Psychological Stress-Induced Modulation of Interleukin 2 Receptor Gene Expression and Interleukin 2 Production in Peripheral Blood Leukocytes

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*We explored the expression of the interleukin 2 receptor (IL-2R) and the synthesis of IL-2R messenger RNA by peripheral blood leukocytes obtained from medical students experiencing examination stress in three independent studies. The peripheral blood leukocytes obtained at low-stress baseline periods had significantly higher percentages of IL-2R-positive cells when compared with cells obtained from the same individuals during examinations. In addition, IL2-R messenger RNA in peripheral blood leukocytes decreased significantly during examination periods in a subset of 13 subjects. In one study, we found an increase in the accumulation of interleukin 2 in cultures of cells showing down regulation of IL-2R expression and IL-2R messenger RNA levels. While there are ample data demonstrating stress-associated decrements in the immune response in humans and animals, these data provide the first evidence that this interaction may be observed at the level of gene expression. The data suggest one mechanism whereby the central nervous system modulates the Immune response during psychological stress.

(*Arch Gen Psychiatry*. 1990;47:707-712)

The mechanisms underlying the many possible interactions between the central nervous system (CNS) and the immune system are an area of intense study. It is generally thought that hormones are key mediators of many of these interactions. For example, it is known that glurocorticoids have immunoregulatory activities across a broad spectrum of the cellular immune response. Moreover, glucocorticoids control their own plasma concentrations through the interaction with hypothalamic corticotropin - releasing factor and pituitary corticotropin.[1] In addition, there is now evidence to suggest that there is actually "hard wiring" between the CNS and the immune system as demonstrated by the observation that nerve endings are in direct contact with T lymphocytes in the spleens of rats.[2,3]

The interaction between the CNS and immune systems is not one-way but is functionally a loop with feedback from the immune system to the CNS. For example, interleukin 1, produced by macrophages, has the ability to stimulate hypothalamic activity, promoting corticotropin-releasing factor secretion[4] and corticotropin stimulation, which in turn stimulates the production and secretion of cortisol.[5-8]

Previous studies from our laboratory have explored the mechanisms underlying stress-induced changes in immune function and have shown that the levels of two lymphokines decrease during periods of academic stress. Peripheral blood leukocytes (PBLs) obtained from medical students during examination periods synthesized less interferon gamma (IFN-γ) after stimulation with concanavalin A (ConA) than PBLs from the same individuals taken at a baseline period 1 month before examinations.[9] In addition, leukocyte migration inhibition factor, a lymphokine that is suppressed during recrudescence of herpes simplex virus type 2 infections,[10] was also down regulated in individuals experiencing academic stress.[9]

It is possible that a stress-induced rise in intracellular cyclic adenosine monophosphate (cAMP) levels may be partially responsible for the stress-associated suppression of immune function that we and others have observed.[11-15] For example, it has been shown that cAMP inhibits lymphocyte proliferation, cytotoxic reaction, lymphokine and antibody production, and cell motility.[16] Moreover, it has been shown that stress stimulates catecholamine synthesis, resulting in increased intracellular levels of camp.[17] In the same individuals in whom we observed decreased levels of IFN-y and leukocyte migration inhibition factor, we also found increases in plasma and in intracellular levels of cAMP in PBLs,[9] which is consistent with these previously published data.

Interleukin 2 (IL-2) is a lymphokine that is produced by activated T lymphocytes. Its production is dependent on antigen presentation by macrophages and the subsequent release of interleukin 1 from macrophages that signals T lymphocytes to release IL-2 and concomitantly express IL-2 receptors (IL-2Rs). The IL-2 released by lymphocytes stimulates further proliferation of T lymphocytes bearing IL-2Rs. Both IL-2 production and the expression of the IL-2R can be modulated in vitro by stimulating T lymphocytes with mitogens such as phytobemagglutinin (PHA) or ConA.[18-22]

In this study, we explored the interactions between the CNS and immune system by examining the expression of the IL-2R the level of IL-2R messenger RNA (mRNA), and the produc

Accepted for publication December 7, 1989.
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tion of 11L-2 by PBLs obtained from medical students experiencing a psychological stressor, academic examinations.

SUBJECTS AND METHODS

Subjects end Design

We performed several studies using first-year medical students at The Ohio State University College of Medicine, Columbus, who volunteered for a research project on stress and the immune response. Inducements for participation included feedback on their immunological data, as well as a payment of $8 for each blood sample. The participants in this study gave informed consent after the purpose of the study was fully explained. The medical student curriculum is such that the students have seven or eight 3-day examination blocks during the academic year. Thus, the medical student class as a whole cycles through these examination periods, and they are aware of all examination dates at the beginning of the academic year. In each of the examination periods reported, the students had a day of examinations on Wednesday, a day to study on Thursday, and examinations again on Friday; all blood samples were obtained at midday on Friday. Thus, the blood samples were drawn near the end of the 3-day examination sequence.

The average age of the medical students in each of the three classes was 22 years. There were 22 men in study 1, 16 men and 9 women in study 2, and 31 men and 13 women in study 3. There were no significant differences in the immunological data as a function of gender.

Blood samples were obtained at two low-stress baseline periods (January and April) and two higher-stress examination periods (February and May) for study 1. For the second study, performed 1 year later, data were collected at a baseline (April) and an examination period 1 month later (Mays The following year, data were collected from a third group at baseline (September) and during an examination period (October; study 3).

The decision to study examination stress in medical students was based both on prior immunological and psychological data from studies in our laboratory, and on data from a number of other laboratories. For example, there are several studies showing a variety of endocrine and other changes in medical students during examinations, including changes in plasma catecholamines and lipoproteins.[23,24]

Self-report Data

The Brief Symptom Inventory (BSI) provided data on changes in distress from baseline to examination.[25] The Anxiety scale was used because it is the most responsive scale for assessing short-term examination distress. Each of the 53 BSI items was rated on a five-point scale from 0 ("not at all") to 4 ("extremely"), based on the amount of associated discomfort during the previous week.

Students also completed the survey form of the Perceived Stress Scale' at baseline and during examinations. The Perceived Stress Scale is designed to assess the degree to which individuals describe their lives as currently stressful; subjects rate the extent to which they have felt each item is true for them from 0 to 4 (very often) in the last week, with items that assess how unpredictable, uncontrollable, and overloaded respondents find their lives. While there is some overlap between the Perceived Stress Scale and measures of psychological distress such as the BSI, several studies that have assessed psychological symptoms and partialed out its effects still show that the Perceived Stress Scale is a good independent predictor of physical symptoms and health service utilization.[35]

Students were asked a number of health behavior questions each time blood was drawn. They were asked to indicate how many hours of sleep they had had in the last 3 days. any change in their weight in the last week, and how many alcoholic drinks they had had in the last 48 hours as well as in the last week. They were also asked to list any medications taken in the last week, either prescription or nonprescription.

Health data were collected using the Health Review developed by Rose et al.[27] The Health Review provides a checklist of specific illness symptoms related to infectious disease. Subjects were asked to indicate which symptoms occurred as part of an illness episode, as isolated symptoms, or as more chronic problems, with operational definitions provided for these categories. Subjects were also asked whether they saw a physician for the problem(s) and how many days they reduced their activity because of each health change. The Health Review is reviewed by our project nurse, who uses preestablished criteria to decide if the subject has had an infectious illness. When necessary, follow-up telephone calls are made to subjects to collect additional information. Students were also asked to describe any chronic health problems.

Measurement of IL 2R Expression

We examined the expression of the IL-2R on the surface of mitogen-stimulated PBLs after separation of mononuclear cells from whole blood using standard procedures.' The PBLs were stimulated with either 5 wg/mL of ConA (Sigma Chemical Co, St Louis, Mo) for 72 hours (study 1) or with 5 p g/mL of PHA (Sigma Chemical Co) for 96 hours (studies 2 and 3). By fluorescent flow cytometry, the percentage of immunofluorescent (IF)-positive unstimulated cells (1×10^6) was subtracted from the percentage of IF-positive PBLs (in study 1). In general, resting T lymphocytes do not express IL-2R..[28] The TAC monoclonal antibodt(MAb) was used to detect IIr2Rs (study 1) using the indirect IF test.[29] This MAb is directed to the IIr2at (55-kd. CD25) subunit of the IL-2R.[30] In later experiments (studies 2 and 3), 1×10^6 PBLs were stimulated with 5 lLg/mL of PHA for 96 hours; the IL-2R1 MAb, isotype IgG2a, fluorescein isothiocyanate conjugate (Coulter Immunology, Hialeah, Fla), was used in the direct IF test[31] to detect IL-2Rs; an isotype antibody, mouse IgG2a fluorescenn isothiocyanate conjugate (Coulter), was used as the control. The percentage of IF-positive (IL-2R-positive) cells was determined using a fluorescence-activated cell sorter (Ortho, Raritan, NJ, or Coulter Epics C).

Measurement of IL-2 mRNA Levels in PBLs

Peripheral blood leukocytes were obtained at baseline (September) and from an examination blood sample taken 1 month later (October) from 13 students, 4 men and 9 women (study3); cytoplasmic RNA was extracted from unstimulated PBLs (0 hours) and ConA-stimulated PBLs (48 hours). A DNA probe to the IL-2R (Oncor Inc, Gaithersburg, Md) containing the fourth exon of the IL-211a gene was radiolabeled with phosphorus 32 by random primer labeling.

Briefly, 6×10^6 cells were stimulated with 5 µg/mL of ConA. Cytoplasmic RNA was isolated by the procedure of Maniatis et al.[32] Cytoplasmic RNA was dissolved in sterile distilled water and the quantity of RNA was determined measuring the optical density at 260 run. Two micrograms of RNA in 50 WL of water was denatured by the addition of 20µL of 37% formaldehyde and 30 wL of 20 x standard saline citrate (1 x SSC is 0.15 mol/L of sodium chloride, 15 mmol/L of sodium citrate, pH 7.0). Samples were incubated at 65°C for 15 minutes and applied immediately to nitrocellulose membranes using a slot blotting manifold (Schleicher and Schuell Inc, Keene, NH). Baseline and examination (stress) samples were applied to the same nitrocellulose membrane. Ribonucleic acid was fixed to the membrane by heating at 80'C in a vacuum oven. Membranes were prehybridized at 42°C for 16 hours in buffer containing 50% formanude, 5 x Denhait's solution, 5 x SSPE (1 x SSPE is 0.18 mol/L of sodium chloride, 10 mmol/L of sodium phosphate, pH 7.7, 1 mmol/L of edetic acid [EDTAJ), 0.10% sodium dodecyl sulfate (wt/vol), and 200 mg/L of heat-denatured herring sperm DNA. Membranes were hybridized at 42°C for 16 hours in the above buffer containing 10% dextran sulfate and 1×10^6 cpm/mL of radiolabeled DNA probe. The filters were washed under high-stringency conditions using 0.20 x SSPE, 0.50% sodium dodecyl sulfate at 42°C. Membranes were exposed to x-ray film for 3 to 4 days at -80°C. Relative intensity was determined by densitometric scanning (Biorad model 620, BiorRd, Richmond, Calif) and calculated as peak height (optical density) x peak width (millimeters). All samples were run in duplicate on the same day and on the same filter.

Measurement of IL-2 Synthesis In PBL Culture Supernatants

The PBLs obtained from medical students in study 3 were induced in vitro with PHA to produce IL-2. Briefly, $3x\ 10^6$ PBLs were cultured in 1 ml, of RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum, 50 Wg/mL of gentamicin sulfate, and PHA at a concentration of 5 Wg/mL for 48 hours at 37°C. Culture supernatants were removed and stored at -20°C until the time of assay for IL-2.

Levels of IL-2 in the culture supernatants were quantitated by determining the proliferative capacity of the 1L-2-dependent cell line, CTLL-20, in the presence of the culture supernatants. The

CTLL-20 cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 25 gg/mL of streptomycin sulfate, 25 U/mL of penicillin, 2 mmol/L of glutamine, 0.225% (wt/vol) sodium bicarbonate, 20 mmol/L of HEPES buffer, and 2 x 10^{-6} moVL of]3-mercaptoethanol plus a 59E (vol/vol) concentration of a cell supernatant from ConA-stimulated rat splenocytes. This supernatant contained approximately 6000 U of IL-2 per milliliter along with other undefined factors that are necessary for the long-term maintenance of CTLL-20 cells. The CTLL-20 cells were washed free of residual IIr2 and resuspended in complete RPMI 1640 medium at a concentration of 1 x 10^6 cells per milliliter. Into each of three wells of a 96-well U-bottomed plate (Nunclon Genzyme, Kneeland, Mass) was placed 5 x 10^4 CTLL-20 cells in a volume of 50 µL. Into each of these wells either 50 µL of the supernatants from the PHA-stimulated PBL cultures or 50 µL of a dilution of a recombinant IL-2 standard (Genzyme) was added. To each well an additional 100 µL of complete RPMI 1640 medium was added. Tb determine the specificity of IL-2-dependent proliferation, 100 µL of a dilution of the anti-IL-2R MAb PC 61.5.3[33] cell line was added in place of complete medium. The cells were incubated at 37°C in 5% carbon dioxide for 20 hours, at which time 1 µCi of tritiated thymidine (specific activity, 6.7 Ci/mmol per liter, ICN, Costa Mesa, Calif) was added in a volume of 50 µL. The cultures were incubated for an additional 4 hours, and the cells were then collected on glass fiber filter disks using an automatic cell harvester (Skatron, Sterling, Va). Cellular incorporation of tritium was determined by liquid scintillation analysis.

The proliferative capacity of the CTLL-20 cells in the presence of the recombinant IL-2 was used to generate a standard curve, which in turn was used to determine the levels of IL-2 in the PHA-stimulated PBL culture supernatants based on their ability to support the proliferation of the CTLL-20 cells. The standard curve, along with its corresponding third-order polynomial equation (R =1.00). was computer generated (Cricket Graph, Cricket Software Inc, Malvern, Pa).

Nutrition

Because poor nutrition can have adverse affects on immunity,[34] we measured serum albumin levels to provide an objective assessment of nutritional adequacy, since serum proteins such as albumin tend to decrease in protein-energy malnutrition. The procedure used to measure albumin was a bromcresol green dye-binding method as adapted by Doumas et al.' It is the method of choice because of its specificity.

RESULTS

Self-report Data

Consistent with our interpretation of the examination periods as higher-stress periods, scores on both the BSI Anxiety scale and the Perceived Stress Scale increased during examinations across these studies. The data were analyzed using repeated-measures analysis of variance with one within-subjects variable, change from baseline to examinations. Data for study 1 have been previously published.' In study 2, BSI Anxiety T scores increased from a mean (±-SD) of 50.12 ± 8.95 during April to 57.44 ± 11.98 during examinations in May (F[1,24]=14.62, P<.001); during the same interval, scores on the Perceived Stress Scale increased from a mean of 3.3l•_2.69 to 4.20±2.80 (F[1,24]=4.80, P<.05). Similarly, the values for the Perceived Stress Scale in study 3 at the beginning of the academic year were 4.98 ±-2.74 at baseline and 6.14 ±2.62 during the students' first set of examinations (F[1,43]=44.76, P<.001 t The BSI was not administered in study 3.

The correlations between these self-report stress measures and the immunological values were small and nonsignificant. This is not surprising since we have an acute stressor, relatively small samples in each case, and relatively small ranges for stress responses in each case, leading to the truncation of range that lowers correlations. In addition, however, the correlations between physiological responses and self-reports during acute strewors are low in most related literature.[36] Correlations between measures of autonomic arousal. such as heart rate or skin conductances. and simultaneous self-reports of anxiety or stress are generally around .30 or lower[-36] The immunology literature suggests that the changes we are measuring probably occur over the course of several days to a week or more. Thus, we would be surprised to find that there were substantial, large, and robust correlations, when literature that looks at much more immediate arousal typically finds low correlations.

Table 1.—Changes in Percent IL-2R–Positive Peripheral Blood Leukocytes Obtained From Medical Students During Low- and High-Stress Periods*

Sample	Period	Change, Mean ± SD	F	df	P
Study 1	Baseline (January)	33.71 ± 12.82	17.89	1,18	.001
	Examinations (February)	14.44 ± 19.64			
	Baseline (April)	18.61 ± 11.91	5.76	1,21	.05
	Examinations (May)	12.31 ± 7.88			
Study 2	Baseline (April)	75.96 ± 10.45	28.19	1,24	.001
	Examinations (June)	54.63 ± 18.79			
Study 3	Baseline (September)	47.63 ± 14.63	6.53	1,21	.02
	Examinations (October)	34.10 ± 19.54			

*For explanation of studies 1 through 3, see "Subjects and Methods" section. IL-2R indicates interleukin 2 receptor.

Students regularly reported less sleep in the 3 days before an examination blood draw than they had experienced before the low stress baseline period. However, the differences between the two periods tended to be relatively small, in part because the examinations were scheduled for Wednesday and Friday, with Thursday as a day off when students typically got additional sleep. In study 1, the students reported a mean (±SD) of 22.12±1.88 hours of sleep in January, compared with 18.92±5.81 hours during the February examinations (F[1,18]= 23.94, P<.001); means (±SDs) for the next pair were 23.57±0.43 hours in April and 20.17±3.83 hours in May (F[1,21]=16.28, P<.001). The mean (t SD) sleep time in study 2 at baseline was 23.08 ± 1.63 hours, compared with 20.36±3.12 hours in the 3 days before examinations (F[1,24]=13.83, P<.001). In study 3, the average hours of sleep at baseline was 21.6 ± 2.71 hours compared with 17.84±4.84 hours during examinations (F[1,43]=26.11, P<.0001~ Correlations between amount of sleep and immunological values during examinations were nonsignifibant, ranging from r= -.15 with 11-2 in study 3 to r=.24 for IL-211 in the same study.

No subject reported any chronic health problems that were likely to have an endocrinological or immunological component other than allergies. Two subjects reported symptoms consistent with upper respiratory tract infections during examination periods in study 1, while one subject reported upper respiratory tract infection symptoms in study 3. Deletion of their data did not alter any of the significant differences reported across the various assays. No medications were reported that are known to have immunological consequences, other than birth control pills, reported by several women; however, their use was constant across samples. Minimal alcohol use was reported, with a maximum of eight drinks reported by any subject in the week before any blood sample, with average alcohol intake of one to three drinks per week.

Expression of IL-2R on the Surface of PBL9

Blood samples were obtained from subjects at two low-stress baseline periods (January and April) and two higher-stress examination periods (February and May; study 1) and were examined for the expression of the IIr2R on the surface of ConA-stimulated PBLs, using the TAC MAb. Peripheral blood leukocytes obtained from the low-stress baseline blood samples had higher percentages of IL 2R-positive cells when compared with cells obtained from the same individuals during the subsequent examination periods, as shown in Table 1. For the January-February pair, 15 of the 19 students showed a decrease from baseline to examinations, while only 3 showed an increase. For the April-May pair, 14 decreased while 8 increased.

We repeated the experiment with a different group of medical students, obtaining PBLs at a baseline (April) and an examination period approximately 1 month later (May; study 2) the following year. To confirm the results obtained in study 1, we used a different mitogen, PHA, and a different MAb to the 11,2a subunit of the IL-2R (Coulter, IL-2R1). We again found a significant decrease in the percentage of PBLs expressing the IL-2R during examinations as compared with the baseline period (F[1,24] = 28.19, P<.001; Table 1); 22 students showed a decrease from baseline to examination, while only 3 showed an increase.

Table 2.—Changes in Levels of mRNA to IL-2R in Peripheral Blood Leukocytes Obtained From Medical Students During Low- and High-Stress Periods*

Sample	Relative Intensity, Mean ± SD	
	0 h	48 h
Baseline (September)	0.216 ± 0.02	0.490 ± 0.51
Examinations (October)	0.077 ± 0.09	0.099 ± 0.14

*mRNA indicates messenger RNA; IL-2R, interleukin 2 receptor. For explanation of methods, see "Subjects and Methods" section. $F(1,12) = 4.53$, $P<.05$. The increase in signal of IL-2 mRNA from 0 to 48 hours for both samples was significant, $P<.005$.

Table 3.—Changes in IL-2 Levels in Supernatants of Peripheral Blood Leukocytes Obtained From Medical Students During Low- and High-Stress Periods*

Sample	Mean ± SD Level, U/mL
Baseline (September)	56.60 ± 40.67
Examinations (October)	101.37 ± 47.86

*IL-2 indicates interleukin 2. For explanation of methods, see "Subjects and Methods" section. $F(1,43) = 22.36$, $P<.001$.

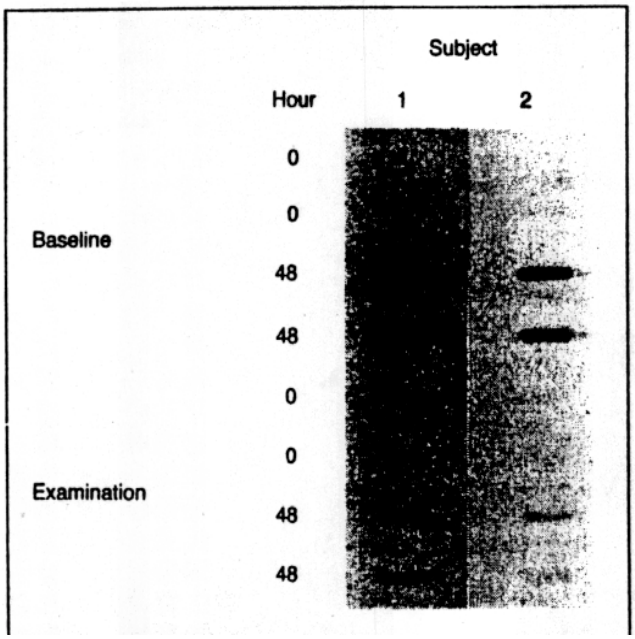

Fig 1.-Relative intensity of interleukin 2 receptor messenger RNA (IL-2R mRNA) in peripheral blood leukocytes (PBLs) from two representa tive medical students (1 and 2). Unstimulated PBLs (0 hours) and mitogen-stimulated PBLs (48 hours) were assayed in duplicate for the expression of IL-2R mRNA using the DNA probe as described in the "Subjects and Methods' section.

We then performed a third study the following year with PBLs obtained from a third medical student class in September (baseline) and October (examination). Of the 44 students for whom data were collected for IL-2R, 22 subjects had data for both IL-2 and IL-2R, and 6 subjects had data for 11-2,11,2R, and mRNA to IL-2R. The lesser numbers for the latter assays were a function of insufficient cell numbers and the labor-intensive nature of the mRNA slot blot hybridization assay, which limited the number of assays that could be run. The expression of the IL-2R on PBLs stimulated with PHA obtained from the examination blood sample was once again suppressed ($F[1,21]=6.53$, $P<.02$; Table 1), confirming the data obtained in studies land 2, with 15 students of the 22 showing a decrease during examinations.

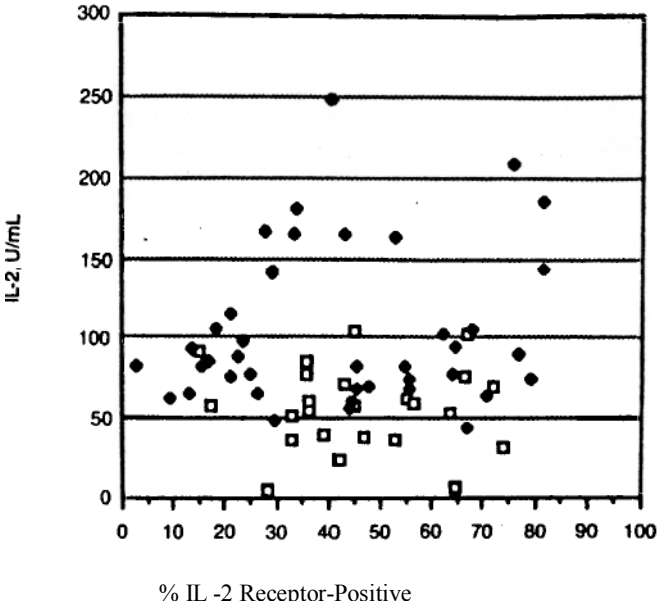

Fig 2.-The level of interleukin 2 (IL-2) in phytohemagglutinin-stimulated peripheral blood leukocyte cell cultures as a function of the percent IL -2 receptor-positive cells at baseline (open squares) and examination (dosed diamonds).

Expression of 11r2R mRNA In

In preliminary experiments, the time of maximal IL-2R mRNA levels following ConA stimulation was determined by incubating PBLs with 5 p.g/mL of ConA for 0, 24, 48, and 72 hours. The IL-2R mRNA levels were determined by slot blot hybridization with a DNA probe to the IL-2Rα gene and quantitated by densitometer scanning using PBLs from a subset of 13 students selected at random. Maximal levels of IL-2R mRNA were observed 48 hours after ConA stimula tion. To confirm specificity, Northern blots were also performed. The IL-2R probe hybridized with mRNA of 3.5 and 1.5 kb, consistent with previous reports (data not shown)." Using slot blot hybridization to measure IL-2R mRNA levels in PBLs from medical students, we found very low levels of mRNA to the IL-2R in resting PBLs (0 hours)_ However, there was a significant increase in the levels of IL2R mRNA 48 hours after stimulation with ConA when IL-2Rs were expressed ($F[1,12] =11.51$, $P<.005$; Table 2, Fig 1).

When IL-2R mRNA levels in PBLs obtained from baseline blood samples were compared with IL-2R mRNA levels in PBLs obtained at the time of examinations (study 3), we found a significant decrease in the relative intensity of the signal to IL-2R mRNA in the PBLs from the examination samples ($F[1,12]=4.53$, $P<.05$). There was also a significant interaction between these variables ($F[1,12] =8.45$, $P<.01$), with a greater response to stimulation with mitogen at baseline than during examinations; of the 13 subjects, 10 showed this change. Although this study involved a small subset of the students in study 3, the decreased 11,2 mRNA levels in PBLs from examination samples is significant and consistent with the decreased cell surface expression of the IL-2R in examination samples.

IL-2 Synthesis by Mitogen-Stimulated PBLs (Study 9)

The ability of PBLs to produce IL-2 in response to in vitro mit:ogen ic stimulation with PHA was determined. The PBLs from medical students undergoing examinations were able to produce a significant ly higher level of IIr2 as compared with those PBLs obtained from students during the September baseline period ($F[1,43]=22.36$, $P<.001$) (Table 3). Examination of IL-2 production revealed that 37 of the 44 students showed an increase in 11,2 production between the baseline and examination periods. Of the remaining 7 students, 5 showed a decrease in IL-2, while the levels of IL-2 produced by the other 2 students remained unchanged. The level of IL-2 activity in the PBL cultures was independent of the percent of IL-2R-positive cells (Fig 2). These results provide evidence that the general increase in IL-2 production between the baseline and examination periods was not due to differing culture conditions between these two periods for the stimulation of PBLs. In addition, among the students whose

PBLs showed increases in IL-2 production between the two blood samples (baseline and examination), those whose PBLs produced relatively high levels of IL-2 at the baseline period were still capable of showing an enhancement of 11,2 production despite their relative ly high background levels at the baseline period.

Although CTLI,20 cell proliferation is dependent on the presence of IL-2, another lymphokine, interleukin 4 (IL-4), has also been shown to induce proliferation of CTLL-20 cells. To determine if the proliferation in response to the PBL culture supernatants was due entirely to IL-2, the MAb PC 61.5.3 was employed. This rat MAb recognizes and binds to a determinant on the IL-2R that is distal to the actual 11,2 binding site but still is able to block both the high- and low amity IL-2R, most likely by changing the configuration of the IL-2R." Although the antibody was unable to provide total inhibition of CTLL-20 cell proliferation in the presence of recombinant IL-2, especially at high concentrations of IL-2, the percentage of the inhibi tion provided by the antibody was similar to that observed in CTLL 20 cultures containing recombinant IL-2. In addition, significant amounts (2000 U) of recombinant IL-4 have been shown to be unable to stimulate significant proliferation of the CTLL-20 cells (John Sheridan, PhD, oral communication, October 12, 1989). Thus, the levels of IL-2 measured under our conditions represent actual IL-2 levels in the cell supernatants rather than the sum of multiple lym phokines that can stimulate CTLL-20 cell proliferation.

Nutrition

All values from the nutritional assays were within normal limits for all subjects at each data point, suggesting that nutritional deficits were not a factor in the observed cellular changes. While it is possible that there were more acute changes in nutrition that were not reflect ed by albumin since its half-life is 2 to 3 weeks, we have consistently failed to find nutrition-related differences in our series of medical student studies using plasma protein markers with relatively shorter half-lives, such as total iron-binding protein, retinol-binding protein, and transferrin.[9,14,15] Consistent with this finding was the absence of a significant change in students' weight from baseline to examinations across studies; students typically reported slight weight gains before examinations.

COMMENT

The interaction among the CNS, the endocrine system, and the immune system is an area of increasing interest. It is well established that different kinds of psychological stressors have an impact on this interaction, resulting in a suppression of certain aspects of the immune response. The nature of the interactions among the CNS, endocrine, and immune systems is just beginning to be understood.[39] The impact of psychologi cal stress on these interactions in both animals and humans is also being explored.[40,41]

We studied the interactions between the CNS and the immune system by examining the effect of academic stress on the expression of the IL-2R and, in one study, the steady state level of IL-2R mRNA and the production of IL-2 by PBLs obtained from medical students during examinations and at a baseline period approximately 1 month before exami nations. Using two different MAbs to the IL-2R and PBLs stimulated with two different mitogens, we found that PBLs obtained at low stress baseline periods in three different studies had significantly higher percentages of IL-2R-posi tive PBLs when compared with similar cells obtained from the same individuals during examinations. In addition, there was a significant decrease in the level of IL-2R mRNA in PBLs obtained during the examination period. These func tional changes were not due to changes in the percentage of T3+ T lymphocyte, or the percentage of T4+ helper-inducer or T8+ cytotoxic-suppressor cells, since the percentage of these populations did not change significantly from baseline to ex amination. These data are consistent with previous studies from our laboratory using a similar paradigm that have dem onstrated significant changes in some aspect of the cellular immune response associated with academic stress.[9,11,13-15]

The data suggest that the inhibition of the expression of the IL-211 on the surface of these PBLs was due to a decrease in mRNA levels to the IL-2R, possibly as a result of the down regulation of gene expression for that product or changes in the stability or degradation of the mRNA. Whether further inhibition or modification of the protein composing the IL-2R takes place posttranslationally is not known.

The accumulation of IL-2 in the supernatants of the same cultures assayed in study 3 was measured; IL-2 bioassays were performed both with and without antibody to IL-2 to confirm that we were specifically measuring IL-2 and not some other lymphokine, eg, IL-4, in the supernatants. There was a significantly higher level of IL-2 in the culture supernatants of the PBLs obtained from the examination blood sam ple as compared with PB Ls obtained from the baseline control sample.

Since the IL-2R was expressed, we know that sufficient IL 2 was synthesized to stimulate polyclonal activation in re sponse to the mitogen. It is not clear from these data, howev er, if the synthesis of IL-2 was affected in any way, and further studies will be needed to determine whether this is the case.

Our observation that the physiological changes associated with academic stress inhibit IL-2R expression while allowing accumulation of IL-2 in culture supernatants, however, is consistent with IL-2 and IL-2R synthesis being controlled by different regulatory cascades. Triggering of T lymphocytes through the Tell receptor complex gives rise to the intracel lular second messengers inositol 1,4,5-triphosphate and dia-cylglycerol.[42,43] Diacylglycerol activates protein kinase C[44] and 1,4,5-triphosphate increases intracellular Ca^{2+} levels.[45] Stud ies with the pharmacological agents 12-0-tetradecanoylphorbol-13-acetate, which activates protein kinase C, and ionomycin, a Ca^{2+} ionophore, have shown that both signals are required to induce IL-2 mRNA synthesis.[46] In contrast, pro tein kinase C activation by 12-0-tetradecanoyl-phorbol-13 acetate is sufficient for IL-2R expression. Aune and Pogue[47] have also shown that human antigen nonspecific CD8+ T-cell lines, which inhibit proliferation of CD4' T cells by pokeweed mitogen, inhibit IL-2R expression on the surface of CD4+ T cells but have no effect on the secretion of 11,2 by these cells.

Interleukin 2 is involved in the regulation of IL-2R expres-sion, since studies have shown that addition of IL-2 to mito gen-stimulated PBLs augments IL-2R expression.[48,49] The fact that we have found reduced IL-2R expression in the presence of increased accumulation of IL-2 suggests that IL 2-induced up regulation of IL-2R expression is not sufficient to overcome the stress-induced inhibition of IL-2R expression.

Since hormones such as glucocorticoids that are frequently elevated during periods of psychological stress have been shown to regulate IL-2 and IFN-y gene expression, the inter-action of these hormones may be part of the mechanism whereby psychological stress mediated through the CNS-endocrine-immune axis suppresses cellular immunity. Gluco-corticoids can bind to cellular DNA as a hormone-receptor complex controlling gene expression.[50] Data suggest that dexamethasone can completely inhibit mitogen-induced IL-2 and IFN-y synthesis by T lymphocytes[22] but only inhibits IL 2R expression by 5096." Since addition of IL-2 blocks the inhibition of IL-2R expression by dexamethasone,[22,51] it is likely that the effect of dexamethasone on IL-2R expression is the result of inhibition of IL-2 synthesis and the resulting absence of up regulation of IL-2R expression by IL-2. As previously mentioned, we have found an inhibition of IFN--i synthesis in earlier academic stress studies with a similar paradigm.' It is possible that the effects of academic stress on IFN-y synthesis observed may be due to the interaction of

glucocorticoids with T lymphocytes, and studies are under way to explore this possibility. However, the observation in the current study that academic stress inhibits IL-2R expression is inconsistent with what is thought to be the role of glucocorticoids in mediating the down regulation of cellular immunity, suggesting that other peptides and/or steroid hormones may be participating in IL-2R/IIr2 regulation in periods of psychological stress. Further studies are needed to examine this question.

This study was supported in part by grants MH 40787, MH 18831, and MH 44660 from the National Institute of Mental Health. Bethesda. Md. and by Ohio State University Comprehensive Cancer Center grant CA 16058 from the National Cancer Institute, National Institutes of Health, Bethesda.

The PC 61.5.3 cell line was kindly provided by Stephen R. Jennings, PhD, Louisiana State University Medical Center, Shreveport. Tom Waldmann, PhD, National Institutes of Health, provided the TAC MAb.

We thank Harry Laufman, Jane Holliday, Mark Kotur, Lynn Castle, Aimee Cowans, Dirk Gottman, and Cathie Atkinson for excellent technical assistance, and the CRC nursing staff for phlebotomy assistance. We thank William Malarkey, John Sheridan, and Caroline Whitacre for reviewing the data We thank the medical students of the classes of 1989. 1990. and 1991 for their enthusiastic participation that made these studies possible.

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