

Effects of Social Reorganization on Cellular Immunity in Male Cynomolgus Monkeys

SCOTT W. LINE¹, JAY R. KAPLAN¹, EUGENE R. HEISE², JULIA K. HILLIARD³, SHELDON COHEN⁴, BRUCE S. RABIN⁵, AND STEPHEN B. MANUCK⁶
Departments of ¹Comparative Medicine and ²Microbiology/Immunology, Bowman Gray School of Medicine, Winston-Salem, North Carolina; ³Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas; ⁴Department of Psychology, Carnegie-Mellon University, Pittsburgh, Pennsylvania; ⁵Division of Clinical Immunopathology, Department of Pathology, and ⁶Department of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania

Exposure to acute stressors has been shown to impair cellular immunity in human beings and other animal species. Comparatively little is known, however, about the effects of long-term stressors on immune function and how individual behavioral characteristics may mediate differences in immune function and clinical disease susceptibility. To determine the effects of social stress on cellular immunity and reactivation of a latent herpesvirus, 20 Herpes B virus-positive male cynomolgus monkeys were exposed to four periodic reorganizations of social group memberships over 5 months. Observations were made to categorize individuals as high or low in expression of aggressive, fearful, and affiliative behaviors. Complete blood counts, lymphocyte proliferation tests, and natural killer cell cytotoxicity assays were performed immediately before and 4 days after reorganizations. Herpesvirus-specific immunoglobulin G antibody levels were measured, and oral and conjunctival swabs were cultured for virus. Reorganization was associated with increased lymphocyte counts ($P = 0.0009$) and decreased lymphocyte proliferation in response to phytohemagglutinin ($P < 0.005$), particularly among monkeys showing high levels of fear ($P = 0.0137$). High-aggressive monkeys showed lower baseline natural killer cell activity ($P = 0.0013$) and higher lymphocyte counts ($P = 0.013$) than low-aggressive monkeys. Herpesvirus antibody titers decreased over time ($P < 0.004$) and no positive virus cultures were obtained. Measures of cellular immunity and behavior were unrelated to virus-specific antibody titers. These results suggest that repeated exposure to a social stressor alters several measures of cellular immunity, and that some of these changes may be predicted by individual differences in agonistic behavior. In contrast to human studies, the results suggest that some psychological stressors may not cause reactivation of a common herpesvirus in this species. © 1996 Wiley-Liss, Inc.

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Address reprint requests to Dr. Jay Kaplan, Comparative Medicine Clinical Research Center, Department of Comparative Medicine, Bowman Gray School of Medicine of Wake Forest University, Medical Center Boulevard, Winston-Salem, NC 27157-1040.

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INTRODUCTION

Exposure to both acute and chronic stressors has been shown to impair cellular immune function in human beings [Dobbin et al., 1991; Sieber et al., 1992; Herbert & Cohen, 1993] and other animal species [Steplewski et al., 1985; Kusnecov et al., 1992]. Not all individuals who are exposed to stress experience deleterious effects on immune function, however. These individual differences may be explained in part by differences in personality or styles of coping with stressful events. Psychological variables, including measures of attachment and depression, have been related to measures of immune function in human studies [Kennedy et al., 1988]. In experiments with nonhuman primates, individuals of low social rank [Gust et al., 1991] and those showing more agonistic behavior [Boccia et al., 1992] tend to show impaired immune responses to stress, while individuals displaying high levels of affiliative behavior are more likely to be resistant to the effects of stress [Cohen et al., 1992].

Impairments in cellular immunity have been postulated to increase susceptibility to certain viral infections and cancers. Herpesviruses, for example, generally persist in a latent state after primary infection, and may be reactivated when the host is immunosuppressed [Friedmann et al., 1977; Goldmeier & Johnson, 1982; Kiecolt-Glaser et al., 1991]. Reactivation may be detected by recurrence of clinical signs, an increase in antibody titer to the virus [Glaser et al., 1987], partial expression of the viral genome [Glaser et al., 1991], or theoretically the secretion of complete virus.

Although social stressors may cause decreases in immune function and subsequent herpesvirus reactivation, not all individuals at risk show evidence of recurrent infection [Glaser et al., 1985]. Whether this is due to variations in how the stressor is perceived, differences in how individuals cope with the stressor, or differences in immune function is unclear. Due to their complex social behaviors and close phylogenetic relationship to human beings, nonhuman primates can serve as excellent models in which to study the interactions between these factors and the expression of herpesvirus infections.

Using a well characterized nonhuman primate model of social stress [Kaplan et al., 1991], we examined the relationships between social stress, individual behavioral characteristics, and cellular immune function. In this system monkeys are stressed by reorganizing them into new social groups at approximately monthly intervals. This type of manipulation disrupts patterns of social interaction, and has been associated with exacerbated atherogenesis [Kaplan et al., 1982] and with altered specific antibody production in response to immunization with tetanus toxoid [Cunnick et al., 1991].

In addition to evaluating cell-mediated immunity, the effects of acute stress on reactivation of Herpes B virus (*Cercopithecine herpesvirus 1*), a virus with important public health significance for nonhuman primate handlers, were examined. This herpesvirus is endemic in several macaque species and is capable of causing fatal encephalitis in human beings [Weigler, 1992]. Based on studies with human beings [Glaser et al., 1987] and rodents [Bonneau et al., 1991a], we hypothesized that the acute stresses associated with reorganization of social groups of male cynomolgus monkeys (*Macaca fascicularis*) would lead to impaired cellular immune function, and an increase in viral activity. Based on previous experiments with cynomolgus monkeys [Kaplan et al., 1991; Cohen et al., 1992], we expected that individuals showing high levels of affiliative behavior would be buffered from

the effects of the stressor, and less likely to show immune suppression and virus reactivation. Although reorganization led to several changes in cellular immunity associated with behavioral differences between monkeys, evidence of B virus reactivation was not found.

METHODS

Subjects

Subjects were 20 adult male cynomolgus monkeys recently imported from Indonesia. All had positive antibody titers to B virus before the start of the experiment. The mean estimated age was 5 years, with individual ages ranging from 4 to 6 years. They weighed a mean of 4.75 ± 0.15 (SEM) kg at the start of the experiment. After quarantine in single cages, which included regular tuberculosis testing and routine treatment for potential parasitic infections, all monkeys were randomly assigned to social groups. They were placed in groups of five monkeys in indoor pens, along with 55 other adult males from the same source. Indoor lights were controlled automatically on a 12 hr cycle; daylight was also visible through large windows on both sides of the building. They were fed a diet containing 0.05 mg cholesterol/kilocal that consisted of 30% of calories from fat, 21% from protein, and 49% from carbohydrates. Continuous access to automatic watering devices was provided.

Social Stressor

Reorganization of group membership was performed four times at approximately 6 week intervals from January to May 1992. "Reorganization" is defined as the point at which group membership was changed, and "social grouping" as the 6 week period during which individuals were housed in a particular cohort. After reorganization, each monkey was housed with three or four new animals. Each reorganization created unique combinations of subjects. The choice of group members was designed to maximize the differences in group composition between the old and new groups for each individual. (The 20 subjects of this experiment were mixed among the other 55 monkeys as part of another study.)

Data Collection

Samples were collected immediately before the first, second, and fourth reorganizations, as well as 4 days after each of these reorganizations. Blood samples for immune assays and serum albumin determination were taken after the subjects were sedated with ketamine hydrochloride (15 mg/kg body weight). Buccal and conjunctival swabs for virus culture, oral examination for herpetic lesions, and measurement of body weight were performed at the same time. Serum albumin was included as a control for nutritional status.

Social Status and Behavior

Behavior of each subject was recorded 12 times during each social grouping throughout the experiment. Social and nonsocial behaviors were recorded using a portable computer during 15 min focal observations [Altmann, 1974]. A total of 240 hr of focal behavioral data was collected. Individual social behaviors were summed into aggressive, fearful, and affiliative categories (Table I), and the frequency of bouts/hr for each category was calculated. Individual monkeys were grouped as high or low for each of the three behavioral categories based on whether they were above or below the median score for the behavior.

Determination of each monkey's rank within its group was determined by observation of fight outcomes and displacements during each social grouping. The

TABLE I. Individual Behaviors Making Up the Behavioral Categories

Aggressive	Fearful	Affiliative
Slap/grab/push/pull	Submissive present	Nonagonistic present
Bite	Crouch	Social play
Grapple	Flee	Initiate friendly interaction
Open-mouth threat	Move away	Body contact
Stare threat	Grimace	Allogroom
Chase/charge/lunge	Submissive lipsmack	Mutual friendly lipsmack
Displace	Scream/squeal	
Initiate agonistic interaction		

monkeys were ranked 1–5, with the dominant animal (rank 1) being the winner of fights with all other animals in that group. Rank determinations were done independently of the focal behavioral observations. As we have done with previous studies of small social groups [Cunnick et al., 1991], monkeys with a social rank above 3 were considered high ranking, while those ranked 3 or below were considered low ranking (dominant: $n = 7$; subordinate: $n = 13$).

Immune Measures

Complete blood counts (CBCs), nonspecific lymphocyte blastogenesis, and natural killer (NK) cell cytotoxicity assays were performed at the Bowman Gray School of Medicine. Total white blood cell and differential counts were determined by standard techniques.

Lymphocyte blastogenesis was evaluated with a whole blood technique [Keller et al., 1981]. This technique requires a smaller amount of blood and better reflects *in vivo* conditions since it preserves cellular and humoral elements not present in separated cell techniques [Fletcher et al., 1987]. Concanavalin A (Con A) was prepared in concentrations of 1, 5, 10, and 20 $\mu\text{g}/\text{dl}$ and 100 μl dispensed in triplicate into 96-well culture plates. Phytohemagglutinin (PHA) was prepared in concentrations of 1, 5, 10, 20, and 30 $\mu\text{g}/\text{dl}$ and similarly dispensed. Three control wells containing 100 μl of assay medium (RPMI 1640 supplemented with 25 mM HEPES, gentamycin, and L-glutamine) were prepared for each sample. Heparinized blood samples were diluted 10-fold in assay medium, after which 100 μl was dispensed into the wells of the culture plates. These were incubated for 96 hr at 37°C and 5% CO_2 . Eighteen hours before the end of incubation, 1 μCi of ^3H -thymidine was added to each well. Cells were harvested onto fiberglass filters using a semi-automatic harvester, and the filters counted using a liquid scintillation counter. Net counts/min (mean stimulated – mean control counts) were calculated using a microcomputer, then expressed as net counts/ 10^5 lymphocytes using the total lymphocyte count from the CBC.

The NK cell activity was assessed immediately before and 4 days after the fourth reorganization using a sodium- ^{51}Cr release assay [Kaplan et al., 1991]. Heparinized blood samples were diluted in 3% dextran and allowed to stand 1 hr at room temperature. The white blood cell-enriched upper layer was transferred to a second tube and underlayered with lymphocyte separation medium (Organon Technica Corp., Durham, NC). After centrifugation for 20 min at 300 g , the mononuclear cell band was collected from the interface and washed twice in Hanks' Buffered Salt Solution. The cells were then resuspended in assay medium (RPMI 1640 supplemented with 25 mM HEPES, gentamycin, L-glutamine, and 10% fetal calf serum), counted, and checked for viability by trypan blue exclusion. The ef-

effector cells were then dispensed in volumes of 100 μ l in triplicate into 96-well culture plates at concentrations that yielded effector:target ratios of 100:1, 50:1, 25:1, and 12.5:1. Spontaneous release of ^{51}Cr was determined from wells containing assay medium; total release of ^{51}Cr was determined from wells containing 5% Triton-X.

Target cells used were from the K562 human myelogenous leukemia cell line, and were grown in assay medium. Cells in the log phase of growth were incubated for 45 min with ^{51}Cr , washed three times in assay medium, and added to the culture plates. The plates were centrifuged for 3 min at 100 g to assure effector:target cell contact, then incubated for 4 hr at 37°C and 5% CO_2 . After incubation, the plates were centrifuged for 10 min at 400 g . Supernatants were harvested and counted in a γ counter. The percent cytotoxicity was calculated from the following formula:

$$\% \text{cytotoxicity} = \frac{E - S}{T - S} \times 100$$

where E is the mean cpm from the experimental wells, S is the mean cpm from the spontaneous release wells, and T is the mean cpm from the total release wells.

Virus Titers and Cultures

Determination of B virus-specific immunoglobulin G (IgG) levels as well as virus cultures was performed at the Southwest Foundation for Biomedical Research. B virus-specific antibody was measured in serum samples by a previously described enzyme-linked immunosorbent assay [Katz et al., 1986] and in certain cases also by Western blot. Transport medium containing swabs used to collect mucosal surface secretions was adsorbed onto monolayers of Vero cells for 1 hr. Subsequently, Dulbecco's modified medium with 1.5% fetal calf serum was added to culture wells after removal of the transport medium. Monolayers were monitored daily over a 7–10 day period for evidence of any cytopathic effect. In the event of a cytopathic effect, samples were replated and radiolabeled metabolically with ^{35}S -methionine. After 24–48 hr postinfection, monolayers were scraped and solubilized in a 1% final concentration of Tween 20 and deoxycholate at 37°C for 60 min. Polyacrylamide gel electrophoresis was performed on samples further treated to a final concentration of 1.5% sodium dodecyl sulfate. Dried gels were exposed to Kodak RP-X-Omat film. Autoradiographs were examined for the presence of B virus-specific polypeptides [Hilliard et al., 1987].

Data Analysis

To determine whether the aggressive, fearful, and affiliative behavior categories described independent characteristics, Pearson correlation coefficients between pairs of variables were calculated.

Within-subject comparisons of pre- and postreorganization samples were made by repeated measures analysis of variance (ANOVA). Within-subject independent variables included reorganization number (i.e., 1, 2, and 4), time of sample in relation to reorganization (before and after), and mitogen concentration. Social rank and levels of affiliative, aggressive, and fearful behaviors were between-subjects independent variables. Dependent variables were CBC differential cell counts, nonspecific lymphocyte blastogenesis, NK activity, and B virus-specific IgG titers. Data were log- or square root-transformed where necessary to meet the assumption of normality of distribution of variance. Separate ANOVAs were performed for each of the between-subjects behavioral variables. The within-subject

factors were the same for each of these ANOVAs, and are not reported redundantly. Power calculations were made to determine the probability of committing a Type II error when testing the effects of rank, aggression, fear, and affiliation on the immunologic responses [Glantz, 1992].

RESULTS

Although there were a few minor injuries associated with reorganization of social groups, mean body weights and albumin levels did not change significantly across the social groupings, and the monkeys remained in generally good health.

Behavior

Repeated measures ANOVAs of the rates of aggressive, fearful, and affiliative behaviors during each social grouping showed no significant variations across social groupings. Consequently, the mean rates across groupings of each behavioral category were used to determine the relationships between behavioral categories and between behavior and measures of immune function.

The median rates of aggressive, fearful, and affiliative behaviors were 1.8, 1.9, and 2.8 bouts per hour, respectively. Rates of aggressive and fearful behavior were negatively correlated ($r = -0.63$, $P < 0.005$), while the rate of affiliative behavior was unrelated to either aggressive ($r = 0.03$, $P > 0.1$) or fearful behavior ($r = -0.26$, $P > 0.1$).

Dominance ranks for individual monkeys were stable, with no significant variation across the four social groups (repeated measures ANOVA, $P > 0.3$). Six monkeys had an average rank above 3 and were considered high ranking, while 14 had an average rank of 3 or below and were considered low ranking. There were no significant differences between high- and low-ranking monkeys in rates of aggressive, fearful, or affiliative behaviors (all P s > 0.3). There were also no statistically significant effects of dominance rank on any of the measures of immune function or herpesvirus reactivation.

CBC

Among the components of the CBC, only the lymphocyte count showed effects related to manipulation of the social environment (Table II). In an aggression_{hi,lo} × time_{before,after} × reorganization number_{1,2,4} ANOVA, there were significant main effects for aggression ($F_{1,18} = 7.60$, $P = 0.013$), reorganization number ($F_{2,17} = 4.74$, $P = 0.0149$), and time ($F_{1,18} = 15.58$, $P = 0.0009$). There were no significant interactions. Total lymphocytes increased by approximately 1,000/ μ l by 4 days after each reorganization (Fig. 1). Lymphocyte counts were higher at the first reorganization, and were higher in high-aggressive monkeys than in low-aggressive monkeys throughout the experiment.

NK Assay

Natural killer cell cytotoxicity assays were performed only at the fourth reorganization due to technical problems at the earlier sampling points. There was a significant increase in the percent of target cells lysed after reorganization ($F_{1,18} = 10.16$, $P = 0.0051$), and a significant interaction between time of sample and aggressive behavior ($F_{1,18} = 14.45$, $P = 0.0013$; Table II). High-aggressive monkeys showed lower NK activity than low-aggressive monkeys before reorganization, and increased to levels equivalent to low-aggressive monkeys at 4 days post-reorganization (Fig. 2). The results were similar at all four effector: target ratios.

TABLE II. Relationships Between Social Rank (High and Low), Individual Behavioral Characteristics (High and Low), Time (Before and After), and Reorganization Number (1, 2, and 4) and Immune Responses*

	PHA-stimulated proliferation	Con A-stimulated proliferation	NK activity	B virus titer	Lymphocyte count ¹
Social rank	NS ²	NS	NS	NS	NS
Aggression	NS ³	NS	0.0013	NS	0.013
Fear	0.0137	0.0025	time × aggr. NS ³	NS	NS
Affiliation	NS	NS	NS ³	NS ³	NS
Time	0.0044	NS	0.0051	0.0035	0.0009
Reorganization number	0.0001	0.0003	— ⁴	0.0001	0.0149

*Numbers represent significance levels for main effects (or interactions) in ANOVAs as described in the text
¹Remainder of CBC components did not show significant results
²NS, not significant
³Power < 0.80
⁴NK activity was measured only at the fourth reorganization.

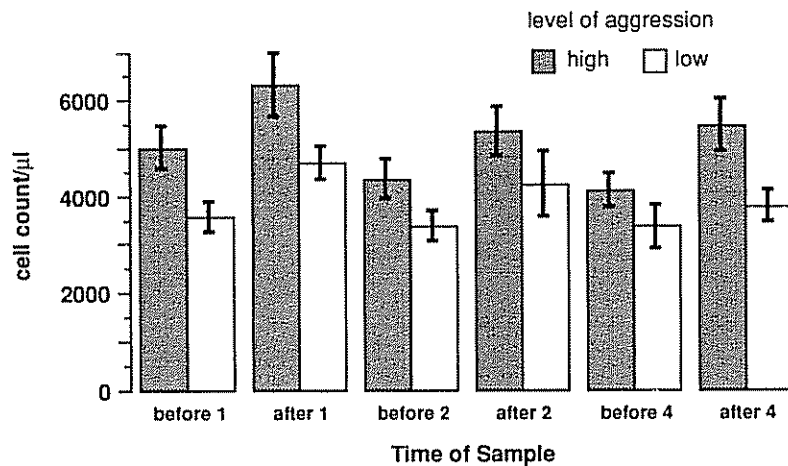


Fig. 1. Effects of reorganization on lymphocyte count. Each bar is mean (\pm SEM) for ten monkeys rated above (gray bars) or below (open bars) the median for rate of aggressive behavior. Samples were taken immediately before and 4 days after first, second, and fourth social reorganizations. Lymphocyte counts increased after each reorganization ($P = 0.0009$), and were higher among high-aggressive monkeys ($P = 0.013$).

Mitogen Responses

Lymphocyte proliferation in response to mitogen stimulation increased over the course of the experiment (Table II; Fig. 3). The main effect of reorganization was significant for both PHA ($F_{2,17} = 28.77, P < 0.0001$) and Con A ($F_{2,17} = 12.42, P = 0.0003$). The main effect of time was also significant for PHA. Four days after each reorganization the PHA response was lower ($F_{1,18} = 14.98, P = 0.0044$), while the Con A response was unchanged ($P > 0.1$).

Among the behavioral variables, there were significant interactions between time of sample and fear for both PHA ($F_{1,18} = 7.46, P = 0.0137$) and Con A ($F_{1,18} = 12.29, P = 0.0025$). High-fear monkeys showed a decreased response to PHA

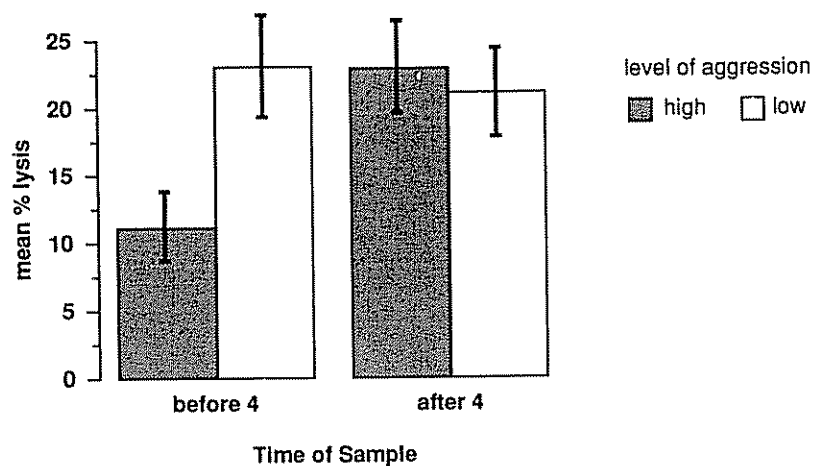


Fig 2. Natural killer (NK) cell activity in high- (gray bars) and low- (open bars) aggressive monkeys at the fourth reorganization. Each bar is percent cytotoxicity for effector:target ratios of 100, 50, 25, and 12.5:1 averaged together. Other details are as in Figure 1. High-aggressive monkeys had lower baseline NK activity ($P = 0.0013$)

after reorganization (before, $109,600 \pm 6,900$ net cpm mean \pm SEM; after, $83,000 \pm 6,700$), while low-fear monkeys did not change (before, $107,900 \pm 6,500$; after, $106,300 \pm 7,200$). With Con A stimulation, high-fear monkeys showed a small decrease after reorganization (before, $136,800 \pm 9,300$; after, $125,600 \pm 9,000$), while low-fear monkeys showed an increase (before, $106,900 \pm 7,500$; after, $134,200 \pm 11,100$).

B Virus Antibody and Virus Culture

There were no positive virus cultures obtained during the experiment. Further, no oral lesions consistent with active herpesvirus infection were observed during physical examination at each sampling point. B virus-specific IgG titers decreased for most monkeys during the experiment (Table II; Fig. 4). There were significant main effects of time ($F_{1,18} = 11.28$, $P = 0.0035$) and reorganization ($F_{2,17} = 13.38$, $P < 0.0001$). Contrast comparisons showed that titers were lower 4 days after the second and fourth reorganizations (P s < 0.0025), and that titers at the fourth reorganization were lower than at the first or second (P s < 0.001). Although half of the subjects did have an increased titer after the first reorganization, the magnitude of the change was relatively small (less than one order of magnitude in each case). Increases were shown by even fewer subjects after the second and fourth reorganizations. There were no significant associations between behavioral characteristics or social rank and virus titers.

Ten of the 20 monkeys showed a small increase in B virus titer after the first reorganization, five after the second reorganization, and only one after the fourth reorganization. All increases were less than one order of magnitude, and none was considered significant.

Although we found no evidence of reactivation of B virus in the 20 monkeys that were subjects of this experiment, there were signs of herpesvirus transmission in the building during this time. Four of the 75 male cynomolgus monkeys in the building were seronegative for B virus when first placed into social groups. By the

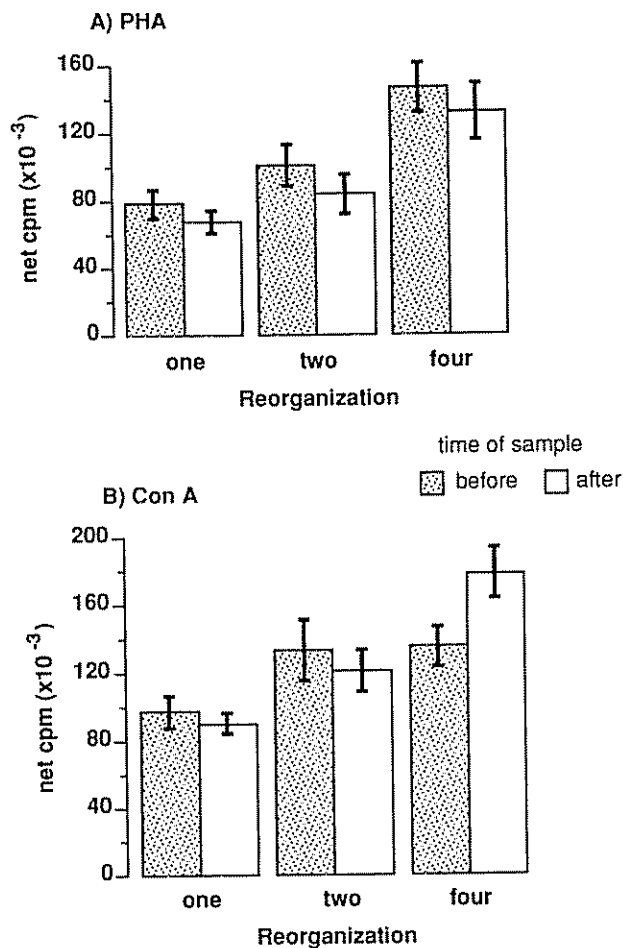


Fig 3. Effects of reorganization on nonspecific lymphocyte blastogenesis measured before (dotted bars) and after (open bars) first, second, and fourth social reorganizations. Each bar is mean (\pm SEM) for 20 monkeys. A: Mean of responses to 5, 10, and 20 μ g/ml phytohemagglutinin (PHA) stimulation. Responses increased during the study ($P < 0.0001$) and were decreased 4 days after each reorganization ($P = 0.0044$). B: Mean of responses to 5, 10, 20, and 30 μ g/ml concanavalin A (Con A) stimulation. Responses increased during the study ($P = 0.0003$).

end of the experiment, three of the four had developed positive B virus-specific IgG titers (all were $> 1:3,800$).

Power Calculations

Power calculations showed that more than 3/4 of the tests performed had a power greater than 0.80. The tests with lower power were for affiliation and B virus titer, affiliation and NK activity, aggression and PHA response, and fear and NK activity (Table II).

DISCUSSION

Social reorganization led to several changes in the cellular immune function of these monkeys indicative of a response to stress. Although decreases in lymphocyte counts are more commonly reported after acute stress in human beings [Herbert &

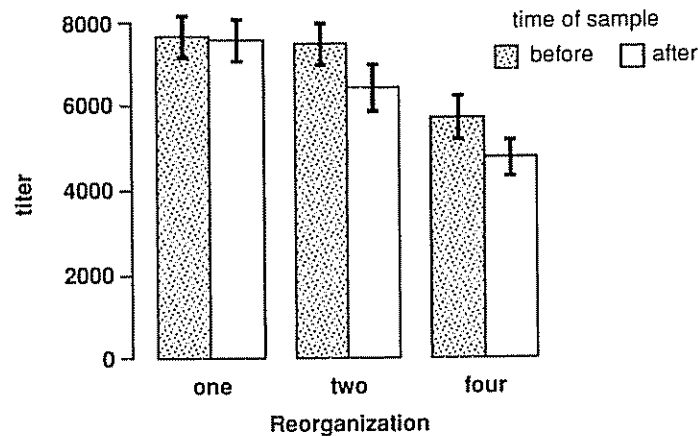


Fig. 4. Mean B virus-specific IgG titers. Each bar is mean (\pm SEM) for 20 seropositive monkeys taken immediately before (dotted bars) and 4 days after (open bars) first, second, and fourth social reorganizations. Mean titer decreased during the experiment ($P = 0.0035$) and was lower 4 days after the second and fourth reorganizations ($P_s < 0.0025$).

Cohen, 1993], increases have also been seen [Landmann et al., 1984; Knapp et al., 1992]. Decreases in PHA-stimulated lymphocyte proliferation [Bachen et al., 1992] and increases in NK activity have also been observed [Knapp et al., 1992]. In pig-tailed macaques, poststress increases in NK activity have also been found [Boccia et al., 1992].

Consistent with earlier studies in our laboratory [Kaplan et al., 1991; Cohen et al., 1992], we found no relationship between social rank and measures of cellular immune function. We found several associations between agonistic behavior patterns and immune function, but not the increased immune responses among highly affiliative monkeys observed in previous studies. The differences may reflect the fact that monkeys in the earlier experiments had been reorganized monthly for over 2 years, while in the present experiment reorganizations had gone on for only 5 months.

The increase in lymphocyte count after each reorganization was probably due to changes in cell migration patterns, an effect similar to that suspected of causing lymphocytosis in human beings after physical or psychological stressors [Soppi et al., 1982; Landmann et al., 1984; Vas et al., 1990; Manuck et al., 1991]. Other investigators have noted decreased lymphocyte counts 24 or 48 hr after social stress in pig-tailed macaques [Boccia et al., 1992] and in rhesus macaques [Gust et al., 1991]. The differences may be a consequence of the type of stressor employed, the species studied, the timing of the sample collection after the stressor, or a combination of these factors. Perhaps an early decrease in the peripheral blood lymphocyte count is followed by an increase, with a subsequent return to the baseline level.

The significance of the higher lymphocyte counts in more aggressive monkeys is not readily apparent. Since the difference was noted at all sampling points, it is unlikely to be due simply to temporary shifts of lymphocytes into the circulation from other compartments of the immune system following the acute reorganization stressor. Further studies that identify changes in lymphocyte subsets could help establish the meaning of this association between behavior and immune cell number.

The decrease in PHA-stimulated blastogenesis after reorganization among high-fear monkeys was consistent with the hypothesis that social stressors and subsequent emotional states would impair immune responses. Similar findings have been reported in human beings [Bachen et al., 1992; Knapp et al., 1992]. This finding indicates that high-fear monkeys were more susceptible to the disruption of social ties caused by reorganization. In other words, high-fear monkeys may have been more likely to interpret reorganization as a threatening event [Lazarus & Folkman, 1984].

A different sort of interaction between reorganization and fear was found for the other nonspecific mitogen. In the case of Con A stimulation, low-fear monkeys had a lower baseline level of blastogenesis, and increased to the level of high-fear monkeys after reorganization. The differences in the interactions may reflect the fact that Con A and PHA induce proliferation by attaching to different cell surface molecules [Kanellopoulos et al., 1985], which potentially could be differentially affected by input from the nervous system.

We anticipated there might be cumulative effects of the repeated reorganizations on the cellular immune system. The progressive increase in mitogen-stimulated lymphocyte proliferation across reorganizations could reflect such an effect. However, chronic stress generally has a suppressive effect on immune function, whereas the change in this experiment was in the opposite direction. Alternative explanations for the increase include seasonal shifts in lymphocyte proliferation, or a drift in laboratory assay conditions. There were no unreorganized control monkeys available, so we were unable to differentiate between these hypotheses.

The low prereorganization NK activity in high-aggressive monkeys could have been due to either differences in cell circulation patterns or in intrinsic activity of NK cells. Since we measured only numbers of total lymphocytes, not NK cells specifically, we cannot differentiate between these possibilities. In either case, the functional consequences of lower NK cell activity were probably small, since the high-aggressive monkeys showed a cytotoxicity response equivalent to the low-aggressive monkeys after reorganization.

Cell-mediated immune responses, and in particular NK cell activity, are thought to be important mechanisms for keeping latent herpesviral infections under control [Glaser et al., 1987]. Decreased NK activity has been associated with reactivation of Varicella-Zoster virus infection in human beings [Saibara et al., 1993] and with enhanced local *H. simplex* infection in mice [Bonneau et al., 1991a]. These authors also found that stress impaired the generation and activation of *H. simplex*-specific cytotoxic T lymphocytes in mice [Bonneau et al., 1991b]. These effects are mediated in part by the adrenal gland [Bonneau et al., 1993].

For this experiment we predicted that acute stress associated with each reorganization, or chronic stress due to repeated reorganization, would lead to suppression of cell-mediated immunity and allow reactivation of B virus infection. The results of this study indicate that the stresses associated with social reorganization affected several aspects of cellular immune function, but that the changes in immunity did not appear to be associated with reactivation of the latent herpesvirus. The failure to obtain positive virus cultures could mean no active virus replication occurred, or that we were simply unable to detect it. Like other alphaherpesviruses, B virus has been found in neural tissues including the trigeminal and lumbosacral ganglia [Boulter, 1975; Zwartouw & Boulter, 1984]. It may be that herpesviruses are sheltered from the immune system while residing in nervous tissue, providing a source for reactivation when cell-mediated immunity is suppressed. Partial reactivation of herpesviruses may also occur, as evidenced by selective expression of the viral genome [Glaser et al., 1985; 1991]. Assays for B

virus-specific proteins or DNA could be used in future studies to detect incomplete reactivation of virus in monkeys.

There was indirect evidence of virus shedding among the 75 monkeys that were housed in the building during this experiment. Three of four seronegative monkeys in this group converted during the course of the experiment. In any case, it appears that active virus shedding was infrequent, since none of the 240 swabs from the 20 monkeys of this experiment yielded virus in cell culture. These results are consistent with those of Weigler et al. [1993], who reported 14 isolations of B virus from a total of 5,036 samples among 157 group-housed rhesus monkeys.

This experiment is the first to report longitudinal data on the B virus status of seropositive macaques. It showed that titers among individual monkeys changed over the course of 5 months, and confirmed an earlier report that the rate of shedding of infectious virus was apparently low. There was no sign that the acute stress of social reorganization increased the level of virus reactivation. These findings are consistent with the view that the risk of human exposure to B virus from seropositive monkeys is small. Nevertheless, the serious consequences of human infection with the virus dictate that proper precautions be observed whenever handling potentially infectious nonhuman primates or their tissues.

The changes in herpesvirus antibody titers were unlike those reported among medical students after examination-related stress [Glaser et al., 1987, 1991]. In that model, students had increased IgG titers to *H. simplex* during periods of stress, compared to baseline levels taken during nonstress periods. In our experiment we found a continual decrease in titers instead of an increase in herpesvirus-specific IgG shortly after exposure to the stressor, and a decrease during nonstress periods. These different results may reflect differences in the biology of the two viruses and their respective hosts, or could suggest that the reorganization stress was insufficient to provoke a reactivation of B virus in the cynomolgus monkeys.

Power calculations suggested that in most cases the experiment had adequate power to detect effects of the behavioral measures on immune responses. The tests with low power ($\beta > 0.20$) were for affiliation and B virus titer, affiliation and NK activity, aggression and PHA response, and fear and NK activity. The conclusion that these behavioral variables were not related to the immunologic responses should be considered tentative.

Although one measure of cell-mediated immunity (PHA-stimulated blastogenesis among high-fear monkeys) was decreased after reorganization, other aspects of cellular immunity were not suppressed. Considered with the decreases in virus antibody titers, the absence of oral ulcerative lesions, and the lack of virus-positive cultures, the data suggest that while social stress altered cellular immune function, it did not induce reactivation of latent B virus infections among these monkeys.

CONCLUSIONS

1. Reorganization of social groups led to changes in several measures of cellular immune function among male cynomolgus macaques, including increases in total lymphocyte counts and NK cell cytotoxicity and decreases in PHA-stimulated lymphocyte blastogenesis.
2. Individual behavioral characteristics modulated these changes in immune status. High rates of aggressive behavior were associated with higher lymphocyte counts and lower baseline NK cell cytotoxicity. High rates of fearful behavior were associated with a decrease in PHA-stimulated blastogenesis after reorganization.
3. Despite the changes in cellular immunity, there was no evidence of reactivation of latent B virus infection subsequent to reorganization. Mean B virus-

specific IgG titers among seropositive monkeys declined over a period of 5 months. There was minimal evidence that the stress of social reorganization led to active shedding of infectious virus.

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REFERENCES

- Altmann, J. Observational study of behavior: Sampling methods. *BEHAVIOUR* 49: 227-265, 1974.
- Bachen, E.A.; Manuck, S.B.; Marsland, A.L.; Cohen, S.; Malkoff, S.B.; Muldoon, M.F.; Rabin, B.S. Lymphocyte subsets and cellular immune responses to a brief experimental stressor. *PSYCHOSOMATIC MEDICINE* 54:673-679, 1992.
- Boccia, M.L.; Laudenslager, M.L.; Broussard, C.L.; Hijazi, A.S. Immune responses following competitive water tests in two species of macaques. *BRAIN, BEHAVIOR, AND IMMUNITY* 6:201-213, 1992.
- Bonneau, R.H.; Sheridan, J.F.; Feng, N.; Glaser, R. Stress-induced suppression of Herpes simplex virus (HSV)-specific cytotoxic T lymphocyte and natural killer cell activity and enhancement of acute pathogenesis following local HSV infection. *BRAIN, BEHAVIOR, AND IMMUNITY* 5:170-192, 1991a.
- Bonneau, R.H.; Sheridan, J.F.; Feng, N.; Glaser, R. Stress-induced effects on cell-mediated innate and adaptive memory components of the murine immune response to Herpes simplex virus infection. *BRAIN, BEHAVIOR, AND IMMUNITY* 5:274-295, 1991b.
- Bonneau, R.H.; Sheridan, J.F.; Feng, N.; Glaser, R. Stress-induced modulation of the primary cellular immune response to herpes simplex virus infection is mediated by both adrenal-dependent and independent mechanisms. *JOURNAL OF NEUROIMMUNOLOGY* 42:167-176, 1993.
- Boulter, E.A. The isolation of monkey B virus (*Herpesvirus simiae*) from the trigeminal ganglia of a healthy seropositive rhesus monkey. *JOURNAL OF BIOLOGICAL STANDARDIZATION* 3:279-280, 1975.
- Cohen, S.; Williamson, G.M. Stress and infectious disease in humans. *PSYCHOLOGICAL BULLETIN* 109:5-24, 1991.
- Cohen, S.; Kaplan, J.R.; Cunnick, J.E.; Manuck, S.B.; Rabin, B.S. Chronic social stress, affiliation, and cellular immune response in nonhuman primates. *PSYCHOLOGICAL SCIENCE* 3:301-304, 1992.
- Cunnick, J.E.; Cohen, S.; Rabin, B.S.; Carpenter, A.B.; Manuck, S.B.; Kaplan, J.R. Alterations in specific antibody production due to rank and social instability. *BRAIN, BEHAVIOR, AND IMMUNITY* 5:357-369, 1991.
- Dobbin, J.P.; Harth, M.; McCain, G.A.; Martin, R.A.; Cousin, K. Cytokine production and lymphocyte transformation during stress. *BRAIN, BEHAVIOR, AND IMMUNITY* 5:339-348, 1991.
- Fletcher, M.A.; Baron, G.C.; Ashman, M.R.; Fischl, M.A.; Klimas, N.G. Use of whole blood methods in assessment of immune parameters in immunodeficiency states. *DIAGNOSTIC AND CLINICAL IMMUNOLOGY* 5:69-81, 1987.
- Friedmann, E.; Katcher, A.H.; Brightman, V.J. Incidence of recurrent herpes labialis and upper respiratory infection: A prospective study of the influence of biologic, social and psychological predictors. *ORAL SURGERY, ORAL MEDICINE AND ORAL PATHOLOGY* 43:873-878, 1977.
- Glantz, S.A. *PRIMER OF BIostatISTICS*. New York, McGraw Hill, 1992.
- Glaser, R.; Kiecolt-Glaser, J.K.; Speicher, C.; Holliday, J.E. Stress, loneliness, and changes in herpesvirus latency. *JOURNAL OF BEHAVIORAL MEDICINE* 8:249-260, 1985.
- Glaser, R.; Rice, J.; Sheridan, J.; Fertel, R.; Stout, J.; Speicher, C.; Pinsky, D.; Kotur, M.; Post, A.; Beck, M.; Kiecolt-Glaser, J.

- Stress-related immune suppression: Health implications. *BRAIN, BEHAVIOR, AND IMMUNITY* 1:7-20, 1987.
- Glaser, R.; Pearson, G.R.; Jones, J.F.; Hillhouse, J.; Kennedy, S.; Mao, H.; Kiecolt-Glaser, J.K. Stress-related activation of Epstein-Barr virus. *BRAIN, BEHAVIOR, AND IMMUNITY* 5:219-232, 1991.
- Goldmeier, D.; Johnson, A. Does psychiatric illness affect the recurrence rate of genital herpes? *BRITISH JOURNAL OF VENEREAL DISEASE* 54:40-43, 1982.
- Gust, D.A.; Gordon, T.P.; Wilson, M.E.; Ahmed-Ansari, A.; Brodie, A.R.; McClure, H.M. Formation of a new social group of unfamiliar female rhesus monkeys affects the immune and pituitary adrenocortical systems. *BRAIN, BEHAVIOR, AND IMMUNITY* 5:296-307, 1991.
- Herbert, T.B.; Cohen, S. Stress and immunity in humans: A meta-analytic review. *PSYCHOSOMATIC SCIENCE* 55:364-379, 1993.
- Hilliard, J.K.; Eberle, R.; Hubbard, T.E.; Lipper, S.L.; Munoz, R.; Weiss, S.A. *Herpesvirus simiae* (B virus): Replication of the virus and identification of viral polypeptides in infected cells. *ARCHIVES OF VIROLOGY* 93:185-198, 1987.
- Kanellopoulos, J.M.; De Petris, S.; Leca, G.; Crumpton, M.J. The mitogenic lectin from *Phaseolus vulgaris* does not recognize the T3 antigen of human T lymphocytes. *EUROPEAN JOURNAL OF IMMUNOLOGY* 15:479-486, 1985.
- Kaplan, J.R.; Manuck, S.B.; Clarkson, T.B.; Lusso, F.M.; Taub, D.M. Social status, environment and atherosclerosis in cynomolgus monkeys. *ARTERIOSCLEROSIS* 2:359-368, 1982.
- Kaplan, J.R.; Heise, E.R.; Manuck, S.B.; Shively, C.A.; Cohen, S.; Rabin, B.S.; Kasprovicz, A.L. The relationship of agonistic and affiliative behavior patterns to cellular immune function among cynomolgus monkeys (*Macaca fascicularis*) living in unstable social groups. *AMERICAN JOURNAL OF PRIMATOLOGY* 25:157-173, 1991.
- Katz, D.; Hilliard, J.K.; Eberle, R.; Lipper, S.L. ELISA for detection of group common and virus specific antibodies in human and simian sera induced by herpes simplex and related simian viruses. *JOURNAL OF VIROLOGICAL METHODS* 14:99-109, 1986.
- Keller, S.E.; Schleifer, S.J.; Sherman, J.; Camerino, M.S.; Smith, H.; Stein, M. Comparison of a simplified whole blood and isolated lymphocyte stimulation technique. *IMMUNOLOGICAL COMMUNICATIONS* 10:417-431, 1981.
- Kennedy, S.; Kiecolt-Glaser, J.K.; Glaser, R. Immunological consequences of acute and chronic stressors: Mediating role of interpersonal relationships. *BRITISH JOURNAL OF MEDICAL PSYCHOLOGY* 61:77-85, 1988.
- Kiecolt-Glaser, J.K.; Dura, J.R.; Speicher, C.E.; Trask, O.J.; Glaser, R. Spousal caregivers of dementia victims: Longitudinal changes in immunity and health. *PSYCHOSOMATIC MEDICINE* 53:345-362, 1991.
- Knapp, P.H.; Levy, E.M.; Giorgi, R.G.; Black, P.H.; Fox, B.H.; Heeren, T.C. Short-term immunological effects of induced emotion. *PSYCHOSOMATIC MEDICINE* 54:133-148, 1992.
- Kusnecov, A.V.; Grotta, L.J.; Schmidt, S.G.; Bonneau, R.H.; Sheridan, J.F.; Glaser, R.; Moynihan, J.A. Decreased herpes simplex viral immunity and enhanced pathogenesis following stressor administration in mice. *JOURNAL OF NEUROIMMUNOLOGY* 38:129-138, 1992.
- Landmann, R.M.A.; Muller, F.B.; Perini, C.H.; Wesp, M.; Erne, P.; Buhler, F.R. Changes of immunoregulatory cells induced by psychological and physical stress: Relationship to plasma catecholamines. *CLINICAL AND EXPERIMENTAL IMMUNOLOGY* 58:127-135, 1984.
- Lazarus, R.S.; Folkman, S. *STRESS, APPRAISAL, AND COPING*. New York, Springer, 1984.
- Manuck, S.B.; Cohen, S.; Rabin, B.S.; Muldoon, M.F.; Bachen, E. Individual differences in cellular immune response to stress. *PSYCHOLOGICAL SCIENCE* 2:111-115, 1991.
- Sieber, W.J.; Rodin, J.; Larson, L.; Ortega, S.; Cummings, N. Modulation of human natural killer cell activity by exposure to uncontrollable stress. *BRAIN, BEHAVIOR, AND IMMUNITY* 6:141-156, 1992.
- Soppi, E.; Varjo, P.; Eskola, J.; Laitinen, L.A. Effect of strenuous physical stress on circulating lymphocyte number and function before and after training. *JOURNAL OF CLINICAL AND LABORATORY IMMUNOLOGY* 8:43-46, 1982.
- Steplewski, Z.; Vogel, W.H.; Ehya, H.; Poropatich, C.; Smith, J.M. Effects of restraint stress on inoculated tumor growth and immune response in rats. *CANCER RESEARCH* 45:5128-5133, 1985.
- Vas, M.R.; Pantalony, D.; Carstairs, K.C. Transient "stress lymphocytosis." *ARCHIVES OF EMERGENCY MEDICINE* 7:126-127, 1990.
- Weigler, B.J. Biology of B virus in macaque and human hosts: A review. *CLINICAL INFECTIOUS DISEASE* 14:555-567, 1992.
- Weigler, B.J.; Hird, D.W.; Hilliard, J.K.;

Lerche, N.W.; Roberts, J.A.; Scott, L.M. Epidemiology of Cercopithecine Herpesvirus 1 (B virus) infection and shedding in a large breeding cohort of rhesus macaques. *JOURNAL OF INFECTIOUS DISEASES* 167:257-263, 1993.

Zwartouw, H.T.; Boulter, E.A. Excretion of B virus in monkeys and evidence of genital infection. *LABORATORY ANIMALS* 18: 125-130, 1984.