

Hypnosis, Differential Expression of Cytokines by T-Cell Subsets, and the Hypothalamo-Pituitary-Adrenal Axis

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This investigation tested the hypothesis that hypnosis can differentially modulate T-cell subsets, and that this effect is mediated by changes in hypothalamo-pituitary-adrenal (HPA) mediators. Seven healthy, highly hypnotizable volunteers participated in three one-day sessions, a baseline and two intervention sessions. Hypnosis intervention entailed a standardized induction, suggestions for ego strengthening and optimally balanced functioning of the immune and neuroendocrine systems, and post-hypnotic suggestions for stress management and continued optimal balance of bodily systems. Blood samples were drawn at five time points between 8:00 a.m. and 3:00 p.m. and were analyzed for T-cell activation and intracellular cytokine expression (Interferon (IFN)- γ , Interleukin-2, Interleukin-4,) and HPA axis mediators (ACTH, cortisol, and β -endorphin). Following hypnosis intervention, statistically significant immunological effects were noted. Specifically, the proportion of T-cells expressing IFN- γ ($p=.0001$) and IL-2 ($p=.013$) were lower after hypnosis. T-cell activation response to polyclonal stimulation was positively correlated with ACTH ($p=.01$) and β -endorphin ($p=.001$) while IFN- γ expression was correlated with levels of cortisol ($p<.001$). Further controlled studies utilizing hypnosis with patients in treatment are warranted in order to examine whether an altered T-cell response can be replicated in the presence of disease.

Keywords: Cytokines, T-cell, hypnosis, hypothalamo-pituitary-adrenal axis, immunity, neuro-endocrine, psychoneuroimmunology

Introduction

T-lymphocytes are a central component of the adaptive immune response, since these cells have a myriad of functions, including antigen recognition, regulation of antibody and cell-mediated immunity, as well as effector functions, e.g. killing of virally-infected or neoplastic target cells. These functions are essential for maintenance of immunological homeostasis. Moreover, T-cells have been implicated in the

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pathogenesis of a variety of autoimmune and infectious diseases including rheumatoid arthritis, diabetes mellitus, AIDS, tuberculosis, and periodontitis. Consequently, the ability to modulate the T-cell response will be of interest in the maintenance of health as well as the treatment of disease states.

A variety of pharmacologic (Keown, 1998; Levitsky, 2000; Simmons et al., 2001; Watschinger, Wenter, & Demetriou, 2000), as well as non-pharmacologic (Bongartz, Lyncker, & Kossman, 1987; Gruber, Hall, Hersh, & Dubois, 1988; Gruber et al., 1993; Hall, 1982-83; Hall, Mumma, Longo, & Dixon, 1992; Rider & Achterberg, 1989; Ruzyla Smith, Barabasz, Barabasz, & Warner, 1995; Taylor, 1995; Teshima, Sogawa, Mizobe, Kuroki, & Nakagawa, 1991; Zachariae et al., 1994) approaches to modulate the T-cell response have been reported in the literature. Exploration of the effects of hypnosis or closely related interventions (e.g., relaxation with imagery) on T-cells has been limited, with contradicting findings at times. Three studies suggest that hypnosis may facilitate an increase in T-cells or total lymphocytes; two studies provide evidence of a decrease in lymphocytes; and one suggests differential modulations of T-cell subsets. Additionally, the role of hypnotizability has also been determined to be an important factor in some studies.

Hypnosis has evidenced significantly elevated total lymphocyte counts (Hall, 1982-83) and also significantly increased total number of T-cells in high but not low hypnotizable subjects (Ruzyla Smith et al., 1995). Further, a combination of hypnosis, progressive muscle relaxation, biofeedback relaxation, and meditation has been demonstrated to induce an increase in T-cells in HIV-positive subjects with low T-cell counts (Taylor, 1995). Hypnosis has also demonstrated significantly increased numbers of CD4 cells in both high and low hypnotizable subjects compared with control subjects who were exposed to relaxation and suggestion or suggestion only (Ruzyla Smith et al., 1995). Thus hypnosis appears to be associated with a specific immune response in comparison with relaxation and suggestion.

On the other hand, significant decreases in lymphocytes in peripheral blood following hypnosis have also been reported for high hypnotizable subjects (Bongartz et al., 1987) with similar significant reductions in lymphocytes noted following a combination of progressive muscle relaxation and music assisted imagery specific to lymphocytes (Rider & Achterberg, 1989). The decrease in lymphocytes was hypothesized to reflect increased lymphocyte migration into tissues and thus their unavailability to be counted in the peripheral blood. Lastly, a combination of relaxation therapy, specific somatic-oriented imagery, and small doses of immunosuppressant drugs was found to significantly increase the percentage of CD8 cells, decrease the CD4/CD8 ratio, and decrease the CD45RO positive cells (active/memory T-cells) in patients with *Alopecia universalis*. Patients receiving immunotherapy by itself were found only to have a significant increase in the percentage of CD8 cells (Teshima et al., 1991).

The functional responses of lymphocytes when stimulated *in vitro* have also been shown to be affected by imagery or relaxation interventions, again with mixed results. The blastogenic response to one mitogen increased significantly following the induction of a relaxation and imagery intervention (Hall et al., 1992). Similar significant increases in responsiveness to two mitogens have been shown following protocols consisting of modified progressive muscle relaxation, guided imagery and biofeedback

with cancer patients (Gruber et al., 1988; Gruber et al., 1993). On the other hand, both increases and decreases in the proliferation response of lymphocytes to mitogens following imagery and relaxation interventions have been reported. There was a greater tendency toward a decrease in the response, which was significantly greater in high compared to low hypnotizable subjects (Zachariae et al., 1994).

Traditionally, T-cells have been classified into two subsets of cells, based on the expression of CD4 and CD8 cell surface molecules. Initial attempts to attribute function to these T-cell subsets characterized the CD4 subset as T helper and the CD8 as suppressor and/or cytotoxic. It is now clear that T-cells are functionally heterogeneous, and division of T-cells based on cell surface expression of CD4 and CD8 does not define their functions. More recent studies have assigned T-cells into distinct subsets based on the expression of cytokines. These studies have suggested that T-cells expressing interleukin (IL)-2 and interferon (IFN)- γ belong to the inflammatory subset that regulate cell-mediated immunity and are known as Type 1 helper (Th1) cells. Conversely, T-cells expressing IL-4 and IL-10 belong to the helper subset that regulate humoral immunity and are referred to as Type 2 helper (Th2) cells. Inasmuch as previous studies have not investigated the effects of hypnosis on functional subsets of T-cells, the present study sought to bridge this gap.

While the immune system was previously thought to function autonomously, cumulative evidence over the past two decades indicates an interrelationship between psychosocial, neuroendocrine, and immune systems (Ader, Cohen, & Felten, 1995; Pert, Dreher, & Ruff, 1998). There is evidence to suggest that the various subsets of T-cells are differentially modulated by hypothalamo-pituitary-adrenal (HPA) axis mediators. This has been documented in a model studying soluble cytokines in the serum (Petrovsky & Harrison, 1997). It appears that the mediators of HPA axis down-regulate Th1 cell activity. This differential effect of mediators on T-cell subsets may be a reflection of differential expression of neuroendocrine receptors on T-cell subsets. Hence, it is reasonable to hypothesize that modulation of neuroendocrine mediators by hypnosis is likely to have different effects on Th1 and Th2 cells. This potential shift of T-cell activity can have a potentially significant influence on diseases in which T-cells have been implicated.

Documentation of a neuroendocrine response to hypnosis is also limited. Cortisol has been shown to decrease to very low levels during extended hypnosis for some, but not all, highly hypnotizable subjects (Sachar, Fishman, & Mason, 1965; Sachar, Cobb, & Shor, 1966). Significant differences in the levels of cortisol were also found in highly hypnotizable subjects when they re-experienced intense emotional states induced under hypnosis (Zachariae et al., 1991). Cortisol was significantly lower following the last suggested emotional state of happiness in comparison with the emotional state before hypnosis or the preceding emotional states of anger and depression. Lastly, the mean plasma level of β -endorphin was significantly higher following hypnotically induced analgesia in patients with arthritic pain (Domangue, Margolis, Lieberman, & Kaji, 1985). Although very limited, this evidence suggests that hypnosis has the potential to modulate the HPA axis in certain subjects.

This paper reports on an exploratory study to investigate the psychoneuroimmunological effects of hypnosis in healthy, high hypnotizable subjects, in an attempt to discern the mechanism through which hypnosis may affect physiological

responses. This investigation was carried out to test the hypothesis that hypnosis can differentially modulate T-cell subsets. It was further hypothesized that a decrease in the HPA mediators, namely ACTH, cortisol, and β -endorphin would be found following hypnosis and that the levels of these mediators would correlate with T-cell subsets.

Methods

Subjects

Seven healthy and highly hypnotizable volunteers were selected as subjects for this study. Five of the subjects were female and two were male. Their age range was between 24 and 42 years of age ($M = 32$, $SD = 7.2$). Three of the subjects were Caucasian, two were African American, one was Asian American and one was Native American. One subject (female) exited from the study because she had borderline anemia following the first week of sampling.

Prior to the study, subjects signed informed consents and were screened for health status with a self-report medical questionnaire. Subjects were excluded from the study if they reported current psychiatric treatment, the presence of systemic diseases (such as a cardiovascular disorder, seizure disorders, recent cerebrovascular events, diabetes mellitus, autoimmune or any other disorder of the immune system), current tobacco use, systemic antibiotic treatment within the past 3 months, or if they were using any medication that could interfere with the planned endocrine or immune assays. Subjects were also excluded if they had a previous bad experience with hypnosis. In addition, proficiency in English was required in order to ensure that the verbal hypnotic suggestions could be followed. During the screening session, subjects had the specifics of the project explained in detail, as laid out in the informed consent. This included the purpose of the study: a study of the effects of hypnosis on the immune and endocrine systems. The explanation also included the amount and scheduling of hypnosis and blood sampling sessions, and the physical layout of the Clinical Research Center where the project was conducted. There was no education provided about the immune or endocrine systems, and no information given about the types of effects that might be found. Subjects had a brief explanation of hypnosis as part of the hypnotizability testing described below but were not informed that they needed to be highly hypnotizable prior to the hypnotizability screening. No additional hypnosis was conducted with subjects prior to the beginning of the research project.

Hypnotizability

Subjects were screened for hypnotic susceptibility with the Harvard Group Scale of Hypnotic Susceptibility (HGSHS; Shor & Orne, 1962). Subjects who were high in hypnotizability were purposely selected for this study in order to maximize any potential effect of hypnosis. Our intention was to see if any effects following hypnosis could be detected, and as such there was no attempt to differentiate between responses of high and low hypnotizable subjects. Only individuals who scored nine or greater on the HGSHS were included in the study.

Procedures

After subjects were determined to be in good health and high in hypnotic susceptibility, they were scheduled to attend three one-day sessions as outpatients at

a General Clinical Research Center (GCRC). The three sessions were scheduled to occur at one week intervals. Subjects were instructed to eat or drink nothing but water after midnight on study days and arrive at the GCRC by 7:30 a.m. where they remained until 3:00 p.m. on each of the days. The first of the three days functioned as a baseline recording day, with no intervention involved.

Upon arrival on the first day, the subject's height and weight was recorded, and the subject was admitted to a private room in which heat and light were constant throughout the day. The subject had an indwelling intravenous catheter inserted into one forearm at 7:30 a.m., which was followed by 30 minutes of supine rest. Blood samples were drawn at 8:00, 8:30, 9:00, 11:00 a.m. and 3:00 p.m.. The subject was supine between 8:00 and 9:00 a.m. and 30 minutes prior to each blood draw. A standard weight maintaining diet was provided during the day with breakfast following the 9:00 a.m. blood collection and lunch at noon. The indwelling catheter was removed following the final blood collection at 3:00 p.m. and the subject was dismissed.

The second and third days of the protocol were identical to the first with the exception that a one-hour hypnosis intervention was provided to subjects following the first blood draw at 8:00 a.m. The hypnosis concluded at 9:00 a.m., with the subject re-alerted just prior to the 9:00 a.m. blood draw.

Blood samples were drawn into the appropriate vacutainer tubes (Becton Dickinson, San Jose, CA) as described by the manufacturer. Blood samples were drawn at room temperature, refrigerated immediately, and centrifuged to separate plasma from cells. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation (Boyum, 1968). Isolated PBMC were counted, using a hemocytometer slide chamber. Trypan blue dye exclusion was used, which demonstrated that viability of cells was greater than 95% (Altman, Randers & Rao, 1993). Cells were cultured, as described below for intracellular cytokine detection assay and analyzed within 24 hours of collection. The plasma and serum samples were frozen at -70 degrees Centigrade until assayed. Immunological analysis of these subjects entailed processing of blood samples taken at five time points during three sessions, analyzed for five different markers (CD3, CD69, IL-2, IL-4 and IFN- γ). Therefore, because of the large number of samples generated per subject, cells for only four of the subjects were analyzed in this preliminary study. Plasma for five subjects was assayed for ACTH, and all seven subjects for β -endorphin. Serum for all seven subjects was assayed for cortisol.

Intracellular Cytokine Detection Assay

PBMC were cultured at a concentration of 2×10^6 cells/ml with the stimulants for various intervals at 37°C. The medium consisted of RPMI-1640 supplemented with 10% FBS and penicillin (100 units/ml)/streptomycin (100 : g/ml) and 25mM HEPES buffer. The stimulatory agents were phorbol 12-myristate 13-acetate (PMA, 25 ng/ml) in conjunction with ionomycin (1 : g/ml). Brefeldin-A (BFA, 10 : g/ml) was included in cultures to prevent secretion of cytokines and retain them intracellularly. Cells were harvested and intracellular expression of cytokines (IL-2, IL-4, and IFN- γ) was detected by immunofluorescent labeling with specific monoclonal antibodies (Becton Dickinson) and flow cytometry as described by Zadeh, Tanavoli, Haines, and Kreutzer (2000). In this manner, the proportion of all T-cells (CD3⁺) that express the prototypical cytokines of type 1 (IL-2⁺ and IFN- γ ⁺), type 2 (IL-4⁺) or type 0 (IL-4⁺ and IFN- γ ⁺) subsets was

defined. In addition, labeling with mAb specific for CD69, which is a very early activation marker, was used to determine the T-cell activation response (Maino, Suni, & Ruitenberg, 1995). Briefly, cultured cells were incubated for 10 minutes with 10 : 1 of 1:10 diluted permeablizing solution (Becton Dickinson) at room temperature. The cells were then washed with PBS containing 0.1% sodium azide and 0.5% Bovine Serum Albumin (BSA) and incubated with a mixture of fluorochrome-conjugated monoclonal antibodies (10 : 1 of each) in microtiter plates for 30 minutes at room temperature. Following two washes, the cells were fixed with 2% buffered paraformaldehyde and analyzed by flow cytometry.

Measurement of HPA Axis Mediators

ACTH was measured using Enzyme Linked Immuno-Sorbent Assay (ELISA). The ACTH ELISA assay is based on a sandwich immunoassay methodology. The samples are incubated with biotinylated anti-ACTH antibody of defined specificity in wells coated with goat anti-human ACTH antibody with predetermined and unique epitope specificity. The wells are then treated with streptavidin labeled with horseradish peroxidase and incubated with tetranethylbenzidine (TMB) substrate. The reaction is stopped with a 0.2M sulfuric acid solution and absorbance is measured using a dual wavelength at 450 and 620 nm.

The serum and plasma samples collected were submitted to Nichols Institute (Quest Diagnostics, San Juan Capistrano, CA) for measurement of total serum cortisol and plasma β -endorphin levels. Serum samples submitted for cortisol measurements were first diluted 1:30 and heat-treated to eliminate binding proteins and potentially cross-reacting steroids present in the sample. The samples were then analyzed by competitive radioimmunoassay (RIA). Plasma samples submitted for β -endorphin analysis were first extracted with silica prior to assessment by RIA.

Hypnosis Intervention.

A semi-standardized hypnotic protocol was followed which consisted of the following. The induction consisted of Finkelstein's (1990) private refuge induction, which was followed by a further deepening by counting backward from 5 to 1. A modified and shortened version of Hartland's (1971) ego strengthening suggestions was provided followed by instructions to continue deepening in whatever manner was best for the subject to relax most thoroughly. This period lasted ten minutes during which only minimal suggestions were provided to continue deepening. Following this, a suggestion protocol which focused on the theme of balancing the neuroendocrine and immune systems was provided. This consisted of four metaphorical image-oriented suggestions, each emphasizing one of the senses of sight, hearing, taste/smell, or touch. This was followed by direct suggestions to balance the neuroendocrine and immune systems, and post-hypnotic suggestions to effectively deal with life stress and to continue to balance him/herself. Exact wording of the four metaphorical image suggestions and the direct balance suggestions are published as an appendix to this article. A second ten-minute period with minimal suggestions was provided following instructions for the subject to begin and continue to balance his/her system in the manner that was best for him/herself. Finally, the direct and post-hypnotic suggestions for continuing balance were repeated and the subject was then re-alerted. Although

imagery has been used in previous studies to suggest both relaxation and immune system changes, the imagery used in this study differed in that it was metaphorical and did not have subjects imagining specific immune changes (e.g., white blood cells attacking virus or tumor cells).

Statistical Analysis

The dependent variables included the HPA mediators (ACTH, cortisol and β -endorphin), T-cell activation (CD69+), and intracellular cytokine production (IFN- γ , IL-2, IL-4). The data were analyzed using individual repeated measures analysis of variance (ANOVA) for each dependent variable. Main effects included time of day and hypnosis intervention. An interaction effect between time of day and intervention was also considered. Separate ANOVA's were performed since there were more dependent variables than subjects and any multivariate ANOVA would lack statistical power. Accordingly, a higher experiment-wise error rate was considered appropriate. A significance level of 0.05 was used for all tests. When main effects were significant, multiple comparison tests were performed using the Student-Newman-Kuels method with a significance level of 0.05. For exploratory purposes, correlation coefficients between neuroendocrine and immune variables were calculated in order to investigate the possible magnitude and direction of any relationships between HPA mediators and T-cell activation and cytokine expression. The correlations were calculated by combining all time points for all subjects.

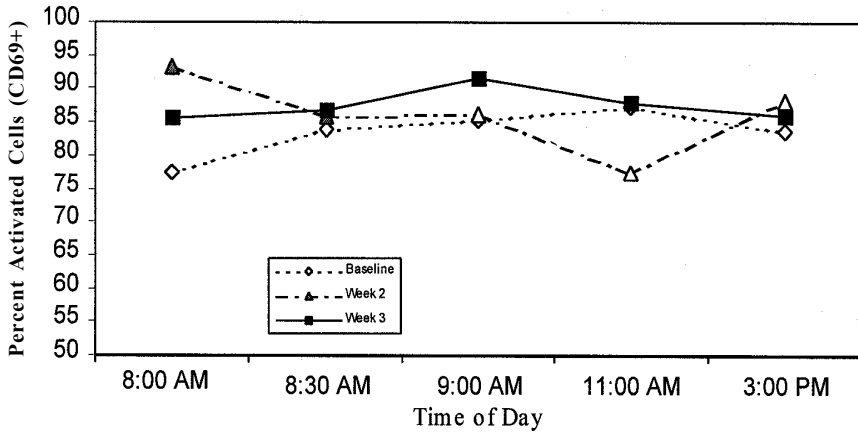
Results

Hypnosis and T-Cell Response

To investigate possible effects of hypnosis on the functional responses of T-cells, the activation response, and the pre-, intra- and post-intervention expression of T-cell cytokines were determined. The peripheral blood cells obtained from subjects at various time points were activated with PMA/ionomycin and analyzed for cell surface expression of CD69, as well as intra-cellular expression of IFN- γ , IL-2 and IL-4. The data in Figures 1 and 2 show the mean percentage of T-cells that express CD69, and cytokines (IFN- γ , IL-2 and IL-4), respectively. For T-cell activation, there were no significant effects for time of day, intervention, or the interaction of the two. For IFN- γ there was a significant effect for time of day ($F_{4,43} = 3.39, p = .017$), with the level at the last time point of the day (3:00 p.m.) significantly higher than all other times. There was also a significant effect for intervention ($F_{2,43} = 16.28, p = .0001$), with the level of IFN- γ significantly lower after both the first and second intervention sessions. There was no significant interaction between time of day and intervention for IFN- γ . For IL-2 there was a significant effect for intervention ($F_{2,43} = 4.82, p = .013$), with the level of IL-2 significantly lower after two intervention sessions. There was no significant effect for time of day, or the interaction between time of day and intervention. For IL-4 there were no significant effects for time of day, intervention, or the interaction of the two.

Hypnosis and Hypothalamo-Pituitary-Adrenal Axis Mediators

The mean levels of ACTH, cortisol, and β -endorphin, are displayed in Figure 3. For ACTH, there were no significant effects for time, intervention, or their interaction.

Figure 1: Percent Activated Cells (CD69+) at Baseline and Weeks 2 and 3

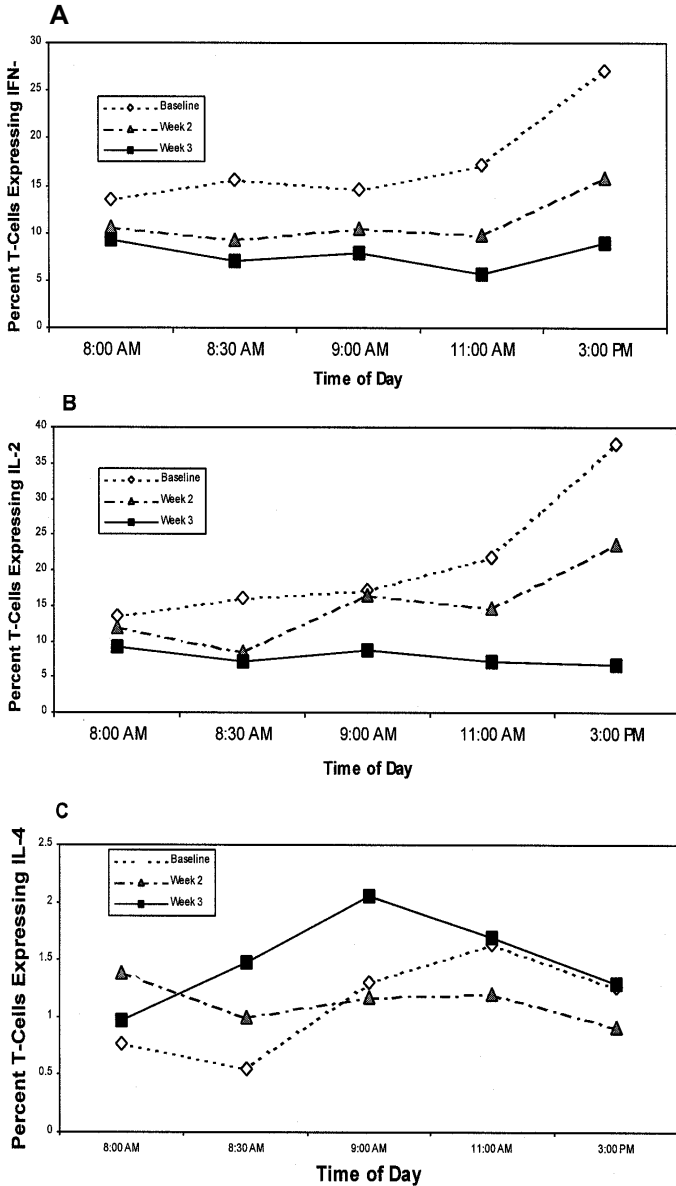
Percent expression of the T-cell activation marker (CD69) following stimulation with PMA+ ionomycin. Mean % of CD69⁺ T-cells (CD3⁺) for 4 subjects is illustrated. The proportion of T-cells expressing CD69 was determined by immunofluorescent labeling with specific monoclonal antibodies for CD3 and CD69 followed by flow cytometry. The first week functioned as a baseline recording day, with no intervention involved. On the second and third consecutive weeks a one-hour hypnosis intervention (8:00 - 9:00 a.m.) was provided to all subjects.

For cortisol, there was a significant effect for time of day ($F_{4,78} = 9.57, p = .0001$), with the mean level of cortisol decreasing during the course of the day, as expected for diurnal variation of this hormone. There was not a significant intervention or interaction effect for cortisol. For β -endorphin, there were no significant effects for time of day, intervention, or the interaction of time and intervention.

Relationship of HPA Mediators and T-cell Activation and Cytokine Expression

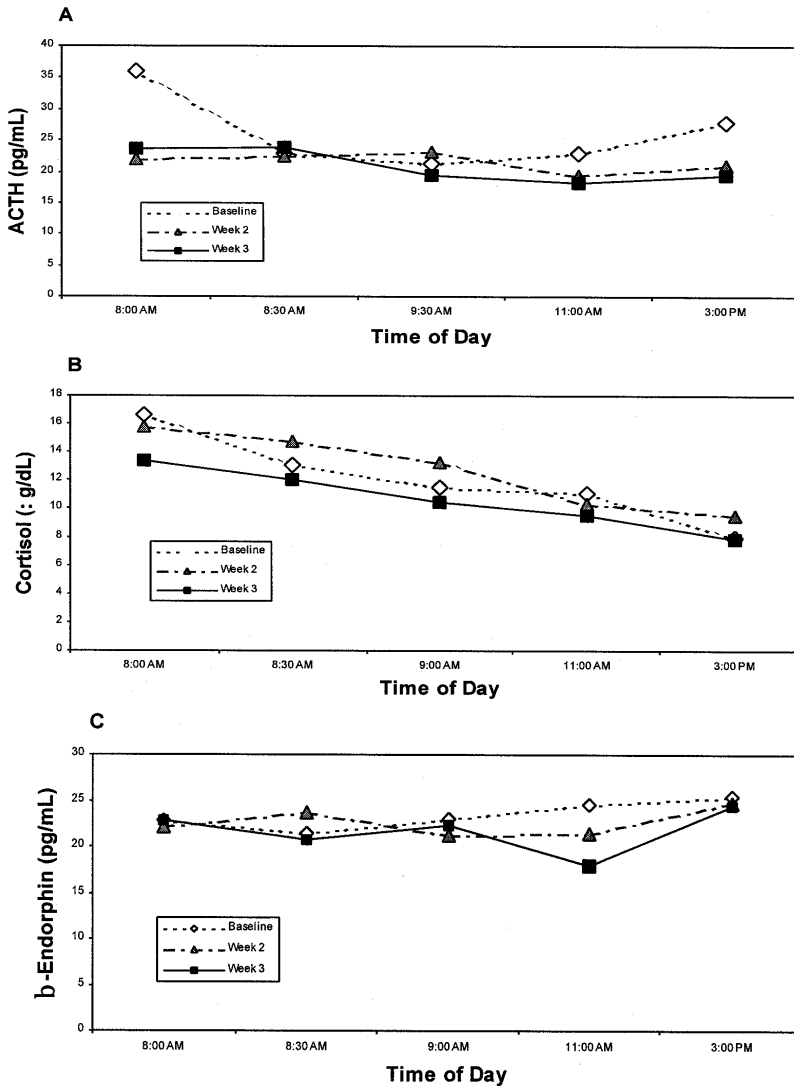
The Pearson correlation coefficients illustrating the relationship between plasma and serum levels of HPA mediators and the T-cell expression of activation marker and cytokines for all time points combined are presented in Table 1. Cortisol was significantly and positively correlated with IFN- γ ($r = .460, p < .001, n = 60$ observations). Moreover, T-cell activation was significantly positively correlated with ACTH ($r = .455, p = .01, n = 30$ observations) and β -endorphin ($r = .473, p = .001, n = 45$ observations). All other correlations were not statistically significant, although the relationships between cortisol and IL-4 ($r = -.23, n = 60$ observations) and between β -endorphin and IL-2 ($r = .23, n = 60$ observations) approached significance ($p = .07$).

Figure 2 (A,B,C): Percent Cytokine Expression at Baseline and Weeks 2 and 3



Percent expression of T-cell cytokines following stimulation with PMA+ionomycin. Mean % of T-cells expressing IFN- γ (A), IL-2 (B) and IL-4 (C) for 4 subjects is illustrated. The proportion of T-cells expressing each cytokine was determined by immunofluorescent labeling and flow cytometry. The first week functioned as a baseline recording day, with no intervention involved. On the second and third consecutive weeks a one-hour hypnosis intervention (8:00- 9:00 a.m.) was provided to all subjects.

Figure 3(A, B, C): Mean Levels of Hypothalamo-Pituitary-Adrenal Axis Mediators at Baseline and Weeks 2 and 3



Mean levels of hypothalamo-pituitary-adrenal (HPA) axis mediators at baseline and following hypnosis intervention. Mean levels of plasma ACTH for 5 subjects (A), serum cortisol for 7 subjects (B) and plasma β -endorphin for 7 subjects (C) are displayed. ACTH levels were determined by ELISA. Cortisol and β -endorphin levels were determined by radioimmunoassay (RIA). The first week functioned as a baseline recording day, with no intervention involved. On the second and third consecutive weeks a one-hour hypnosis intervention (8:00 - 9:00 a.m.) was provided to all subjects.

Table 1: Pearson Correlation Coefficients of HPA Axis Mediators and T-cell Activation and Cytokine Expression^a

	% T-cell ^b activation	% IFN- ζ	% IL-2	% IL-4
ACTH	.455 ^c	-.037	.024	.028
Cortisol	.081	.460 ^d	-.083	-.233 ^e
β -endorphin	.473 ^f	-.083	.233 ^e	.027

^aCorrelations are for all time points combined

^b%CD69+CD3+T-cells following stimulation with PMA/ionomycin

^c $p = .01$ ^d $p < .001$

^e $p = .07$ ^f $p = .001$

Discussion

Previous investigations of the effects of hypnosis on T-cells have demonstrated a quantitative increase in the proportion of T-cells and their CD4 subset (Ruzyla Smith et al., 1995). The present study sought to determine qualitative changes within functional subsets of T-cells. The first hypothesis pursued in this study was the differential effects of hypnosis on T-cell subsets. In that respect, the present study demonstrated a significant decrease in the expression of the prototypical cytokines of Type-1 T-cells, i.e., IFN- ζ and IL-2, following hypnosis intervention. These data provide evidence to support the proposed hypothesis. However, statistically significant changes in IL-4 expression were not found. This could be due to the true inability of hypnosis to modulate the Type-2 subset of T-cells. Alternatively, it is possible that this lack of effect is due to technical reasons. There is a number of factors that could explain this lack of detectable IL-4 change. Firstly, IL-4⁺ T-cells are generally far fewer in proportion (approx. 1% of all T-cells) compared with IL-2⁺ (8-21%) or IFN- ζ ⁺ (8-18%) T-cells observed in this study. Consequently, changes in the IL-4⁺ subset are more difficult to detect. Secondly, the small sample size makes it difficult to detect such subtle changes. Thus, further investigation with larger sample sizes is required in order to provide more definitive evidence for the proposed hypothesis.

In the present study, no intervention was administered on the first day of sampling and the immunological and neuroendocrine measures on that day were used as baseline for each patient. In order to control for the intervention administered vs.

potentially spontaneous changes or the possibility of statistical regression to the mean, future studies also need to include a second subject group who receive control intervention. Moreover, it is unclear whether the findings of the present study are unique to the highly hypnotizable subjects investigated here, or can be reproduced in subjects with various levels of hypnotizability. Future studies may be expanded to include subjects of low or moderate hypnotizability.

The clinical significance of the ability of hypnosis to alter the T-cell response is unclear. It is important that future studies investigate the utility of hypnosis-mediated T-cell alteration in the treatment of diseases. While hypnosis has been used in medical treatment for over 150 years, documented immunological effects of this intervention modality are scant. Controlled studies are warranted, utilizing hypnosis in disease models where immuno-modulation has been proposed to be beneficial. In this manner, alteration of the T-cell response can be replicated in the presence of disease, and the potential of this alteration to produce longer-term clinical benefits can be studied. If it is confirmed that hypnosis leads to a decrease in the Th1 (inflammatory) subset of T-cells, this intervention modality may potentially be used in clinical therapy involving diseases where the over expression of Th1 cells occurs (rheumatoid arthritis, insulin dependent diabetes mellitus, etc.).

An additional hypothesis tested in the present study proposed that HPA axis mediators are involved in the mechanism of T-cell functional changes associated with hypnosis. The results of this preliminary study utilizing a small sample size failed to demonstrate statistically significant effects of hypnosis on the three HPA mediators tested, i.e., ACTH, cortisol, or β -endorphin. In view of previous reports suggesting that hypnosis can lower cortisol levels in highly hypnotizable subjects (Sachar, Fishman, & Mason, 1965; Sachar, Cobb, & Shor, 1966), further investigation of our proposed hypothesis, using larger sample sizes, is merited.

The ACTH levels in weeks 2 and 3 were lower than baseline not only at 8:00 a.m. but also at 11:00 a.m. and 3:00 p.m. These changes suggest that hypnosis may attenuate ACTH release, most likely mediated via a decrease in corticotropin releasing hormone (CRH) secretion. Since ACTH is under CRH control, which is under tonic inhibition from hippocampal areas, a lower ACTH in the morning during the second and third weeks of the study may suggest either attenuation of the diurnal variations due to an anticipated state of relaxation, or a secondary response to a decreased level of anxiety on weeks 2 and 3 compared with the initial visit. These changes were not significant due to the small number of subjects in the study. We did not, however, observe similar changes in the cortisol levels, probably since ACTH response to hypnotic intervention is more sensitive than the changes in cortisol level. Moreover, the cortisol tested in this study was total serum cortisol, which includes both free, as well as bound cortisol. Inasmuch as more than 90% of circulating cortisol is bound to cortisol-binding globulin (CBG) or albumin, and the free or unbound cortisol is the physiologically active form, it will be important for future studies to test free cortisol. This unbound cortisol is more likely to be available for biologic interaction with T-cells. These findings warrant further investigation using a larger number of subjects and measurements of ACTH and cortisol (total and free), as well as CRH levels.

The exploratory correlational analysis indicated that cortisol and IFN- γ appear

to be positively associated. However, the causality of this association cannot be ascertained from the current evidence. There are several possibilities as to the nature of the causal association, if any, between cortisol and IFN- γ . Although it is possible that cortisol may have a positive effect on IFN- γ expression, previous studies predominantly report an inhibitory effect of cortisol on T-cells (Kronfol, Nair, Zhang, Hill, & Brown, 1997; Nair & Schwartz, 1984). Hence, it is possible that the positive association between cortisol and IFN- γ is the result of an indirect effect of cortisol on other mediators. The hypothesis that we are currently pursuing is that cortisol has a negative effect on the Th2 subset of T-cells. In that regard, our data indicate a trend toward a negative correlation between cortisol and IL-4 ($r = -.23, p = .07$). Thus, we have hypothesized that cortisol has an inhibitory effect on Th2 cells that express IL-4. Normally, IL-4 produced by the Th2 subset of T-cells has an inhibitory effect on IFN- γ production by Th1 T-cells. Thus, it is possible that the removal of the inhibitory feedback of IL-4 leads to a rise in IFN- γ production, or vice versa.

The present study introduces a model for studying non-pharmacological modulation of the T cell response and the cellular and molecular mediators involved. This model is of possible interest not only for understanding the basic psychoneuroimmunological interactions involved, but also potential clinical applications of this form of immuno-modulation. Experiments are currently in progress to address some of the issues raised by the present study.

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**Appendix 1: Hypnosis Induction and Suggestion Protocol
Immune and Endocrine Balancing
G.J. Wood, Ph.D.**

1. Induction and deepening: The Private Refuge, Selig Finkelstein (1990) (a progressive relaxation procedure followed by downward movement and imagery of a safe place).
2. Ego supportive suggestions - modified version of original by John Hartland (1971) (direct suggestions of a very general nature to enhance ego functioning currently and in the future).
3. Introduction of the balance concept within physiological systems
4. Balance metaphors—indirect suggestions, indirect post-hypnotic suggestions
5. Direct suggestions for optimal balancing of the immune and neuroendocrine systems
6. Post-hypnotic suggestions for continued relaxation, stress reduction/management, and optimal balancing of the immune and neuroendocrine systems.
7. Alerting, reverse of imagery involved in the Private Refuge.

After the Induction and Deepening of Hypnosis and General Ego-Strengthening Suggestions:

3. Introduction of the Balance Concept

Even as your mind wanders, continuing to relax even more deeply, your body continues to do all of the things it needs to do to keep you strong and promote health. There are thousands of actions that your body makes each day, without your even having to think about them. For instance there's no real need to think about your breathing or your heart beating, since your mind and body adjusts each of these so that they occur at just the right pace. Other systems in your body also function quite wonderfully, with little or no conscious thought. For instance, the immune system works hard to defend your body against bacteria and viruses. Working with white blood cells and special chemicals, your immune system not only fights to keep you healthy, but also communicates with the brain and other body systems. And through these various reactions and changes, your mind and body, without even thinking about it, work to find a perfect balance between all these systems and cells and molecules.

4. Balance Metaphors

Many aspects of life require a sense of balance. In actuality, balance is a rather complex thing, and it may surprise you, when you think about it, how amazingly you balance many aspects of your life. This has really been going on for quite some time now. Think back to when you were a child. You can probably remember times when you were playing when you balanced yourself just perfectly. Perhaps it was learning to walk along a raised curb, or along a board or a low wall. Remember how it seemed hard at first, trying to stay on the curb; as you would take a step forward it felt as if you might fall off one side or the other. But you instinctively responded by shifting your weight in the right direction and putting your arms out to the sides to gain a sense of stability.

This was not something you thought about, your mind and body just responded. It is wonderful how, without really thinking about things, our minds and bodies naturally respond to bring us into balance. And the more you practice at balancing yourself, the better you become. Each day practicing in so many little ways you become increasingly more balanced, so that just like when you were a child walking along the curb and you felt yourself losing balance, you would make a correction and rebalance yourself. This is something that your mind and body do continually and which you can find yourself doing an even better job of each day.

A sense of balance is also used in cooking to get just the right proportions of flavors. But how do you get just the right balance? And isn't the right balance of flavors an individual thing? What may be the perfect proportions of seasoning for one may not be the same for another. Consider, for instance, that you were making the perfect spaghetti sauce. What would be the right balance of flavors for you? Tomatoes? Garlic? Oregano? Basil? Salt? Pepper? Onion? Mushrooms? Meat? Or other vegetables? Maybe there are other ingredients that only you know. There might even be a secret family ingredient that you might put in, or leave out. Each flavor helps to balance another. Some are very aggressive flavors, some more mild. The right balance of these flavors is critical, and you know just the right amounts of each to add. Sometimes one of the stronger flavors starts to overpower a milder flavor, and you balance it out by adding more of the mild. And as you cook you can further balance the flavors of the sauce in small, seemingly unnoticeable ways, yet each of your actions help to create a fully balanced and truly remarkable sauce. It is exactly balanced the way it needs to be for you.

A sense of balance is also used by us in many other ways each day. Listening to music is a good example. On your stereo, there's even a knob or a lever marked balance, and you've probably noticed how different sounds may come out of the right speaker from what comes out of the left speaker and you find the right proportion of the two so they are complementary. Now have you ever had the music go way out of balance? Doesn't the music sound wrong? Like something's missing? All the sound is coming from just one side. Not only does the music sound wrong but it's rather irritating. It's like only hearing from the right ear, or the left ear, when our brain needs to use both right and left parts to achieve a harmonious balance. So of course the natural thing to do, almost without thinking about it, is to reach over and move the balance control so that it's balanced just right for you. That's a much more pleasant way to listen. Both sides working in harmony to complement each other.

Another example of balance in our daily lives can be seen in our favorite room at home. This room is probably balanced in many ways, and it's precisely these ways which contribute to your level of comfort, health and relaxation. You probably balanced this room without giving a whole lot of thought to balance, but rather to what brings you comfort. Is there a special place where a chair, or bed, or desk really needs to be exactly? That moving it somewhere else wouldn't be quite right? Even the various colors of the room are probably balanced to complement each other. Now imagine this room with all of the furniture in one half of the room, or pushed over to one side. See the various pieces of furniture, shoved close together, and nothing but empty floor in the rest of the room. How does it look and feel to you? Isn't the room out of balance? Isn't it uncomfortable? Now let your mind rebalance everything. Put everything back just as

it needs to be. Balance yourself fully and completely. And let your room return to its balanced relaxed and comforting condition.

5 & 6. Direct and Post-Hypnotic Suggestions to Mediate the Neuroendocrine and Immune Systems

Just as there are so many aspects of life that require balancing, so too our bodies work to achieve an optimal balance. Your mind has the ability to assist your body in achieving this state of balance. Allow your mind to help your body to make changes to achieve this optimal balance of the immune and endocrine (or hormone) systems. Just as your unconscious mind helps to control your breathing and the beating of your heart, so too can your unconscious mind help to balance your immune and endocrine systems. Its not something you have to think about, just allow your mind to do it. Optimal balance, everything working just right, in perfect harmony. And each day, your mind can help your body to fine-tune all of the actions and reactions necessary so that you can maintain an optimal balance. Likewise each day your mind can assist you in dealing with any stresses that arise so that you can deal with them in a healthy manner and calm and relax yourself afterward. Each day you can find yourself feeling more and more in balance, both in your external world and within your mind and body.

7. Alerting

Alerting should be done within the context of the procedure and the patient's needs with sounds, sensations and/or images as well as direct suggestions.