

## Alterations in Specific Antibody Production Due to Rank and Social Instability

JOAN E. CUNNICK,<sup>\*†1</sup> SHELDON COHEN,<sup>\*†</sup> BRUCE S. RABIN,<sup>\*</sup>  
A. BETTS CARPENTER,<sup>\*</sup> STEPHEN B. MANUCK<sup>‡</sup> AND JAY R. KAPLAN<sup>§¶</sup>

<sup>\*</sup>*Brain, Behavior and Immunity Center, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261; †Behavioral Physiology Laboratory, Department of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260; ‡Department of Psychology, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213; §Department of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103; and ¶Department of Anthropology, Wake Forest University, Winston-Salem, North Carolina 27103*

Separate studies examined the influence of the social environment of male cynomolgus macaques on primary and secondary antibody responses to immunization with tetanus toxoid. All animals showed evidence of both primary and secondary anti-tetanus antibody response. In the first study, subordinate animals had a greater primary antibody response to tetanus toxoid, while a single social reorganization (acute stressor) did not influence the response. In the second study, social rank was not associated with the secondary antibody response but repeated social reorganizations (chronic stressor) resulted in a greater level of specific antibody production in comparison to nonreorganized controls. These effects could not be accounted for on the basis of nonspecific differences in total serum IgG or serum albumin. © 1991 Academic Press, Inc

### INTRODUCTION

Studies of both human and nonhuman primates indicate that changes in the social environment have an influence on the immune response. For example, alterations in cellular function have been found among the bereaved (Bartrop, Lazarus, Lockhurst, Kiloh, & Penny, 1977; Schleifer, Keller, Camerino, Thornton, & Stein, 1983), persons taking important examinations (Dorian, Garfinkel, Brown, Shore, Gladman, & Keystone, 1982; Glaser, Rice, Speicher, Stout, & Kiecolt-Glaser, 1986; Glaser, Kennedy, Lafuse, Bonneau, Speicher, Hillhouse, & Kiecolt-Glaser, 1990), and individuals caretaking for Alzheimer's disease victims (Kiecolt-Glaser, Glaser, Shuttleworth, Dyer, Ogrocki, & Speicher, 1987). Non-human primates similarly show modulation of both cellular and humoral immunity in response to acute social change. For example, antibody production in response to an antigenic challenge is attenuated when animals are separated from parents or peers (Laudenslager, Reite, & Held, 1986; Coe, Rosenberg, Fischer, & Levine, 1987; Coe, Rosenberg, & Levine, 1988).

Less studied with respect to effects on immune response is social status. It might be expected that animals of different social status would respond differently to changes in the social environment since such changes may impose a variety of

<sup>1</sup> To whom correspondence and reprint requests should be addressed at the Department of Microbiology, Immunology, and Preventive Medicine, 205 Science Building I, Iowa State University, Ames, IA 50011.

behavioral requirements, depending on status. Moreover, social status itself is associated with different behavioral repertoires and hence may influence endocrine and immune responses independently of stressful social environmental changes.

The current studies employ group-housed cynomolgus macaques (*Macaca fascicularis*), a species of monkey often used in studying psychosocial influences on coronary artery disease, adrenal function, and heart rate (Kaplan, Manuck, Clarkson, Lusso, & Taub, 1982; Kaplan, Manuck, Clarkson, Lusso, Taub, & Miller, 1983; Shively & Kaplan, 1984; Kaplan, Manuck, Adams, Weingand, & Clarkson, 1987; Kaplan, Manuck, & Gatsonis, 1990). In this investigation we used a social change manipulation which involved the reorganization of the membership of social groups, while nonchange control animals remained in stable conditions. Additionally, each animal's social status was repeatedly evaluated. Earlier work has shown this paradigm to be ecologically valid with reorganized animals showing substantial behavioral responses and with multiple reorganizations contributing to the development of coronary artery disease (Kaplan et al., 1982, 1983).

The first study examines the effects of an acute social stressor and social status on the primary antibody response to a tetanus toxoid immunization. The second study examines the effects of a chronic-repetitive social stressor and social status on secondary antibody response to the same antigenic challenge. In both cases, the social stressors are experimentally manipulated and social status is based on observed, stable individual differences in animal behavior across time.

#### MATERIALS AND METHODS

*Animals.* The subjects were 79 male, cynomolgus monkeys (*M. fascicularis*) imported from Indonesia. Animals were held in quarantine and subjected to repeated physical exams for a 3-month period before being placed in social groups of four or five monkeys per pen. The animals remained in these social groups for 7 months prior to reorganization No. 1. After reorganization No. 1, 54 animals were maintained as stable social groups (SSS) of four or five animals per group for 36 weeks, while 25 animals were maintained in unstable social groups (USS) of five animals per group for a total of 10 monthly reorganizations. All monkeys (SSS and USS groups) were part of a study designed to investigate the influence of stress on the development of atherosclerosis. Hence, all monkeys were fed a moderately atherogenic diet containing 0.25 mg cholesterol/Cal, which resulted in total serum cholesterol concentrations of 200–400 mg/dl.

*Social environment.* The social reorganization manipulation involved monthly redistribution of animals across groups being reorganized. Each monkey in the reorganized groups was housed with three or four new animals in each reorganization. Study I examined the influence of the first reorganization, while study II was conducted after 10 reorganizations.

*Determination of social status.* Clearly identifiable, dominance hierarchies are formed by male cynomolgus macaques (Kaplan et al., 1982). Behavioral observations of 30 min in length were made on each social group, twice per week, during which time aggressive, submissive, affiliative, and nonsocial behaviors were recorded by a combination of *ad libitum* and scan sampling (Altman, 1974)

and  
for  
indi  
seve  
don  
soc.  
anir  
reor  
dat:  
don  
for  
(Do  
dat:  
for  
S  
the  
imn  
acu  
cha  
imn  
F  
one  
gan  
tibe  
reo  
niz:  
No  
Ha  
ant  
tur

—  
—5

—  
N  
sam



postimmunization. Blood samples were allowed to clot and then centrifuged to obtain serum. The serum was aliquoted in 1-ml polypropylene tubes and frozen at  $-70^{\circ}\text{C}$  until all samples for the primary immunization and 4 weeks postimmunization were collected.

*Study II: Secondary tetanus immunization.* The immunizations for the secondary antibody production (study II) occurred 9 months after reorganization No. 1 for those animals in the SSS condition ( $n = 53$ ) and 1 week after reorganization No. 10 (chronic stressor) for those animals in the USS condition ( $n = 23$ ). All animals were immunized with .13 ml of tetanus toxoid (Super-tet, Haver, KS), im. Serum samples were obtained as described above at the time of immunization, 1 week postimmunization, and 4 weeks postimmunization. As in study I, serum samples were assayed for specific antibody to tetanus toxoid, total IgG, and albumin.

*Tetanus antibody determination.* IgG antibody to tetanus toxoid was performed by the method of Sedgewick, Ballou, Sparks, and Tilton (1983). Briefly, the procedure was a solid phase indirect enzyme-linked immunoassay (EIA). Tetanus toxoid concentrate (420 flocculating units/ml, Commonwealth of Massachusetts Department of Health, Boston, MA) was diluted 1:1000 in a carbonate coating buffer (pH 9.6) and 0.1 ml was added to the wells of a microtiter plate (Nunc, Immunolon II, Vangard International, Neptune, NJ). Following three washes with phosphate-buffered saline-0.5% Tween 20 (PBS-T), unreacted sites on the plates were blocked with the addition of 0.1 ml PBS, 2% bovine serum albumin (BSA) to the wells. After a 2-h incubation at  $37^{\circ}\text{C}$ , excess blocking buffer was removed by three washes with PBS-T. The test sera were diluted 1:100 in PBS-T-1% BSA and 0.1 ml was added to each well in duplicate. In addition, serial dilutions (from 2 to 0.008 U/ml) of a tetanus immune globulin (250 U/ml Cutter Biological, Elkhart, IN) were added to duplicate wells (0.1 ml each) to make a standard curve. Following a 1-h incubation at  $37^{\circ}\text{C}$ , the plates were again washed three times with PBS-T. Affinity-purified goat anti-human IgG-peroxidase conjugate (Tago, Inc. Burlingame CA) was added (0.1 ml) at a 1:50,000 dilution in PBS-T-1% BSA. Plates were again incubated for 1 h at  $37^{\circ}\text{C}$  and then washed with PBS-T three times. The substrates used were 3, 3<sup>1</sup>, 5, 5<sup>1</sup> tetramethyl benzidine (TMB Calbiochem, Inc., La Jolla, CA) and hydrogen peroxide. TMB was dissolved in 1 ml dimethyl sulfoxide and added to 99 ml of 0.1 M sodium acetate buffer (pH 6.0). Immediately prior to use, 0.013 ml of 3%  $\text{H}_2\text{O}_2$  was added to the TMB buffer solution. One hundred microliters of the TMB solution was added to the wells of the microtiter plate and this was incubated at room temperature for 30 min. The reaction was stopped with the addition of 0.05 ml 2.5 M  $\text{H}_2\text{SO}_4$  to each well. The absorbance was read at 450 nm on an automated EIA photometer.

For calculation of assay results, the log value of the standard tetanus concentration was calculated and plotted versus the absorbance value. The best fit straight line was calculated with linear regression analysis. Test sample values were extrapolated from the standard curve and multiplied by the dilution factor to obtain the final value of tetanus antibody in U/ml. Any samples whose absorbance did not fall on the linear portion of the standard curve were repeated using a different dilution. Test samples with a value greater than or equal to 0.03 U/ml

were considered positive, as suggested by Sedgewick et al. (1983). Both the interassay and the intraassay coefficients of variation of the assay were always less than 10%. In addition, no assays were considered acceptable unless the regression coefficient for the standard curve was  $\geq 0.98$ .

*Total IgG and albumin measurements.* After testing serum samples for specific antibodies, all serum specimens were tested for total IgG and albumin content to assess for nonspecific differences in serum proteins. Total IgG and albumin concentrations were assessed using a fully automated nephelometer, the Beckman Protein Array System. The reagents for these measures were the Beckman immunochemistry systems immunoglobulin G reagent kit and albumin kit. Assays were performed using 1:216 and 1:1296 dilutions of serum for IgG and albumin, respectively. Although the antibodies used in the assays are for human IgG and human albumin they are cross-reactive (66 and 83%, respectively) with similar monkey proteins.

*Statistical treatment of data.* Analysis of covariance was used to assess differences among the animals in both studies. The model covaried for the specific antibody, total IgG, or albumin level prior to each immunization and analyzed differences based on a dichotomized dominance level (animals ranked 1 or 2, dominant; animals ranked 3, 4, or 5, subordinate) and the condition. In study I the condition referred to the time of primary immunization relative to the first social reorganization (three levels: immunization 4 weeks or 1 week prior to reorganization or on the day of reorganization). In study II the condition was social stability (two levels: SSS or USS), and time (1 and 4 weeks postimmunization) was entered as a repeated measure. For study II, the data for the specific tetanus antibody was log transformed prior to analysis to meet the statistical criteria of homogeneity of variance. The significance level was set at  $\alpha = .05$ .

## RESULTS

*Evidence for a primary and secondary immune response.* Figure 1A presents the specific antibody level collapsed across all monkeys measured at the time of primary immunization, 4 weeks postimmunization, and 36 weeks postimmunization. Standard errors are indicated at each point. At the time of primary immunization all monkeys had antibody levels below 0.026 U/ml with 40% of the monkeys below a detectable level (0.008 U/ml). These levels are within the range expected for animals not previously immunized or exposed to tetanus. Measurable antibody levels between .008 and .026 U in unimmunized animals are probably due to cross-reacting immunoglobulins. All monkeys showed a positive response to the primary tetanus immunization (mean  $\pm$  SE change was  $.313 \pm .015$  U at week 4).

Figure 1B presents the specific antibody level collapsed across all monkeys measured at the time of secondary immunization (36 weeks after primary immunization), 1 week later, and 4 weeks later. Standard errors are indicated at each point. The response to the secondary immunization was much greater than the primary immune response and peaked at 1 week (mean  $\pm$  SE change was  $6.01 \pm .50$  U at week 1).

Thus, it can be seen that all monkeys responded in an adequate fashion to both

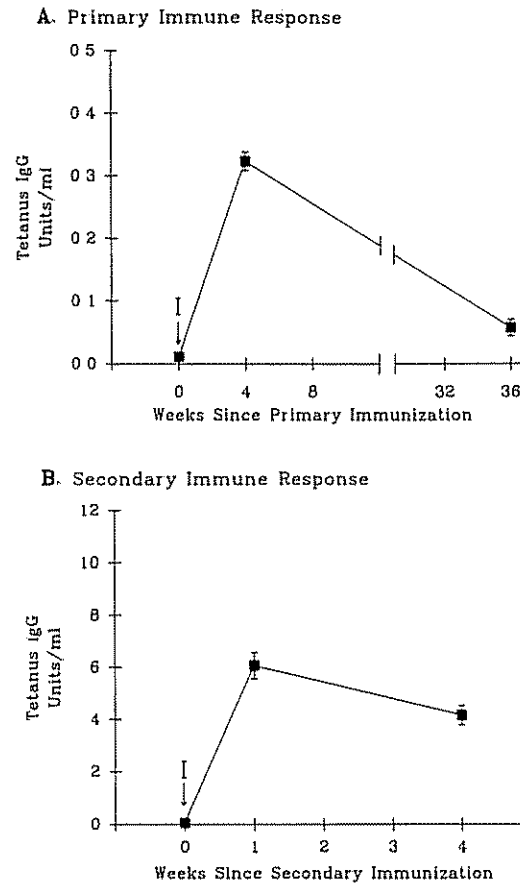


FIG. 1. Mean levels ( $\pm$ SE) of specific antibody to tetanus toxoid for primary and secondary immunizations for all animals immunized. (A) Primary immunization (Study I): Units per milliliter of specific IgG antibody to tetanus immediately prior to immunization (I), 4 weeks postimmunization, and 36 weeks postimmunization. (B) Secondary immunization (Study II): Units per milliliter of specific IgG antibody to tetanus immediately prior to immunization (I), 1 week postimmunization, and 4 weeks postimmunization.

the primary and the secondary immunizations with specific levels of antibody to tetanus toxoid.

*Study I: Effects of rank on the primary immune response.* Although all monkeys responded to the primary immunization with detectable levels of specific antibody, the ANCOVA (covarying for baseline levels of specific antibody) indicated a main effect for rank. Figure 2A presents the significant data from the analysis of the primary immune response. There was a significantly higher level of specific IgG to tetanus in the subordinate monkeys ( $n = 46$ ) than in the dominant monkeys ( $n = 33$ ),  $F(1,72) = 5.49$ ,  $p < .03$ . There was no significant effect for condition (time of reorganization) or for the interaction of rank and condition,  $F$ 's  $< 1$ . A separate ANCOVA was used to determine if rank at primary immunization could

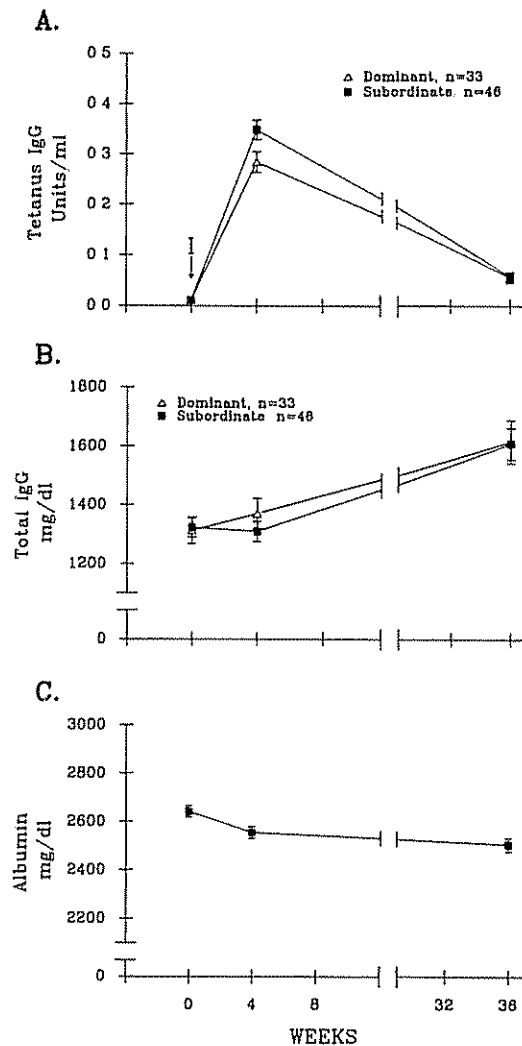


FIG. 2. Results of study I collapsed across condition. (A) Serum levels of specific IgG to tetanus (units/ml) in dominant and subordinate monkeys. (B) Serum levels of total IgG (mg/dl) in dominant and subordinate monkeys. (C) Serum levels of albumin (mg/dl) collapsed across all conditions and both ranks.

predict basal tetanus antibody levels at 36 weeks. This analysis showed no significant effect of rank,  $F < 1$ .

In order to determine whether the differences in specific antibody levels were due to general differences in total levels of IgG, we analyzed the data for differences in rank. Figure 2B presents the data for total IgG during the primary immunization. The ANCOVA indicated a marginally significant main effect for rank,  $F(1,72) = 3.95$ ,  $p = .051$ . However, for this data the dominant monkeys had the highest levels of total IgG. Therefore, the changes found in specific tetanus antibody levels can not be explained by general changes in total IgG.

Albumin was measured at all time points during the primary immunization to determine a general index of nutritional status which could impact on a monkey's ability to mount an immune response and produce specific antibodies. Figure 2C presents the data for the levels of albumin collapsed across all groups, as there were no significant differences among the groups,  $F$ 's  $< 1$ . Consequently, measurement of serum albumin levels did not indicate any differences among the groups which could account for differences observed for specific or total IgG.

*Study II: Effects of condition on the secondary immune response.* All monkeys were randomly assigned to either stable social groups ( $n = 54$ ) or unstable social groups ( $n = 23$ ) for the subsequent 9 months. For the unstable social groups, the monkeys were reorganized into new social groups once every 4 weeks. One week after the 10th reorganization (the first reorganization occurred at the beginning of Month 1), the monkeys in unstable social groups were immunized for the second time with tetanus toxoid. At the same time the monkeys in stable social groups were also immunized for the second time.

All monkeys responded with a significant increase in antibody levels 1 week after the secondary immunization. The data for the secondary antibody response to tetanus toxoid are presented in Fig. 3A. Three monkeys were excluded from the analysis. One was excluded due to an antibody level that was greater than 3 standard deviations above the mean even after log transformation of the data, while two monkeys were housed individually at the time of immunization due to a prior illness. The ANCOVA indicated a significant main effect for condition (social stability),  $F(1,71) = 7.18$ ,  $p < .009$ , and a significant main effect for time,  $F(1,71) = 34.19$ ,  $p < .001$ . There was no significant effect of rank, nor were there any significant interactions of condition, rank, and time,  $F$ 's  $< 1$ . The condition effect was due to a higher specific antibody level in those animals in the USS. The time effect was due to a decrease in specific antibody from Week 1 to Week 4 postimmunization.

In order to determine whether the differences in specific antibody levels were due to general differences in total levels of IgG, we analyzed the data for differences in condition and time. Figure 2B presents the data for total IgG during the primary immunization. The ANCOVA indicated no main effects for condition, rank, or time,  $F$ 's  $< 1$ . However, there was an interaction of condition and time,  $F(1,72) = 6.14$ ,  $p < .02$ . For these data the monkeys in the stable social groups had increasing levels of total IgG from Week 1 to Week 4, while the monkeys in the unstable social groups had decreasing levels of total IgG from Week 1 to Week 4 postimmunization. Thus, the changes found in specific antibody levels cannot be explained by general changes in total IgG.

As for study I, albumin was measured at all time points during the secondary immunization to determine a general index of nutritional status. Figure 3C presents the data for the levels of albumin collapsed across all groups, as the only significant effect was time,  $F(1,72) = 5.96$ ,  $p < .02$ . This effect was due to a slight decrease in serum albumin. Therefore, measurement of serum albumin levels did not indicate any differences among the groups which could account for differences observed for specific or total IgG.

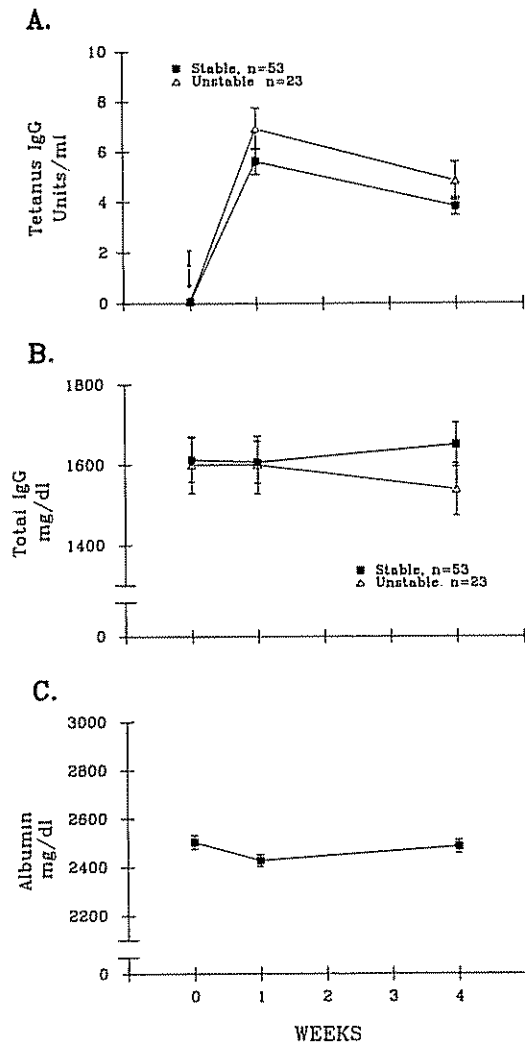


FIG. 3. Results of study II collapsed across tank. (A) Serum levels of specific IgG to tetanus (units/ml) in monkeys housed in stable and unstable social groups. (B) Serum levels of total IgG (mg/dl) in monkeys housed in stable and unstable social groups. (C) Serum levels of albumin (mg/dl) collapsed across all conditions and both ranks.

### DISCUSSION

The present data indicate that monkeys immunized with tetanus toxoid mounted both a primary and a secondary immune response. Moreover, both rank and stability of social condition influenced the magnitude of the specific immune response to tetanus toxoid as measured by the production of specific IgG antibodies.

In study I there was an effect of rank in which subordinate monkeys demon-

strated greater specific antibody production to tetanus toxoid. This was not due to a general effect on serum protein levels as dominant monkeys demonstrated greater levels of total serum IgG and both groups had similar levels of serum albumin. Furthermore, the acute stress of a single social reorganization was not sufficient to influence the primary immune response.

Although the effects of social rank on antibody production in study I appear contrary to expectations, the literature on the effects of dominance hierarchies as factors in antibody responses is small and contradictory. Fauman (1987) found that subordinate mice had greater antibody levels after KLH immunization, while Vessey (1964) found that dominant mice had higher antibody titers after immunization.

In the present set of experiments the secondary immune response was determined among animals exposed to chronic social stress (repeated group reorganization) or stable social conditions. In study II monkeys had been in stable or unstable social conditions for 9 months. These data demonstrated a greater increase in antibody titer in response to the secondary immunization in those monkeys exposed to the chronic stress of social reorganization (USS). The difference between the USS and the SSS was not affected by rank and could not be explained by differences in total serum IgG or by serum albumin levels.

The results of study II are particularly striking in that we find effects of reorganization after 9 months. This suggests a lack of habituation to the chronic social stress employed in this study. This contradicts several animal studies which indicate that repeated presentations of an acute stressor can cause habituation of stressor-induced immune changes (Lysle, Lyte, Fowler, & Rabin, 1987; Lysle, Cunnick, Fowler, & Rabin, 1988a; Lysle, Cunnick, Wu, Caggiula, Wood, & Rabin, 1988b). However, those studies examined repeated presentations of an acute stressor over a short time, while our study examined a complex social stressor which probably varied across reorganizations based upon the interactions of the individual animals in each new social group.

A second important feature of study II is that the social stress condition was capable of inducing a difference in a secondary antibody response. Most studies examining the effects of stress on antibody production report differences in the primary response. Differences in a secondary response have been reported using low-dose antigen administration with an acute physical stressor (Moynihan, Ader, Grota, Schachtman, & Cohen; 1990) and in response to crowding (Solomon; 1969).

Although the results of the primary and secondary immunizations differ with respect to the effect of rank and social reorganization on the development of a specific antibody response, it is important to emphasize that the stressors in the two studies were different. The stressor used in the first experiment involved an acute exposure of a single social reorganization to monkeys which had been in stable social groups for 7 months. On the other hand, the stressor in the second study was the 10th such reorganization, representing a chronic condition of social instability for those animals exposed to it. Moreover, the timing of the reorganization in relation to the primary and secondary immunizations was not identical as the immunizations occurred prior to reorganization for the primary immunizations

anc  
tio  
rel  
res  
Sir  
198  
tet  
sho  
Wi  
du  
&  
ati  
Me  
lar  
siv  
ch  
the  
the  
de  
inc  
sp  
va  
hig  
ing  
im  
wi  
ing  
is  
be  
ha  
hu  
all  
go  
to  
tic  
ar  
Th  
S C

and subsequent to the reorganization for the USS in the secondary immunizations. There have been many reports which indicate that timing of a stressor in relation to an immunization will determine its ability to modulate an antibody response (Esterling & Rabin, 1987; Laudenslager, Fleshner, Hofstadter, Held, Simons, and Maier, 1988; Zalcman, Minkiewicz-Janda, Richter, & Anisman, 1988; Zalcman, Richter, & Anisman, 1989).

The mechanisms by which social stress can modulate the antibody response to tetanus are unknown. However, this primate model of social stress has been shown to alter hormones such as cortisol and catecholamine (Herd, 1981, Williams, Lane, Kuhn, Melosh, White, & Schanberg, 1982). Cortisol is also produced excessively by the enlarged adrenal gland of subordinate animals (Shively & Kaplan, 1984). Each of these hormones is capable of inducing immune alterations (Munck, Guyre, & Holbrook, 1984; Byron, 1972; Bourne, Lichtenstein, Melmon, Henney, Weinstein, & Shearer, 1974). Furthermore, sympathomedullary reactivity *in vivo* has been shown to significantly influence immune responsiveness (Cunnick, Lysle, Kucinski, & Rabin, 1990).

Alterations in the production of specific antibodies due to stress may indicate changes in immune regulation. The immune system is regulated, in part, through the interaction of T-helper and T-suppressor lymphocytes. Hormones, such as those altered by stress, affecting both types of T-cells would produce effects dependant upon the extent to which each cell type was affected.

The view that these data are counter intuitive may be an oversimplification and incorrect. It is unclear that an increased antibody level indicates a better response. The increase in antibody levels does indicate a change in regulation that varied dependant upon the different social conditions. Moreover, in a system as highly controlled as the immune system there may be some cost for overresponding.

Our choice of tetanus toxoid as an antigen was made based upon its utility in immunizing the monkeys to a possible pathogen. As tetanus toxoid is premixed with aluminum phosphate, an adjuvant to immunization therapy, it is not surprising that all animals demonstrated an immune response to the toxoid. However, it is interesting that even in the presence of an adjuvant there were differences between the groups in both studies I and II. The differences may have been larger had the antigenic stimulus consisted of the toxoid alone.

In summary, this study presents the immunological outcome of a unique non-human primate model of chronic social stress. This model is advantageous as it allows the study of a chronic social stressor spanning 9 months and, thus, is a good model for chronic social stress in humans. This report presents the first data to indicate the relevance of chronic social stress as a modulator of immune function and also indicates that social interactions (maintenance of dominance hierarchy) can modulate immune function.

#### ACKNOWLEDGMENTS

The authors thank Ms. Ada Armfield and Mr. Paul Wood for their excellent technical assistance. This work was supported in part by the Pathology Education Research Foundation (Pittsburgh, PA). S.C.'s participation was supported by a Research Scientist Development Award from the National

Institute of Mental Health (K02MH00721). J.R.K.'s participation was supported by Grants HL14164 and HL26561. S.B.M.'s participation was supported by Grant HL40962.

## REFERENCES

- Bartrop, R. W., Lazarus, L., Lockhurst, E., Kiloh, L. G., & Penny, R. (1977). Depressed lymphocyte function after bereavement. *Lancet* 1, 834-836.
- Bernstein, I. S. (1970). Primate status hierarchies. In L. A. Rosenblum (Ed.), *Primate behavior: Developments in field and laboratory research*, Vol. 1, pp. 71-107. Academic Press: New York.
- Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y., & Shearer, G. M. (1974). Modulation of inflammation and immunity by cyclic AMP. *Science* 184, 19-28.
- Byron, J. W. (1972). Evidence for a  $\beta$ -adrenergic receptor initiating DNA synthesis in haemopoietic stem cells. *Exp. Cell Res.* 71, 228-232.
- Coe, C. L., Rosenberg, L. T., Fischer, M., & Levine, S. (1987). Psychological factors capable of preventing the inhibition of antibody responses in separated infant monkeys. *Child Dev.* 58, 1420-1430.
- Coe, C. L., Rosenberg, L. T., & Levine, S. (1988). Effects of separation on humoral immunity in infant primates. *Intern. J. Neurosci.* 40, 289-302.
- Cunnick, J. E., Lysle, D. T., Kucinski, B. J., & Rabin, B. S. (1990). Evidence that shock-induced immune suppression is mediated by adrenal hormones and peripheral  $\beta$ -adrenergic receptors. *Pharmacol. Biochem. Behav.* 36, 645-651.
- Dorian, B., Garfinkel, P., Brown, G., Shore, A., Gladman, D., & Keystone, E. (1982). Aberrations in lymphocyte subpopulations and function during psychological stress. *Clin. Exp. Immunol.* 50, 132-138.
- Esterling, B., & Rabin, B. S. (1987). Stress-induced alteration of T-lymphocyte subsets and humoral immunity in mice. *Behav. Neurosci.* 101, 115-119.
- Fauman, M. A. (1987). The relation of dominant and submissive behavior to the humoral immune response in BALB/c mice. *Biol. Psychiatry* 22, 776-779.
- Glaser, R., Rice, J., Speicher, C. E., Stout, J. C., & Kiecolt-Glaser, J. K. (1986). Stress depresses interferon production by leukocytes concomitant with a decrease in natural killer cell activity. *Behav. Neurosci.* 100, 675-678.
- Glaser, R., Kennedy, S., Lafuse, W. P., Bonneau, R. H., Speicher, C., Hillhouse, J., & Kiecolt-Glaser, J. K. (1990). Psychological stress induced modulation of interleukin 2-receptor gene expression and interleukin 2 production in peripheral blood leukocytes. *Arch. Gen Psychiatry* 47, 707-712.
- Herd, J. A. (1981). Behavioral factors in the physiological mechanisms of cardiovascular disease. In S. M. Weiss, J. A. Herd, & B. H. Fox (Eds.), *Perspectives on behavioral medicine*, pp. 55-65. Academic Press: New York.
- Kiecolt-Glaser, J. K., Glaser, R., Shuttleworth, E. C., Dyer, C. S., Ogrocki, P., & Speicher, C. E. (1987). Chronic stress and immunity in family caregivers of Alzheimer's disease victims. *Psychosom. Med.* 49, 523-535.
- Kaplan, J. R., Manuck, S. B., Clarkson, T. B., Lusso, F. M., & Taub, D. M. (1982). Social status, environment and atherosclerosis in cynomolgus monkeys. *Arteriosclerosis* 2, 359-368.
- Kaplan, J. R., Manuck, S. B., Clarkson, T. B., Lusso, F. M., Taub, D. M., & Miller, E. W. (1983). Social stress and atherosclerosis in normocholesterolemic monkeys. *Science* 220, 733-735.
- Kaplan, J. R., Manuck, S. B., Adams, M. R., Weingand, K. N., & Clarkson, T. B. (1987). Inhibition of coronary atherosclerosis by propranolol in behaviorally predisposed monkeys fed an atherogenic diet. *Circulation* 76, 1364-1372.
- Kaplan, J. R., Manuck, S. B., & Gatsonis, C. (1990). Heart rate and social status among male cynomolgus monkeys (*Macaca fascicularis*) housed in disrupted social groupings. *Am. J. Primatol.* 21, 175-187.
- Laudenslager, M. L., Reite, M., & Held, P. E. (1986). Early mother/infant separation experiences impair the primary but not the secondary antibody response to a novel antigen in young pigtail monkeys. *Psychosom. Med.* 48, 304.
- Laudenslager, M. L., Fleshner, M., Hofstadter, P., Held, P. E., Simons, L., & Maier, S. F. (1988).

Ly:

Ly:

Ly:

Mo

Mu

Sad

Sch

Sed

Shi

Sol

Wil

Ves

Zab

Zab

Rec

- Suppression of specific antibody production by inescapable shock: Stability under varying conditions. *Brain Behav. Immun.* 2, 92-101.
- Lysle, D. T., Lyte, M., Fowler, H., & Rabin, B. S. (1987). Shock-induced modulation of lymphocyte reactivity: Suppression, habituation, and recovery. *Life Sci.* 41, 1805-1814.
- Lysle, D. T., Cunnick, J. E., Fowler, H., & Rabin, B. S. (1988a). Pavlovian conditioning of shock-induced suppression of lymphocyte reactivity: Acquisition, extinction, and reexposure effects. *Life Sci.*, 42, 2185-2194.
- Lysle, D. T., Cunnick, J. E., Wu, R., Caggiula, A. R., Wood, P. G., & Rabin, B. S. (1988b). 2-Deoxy-d-glucose modulation of T-lymphocyte reactivity: Differential effects on lymphoid compartments. *Brain Behav. Immun.* 2, 212-221.
- Moynihan, J. A., Ader, R., Grota, L. J., Schachtman, T. R., & Cohen, N. (1990). The effects of stress on the development of immunological memory following low-dose antigen priming in mice. *Brain Behav. Immun.* 4, 1-12.
- Munck, A., Guyre, P. M., & Holbrook, M. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrinol Rev.* 5, 25-44.
- Sade, D. S. (1967). Determinants of dominance in a group of free ranging rhesus monkeys. In S. Altmann (Ed.), *Social communication among primates*, pp 99-114. Univ. of Chicago Press: Chicago.
- Schleifer, S. J., Keller, S. E., Camerino, M., Thornton, J. C., & Stein, M. (1983). Suppression of lymphocyte stimulation following bereavement. *J. Am. Med. Assoc.* 250, 374-377.
- Sedgewick, A. K., Ballou, M., Sparks, K., & Tilton, R. C. (1983). Rapid quantitative microenzyme linked immunoabsorbent assay for tetanus antibodies. *J. Clin. Microbiol.* 18, 104-109.
- Shively, C., & Kaplan, J. (1984). Effects of social factors on adrenal weight and related physiology of *Macaca fascicularis*. *Physiol. Behav.* 33, 777-782.
- Solomon, G. F. (1969). Stress and antibody response in rats. *Int. Arch. Allergy* 35, 97-104.
- Williams, R. B., Lane, J. D., Kuhn, C. M., Melosh, W., White, A. D., & Schanberg, S. M. (1982). Type A behavior and elevated physiological and neuroendocrine responses to cognitive tasks. *Science* 218, 483-485.
- Vessey, S. H. (1964). Effects of grouping on levels of circulating antibodies in mice. *Proc. Soc. Exp. Biol. Med.* 115, 252-255.
- Zalcman, S., Minkiewicz-Janda, A., Richter, M., & Anisman, H. (1988). Critical periods associated with stressor effects on antibody titers and on the plaque-forming cell response to sheep red blood cells. *Brain Behav. Immun.* 2, 254-266.
- Zalcman, S., Richter, M., & Anisman, H. (1989). Alterations of immune functioning following exposure to stressor-related cues. *Brain Behav. Immun.* 3, 99-109.

Received May 3, 1991