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The Mechanisms of Immune Suppression by High-pressure Stress in Mice

R. FUJIWARA*†‡, H. SHIBATA†, T. KOMORI‡, M. M. YOKOYAMA†, Y. OKAZAKI‡ AND M. OHMORI*

*First Department of Pathology, Kagawa Medical School, Kita, Kagawa, 761-0701, †Department of Immunology, Kurume University School of Medicine, Kurume, 830-0011 Fukuoka, and ‡Department of Psychiatry, Mie University School of Medicine, Tsu, 514-8507 Mie, Japan

Abstract

The effects of high-pressure stress on the induction of anti-sheep red blood cells (SRBC) and of plaque-forming cells (PFC), and on thymus weight, were studied in BALB/c mice in-vivo and in-vitro.

The efficacy of high-pressure stress in suppressing PFC and thymic involution was maximum when the stress was applied 1 h day^{-1} for 2 days before immunization with SRBC. Both effects were blocked by administration of indomethacin, atropine, naloxone or phentolamine before the first application of stress, whereas hexamethonium and propranolol had no such effect. Hexamethonium, naloxone and propranolol administered before the second application of high-pressure stress blocked both effects. Prostaglandin and acetylcholine given 24 h before application of high-pressure stress caused a marked reduction in PFC count, but not in thymus weight. The reduced PFC count caused by acetylcholine was blocked by pretreatment with indomethacin. When adrenaline was injected 24 h after application of high-pressure stress a marked reduction in PFC was observed, but without thymic involution. When adrenaline was injected 24 h after prostaglandin injection the PFC count was also markedly reduced, but not thymus weight. The decrease in PFC caused by two exposures to stress or one exposure to stress plus injection of adrenaline was blocked by diethylcarbamazine before the second exposure to stress or the injection of adrenaline. In addition, normal spleen cells were induced as suppressor cells when incubated with the serum of stressed mice, but not when supplemented with anti-leukotriene C_4, D_4 antibody.

These data suggest that mice fall into a pre-stress condition via the release of prostaglandin after the first stress, and then immunosuppression is induced in these prestressed mice via the release of leukotriene C_4, D_4, caused by the activation of the autonomic nervous system by the second exposure to stress.

Physical and emotional stress has been shown to enhance or depress the immune response, depending on the particular stressors involved (Munster 1976; Bartrop et al 1977; Ader et al 1987; Fujiwara & Orita 1987). Immune response is reduced in mice after surgical operation (Munster 1976; Fujiwara et al 1984), but is enhanced as a result of pain stimulation (Fujiwara & Orita 1987; Fujiwara et al 1989). Immune suppression involving thymic involution from stress would thus seem to result from glucocorticoid released from the adrenal cortex by activation of the hypothalamo–pituitary–adrenal axis. Moreover, immune enhancement not involving such thymic involution might be caused by adrenaline released from the adrenal medulla after activation of the sympathetic nervous system (Fujiwara & Orita 1987). Such stress is sufficient to alter immune response after only a single application. But in the second example, stress must be applied more than once to alter immune response, such as in acupuncture (Fujiwara et al 1991) and the use of high-pressure stress (Shibata et al 1991). In this study a marked reduction in immune response was observed in mice subjected to high-pressure stress.

The aim of this study was to determine, by use of a variety of pharmacological agents, the mechan-

Correspondence: R. Fujiwara, First Department of Pathology, Kagawa Medical University, 1750-1 Ikenobe, Miki-cho, Kita-gun, 761-0701 Kagawa, Japan.

isms of immune suppression resulting from high-pressure stress.

Materials and Methods

Animals

BALB/c male mice, 8–10 weeks, (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan) were used throughout the experiments. Animals were housed in groups of six and had free access to a pellet diet and water. The animal room was maintained at 21–25°C, with a 12 h light–dark cycle (light on at 0700 h). All experiments were performed on groups of three mice, and were repeated four times (n = 12 per experiment group) both in-vivo and in-vitro.

Spleen cell preparation

The spleen and thymus were removed aseptically from mice and wet weight was measured. Spleens were finely minced in RPMI 1640 medium and passed through a no. 150 wire mesh. The cells obtained were washed three times in phosphate-buffered saline (PBS; pH 7·4), and then suspended in RPMI 1640 medium at a concentration of 1×10^7 cells mL^{-1}.

Measurement of plaque-forming cells (PFC) in-vivo

Sheep red blood cells (SRBC; 0·25 mL) at a concentration of 1×10^8 cells mL^{-1} in saline were injected into the tail vein of mice immediately after exposure to high-pressure stress. Five days after immunization the spleens were removed and spleen cells were prepared and assessed for anti-SRBC IgM PFC according to the method of Cunningham & Szenberg (1968). Briefly, spleen cells adjusted to 1×10^7 cells mL^{-1} (0·4 mL), SRBC (50%; 0·05 mL) and guinea-pig serum as a source of complement (0·05 mL) were mixed, then incubated for 1 h at 37°C, in a chamber made on a glass slide with a cover slip, after which the PFC were counted.

Measurement of anti-SRBC PFC in-vitro

Normal mice spleen cells were cultured with regulatory cells treated with mytomycin C (25 μg mL^{-1}, 37°C, for 30 min) and SRBC in RPMI 1640 medium including foetal bovine serum (10%), HEPES (25 mM), penicillin (100 units mL^{-1}), streptomycin (100 μg mL^{-1}) and 2-mercaptoethanol (5×10^{-5} M), according to a modification of the method of Mishell & Dutton (1967). Briefly, 8×10^6 spleen cells from normal mice were cultured with 8×10^6 spleen cells from treated mice (regulatory cells) and 8×10^6 SRBC on a culture plate (Falcon; 24-well culture plate; No. 3047) in 2 mL medium. The cultures were placed in a humidified incubator at 37°C with a constant gas flow (5% CO_2 in air). PFC responses were determined on day 5 by the Cunningham assay.

High-pressure stress

Three mice were put into a chamber (diameter 125 mm, length 180 mm, volume 2210 cm^3) and high pressure (2·2 kg cm^{-2}) compressed air was applied for 60 min once a day for 2 days (Shibata et al 1991). Control mice were placed in this chamber without the exposure to high pressure.

Drug administration

All drugs except indomethacin were dissolved in physiological saline and given to mice orally or subcutaneously. Indomethacin was suspended in 5% saline gum arabic, and given to mice orally. The control group received only physiological saline.

The drugs used were atropine (atropine sulphate; Tanabe), diethylcarbamazine (Sigma), hexamethonium (methobromine; Yamanouchi), naloxone (Sigma), phentolamine (Regitine; Ciba–Takeda) and propranolol (propranolol chloride; Sumitomo).

Injection of autacoids

All autacoids were injected into the tail vein of mice 60 min after pretreatment with subcutaneous hexamethonium (10 mg kg^{-1}), to remove disagreeable stimulation from the intravenous injection (Fujiwara & Orita 1987).

The autacoids used were acetylcholine (Neucholin-A; Zelia), enkephalin (Sigma), adrenaline (Bosmin; Daiichi), noradrenaline (Sankyo) and prostaglandin E_2 (Ono).

Reagent

Anti-leukotriene C_4,D_4 rabbit serum was from Advanced Magnetics.

Statistical analysis

If analysis of variance showed a significant difference ($P < 0.01$), results were further analysed by the Newman–Keuls test. $P < 0.05$ was considered to be indicative of significance.

Results

Influences of different blockers on high-pressure stress

Two applications of high-pressure stress, once a day for 60 min for 2 days (Table 1), were required to bring about reductions in the number of PFC and thymus weight in mice. To determine the mechanisms of this stress-induced effect, the effects of atropine, hexamethonium, indomethacin, naloxone, phentolamine and propranolol were examined. PFC and thymus weight after two applications of stress remained at normal levels when atropine, indomethacin, naloxone or phentolamine were administered before the initial stress; neither hexamethonium nor propranolol had any effect. Normal values were maintained when hexamethonium, naloxone and propranolol were administered before the second application of stress whereas atropine, indomethacin and phentolamine had no effect (Table 2).

Table 1. The effect of high-pressure stress on the production of plaque-forming cells and on thymus weight in mice.

Treatment	Thymus weight (mg; mean ± s.e.m.)	Plaque-forming cells/10^6 spleen cells (mean ± s.e.m.)
Normal control	52·4 ± 3·5	1693 ± 115
High pressure[a]	48·7 ± 3·3	1506 ± 100
High pressure[b]	25·6 ± 1·9*	703 ± 49*
Analysis of variance	$F(2, 33) = 23·65$	$F(2, 33) = 30·86$
P value	$P < 0·01$	$P < 0·01$

[a]Mice were subjected once to high-pressure stress for 60 min and were injected with SRBC immediately after stress. [b]Mice were subjected to high-pressure stress for 60 min once a day for 2 days and were injected with SRBC immediately after the second application of stress. The plaque-forming cells in mice were counted on day 5 after immunization with SRBC. *$P < 0·05$ compared with control (Newman–Keuls post-hoc comparison; $\alpha = 0·05$, n = 12).

Role of different autacoids in high-pressure stress-induced immune suppression

In the study with the neurotransmitters acetylcholine, enkephalin, adrenaline, noradrenaline and prostaglandin E_2, PFC decreased when acetylcholine and prostaglandin E_2 were administered 24 h before stress. Noradrenaline and enkephalin had no effect on PFC. Acetylcholine and prostaglandin E_2 caused no reduction in thymus weight (Table 3). The reduction in PFC elicited by acetylcholine and stress was blocked by pre-administration of indomethacin before the acetylcholine injection (Table 4). Adrenaline injected 24 h after high-pressure stress caused PFC to decrease. Enkephalin had no such effect (Table 5). Prostaglandin E_2 and adrenaline used in place of the two applications of high-pressure stress caused a reduction in PFC, whereas thymus weight was unaffected (Table 6).

Table 2. Effects of different drugs on the suppression of plaque-forming cells by high-pressure stress in mice.

Drug	Plaque-forming cells/10^6 spleen cells (mean ± s.e.m.)		
	Drug only (not stressed)	Drug treatment before: First stress	Second stress
Atropine (1 mg kg^{-1}, subcutaneous)	2109 ± 153	2281 ± 162**	834 ± 53*
Hexamethonium (10 mg kg^{-1}, subcutaneous)	1617 ± 105	799 ± 54*	1563 ± 106*
Indomethacin (10 mg kg^{-1}, oral)	1984 ± 128	1585 ± 103**	752 ± 55*
Naloxone (0·01 mg kg^{-1}, subcutaneous)	1794 ± 116	1699 ± 110**	1941 ± 132**
Phentolamine (5 mg kg^{-1}, oral)	2517 ± 179	2550 ± 171**	833 ± 52*
Propranolol (5 mg kg^{-1}, oral)	1872 ± 124	784 ± 46*	1938 ± 129**
Normal control		1748 ± 121	
Stress control		729 ± 44*	
Analysis of variance		$F(19,220) = 27·57$	
P value		$P < 0·01$	

Atropine, hexamethonium and naloxone were injected 30 min, and indomethacin, phentolamine and propranolol were administered 60 min, before the mice were subjected to the first or the second stress. Stress control mice were subjected to stress twice. *$P < 0·05$ compared with normal control; **$P < 0·05$ compared with stress control (n = 12).

Table 3. The role of neurotransmitters on the first stress.

Treatment	Thymus weight (mg; mean ± s.e.m.)	Plaque-forming cells/10^6 spleen cells (mean ± s.e.m.)
Normal control	48·1 ± 3·2	1521 ± 103
High pressure/ high pressure	22·3 ± 2·9*	696 ± 47*
Acetylcholine/ high pressure	49·3 ± 3·5	832 ± 56*
Enkephalin/ high pressure	46·5 ± 3·1	1704 ± 112
Noradrenaline/ high pressure	47·0 ± 2·8	1557 ± 107
Prostaglandin E_2/ high pressure	47·9 ± 3·3	682 ± 41*
Analysis of variance	F(5, 66) = 12·32	F(5, 66) = 32·72
P value	$P < 0·01$	$P < 0·01$

Acetylcholine, enkephalin, noradrenaline and prostaglandin E_2 (10^{-5} g kg^{-1}) were administered intravenously to mice, instead of the first high-pressure stress, 24 h before application of the second high-pressure stress. *$P < 0·05$ compared with normal control (n = 12). PFC; PFC/10^6 spleen cells.

Table 4. The effect fo acetylcholine and prostaglandin E_2 on the suppression of the production of plaque-forming cells by high-pressure stress in mice

Treatment	Thymus weight (mg; mean ± s.e.m.)	Plaque-forming cells/10^6 spleen cells (mean ± s.e.m.)
Normal control	54·1 ± 3·7	1495 ± 103
High pressure/ high pressure	28·5 ± 2·0*	732 ± 49*
Acetylcholine/ high pressure	52·6 ± 3·4	961 ± 67*
Prostaglandin E_2/ high pressure	57·3 ± 3·8	716 ± 49*
Atropine-treated before first treatment		
Acetylcholine/ high pressure	54·6 ± 3·5	1476 ± 100
Prostaglandin E_2/ high pressure	50·2 ± 3·3	796 ± 54*
Indomethacin-treated before first treatment		
Acetylcholine/ high pressure	56·1 ± 3·2	1523 ± 106
Prostaglandin E_2/ high pressure	51·0 ± 3·5	751 ± 48*
Analysis of variance	F(7,88) = 7·22	F(7,88) = 24·10
P value	$P < 0·01$	$P < 0·01$

Atropine (1 mg kg^{-1}) or indomethacin (10 mg kg^{-1}) were administered subcutaneously and orally, respectively, 30 and 60 min, respectively, before injection of acetylcholine and prostaglandin E_2 instead of the first stress. *$P < 0·05$ compared with normal control (n = 12).

Table 5. The effect of adrenaline and enkephalin on the suppression of the production of plaque-forming cells by high-pressure stress in mice

Treatment	Thymus weight (mg; mean ± s.e.m.)	Plaque-forming cells/10^6 spleen cells (mean ± s.e.m.)
Normal control	49·2 ± 3· 3	1508 ± 106
High pressure/ high pressure	24·5 ± 1·7*	761 ± 52*
High pressure/ enkephalin	50·6 ± 3·5	1884 ± 128
High pressure/ adrenaline	48·9 ± 3·2	759 ± 49*
Analysis of variance	F(3, 44) = 17·38	F(3, 44) = 38·58
P value	$P < 0·01$	$P < 0·01$

Enkephalin and adrenaline (10^{-5} g kg^{-1}) were administered intravenously to mice, instead of the second stress, 24 h after the first stress. *$P < 0·05$ compared with normal control (n = 12).

Table 6. The effect of prostaglandin E_2 and adrenaline on the suppression of the production of plaque-forming cells by high-pressure stress in mice

Treatment	Thymus weight (mg; mean ± s.e.m.)	Plaque-forming cells/10^6 spleen cells (mean ± s.e.m.)
Normal control	52·4 ± 3·6	1599 ± 109
High pressure/ high pressure	23·9 ± 1·6*	705 ± 45*
Prostaglandin E_2/ adrenaline	50·2 ± 3·3	681 ± 41*
Analysis of variance	F(2, 33) = 28·56	F(2, 33) = 52·69
P value	$P < 0·01$	$P < 0·01$

Prostaglandin E_2 (10^{-5} g kg^{-1}) was administered intravenously to mice 24 h before intravenous injection of adrenaline (10^{-5} g kg^{-1}). *$P < 0·05$ compared with normal control (n = 12).

Detection of immune suppressive factor in mice subjected to high-pressure stress

Spleen cells from normal mice as responders were cultured in-vitro with SRBC and regulator spleen cells of mice subjected twice to high-pressure stress. The PFC-suppressing activity of regulatory cells was detected from 8 to 24 h after the second application of stress (Table 7). It was also present in the serum of mice from 8 to 24 h after the second application of stress. Normal spleen cells were incubated at serum concentrations of 50% for 60 min at 37°C, and then used as regulatory cells. When these cells were cultured with SRBC and normal spleen cells for 5 days, PFC in normal spleen cells were reduced (Table 8).

Table 7. The appearance of suppressor cells in the spleen from mice subjected to high-pressure stress.

Time (h) after the second stress (Regulator spleen cells)	Plaque-forming cells/well (mean ± s.e.m.)
0 (normal mice)	1698 ± 118
4	1726 ± 121
8	1209 ± 79*
12	682 ± 49*
16	679 ± 47*
24	708 ± 46*
Analysis of variance	F(5,66) = 36·56
P value	*P* < 0·01

The regulator spleen cells were removed 4, 8, 12, 16 and 24 h after subjection of the mice to the second stress, and were used as regulator cells. Responder spleen cells (spleen cells from normal mice) and mitomycin C-treated regulatory cells were incubated with sheep red blood cells at 37°C for 5 days, and plaque-forming and responder cells were counted. *$P < 0·05$ compared with normal mice (n = 12).

Table 8. Appearance of the suppressor factor in the serum of mice subjected to high-pressure stress.

Treated with serum from:	Time (h) after the second stress	Plaque-forming cells/well (mean ± s.e.m.)
Normal mice (normal control)	–	1609 ± 109
Mice subjected to high pressure	2	1834 ± 127
	4	1583 ± 112
	8	1251 ± 79*
	12	762 ± 51*
	24	739 ± 52*
Analysis of variance		F(5, 66) = 24·58
P value		*P* < 0·01

Spleen cells from normal mice were incubated for 60 min at 37°C with an equal amount of serum either from normal mice or from mice twice subjected to stress, and were used as regulatory cells. *$P < 0·05$ compared with normal mice (n = 12).

Role of adrenaline in high-pressure-stress-induced immune suppression

A suppressor factor in the serum was sought 8 h after the second application of stress, because it was considered that the effect might be slow-reactive-substance anaphylaxis (SRS-A; leukotriene C_4,D_4). Diethylcarbamazine, the agent that blocks leukotriene C_4,D_4 release, was used for this purpose. In mice subjected to two applications of stress, PFC were restored to the normal level by administration of diethylcarbamazine before the second application of stress and the injection of adrenaline (Table 9).

The anti-leukotriene C_4,D_4 antibody was studied for its effects on the suppressor factor in the serum. Spleen cells from normal mice were incubated with serum from mice subjected to two stresses and adrenaline, and then used as regulatory cells. These regulatory cells were subsequently observed to have PFC-suppressing activity whereas normal spleen cells and the serum of mice twice exposed to stress and adrenaline, and then incubated with anti-leukotriene C_4,D_4 serum at a final concentration of 1/500, had no such activity (Table 10).

Table 9. The effect of diethylcarbamazine on the suppression of the production of plaque-forming cells in mice subjected to stress twice or subjected to stress once and then given adrenaline.

Treatment	Thymus weight (mg; mean ± s.e.m.)	Plaque-forming cells/10^6 spleen cells (mean ± s.e.m.)
Normal control	50·8 ± 3·3	1556 ± 105
Stress control		
High pressure/ high pressure	27·3 ± 1·9*	703 ± 52*
High pressure/ adrenaline	53·4 ± 3·7	739 ± 48*
Pretreatment with diethylcarbamazine		
–	49·7 ± 3·5	1625 ± 112
High pressure/ high pressure	48·6 ± 3·0	1572 ± 98**
High pressure/ adrenaline	51·6 ± 3·3	1608 ± 100**
Analysis of variance	F(5, 66) = 9·44	F(5, 66) = 25·18
P value	*P* < 0·01	*P* < 0·01

Diethylcarbamazine (50 mg kg^{-1}) was administered orally to mice 60 min before either the second stress or intravenous administration of adrenaline (10^{-5} g kg^{-1}). *$P < 0·05$ compared with normal control; **$P < 0·05$ compared with stress control (n = 12).

Discussion

Before beginning this study, we supposed that a single application of stress would induce immunosuppression after both high-pressure stress and surgical stress (Fujiwara et al 1984). However, in this study the PFC count and thymus weight of mice exposed twice to high-pressure stress before SRBC immunization markedly decreased, whereas in the mice exposed only once to such stress, no such decrease was noted. The effect of the first application of stress in suppressing PFC and thymic involution was blocked by pretreatment with atropine, indomethacin, naloxone or phentolamine. The effect of the second application of stress was blocked by pretreatment with hexamethonium, naloxone or propranolol. In mice given acetylcholine, enkephalin, noradrenaline and prostaglandin E_2 injections, instead of the first appli-

Table 10. The effect of anti-leukotriene C4,D4 antibody on plaque-forming cell suppression factor in serum from mice subjected to stress twice.

Incubation with serum from	Anti-leukotriene C4,D4 antibody	Plaque-forming cells/well (mean ± s.e.m.)
–	–	1635 ± 104
Normal mice	–	1574 ± 99
Mice subjected to stress twice	–	721 ± 50*
Mice subjected to the first stress and given adrenaline	–	801 ± 56*
Normal mice	+	1763 ± 120
Mice subjected to stress twice	+	1648 ± 114**
Mice subjected to the first stress and given adrenaline	+	1724 ± 110**
Stress control		756 ± 54*
Analysis of variance		F(7, 88) = 26·24
P value		$P < 0{\cdot}01$

Stress control—spleen cells from mice subjected to stress twice were incubated at 37°C for 5 days with sheep red blood cells. Spleen cells from normal mice were incubated at 37°C for 60 min with serum (50%) or with serum plus anti-leukotriene C4,D4 antibody (final concentration 1/500), and used as regulator cells. The responder cells were used the spleen cells from normal mice. Serum was obtained 12 h after the second stress or the injection of adrenaline. Control serum was obtained from normal mice. *$P < 0{\cdot}05$ compared with normal mice; **$P < 0{\cdot}05$ compared with mice subjected to stress twice (free from anti-leukotriene C4,D4 antibody) (n = 12).

cation of stress, 24 h before application of the second stress, the PFC count was reduced by injection of acetylcholine or prostaglandin E_2. However, although the effect of acetylcholine was blocked by pretreatment with indomethacin, the effect of prostaglandin E_2 was not blocked by pretreatment with atropine. The PFC suppression caused by acetylcholine thus seems to have been a result of the release of prostaglandins. The first stress was also blocked by pretreatment with naloxone and phentolamine. Even when enkephalin and noradrenaline were given instead of the first application of stress, because no PFC inhibition resulted it might be considered that enkephalin and noradrenaline participate via the central nervous system in the suppression of PFC by stress. The noradrenergic and opiate reactive nerves in the hypothalamus would seem to be activated by the first application of stress. These nerves in the hypothalamus have been shown to respond to stress (Sternberg et al 1992). Furthermore, the noradrenergic nerve-mediated corticotropin-releasing hormone released from hypothalamic neurons might also be regarded as a possible modulator of stress response (Calogero et al 1988).

PFC counts have been shown to be unaffected by enkephalin and noradrenaline (Fujiwara & Orita 1987; Matsuo et al 1990). Accordingly, when mice were injected with enkephalin and adrenaline 24 h after the first application of high-pressure stress, the PFC count was reduced by adrenaline, but not by enkephalin. It seems that the effect of the second application of stress was a result of adrenaline released from the adrenal gland. The PFC count decreased markedly in mice given prostaglandin E_2 instead of the first application of high-pressure stress or given adrenaline instead of the second application of stress. This implies that the suppression of PFC production is a result of the release of prostaglandin after the first application of stress, and the release of adrenaline after the second application.

Suppressor cells in the spleen and the suppressor factor in the serum appeared 8 h after the second application of stress or injection of adrenaline. Had the presence of the suppressor cells and suppressor factor been elicited by adrenaline, these should have been detected 2 h after the application of stress or after adrenaline treatment. However, in our previous study we demonstrated that adrenaline caused an increase in PFC in-vivo and in-vitro. Moreover, there have been no reports that PFC is reduced by adrenaline. The suppression of PFC might thus be considered to be caused by SRS-A released by adrenaline. The suppression of PFC by two exposures to stress or by stress and adrenaline injection was blocked by the administration of diethylcarbamazine, the agent that blocks leukotriene C_4,D_4 release, before the second application of stress or the injection of adrenaline. Induction of suppressor-cell formation by incubating normal spleen cells with mouse sera after the second application of stress or the injection of adrenaline was blocked by anti-leukotriene C_4,D_4 rabbit serum.

PFC might possibly have been suppressed by two applications of high-pressure stress. Although the first and second stresses were identical, the

response of the mice given the first stress was different from that of the mice given the second stress. We have considered that the change of reactivity after mice were given the first stress might change the expression of receptors against transmitters. Thus, we considered this to be a pre-stressed state. The first exposure to high-pressure stress induced a pre-stressed state in mice via the release of prostaglandin. With the second stress, the pre-stressed mice entered an immunosuppressive state caused by leukotriene C_4,D_4 released by the β-effect of adrenaline released from the adrenal gland. This immunosuppression was because of the activation of suppressor T cells by leukotriene C_4,D_4.

Thymus weight was markedly reduced by two exposures to stress, but not by a single application of stress and injection of prostaglandin or adrenaline, or by injection of prostaglandin and adrenaline. Immune suppression and thymic involution might thus be considered to occur by different mechanisms, thymic involution being possibly attributable to the activation of the pituitary–adrenal axis which releases glucocorticoid (Sternberg et al 1992).

Immune suppression by stress is considered to be elicited by glucocorticoids released from the adrenal cortex. That lymphocyte function and thymus weight were reduced by application of glucocorticoids in-vivo and in-vitro supports this possibility (Cupps & Fauci 1982; Snyder & Unanue 1982; Staruch & Wood 1985; Del Rey et al 1987; Lee et al 1988; Sternberg & Parker 1988). Immune suppression might also occur in response to accidental trauma (Howard & Simmons 1974), surface burns (Miller & Baker 1979) and surgery (Munster 1976; Fujiwara et al 1984), and it was shown here to be because of the effects of glucocorticoids released from the adrenal cortex as a result of the activation of the pituitary–adrenal axis. From these findings immunosuppressive stress might be considered to be of two types—strong stress, that suppresses immune function and reduces thymus weight with a single application, and weak stress, that must be applied more than once. The weak stress induces a pre-stressed condition via the effects of prostaglandin, and suppression of immune function might subsequently result because of the effects of leukotriene C_4,D_4 after the second application of stress. Thus, we suggest there are two classes of immunesuppressive stress—one involves immune suppression by a single application of stress and the other involves immune suppression via the pre-stressed state as a result of two or more applications of stress. Animals including man are continually exposed to various stresses in their environment, and so might be in the pre-stressed state. In this state, change in animal reactivity might be induced, causing them to fall victim to various diseases. Thus, we consider that the receptors for transmitters might be different in pre-stressed animals, and here investigate and report the various changes in receptors in relation to various transmitters in pre-stressed animals (Shizuya et al 1997, 1998).

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