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KEYWORDS: surgical stress, graft-versus-host reaction, suppressor T cells

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SUPPRESSIVE INFLUENCE OF SURGICAL STRESS ON THE GRAFT-VERSUS-HOST REACTION IN MICE

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Abstract. The influence of surgical stress on the local graft-versus-host reaction (GVHR) in F_1 mice was studied. Skin incision 1 day prior to injection of parental spleen cells produced impairment of popliteal lymph node enlargement; however, this effect was not observed when GVHR was induced 3 and 5 days after operation. Strong GVHR suppressive activity of spleen cells was observed three hours after leg amputation before a decrease in thymus weight became evident. The GVHR suppressive activity declined by six hours later, but a second peak of 60 % inhibition was observed after 24 h. This suppressive activity completely disappeared by treatment with anti-Thy 1.2 and complement. This shows that the GVHR is suppressed by surgical stress, and that this suppression is due to suppressor T lymphocytes.

Key words.: surgical stress, graft-versus-host reaction, suppressor T cells.

Severe immunosuppression occurs after major thermal burns, accidental injuries and extensive surgical operations. For example, phagocytic and intracellular bactericidal activities in both neutrophils and macrophages are frequently impaired (1-3). It has also been shown that specific immunity and especially cell-mediated immunity are suppressed. Thus, injuries have been shown to prolong the survival of skin allografts (4), inhibit graft vs. host reactions (5), suppress delayed type hypersensivity (6, 7), inhibit the lymphoproliferative response to mitogens and antigens (8, 9) and inhibit both the generation and activity of cytotoxic lymphocytes (10-12).

The mechanisms underlying the suppression of immune function following injury are known. The suppression is not, at least not entirely, due to adrenal activity since immunological changes do not correlate with serum cortisol concentrations (13) and lmmunological changes can be produced in adrenalectomized animals (14). Munster proposed a hypothesis that injuries activate the suppressor T cell system, which is involved in normal immuno-regulation (15). Recent reports elucidated that suppressor cell activity may be responsible for suppression of immune function in injury (16, 17). In this study, we present additional evidence suggesting that inhibition of cellular immunity following surgical operation may be due to activation of suppressor T cells.

MATERIALS AND METHODS

Animals. BALB/c and (C57BL/6 × BALB/c) F_1 hybrid (CBF_1) male mice were purchased from Shizuoka Experimental Animals, and were used at 6 to 8 weeks of age.

Preparation of spleen cells. The spleen was removed aseptically, finely sliced in RPMI 1640 (GIBCO) solution, and then passed through No. 150 wire mesh. The cells thus obtained were washed three times with phosphate buffered saline (PBS), and then hemolysed using 0.75 % tris ammonium chloride solution (pH 7.65). Washing with PBS was repeated three times, after which the cells were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units of penicillin and 100 μg of streptomycin per ml.

Induction of the graft-versus-host reaction (GVHR). Measurement of the wet weight of popliteal lymph nodes (PLN) of CBF_1 mice was performed in accordance with the method of Ford (18). Namely, parent strain mouse spleen cells (2×10^7 cells/0.025 ml) were injected subcutaneously into the footpad of one hindleg of CBF_1 mice. In the other leg, the same number of CBF_1 mice spleen cells were injected. On the seventh day, the wet weight of PLN of both leg was measured and used to calculate the stimulation index (S.I.) which expresses the extent of local GVHR :

$$\text{S.I.} = \frac{\text{Wet weight of PLN in the leg injected with parent spleen cells}}{\text{Wet weight of PLN in the leg injected with } F_1 \text{ spleen cells}}$$

Measurement of GVHR suppressive activity. Spleen cells of CBF_1 mice that had received surgical operations were removed at planned intervals, and 2×10^7 spleen cells were mixed with BALB/c mouse spleen cells at ratio of 1 : 1 in a total volume 0.05 of ml. This mixture was injected into the hindleg footpad of a different CBF_1 mouse to trigger a GVHR. As a control, mixture of normal CBF_1 mouse spleen cells and BALB/c mouse spleen cells were used. On the eighth day following induction of GVHR, the wet weight of PLN was measured, and the extent of GVHR suppressive activity was calculated :

$$\text{GVHR suppressive activity (\% control GVHR)} = \frac{\text{Stimulation index of treated group}}{\text{Stimulation index of control group}}$$

Preparation with anti-Thy 1.2.. Spleen cells (1×10^7 cells/ml) were incubated for 30 min at 4℃ with a 1 : 1000 final dilution of monoclonal IgM anti-Thy 1.2 serum (Olac, England), and then centrifuged at 1000 rpm for 5 min. The cell pellet was resuspended with a 1 : 4 dilution of dried guinea pig complement (C) (Kyokuto Seiyaku Industry Co. Ltd.) for 40 min at 37℃. As a control, only the complement was used.

Elimination of adherent cells. Three to 4 ml of FBS previously inactivated were added to each plastic Petri dish (9 cm in diameter, Falcon), which was then tilted to permit the serum to cover its entire bottom surface and allowed to stand over night at 4℃ to be used as a serum-coated plate. To the plate, 6 ml of the spleen cell suspension in medium were added and incubated at 37℃ for 60 min, and the non-adherent cells were removed. The same procedure was repeated 3 times to eliminate adherent cells.

Surgical operations. CBF_1 mice received, under ether anesthesia, either an incision and suture of the skin along the dorsal midline for a length of about 1 cm, or an amputation of one leg after ligature of the femoral reign.

Statistical analysis. Statistical significance was determined with Student's t test.

RESULTS

The influence of sugical operation on thymus weight. Following surgical stress, the

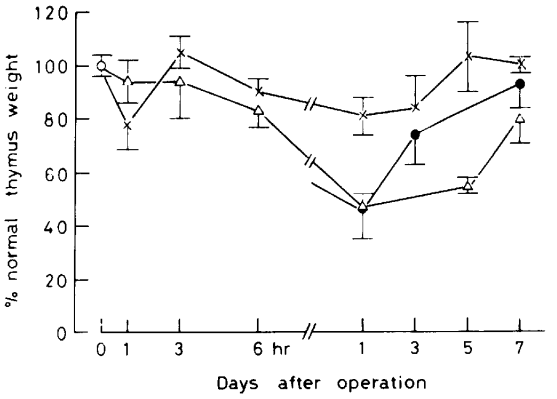

Fig. 1. Effect of surgical operation on thymus weight in mice. Thymus weights of CBF_1 mice anesthetized with ether (×——×), mice with dorsal skin incision (●——●) and those with unilateral hindleg amputation (△——△), expressed as % of thymus weight of normal mice.

weight of the thymus was measuted at fixed intervals of time. After amputation of one leg, there was no decrease in the weight of the thymus one and three hours after operation, but a slight decrose was detected at six hours later. Both the amputation and skin incision groups had a marked decrease of about 50 % in the wet weight of the thymus 24 h after operation. In the skin incision group, the decrease was only 25 % by the third day, and completely disappeared by the seventh day. In the amputation group, the thymus weight was still 45 % less than normal on the seventh day (Fig. 1).

TABLE 1. INFLUENCE OF SURGICAL OPERATION ON GVHR IN MICE.

C Treatment	Stimulation index (mean ± S.E.) GVHR induction after treatment (day)		
	1	3	7
Control	5.30 ± 0.54 (100)	6.02 ± 0.66 (100)	6.28 ±0.5 4 (100)
Ether anesthesia	4.59 ± 0.58 (86.6)	6.24 ± 0.46 (103.7)	8.54 ±1. 13 (136.0)
Skin incision	3.88 ± 0.32* (73.2)	4.68 ± 0.46 (77.7)	6.42 ± 0.61 (102.2)

Degree of GVHR expressed as stimulation index and % of control GVHR (in parentheses).
* $p < 0.05$ as compared with the control group.

The influence of surgical operation on GVHR. When a GVHR was triggered with BALB/c spleen cells in CBF_1 mice at various times after the surgical stress of skin incision, a significant inhibition was only seen in the group that was induced the GVHR on the first day after operation (Table 1).

TABLE 2. GVHR-SUPPRESSIVE ACTIVITY IN SPLEEN CELLS OF NORMAL CBF_1 MICE.

Requlatory cells	GVHR induced by spleen cells from	
	BALB/c	C57BL/6
Non	10.73 ± 0.41 (100.0)	8.96 ± 0.89 (100.0)
Spleen cells from normal CBF_1 mice	6.93 ± 0.79* (64.6)	6.58 ± 0.76** (73.6)

GVHR was elicited by injection of a mixture of 2×10^7 normal F_1 spleen cells and 2×10^7 parental spleen cells. Degree of GVHR is expressed as stimulation index and % of control GVHR. * $p < 0.001$, ** $p < 0.05$

TABLE 3. GVHR-SUPPRESSIVE ACTIVITY IN VARIOUS NORMAL CBF_1 MOUSE SPLEEN CELL POPULATIONS.

Requlatory cells	Stimulation index (mean ± S.E.)	% control GVHR	Significance to control
None	9.62 ± 0.74	100.0	–
Normal CBF_1 mouse spleen cells	6.78 ± 0.94	70.5	$p < 0.05$
Mitomycin C treated cells	6.45 ± 0.77	67.0	$p < 0.01$
Adherent cells	11.59 ± 1.13	120.5	N.S.
Non-adherent cells	6.52 ± 0.44	67.8	$p < 0.01$
Complement treated cells	5.58 ± 0.46	58.0	$p < 0.01$
Anti-Thy 1.2. + complement treated cells	8.79 ± 0.66	91.4	N.S.

These CBF_1 spleen cells (2×10^7 cells) were combined with 2×10^7 BALB/c spleen cells and injected into CBF mice to evoke GVHR. Degree of GVHR expressed as stimulation index and % of control GVHR with BALB/c spleen cells alone.

GVHR-suppressive activity of normal F_1 spleen cells. In CBF_1 mice injected with a mixture of normal syngeneic mouse spleen cells and BALB/c or C57BL/6 mouse spleen cells, the GVH reaction thereby elicited was less than that evoked with the latter cells alone (Table 2). The GVHR-suppressive activity in normal CBF_1 mouse spleen cells was not affected by pretreatment with mitomycin C (25 μg/ml at 37℃ for 30 min), but disappered following treatment of the cells with anti-Thy 1.2 antibody and complement. The suppressive activity was demonstrated in the non-adherent fraction of the cells (Table 3).

Effect of surgical operation on GVHR-suppressive activity of CBF_1 spleen cells. As shown in Fig. 2, mild GVHR suppressive activity was seen 3 h after ether anesthesia. From 1 to 7 days thereafter, there was no difference from that of non-anesthetized mice. In the unilateral amputation group, marked GVHR-suppressive activity became evident 1 h after the operation, reached a first peak within 3 h and declined. This activity showed a bimoidal curve indicating a second peak at 24 h, declined by the fifth day, and returned to the normal level by the

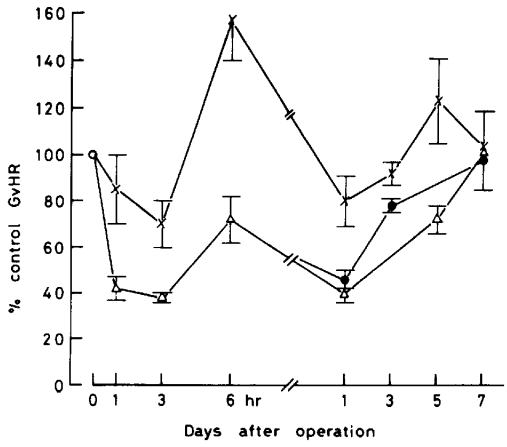

Fig. 2. Effect of surgical operation on GVHR-suppressive activity of CBF_1 spleen cells. GVHR was elicited by injection of BALB/c spleen cells combined with spleen cells from mice with skin incision (●——●), those with unilateral hindleg amputation (△——△) or those with ether anesthesia (×——×), and expressed as % of control GVHR evoked with combined CBF_1-BALB/c spleen cells.

TABLE 4. GVHR-SUPPRESSIVE ACTIVITY OF VARIOUS SPLEEN CELL POPULATIONS FROM MICE 3 H AFTER ETHER ANESTHESIA OR AFTER UNILATERAL HINDLEG AMPUTATION UNDER ETHER ANESTHESIA.

Regulatory cells (2×10^7 cells)	Stimulation index (mean ± S.E.)	% control GVHR	Significance to control
Control	5.41 ± 0.36	100.0	–
Ether anesthesia group			
Whole cells	3.69 ± 0.53	68.2	$p < 0.05$
Complement treated cells	3.62 ± 0.38	66.8	$p < 0.01$
Anti-Thy 1.2.+ complement treated cells	5.00 ± 0.41	92.4	N.S.
Amputation group			
Whole cells	2.55 ± 0.21	47.1	$p < 0.001$
Complement treated cells	1.91 ± 0.20	35.3	$p < 0.001$
Anti-Thy 1.2.+ complement treated cells	5.51 ± 0.36	101.8	N.S.

Degree of reaction expressed as stimulation index and % of control GVHR evoked with a mixture of normal CBF_1 and BALB/c mouse spleen cells.

seventh day. In the skin incision group, the same degree of GVHR-suppressive activity as in the amputation group was detected at 24 h. But there was slight inhibition by the third day, and no inhibition on the seventh day. An increase in GVHR-suppressive activity after operative intervention was thus demonstrated, and the decrease in this activity appeared to correlate with the increase in the weight of the thymus.

TABLE 5. GVHR-SUPPRESSIVE ACTIVITY OF VARIOUS SPLEEN CELL POPULATIONS FROM MICE ONE DAY AFTER UNILATERAL LEG AMPUTATION.

Requlatory cells (2×10^7 cells)	Stimulation index (mean ± S.E.)	% control GVHR	Significance to control
Control	6.49 ± 0.89	100.0	–
Untreated cells	2.60 ± 0.35	40.1	$p < 0.001$
Adherent cells	8.18 ± 1.01	126.0	N.S.
Non-adherent cells	4.12 ± 0.31	63.5	$p < 0.02$
Complement treated cells	3.93 ± 0.24	60.6	$p < 0.01$
Anti-Thy 1.2. + complement treated cells	5.23 ± 0.46	80.6	N.S.

Degree of reaction expressed as stimulation index and % of control GVHR evoked with a mixture of normal CBF_1 and BALB/c mouse spleen cells.

In order to characterize the suppressor cells, elimination of adherent cells or Thy 1^+ cells was performed prior to injection. GVHR suppressive activity after 3 h of anesthesia or operation was completely lost by treatment with anti-Thy 1.2 antibody and complement (Table 4). GVHR suppressive activity on the first day after operation was detected in the non-adherent fraction. This activity was also completely lost after elimination of Thy 1^+ cells (Table 5).

DISCUSSION

It has long been known that the immunological response decreases after surgical operation. Munster has proposed that extensive burns, trauma and operations result in release of antigenic protein rich in tissue-specific antigens from wound (15). If the immune system acts normally, autoimmune disease develops, thus the normal tissue is in danger of being destroyed. He theorized that, in order to forestall such a danger, suppressor T lymphocytes must be activated during stress. We confirmed this theory by mesuaring the GVHR and GVHR suppressive activity after operation. The GVHR was inhibted by sugical operations, and marked GVHR suppressive activity of spleen cells of operated mice was observed. This suppressive activity was displayed by T lymphocytes. These results support the theory of Munster. When physical stress is without causing any injury, the immune response is inhibited (19). Furthermore, the immune response has been reported to decrease with anesthetic procedures (20, 21). In our study, the GVHR was not inhibited by ether anesthesia alone, but the GVHR suppressive activity of spleen cells of anesthetized mice was slightly observed. These suppressor cells were T lymphocytes, as in the case when surgical stress was added, but not adherent cells and non-T lymphocytes. Regardless of whether or not there is a physical injury, under great stress which induce atrophy of the thymus gland in mice (22), the immune response is most likely inhibited. We have investigated the immune response in another experiment in which

pain stimuli is given to mice as stress. In cases of using pain stimuli with no recognizable thymus atrophy, NK activity, antibody production response against SRBC and the GVHR were not inhibited, rather, an increase was seen (23). These facts suggest that changes in the immune response that occur in response to stress should be considered as being of two types : those accompanied by thymus atrophy and those not. In our study, a correlation between recovery in thymus weight after operation and loss of GVHR inhibitory response was seen. Thus, the decrease in thymus weight and the development of immune inhibition effect may be the result of the same mechanism. Since the thymus atrophy after severe stress is due to release of steroid hormones from the adrenal cortex, it is thought that the release of steroid hormones is a major factor in the decrease of immune response occurring after surgery.

But a report states that no clear-cut relationship can be drawn between the serum cortisol concentration and delayed hypersensitivity reaction following trauma and surgery (13). Recently, it was reported that hydrocortisone (Hc) activated presuppressor spleen cells to become suppressor within 48 h, and the Hc-induced suppressor cells are capable of inhibiting *in vitro* the lytic function of NK effectors of the untreated mice spleens. (24).

Constantin (13) demonstrated immune suppressive activity in sera of patients who received surgery or trauma. The inhibitory factor was found in the peptide fraction, with a molecular weight of less than 10,000. Likewise, it was reported that the serum of postoperative patients inhibits the ADCC reaction of lymphocytes from normal persons (11). Such factors may also be related to the generation of suppressor cell activity.

In our study, the activity of T lymphocytes that inhibit GVHR was also recognized in normal mouse spleen cells. It is not clear whether the two types of suppressor T lymphocytes are the same or not. However, we speculate that normally present suppressor T lymphocytes are activated by surgical intervention.

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