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Immunological suppression after surgery in scoliosis patients

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Abstract This is a prospective study to analyze the postoperative immunological changes in scoliosis surgery. Twenty-eight patients who underwent Harrington instrumentation were analyzed. Seven patients who underwent arthroscopic surgery were analyzed as a control group. The objective of this study is to determine whether surgery and surgical stress (minor versus major surgery) changed the immune capability of patients as indicated by the ratio of lymphocyte cell types and interferon induction. Pre- and postoperative blood from scoliosis and knee arthroscopy patients was analyzed at various times using antisera and two-color flow cytometry with monoclonal antibodies. Both scoliosis and knee arthroscopy patients had a postoperative decrease in the population of B-lymphocytes (Leu 12+) and a transient increase in the natural killer

cells (Leu 4-/Leu 19+) and the cytotoxic T-lymphocytes (Leu 2a+/Leu 15-). These changes were significantly greater in the scoliosis patients, who also experienced a prolonged decrease in the inducer T-lymphocytes (Leu 8+/Leu 3a+) for 3 weeks following surgery. Production of interferon- α and interferon- γ by lymphocytes was suppressed postoperatively and returned to preoperative levels within 3 weeks. Immunological depressive changes occurred postoperatively and lasted at least 1 week. These effects are more pronounced with longer surgeries, suggesting greater postoperative vigilance for infections in these cases.

Key words Flow cytometry · Immunoglobulins · Interferon- α · Scoliosis · Harrington instrumentation

Introduction

Mandelbaum et al. [6] reported a decrease in serum total protein, electrolyte, and other indicators of nutritional deficiency after anterior and posterior spinal fusion surgery in scoliosis patients. In addition to these nutrient changes, we noticed a decrease in serum immunoglobulin levels in our clinical practices. This led us to analyze further the immunological changes after surgery in scoliosis patients.

Postoperative immunological changes were investigated by monitoring the production of interferon (IFN) by lymphocytes and in lymphocyte subsets using two-color

flow cytometry in scoliosis patients, with knee joint disorder patients used as a control group.

Materials and methods

Patients

The patients consisted of 28 healthy young individuals with idiopathic scoliosis (Group A) and 7 healthy young individuals with knee joint disorders (Group B) for the comparative study. Mean age, gender, anesthesia, operative time, blood loss, and operative procedure for these two groups are shown in Table 1. Patient age and gender were similar in the two groups. General anesthesia with Enfluren was given in both groups. In the scoliosis group, poste-

Table 1 Characteristics of the 28 patients with scoliosis and the 7 control patients with knee joint disorders

	Group A Scoliosis patients (n = 28)	Group B Control patients (n = 7)
Age (mean)	(14.4) years 14–24 years	(16.7) years 12–24 years
Male/Female	4/24	1/6
Anesthesia	Nitrous oxide- oxygen-enfluren	Nitrous oxide- oxygen-enfluren
Operation time (mean)	2 h 55 min~8 h 14 min (3h 52 min)	29 min~45 min (38 min)
Blood loss (mean)	300-2500 ml (672 ml)	0 ml
Procedure	Harrington sublaminar wiring	Knee joint arthroscopy

rior spine fusion with Harrington instrumentation was performed. In the control group arthroscopic surgery was carried out. The mean operative time was 3 h 52 min in the scoliosis group and 38 min in the “knee joint” group. Mean blood loss was estimated at 672 ml (20% of the total blood volume) in the scoliosis group, but in the control group the operation was done without blood loss.

Statistical analysis was performed using Student's *t*-test and the level of statistical significance was set at $P < 0.05$ (5%).

Experimental protocol

In the scoliosis group, autologous blood transfusion was used. The total volume of preoperative storage was 1300 ml. Blood samples were taken before and after drawing the blood for transfusion, immediately before anesthesia, immediately before operation after intubation, 30 min after surgery, and again every hour for 3 h, and then again on the 1st, 2nd, 3rd, 6th, 9th, 23rd, and 37th postoperative day. Routine peripheral blood cell counts and biochemical studies were conducted. Serum immunoglobulin (Ig) G, A, and M were assayed by Latex photometric immunoassay.

Lymphocytes were obtained by Ficoll-Hypaque gradient centrifugation. Lymphocyte blastogenesis was measured by the stimulation of lymphocytes with phytohemagglutinin (PHA) and expressed as a stimulation index, calculated as the ratio of Cr incorporation in PHA stimulated cell culture versus that in the control culture. Interferon (IFN) induction was carried out in duplicate as previously reported [8, 9].

In brief, lymphocytes were stimulated with the mumps virus, cultured for 7 days in a humidified atmosphere of 5% CO₂. The culture supernatant was harvested for the IFN assay. IFN assay was carried out by the semi-micro dye-binding method. Titers of IFN- α and IFN- γ (type II) were expressed by IFN activity, which was neutralized by the respective antisera against IFN- α and IFN- γ .

Lymphocyte subpopulations were analyzed by two-color flow cytometry, using the following monoclonal antibodies; Leu 2a, 3a, 4, 8, 12, 15, 19, HLA-DR (FACS 440, Becton-Dickinson, Mountain View, Calif.), and 2H4 monoclonal antibodies (Coulter Immunology, Hialeah, Fla., USA) as previously reported [11].

Results

Serum Ig levels

In the scoliosis group, serum albumin was 4.8 ± 0.3 g/dl before surgery, decreasing to 4.2 ± 0.4 g/dl 2 days after

Fig. 1 Changes in serum immunoglobulin. (□, ■; Ig G; △, ▲ Ig M; ○, ● Ig A)

surgery ($P < 0.01$). It increased to 4.4 ± 0.4 g/dl by day 9 and returned to 4.7 ± 0.3 g/dl by day 23. Serum Ig G was 1279 ± 290 mg/dl before surgery, decreasing to 909 ± 262 mg/dl 2 days after surgery ($P < 0.01$), and returning to the preoperative level by day 9 (Fig. 1). Serum Ig A was 184 ± 75 mg/dl before surgery, it decreased to 122 ± 38 mg/dl 2 days after surgery ($P < 0.05$), and increased to 251 ± 84 mg/dl by day 9. Serum Ig M was 232 ± 86 mg/dl before surgery, it decreased to 128 ± 57 mg/dl 2 days after surgery ($P < 0.01$), and increased to 368 ± 143 mg/dl by day 9. In the knee arthroscopy control group, no significant change was observed during this time period.

Peripheral blood counts

In the scoliosis group, autologous banked blood was transfused in a similar volume to the total blood loss during surgery. Despite receiving a blood transfusion, the blood hemoglobin level decreased from 12.8 ± 1.0 g/dl on the

preoperative day to 8.7 ± 1.4 g/dl 2 days after surgery ($P < 0.01$), and it returned to the preoperative level by day 23. Total peripheral lymphocyte count was $1802 \pm 551/\text{mm}^3$ preoperatively, decreasing to approximately $1200/\text{mm}^3$ by postoperative day 1, 2, or 3 ($P < 0.05$), and returning to the preoperative level by day 23. In the control group, no significant change was observed.

Lymphocyte transformation

In the scoliosis group, the stimulation index of lymphocyte transformation was 183 ± 87 preoperatively, following surgery this decreased to 95 ± 57 by day 9 and increased to 137 ± 56 by day 23. In contrast, in the control group it increased postoperatively on day 2 and 9.

Interferon production

The production of IFN- α and IFN- γ decreased on day 2 after surgery in both groups, and the decrease in IFN production was observed more profoundly and more persistently in the scoliosis group (Fig. 2). The production of IFN- α and IFN- γ returned to preoperative levels by day 23 after surgery.

Subpopulation of peripheral lymphocytes

In the scoliosis group, serial peripheral blood specimens were obtained as mentioned before. In the control group, blood samples were examined in a manner similar to the protocol of the scoliosis group, except that no autologous blood was banked. Leu 12+ cells (B cells) accounted for $12.9 \pm 5.6\%$ of the total peripheral lymphocytes at the beginning of the study. The drawing of autologous blood did not affect the proportion of Leu 12+ cells. The percentage of Leu 12+ cells markedly decreased on the operative day before and after anesthesia, especially 30 min after surgery, decreasing to $5.8 \pm 2.1\%$ ($P < 0.05$). It increased to $20.6 \pm 2.3\%$ on postoperative day 1 ($P < 0.01$), and then decreased gradually to the preoperative level by day 9. In the control group, no significant change was observed during the study period. In the scoliosis group, the decrease in the proportion of Leu 12+ cells on the operative day and subsequent increase above preoperative levels from day 1 to day 9 were significant compared to the equivalent values for the knee joint group.

The proportion of Leu 4-/Leu 19+ cells (natural killer cells) was $10.7 \pm 6.1\%$ of the total peripheral lymphocytes at the beginning of the study and increased on the operative day before and after anesthetic procedure, especially at 30 min after surgery, when it reached $24.6 \pm 12.0\%$. It decreased gradually to the lowest point ($7.4 \pm 5.1\%$) on day 3 after surgery and returned to the preoperative level

Fig. 2 Changes in interferon production by lymphocytes stimulated with mumps virus. (△, ▲ IFN- α units; ○, ● IFN- γ units)

on day 6. In the knee joint group, no statistically significant change was found. An increase in the proportion of Leu 4-/Leu 19+ cells at 2 h after surgery in the scoliosis group was of statistical significance compared to the equivalent value for the knee joint group. The proportion of Leu 4+/HLADR+ cells (activated T cells) decreased significantly ($P < 0.05$) from the preoperative level of $3.1 \pm 1.6\%$ to $2.0 \pm 0.8\%$ 3 h after surgery in the scoliosis group, but no change was observed in the knee joint group (Fig. 3).

The proportion of Leu 8+/Leu 3a+ cells (inducer T cells) was $27.3 \pm 9.4\%$ of total peripheral lymphocytes before drawing autologous blood, and it decreased gradually to $15.7 \pm 8.0\%$ 3 h postoperatively ($P < 0.01$) in the scoliosis group. It increased slightly to $21.2 \pm 11.0\%$ on day 2 after surgery, and showed approximately the same level from day 2 to day 9, still being significantly lower than the preoperative state ($P < 0.05$). On day 23, it returned to the preoperative level. In the knee joint group, it decreased transiently on the operative day, without statistical significance, and returned to the preoperative level. The

Fig. 3 Changes in the proportion of B cells (Leu12+), natural killer cells (Leu4-/Leu19+), and activated T cells (Leu4+/HLADR+)

decrease in the proportion of Leu8+/Leu3a+ from the 1st to the 23rd postoperative day in the scoliosis group was statistically significant, compared to the equivalent values for the knee joint group ($P < 0.05$). The proportion of Leu8-/Leu3a+ cells (helper T cells), $9.2 \pm 6.5\%$ of total lymphocytes, did not change during the study period in the scoliosis group. In contrast, a transient increase in the proportion of Leu8-/Leu3a+ cells was observed in the knee joint group, from $10.5 \pm 3.7\%$ before surgery to $20.1 \pm 8.5\%$ 3 h after surgery, but it was not statistically significant (Fig. 4).

The initial proportion of Leu2a+/Leu15- cells (cytotoxic T cells) in the scoliosis group was $20.6 \pm 6.0\%$ of the total lymphocytes. This increased to $31.8 \pm 8.8\%$ 30 min after surgery ($P < 0.01$), and decreased to the preoperative level on postoperative day 1. In the knee joint group, a small transient increase was observed. The proportion of Leu3a+/2H4+ cells (suppressor-inducer T cells) was $22.7 \pm 7.2\%$ in the scoliosis group, and no significant change was observed except on the 1st postoperative day when it decreased to $14.3 \pm 6.9\%$ in the scoliosis group, as compared to $23.0 \pm 11.5\%$ in the knee joint group ($P < 0.01$) (Fig. 5).

Fig. 4 Changes in the proportion of inducer T cells (Leu8+/Leu3a+) and helper T cells (Leu8-/Leu3a+)

Fig. 5 Changes in the proportion of cytotoxic T cells (Leu2a+/Leu15-) and suppressor-inducer T cells (Leu3a+/2H4+)

Discussion

Analysis of lymphocyte subsets has been extensively conducted using flow cytometry during the course of infectious diseases, immunodeficiency, and hematological disorders [2, 5, 10, 12]. An age-related immunological reduction on proliferative response upon stimulation with phytohemagglutinin (PHA) has been reported [3, 4, 7], but little is known about the influence of surgery on the immune system of healthy individuals. In this study, we investigated the changes in immunological parameters before and after surgery in healthy young subjects. In the scoliosis group, surgical stress was considered to be more serious than in the control group who underwent knee joint arthroscopic surgery, because of the longer operative time and the larger volume of blood loss during surgery. In the scoliosis group, all patients had banked autologous blood for transfusion during and after surgery, avoiding the risk of viral infection through homologous blood transfusion. Our results showed that the influence of autologous blood transfusion was negligible.

A significant decrease in serum immunoglobulin (Ig) levels was observed in the scoliosis group and serum level of albumin was significantly decreased on day 2 after surgery compared to the preoperative level. Despite an appropriate compensation of the blood loss, the degree of decrease in serum immunoglobulin was more prominent than that of the serum albumin levels. Though the exudative loss of fluid in the surgical field should be taken into account, we think that the significant changes in serum Ig are attributable to the surgical process rather than simply to blood loss or interstitial fluid loss.

Nakayama et al. reported that IFN- α was produced by non-T cells, whereas IFN- γ was produced by CD 4 cells and CD 8 cells obtained from immune individuals when lymphocytes were stimulated with the mumps virus antigen [9]. The production of IFN- α and IFN- γ decreased after surgery, and it returned to the preoperative levels by day 23 after surgery in both groups. These changes were greater in the scoliosis group. Decreased production of IFN- γ reflects the suppression of virus specific cell-mediated immunities. Decreased production of IFN- α is supposed to reflect a transient dysfunction of a non-specific host defense mechanism.

Precise descriptions of functionally distinct subsets have recently been determined using a large panel of monoclonal antibodies and two-color flow cytometry. On the day of operation, a transient increase in the proportion of natural killer cells and cytotoxic T cells as well as a transient decrease in the proportion of inducer T cells and suppressor-inducer T cells were demonstrated in both groups. These returned to the preoperative levels on postoperative day 1 in the control group. In the scoliosis

group, the changes in these lymphocytes were greater and the decrease in the proportion of inducer T cells was persistently observed from day 1 to day 9 after surgery; a transient increase in the proportion of helper T cells was not observed. In addition, a decrease in the proportion of B cells was marked immediately after surgery in the scoliosis group, but not in the control group. We think that these changes in the proportion of functionally distinct lymphocytes lead to the decrease in the production of IFN and Ig synthesis.

The patients in this study are all healthy individuals without immunological disorders. Yet significant changes in the proportion of immuno-competent lymphocytes during and after surgery occurred. A striking defect in lymphokine production by tumor-associated mononuclear leukocytes among cancer patients has been described [1]. It is also well known that transient immuno-suppression occurs after homologous blood transfusion. Therefore, the postoperative immunological dysfunction should be taken into consideration prior to scheduling major spinal instrumentation surgery in patients with metastatic tumor of the spine. Our study suggests that autologous blood transfusion is preferable for scoliosis surgery as well as metastatic cancer surgery.

In the surgical treatment of scoliosis, the multi-hook system is superior to Harrington instrumentation in obtaining better correction of hump deformity as well as scoliosis. However, Harrington instrumentation is simpler to apply than the recent advanced instrumentation systems, which require longer operation time. It is noteworthy that despite significant immunological changes during and after surgery, no postoperative infection occurred in our series. Among 246 patients who underwent Harrington instrumentation for scoliosis performed by the author (N.S.), postoperative infection occurred in none. In contrast, among 112 patients who underwent ISOLA instrumentation for scoliosis carried out by the same surgeon, postoperative infection occurred in 3 (2.7%), excluding three cases of late infection.

Conclusions

Immunological depressive changes occurred postoperatively and lasted at least 1 week. These effects are more pronounced with longer surgeries, suggesting greater postoperative vigilance for infections in these cases. Although this study did not show which change(s) can predict a definitely increased risk for infection postoperatively, it should be noted that patients who undergo spinal instrumentation surgery are likely to experience immunological suppression for 1 week postoperatively.

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