Lyt antisera. *Cancer Res.*, **43** : 572-576, 1983.
 - 19) Sedman, P.C., Ramsden, C.W., Brennan, T. G., Giles, G.R. and Guillou, P.J.: Effects of low dose perioperative interferon on the surgically induced suppression of antitumour immune responses. *Br. J. Surg.*, **75** : 976-981, 1988.