secretion of TSH and GH by cultured anterior pituitary cells. CRF has effects similar to those of sauvagine (*22*) on blood pressure. Mean carotid blood pressure in a urethan-anesthetized rat fell from 87 mmHg to 72 ± 2 mmHg and remained at that level for 30 minutes after injections of 8 μg of CRF per kilogram of body weight; the subsequent administration of 80 μg of CRF per kilogram of body weight lowered mean blood pressure to 42 ± 3 mmHg for more than 2 hours. The doses of CRF required to lower blood pressure are at least 100 times greater than the amounts that stimulate ACTH and β-End secretion.

Another nonmammalian hypotensive peptide, urotensin I, isolated from teleost urohypophysis (*23*) is closely related structurally (*24*) to sauvagine and thus to CRF.

Although physiologic roles for CRF have yet to be established, the high potency and intrinsic activity to stimulate ACTH and β-End secretion and CRF's presence in the hypothalamus would be consistent with this peptide functioning as a regulator of adenohypophyseal corticotropic cells (*25*). Studies of the interactions of this CRF and known modulators of ACTH and β-End secretion such as glucocorticoids, prostaglandins, norepinephrine, and vasopressin may provide insight concerning corticotropic cells' secretory activities under a variety of physiologic and pathophysiologic circumstances. As with other regulatory peptides, this CRF will probably be found to be distributed outside of the hypothalamus and to possess various extra-hypophysiotropic roles. The ability of CRF to release neurotropic substances such as ACTH and β-endorphin as well as its possible direct brain actions lead us to speculate that this peptide might be a key signal in mediating and integrating an organism's endocrine, visceral, and behavioral responses to stress.

WYLIE VALE, JOACHIM SPIESS
CATHERINE RIVIER, JEAN RIVIER
Peptide Biology Laboratory,
Salk Institute for Biological Studies,
La Jolla, California 92037

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Suppression of Immunity by Stress: Effect of a Graded Series of Stressors on Lymphocyte Stimulation in the Rat

Abstract. *In rats a graded series of stressors produced progressively greater suppression of lymphocyte function, as measured by the number of circulating lymphocytes and by phytohemagglutinin stimulation of lymphocytes in whole blood and isolated cultures. This evidence suggests that stress suppresses immunity in proportion to the intensity of the stressor.*

Stress may contribute to the development and course of a range of illnesses by inducing changes in immune function (*1*). Indirect evidence that stress can influence immunity has come from studies of the effect of stressful conditions on infectious processes, neoplasia, and tissue rejection (*2*). There is also recent direct evidence for this phenomenon (*3*). The present study was undertaken to determine the relation between a graded series of stressors and a direct in vitro measure of immune function, mitogen-induced lymphocyte proliferation.

Forty-eight W/Fu rats (Microbiological Associates) were divided into four groups of 12 each: home-cage controls, apparatus controls, low-shock animals, and high-shock animals. Home-cage controls were housed four to a cage and not disturbed during the experimental period. Apparatus controls were placed in the shock apparatus with electrodes attached to their tails (*4*), but no current was applied. The procedure involved mild restraint, since the animal's tail was kept outside the cage by means of a ring taped to the tail. The two shock groups were placed into the apparatus with electrodes attached to their tails. Low-shock

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rats received 2-second shocks at variable intervals averaging one shock per minute. The current intensity was 0.8 mA for the first 8 hours, 1.0 mA for the second 8 hours, and 1.2 mA for the last 4 hours. High-shock animals were shocked on the same schedule but at higher intensity: 1.6 mA for the first 8 hours, 2.4 mA for the second 8 hours, and 3.0 mA for the final 4 hours. These conditions have been shown to elicit a progressively greater response in such indicants of stress as increased plasma steroid levels (*5*), decreased norepinephrine levels (*6*), and increased development of gastric lesions (*7*). All experiments were conducted at the same time of day to control for possible circadian effects. Following the shock period the rats were randomly removed from the apparatus and exsanguinated by heart puncture under ether anesthesia within 3 minutes. Aliquots of blood from each animal were used for each of the in vitro studies.

Total white blood cell and differential counts were performed by standard techniques (*8*). The percentage and absolute number of T cells were determined by the method of Golub (*9*) with the use of theta antibody to W/Fu T cells (*10*). Stimulation of lymphocytes in whole blood was performed by the method of Han and Pauly (*11*) with several modifications: all cultures were prepared in triplicate; RPMI medium 1640 (Gibco Laboratories) supplemented with penicillin and streptomycin and adjusted to a *p*H of 7.2 to 7.3 was used throughout the study; and cultures were prepared with 0.1 ml of whole blood in 1.0 ml of RPMI medium together with 1.0 ml of phytohemagglutinin (PHA) in medium for a final culture volume of 2 ml.

Phytohemagglutinin concentrations of 2.0 and 4.0 μg/ml were used since they yield a 50 and 100 percent maximal response, respectively. Nonstimulated control cultures were incubated with 2 ml of medium alone. All cultures were incubated at 37°C in humidified air with 5 percent CO_2. After 3 days 0.5 μCi of ^{125}IdUrd (5-iododeoxyuridine; specific activity, 2000 Ci/mmole; New England Nuclear) was added to the PHA-treated cultures and to the control cultures. After 6 hours the cultures were washed four times with saline. The total incubation time was 3 days. To remove unincorporated ^{125}IdUrd, the cells were suspended in saline for 12 hours at 23°C and washed with saline; incorporation of the radioisotope was then measured in a gamma counter. This procedure allows for the passive diffusion of unincorporated radioisotope from the red cells to the saline. The lymphocyte counts are means for triplicate cultures. The data used for the statistical analysis (and represented in Figs. 1 and 2) are counts per minute in the stimulated cultures minus counts per minute in the corresponding unstimulated cultures.

We have developed a micromethod for measuring the stimulation of lymphocytes isolated from rat peripheral blood (*12*). Stock Percoll (Pharmacia) was osmotically adjusted with 9 percent saline before being diluted with normal saline. Blood was diluted 1:6 in normal saline, layered on a 70 percent Percoll gradient, and centrifuged for 30 minutes at 400*g*. The lymphocyte layers were removed and washed three times with medium. Lymphocytes were counted and adjusted to a density of 5×10^6 cells per milliliter in medium containing 20 percent heat-inactivated fetal calf serum (Gibco Laboratories). One-tenth of a milliliter of the cell suspension was placed in each well of a flat-bottom microtiter plate (Falcon 3040) containing 0.1 ml of medium or medium plus PHA at final concentrations of 1.0, 2.0, 4.0, and 6.0 μg/ml. All cultures were prepared in triplicate. The cultures were incubated for 2 days at 37°C in a humidified atmosphere containing 5 percent CO_2 and then labeled with 0.2 μCi of ^{125}IdUrd together with 5-fluorodeoxyuridine (Sigma) at a final concentration of $10^{-6}M$. After 18 hours the cells were collected from the wells onto glass fiber filter paper (Reeve Angel, grade 934AH) with a modified cell harvester (Brandel) and washed with saline for 15 seconds and 3 percent acetic acid for 45 seconds (*11*). Incorporation of the radioisotope was then measured in a gamma counter.

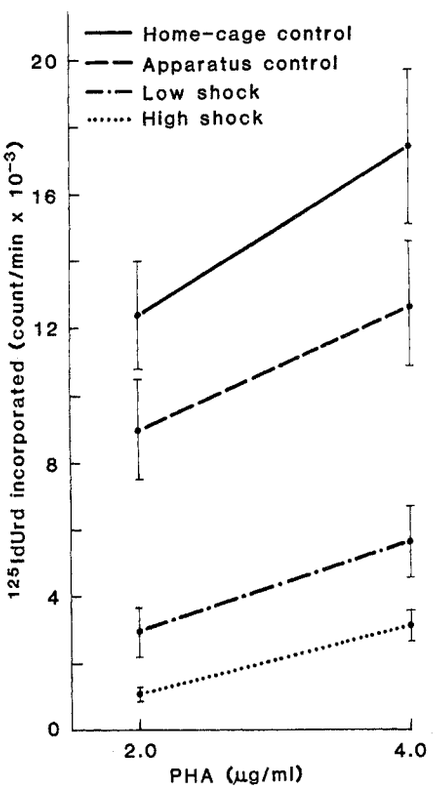

Fig. 1. Stimulation of lymphocytes in whole blood by PHA in the four groups. Data (means ± standard errors) are counts per minute in the stimulated cultures minus counts per minute in the corresponding unstimulated cultures.

In the whole blood assay there was a graduated suppression of lymphocyte stimulation by both doses of PHA in response to graded increases in the intensity of stress (Fig. 1). Placing the animal into the apparatus produced a mild suppression of lymphocyte stimulation compared to that measured in cultures from home-cage controls. Shock greatly increased the suppression, with the response of cells from the high-shock group approaching that observed in cells cultured without PHA. An overall analysis of variance revealed a highly significant difference in lymphocyte stimulation among the four groups [$F(3, 44) = 24.0$, $P < .001$]. There was a significant difference in lymphocyte stimulation between the home-cage control group and each of the shock groups ($P < .05$), between the apparatus control group and each of the shock groups ($P < .05$), and between the shock groups (*13*). The difference between the home-cage and apparatus control groups approached, but did not reach, statistical significance. Incorporation of ^{125}IdUrd by cultures not receiving PHA was as follows (counts per minute): 182.8 ± 26.7 for home-cage controls, 145.8 ± 16.4 for apparatus controls, 159.1 ± 20.0 for low-shock animals, and 156.1 ± 31.7 for high-shock animals. These values are not significantly different from one another.

That stressful conditions led to decreased stimulation of lymphocytes by PHA in whole blood may have been due to decreased numbers of lymphocytes or to altered numbers of other cell types that may influence lymphocyte responses. There were no significant differences in the absolute number of monocytes or polymorphonuclear leukocytes. There was no significant between-group difference in the percentage of T cells; however, a significant lymphocytopenia [$F(3, 43) = 3.86$, $P < .02$] was induced by the stressful conditions (Table 1). The lymphocytopenia may have resulted in a decrease in the number of PHA-reactive lymphocytes in the whole blood cultures.

Adjustment of blood volume in the whole blood cultures to yield specific

cell numbers is not practicable, since it may lead to changes in the ratio of mitogen and medium to cell types, thereby modifying the lymphocyte response. Further studies were, therefore, conducted with a fixed number of isolated lymphocytes. Stressful conditions had a marked effect on PHA-treated lymphocytes in the isolated cell assay (Fig. 2). The response of lymphocytes from the apparatus control group was moderately suppressed, while the response of lymphocytes from the shock groups was suppressed almost to the level observed in unstimulated cultures. An overall analysis of variance revealed a highly significant difference in lymphocyte stimulation among the four groups [$F(3, 44) = 12.2$, $P < .001$]. There was a significant difference ($P < .05$) between the home-cage control group and each shock group, between the apparatus control group and each shock group, and between the control groups. There was no significant difference between the shock groups (*14*).

The unstimulated cultures were also suppressed by stress in the isolated lymphocyte assay. Incorporation of ^{125}IdUrd by these cultures was as follows (count/min): 136.3 ± 13.9 for home-cage controls, 136.6 ± 27.5 for apparatus controls, 63.2 ± 6.9 for low-shock animals, and 89.1 ± 24.0 for high-shock animals. An analysis of variance revealed a significant between-group difference [$F(3, 44) = 3.53$, $P < .03$] (*15*).

It is of note that the two levels of shock produced a graded suppression of lymphocyte stimulation in the whole blood assay but completely suppressed stimulation in the isolated cell assay. This difference may be related to differences in cell numbers, the presence of plasma factors or other cell types in the whole blood cultures, or the additional manipulation of cells during isolation procedures.

The present study also reveals that stressful conditions inhibit spontaneous lymphocyte stimulation, which may be an additional indicator of depressed immune status in vivo. Suppression of unstimulated lymphocytes in the shocked animals was observed in isolated cell but not in whole blood cultures. This may have been due to the fetal calf serum, which was present only in the isolated lymphocyte cultures. Fetal calf serum has mild stimulatory effects (*16*), and processes similar to those involved in stress effects on PHA-induced lymphocyte stimulation may be involved in the suppression of stimulation by fetal calf serum.

Table 1. Peripheral blood lymphocytes in the various groups. Values are means ± standard errors.

Group	Absolute number of lymphocytes (cells per milliliter × 10^{-6})	T lymphocytes (%)
Home-cage control	6.64 ± 0.80	54.09 ± 5.39
Apparatus control	4.84 ± 0.70	58.25 ± 8.33
Low-shock	3.98 ± 1.13	53.41 ± 6.12
High-shock	2.92 ± 0.42	45.09 ± 9.0

Recent studies have demonstrated a balance between inducer and suppressor T cells in the maintenance of immune function (*17*). It is possible that, following stress, functional subpopulations of T lymphocytes are depleted from the circulation, resulting in altered lymphocyte responses. It remains to be determined whether stress, in addition to causing lymphocytopenia, results in a redistribution of subpopulations of T cells, producing a selective decrease in reactive lymphocytes or a functional impairment of the lymphocyte.

The processes that may mediate stressful influences on immune function are unknown. The endocrine system is highly responsive to stress and probably has a significant effect on immune processes. The lymphocytopenia associated with stress has been related to elevated levels of corticosteroids (*18*). Both physiological and pharmacological doses of corticosteroids have been found to diminish lymphocyte stimulation by PHA (*19*). On the other hand, mitogen-induced lymphocyte stimulation has been altered with no changes in corticosteroid levels (*20*). Other hormones (*21*) and neuroregulators (*22*) influence lymphocyte stimulation and may also be involved in the effects of stress on the cell-mediated immune response in the rat.

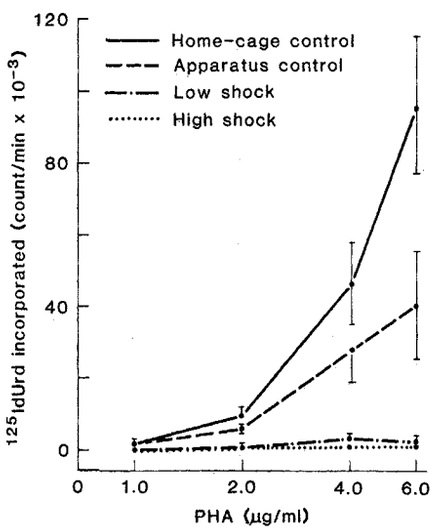

Fig. 2. Stimulation of isolated lymphocytes by PHA in the four groups. Values are as described in the legend to Fig. 1.

STEVEN E. KELLER*
Department of Psychiatry, Mount Sinai School of Medicine, City University of New York, New York 10029

JAY M. WEISS
Rockefeller University, New York 10021

STEVEN J. SCHLEIFER
Department of Psychiatry, Mount Sinai School of Medicine

NEIL E. MILLER
Rockefeller University

MARVIN STEIN
Department of Psychiatry, Mount Sinai School of Medicine

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ods **29**, 133 (1979)]. Lymphocyte viability tested by dye exclusion is > 95 percent.
13. The variances of the home-cage and apparatus control groups were considerably larger than the variances of the shock groups (Fig. 1). Since the estimate of error variance used in making multiple contrasts is derived by averaging the variances in all groups, the larger variance of the control groups would inflate the actual error variance related to the shock groups. This problem was overcome by computing separate analyses of variance contrasting the control groups or the shock groups. The differences in lymphocyte stimulation between the control groups approached significance [$F(1, 22) = 3.94$, $P < .06$], and the low-shock group differed significantly from the high-shock group [$F(1, 22) = 7.27$, $P < .01$].
14. In further studies conducted with the isolated lymphocyte assay, the stressful conditions also suppressed lymphocyte stimulation in PHA-treated cultures incubated for 4 days.
15. In addition to the effects seen with the 3-day incubation, shock also suppressed the unstimulated lymphocyte cultures in the isolated cell assay with a 4-day incubation period [$F(3, 44) = 7.47$, $P < .01$]. Incorporation of ^{125}IdUrd by these cultures was (count/min) 241.3 ± 26.5 for home-cage controls, 149.9 ± 26.5 for apparatus controls, 57.7 ± 5.6 for low-shock animals, and 99.4 ± 18.3 for high-shock animals.
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* Correspondence and requests for reprints should be addressed to S.E.K.

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Preferential Synthesis of the G1m(1) Allotype of IgG1 in the Central Nervous System of Multiple Sclerosis Patients

Abstract. *Quantitations of the G1m(1) and G1m(3) allotypic determinants of human immunoglobulin G were performed by radioimmunoassay on cerebrospinal fluid and serum samples from patients with multiple sclerosis and from patients with other neurological disorders. In multiple sclerosis patients that were heterozygous for these determinants, G1m(1) concentration in the cerebrospinal fluid was greatly increased—reflected by an increased ratio of G1m(1) to G1m(3)—in comparison with that of patients with other neurological disorders. These results suggest that in the heterozygous multiple sclerosis patients, most of the plasma cells in the central nervous system that secrete oligoclonal immunoglobulin G preferentially synthesize G1m(1) IgG1 molecules.*

The *Gm* genes constitute a polycistronic system encoding for either of two allelic γ heavy chains within each immunoglobulin G (IgG) subclass in humans (*1*). Different alleles are present in different ethnic groups. In Caucasians, G1m(1) and G1m(3) are antigenic determinants located on the allelic forms of IgG1 molecules (*2*); homozygotes have either G1m(1) or G1m(3) γ1 chains, whereas heterozygotes have both G1m(1) and G1m(3) γ1 chains. G3m(5) and G3m(21) represent the pseudoallelic forms of IgG3. The Gm^1 and Gm^{21} genes are almost always inherited simultaneously, that is, as a haplotype, as are the Gm^3 and Gm^5 genes. Caucasians are thus either homozygous $Gm^{1;21}/Gm^{1;21}$, homozygous $Gm^{3;5}/Gm^{3;5}$, or heterozygous $Gm^{1;21}/Gm^{3;5}$. In serum from heterozygous individuals, the amounts of the G1m(1) and G1m(3) allotypes of IgG1 are approximately equal (*3*), and each represents about half of the amount of IgG1—either G1m(1) or G1m(3)—present in the corresponding homozygous population. Quantitative variations in G3m(5) and G3m(21) in the different genotypes are more complex (*3*, *4*).

Multiple sclerosis (MS) is a chronic, relapsing, and remitting disease characterized by demyelination in the central nervous system. A well-known feature of MS is an increase of IgG (*5*), particularly IgG1 (*6*, *7*), in the cerebrospinal fluid, resulting from intrathecal synthesis by lymphoplasmocytes in the central nervous system (*8*). The oligoclonal electrophoretic patterns of immunoglobulins from the cerebrospinal fluid or brain tissue of MS patients (*9*) suggest their production by a few B cell clones (*10*). They may originate from a limited number of B cell clones in the central nervous system (*10*). We now report a preferential increase in G1m(1) compared to G1m(3) in the cerebrospinal fluid of MS patients who are heterozygous $Gm^{1;21}/Gm^{3;5}$. This finding is evidence for (i) the oligoclonal character of IgG1 in the cerebrospinal fluid, (ii) the persistent presence of a limited number of (nonsuppressed) B cell clones in the central nervous system, and (iii) the involvement of two unlinked genetic systems in the susceptibility to this disease.

Measurements of G1m(1) and G1m(3) were performed in paired samples of cerebrospinal fluid and serum from patients with MS or other neurological disorders (OND) without demyelinating or infectious disease of the central nervous system (seizure disorder, dementia, ethanol abuse, syncope, cerebrovascular accident, and Parkinson's disease). Probable Gm genotypes were inferred from the Gm phenotypes detected in serum by hemagglutination inhibition on microtiter plates (*11*). Concentrations of G1m(1) and G1m(3) were measured by radioimmunoassay (*4*); the normal values in serum have been published (*4*). Samples of cerebrospinal fluid from healthy donors were not available. The mean content of G1m(1) and G1m(3) and the ratio G1m(1)/G1m(3), if any, were calculated in serum and cerebrospinal fluid for each group (Table 1). The statistically significant differences observed between groups are shown in Table 2.

The serum concentrations of G1m(1) and G1m(3) and the G1m(1)/G1m(3) ratio in Gm homozygous and heterozygous OND patients were not different from those in healthy donors; consequently, the OND group can be considered as a reference population for normal values in the cerebrospinal fluid. The concentrations of G1m(1) or G1m(3) in homozygotes, or of G1m(1) + G1m(3) in heterozygotes, were markedly increased in the cerebrospinal fluid of MS patients as compared with the OND group, in agreement with the previously reported increase of IgG1 in the cerebrospinal fluid of MS patients (*6*, *7*). A modest, not statistically significant, increase in G1m(1) in serum was observed in the $Gm^{1;21}$ homozygous MS population, whereas the $Gm^{3;5}$ homozygous MS population exhibited a mean value of G1m(3) close to that for OND patients and healthy donors.

The most striking quantitative results were obtained in the Gm heterozygous MS population, in which a greatly increased G1m(1) concentration was found in the cerebrospinal fluid, whereas the G1m(3) content was identical to that observed in the OND group. This shift of

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