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The Relationship of Agonistic and Affiliative Behavior Patterns to Cellular Immune Function Among Cynomolgus Monkeys (*Macaca fascicularis*) Living in Unstable Social Groups

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Considerable recent interest has focused on the possibility that behavioral factors may influence immune competence, and hence, potentially, patterns of disease. We report here the relationship between the aggressive and affiliative behavior and the cellular immune responses of 30 adult male cynomolgus monkeys (*Macaca fascicularis*) living in small (n = 5) social groups whose members were periodically redistributed over 26 months. Animals also were subjected to behavioral observation, allowing them to be categorized as either high or low in aggressiveness and affiliation. At the end of the 26 months, lymphocyte proliferation tests were performed on blood samples from all monkeys, using both concanavalin A (ConA) and phytohemagglutinin (PHA) in concentrations of 1, 5, and 10 ug/ml. Two-by-two (Aggressiveness [high, low] X Affiliation [high, low]) analyses of variance performed on these data showed lymphocyte proliferation in response to both ConA and PHA to be greatest (at 1 ug/ml) among highly affiliative animals, albeit only if they were also low in aggressiveness (ConA: Affiliation × Aggression, $P < 0.02$; PHA: Affiliation × Aggression, $P < 0.03$). An additional finding was that natural killer cell activity (at an effector to target ratio of 100:1) was highest among highly affiliative animals, regardless of their aggressiveness ($P < 0.05$). These results indicate that immune competence may be enhanced among monkeys which, in response to a disrupted social environment, spend large amounts of time in affiliation with other animals. Social status, a phenomenon known to influence many aspects of nonhuman primate physiology, was unassociated with nonspecific lymphocyte blastogenesis or natural killer cell activity in this experiment.

Key words: affiliation, aggression, psychoimmunology

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INTRODUCTION

Most studies of the relationship between psychosocial factors and immunity have examined the influences of "stress" or of related affective experiences (e.g., depression) [Tecoma & Huey, 1985; Khansari et al., 1990]. These studies provide substantial evidence that stressful events can trigger alterations in immune function. In humans, for instance, suppression of nonspecific lymphocyte blastogenesis has been associated with bereavement, depression, the stress of academic examinations, loneliness, and an absence of close confidants [Bartrop et al., 1977; Schleifer et al., 1984; Linn et al. 1984; Irwin et al., 1987; Dorian et al., 1981; Kiecolt-Glaser et al., 1984a; Kiecolt-Glaser et al., 1984b; Thomas et al., 1985]. Nonhuman primates subjected to the stress of a "separation" paradigm, which produces behavioral responses in infant monkeys that are reminiscent of human bereavement, loneliness, and depression [Kalin & Carnes, 1984; Mineka & Suomi, 1978; Rosemblum & Paully, 1987], also exhibit altered immune function [Coe et al., 1987; Coe et al., 1988a; Coe et al., 1988b; Reite et al., 1981; Laudenslager et al., 1982; Laudenslager et al., 1985]. And finally, aversive stimulation (shock, loud noise) has been shown to blunt cellular and/or humoral immune responses of mice and rats [Monjan, 1981; Keller et al. 1981; Mormede et al., 1988; Monjan & Collector, 1977]

While much has been described regarding the immunological impact of acutely stressful events, the immunological consequences of chronic or enduring behavioral attributes have not been adequately investigated. Accordingly, in the current study we examined individual differences in behavioral characteristics of socially housed cynomolgus monkeys (*Macaca fascicularis*), as these related to aspects of cellular immune function. The data were derived from long term, direct observation of subjects, in contrast to the self-report questionnaires which form much of the basis for studies examining behavioral influences on immunity among human beings. Analysis focused on individual differences relating to two of the most prominent aspects of primate social behavior—aggression and affiliation. Also considered was the potential effect on immune function of each individual's dominance status or rank within its social grouping.

Our focus on affiliation is motivated by a broad range of work suggesting the importance of affiliative behaviors on general health. Six prospective epidemiologic studies have found that persons with multiple social contacts live longer than their more isolated counterparts [e.g. Cohen, 1988; House et al., 1988]. Moreover, the availability of others in times of stress has been found to ameliorate stress-induced psychological and physical symptomatology [e.g. Cohen & Wills, 1985; Kessler & McLeod, 1985]. Immunologic data relevant to this perspective have also been reported, as for example in the above-cited studies of nonhuman primates which demonstrate that separation from social partners suppresses cellular immune function [Reite et al., 1981; Laudenslager et al., 1985]. Similarly, several studies indicate that persons scoring high on a self-report loneliness scale are characterized by depressed lymphocyte proliferation and elevated antibody response to latent herpes viruses [e.g. Glaser et al., 1985; Kiecolt-Glaser et al., 1984b].

In contrast to affiliation, no studies have examined associations between aggressive behavioral traits and immune function in primates. Our focus on these behaviors is motivated by evidence of associations between aggression and neuroendocrine changes thought to modulate immune reactions. For example, aggression and hostility have been associated with elevated levels of catecholamines and corticosteroids, endocrine changes presumed to suppress immune response [e.g.,

Monjan, 1981; Schleifer et al., in press]. Moreover, it has been suggested that the presence of hostility and aggression constitute elements of a cancer-prone personality [Bacon et al., 1952; Cooper, 1984].

The most commonly reported dependent variables in studies of behavioral influences on cellular immune function include lymphocyte proliferation in response to nonspecific mitogen stimulation and natural killer cell activity. In the current study, we examined natural killer cell activity and lymphocyte blastogenesis in response to nonspecific mitogens among 30 adult male cynomolgus monkeys which had been maintained in groups of five animals each for the previous 26 months. During this period, the animals were routinely monitored for rates and patterns of social interaction. Based on the above-cited findings, we hypothesized that both lymphocyte proliferation and NK cell activity would be higher in monkeys characterized by high affiliation and/or low aggression than among their less affiliative or more aggressive counterparts.

METHODS

Animals

Subjects were 30 male cynomolgus monkeys (*Macaca fascicularis*) imported from Indonesia as adults (average age = 7.0 years, estimated from dentition). As part of a study of behavioral influences on cardiovascular disease, the animals were maintained in 5-member social groups for 26 months. Animals were housed in individual pens measuring 2.0 × 3.2 × 2.5 m and having outdoor exposure during three seasons. During this time the monkeys consumed a diet moderately high in saturated fat and cholesterol, designed to mimic the diet typically consumed by North Americans [Kaplan et al., 1987]. One monkey was lost to the study during the first year; here we report data from the remaining 29 animals. All procedures involving animals were conducted in compliance with state and federal laws, standards of the Department of Health and Human Services, and guidelines established by our institution's Animal Care and Use Committee.

Experimental Design and Procedures

We have observed previously that atherosclerotic disease in male cynomolgus monkeys is exacerbated when animals are exposed to a disrupted social environment [Kaplan et al., 1982; Kaplan et al., 1983]. In those studies, as well as in the current investigation, social disruption or instability was achieved by periodic redistribution of monkeys among social groups. The periodic reorganization of group memberships in the current study was applied to *all* animals and occurred approximately every five weeks, with 20 such reorganizations accomplished over the course of the 26 month experiment. The redistribution of animals across groups followed a schedule causing each monkey to be housed with either three or four new animals on every reorganization.

Behavioral Data Collection and Management

The behavioral repertoire of macaques living in small groups has been described previously, by ourselves and others [Kaplan et al., 1982; de Waal, 1977]. In order to characterize individual behavior in the current study, observations of 15 minutes length were made on each animal, twice per week. In these observations, data relating to aggressive, submissive, affiliative, and nonsocial interactions were recorded on a datamyte device with a focal sampling technique [Kaplan et al., 1982; Altmann, 1974]. These observations were made between 0900 and 1600 h, with times of day balanced across groups. After collection, the data were transmitted to a VAX computer for calculation of either rates of performance (discrete

TABLE I. Behavior Actions and States

Actions		
Aggression	(contact)	slap/grab/push/pull
		bite
		grapple
	(noncontact)	lunge/charge/chase
		open mouth face
		stare
		displace
Submission		flee
		jump away
		crouch/cower
		grimace
		be displaced
		scream
		present
Affiliation		groom
		be groomed
States		groom
		be groomed
		passive contact
		close proximity (touching distance)
		alone at a distance

behavioral acts) or percentages of time spent in various activities (behavioral states) (Table I). For purposes of later analysis, rates of performance of the discrete behaviors representing aggressive actions were summed to yield an overall rate of aggression per hour for each animal, for each reorganization period. An average rate for the experiment was then calculated for each animal. A similar averaging procedure was used with respect to the percentage of time spent in behavioral status (time spent grooming or being groomed, within close proximity, or in passive physical contact, and time spent alone at a distance from other animals).

Determination of Dominance Status

As in previous experiments [Kaplan et al., 1982; Kaplan et al., 1987], social status determinations were based on observed patterns of fight wins and losses among individuals (as determined from aggression and submission matrices constructed from the focal observations), rather than on the frequency or intensity of agonism. The one animal in each group which defeated all others, as evidenced by elicitation of flight gestures [Sade, 1967], was designated as first ranking; the monkey that defeated all but the first ranking animal was labeled second ranking, and so forth. The status of each animal was evaluated every two weeks during the course of the experiment. These two-week values were later used to assign to each animal a rank for each social reorganization period as well as an overall rank for the entire experiment. We have shown previously in experiments of similar design that, despite the repeated exposure of animals to new social groupings, dominance rankings of adult male cynomolgus macaques tend to be relatively stable, with high and low ranking monkeys maintaining their relative positions across groupings [Kaplan et al., 1987]. In fact, we observed in the current study that only four of the 29 animals changed rank substantially (that is, crossed the median from high to low or from low to high ranking) from the first to the second half of the

experiment [Kaplan et al., 1990]. This outcome suggests that the dominance rankings averaged over the entire experiment largely reflect the behavioral status of the animals over the same period.

Evaluation of Immune Function

Immune function was assessed in all animals by evaluating, in vitro, lymphocyte proliferative responses to mitogenic stimulation by concanavalin A (ConA) and phytohemagglutinin (PHA). Additionally, natural killer cell (NK) functional activity was examined. Blood samples for these assays were collected on a single occasion, beginning in the fourth week following the 20th and final social reorganization. At this time, four monkeys per day (randomly chosen) were anesthetized with ketamine HCl (at a dose of 15 mg/kg). Ten mls of whole blood were collected (in tubes containing 10 units of beef lung heparin/ml) from each animal and immediately transported on wet ice to the immunology laboratory; all evaluations began within 2 to 3 hours of sample collection. This procedure continued until all 29 animals were sampled; shortly thereafter all animals were necropsied as part of a study of behavioral influences on cardiovascular disease. All immunologic assessments were conducted at the Bowman Gray School of Medicine.

Cell preparation. Each 10 ml of heparinized blood was diluted in 25 ml of plasmagel (3% dextran in phosphate buffered saline) and allowed to sediment at unit gravity for 1 hour. The white blood cell enriched upper layer was overlayered on lymphocyte separation medium (Organon Technica Corp., Durham, NC). The gradients were centrifuged for 20 min at 300*g*. The mononuclear cell (MNC) band was collected and washed twice with RPMI 1640. The cells were counted on a hemocytometer and viability was determined by trypan blue exclusion. Cell viability was greater than 95% and red cell contamination was less than 2%.

Proliferation assay. The cells were diluted to 2 million MNC/ml in complete medium (RPMI 1640 supplemented with 25mM Hepes, 10% normal monkey serum, 2mM L-glutamine, units/ml penicillin, and 100 ug/ml streptomycin), and 100 ul of cell suspension was dispensed/well in 96 well round-bottomed trays. Fifty ul of PHA and ConA diluted to 1, 5, and 10 ug/ml in complete medium was added per 20,000 cells in triplicate cultures. Mitogen was omitted in control cultures. Trays were incubated at 37°C in a 5% CO_2 atmosphere for 66 hours and pulsed with 1 uCi of tritiated thymidine (specific activity 6.7 Ci/mmol) 18 hr before recovering the cells on glass fiber filters using a cell harvester. Thymidine uptake was determined by liquid scintillation counting. The dependent measure used evaluating the lymphocyte proliferation assays was the change, in radioactive counts per minute, between the control and stimulated conditions.

Natural killer cell assay. Target cells were the K562 human myelogenous leukemia cell line propogated in RPMI 1640 containing 25 mM hepes, 10% fetal calf serum, penicillin, and streptomycin. Pelleted cells were washed twice in complete medium except that 2 g/l of sodium carbonate was substituted for 25 mM Hepes buffer. The cells were labeled by incubating with 100 uCi (1 mCi/ml) sodium 51-chromate (New England Nuclear Corp.) at 37°C for 45 min. Targets were then washed twice, counted, and resuspended in bicarbonate buffered complete medium at one million cells/ml. Ten thousand target cells were plated in triplicate. Effector cells were added to the round-bottomed trays to obtain four effector/target ratios (12.5, 25, 50, and 100 E:T). Controls for total incorporation, spontaneous release, and maximum release were included on each plate. All trays were spun for 10 min at 1000 rpm at room temperature and were incubated for 4 hr at 37°C in a 5% CO_2:95% air humidified incubator. After incubation, controls for total incorporation were resuspended and harvested, the plates were centrifuged for 10 min at

4°C, and 10% triton-X in water was added to the maximum release control. The trays were centrifuged at 4°C and the supernatants were harvested for counting in an auto-gamma counter. The percentage of specific 51-chromate release at each E:T ratio was then calculated by the formula:

$$\% \text{ lysis} = \frac{\text{mean cpm experimental} - \text{mean cpm SR}}{\text{mean cpm total counts} - \text{mean cpm SR}}$$

cpm = counts per minute; SR = spontaneous release.

Percent specific release values were plotted against the E:T ratios for each animal

Data Analysis

Data analysis involved a test of the hypothesis that individual differences in the rates of performance of affiliative or agonistic behaviors were associated with indices of cell-mediated immune function. The first step in the analysis was to calculate, for the entire experiment, the average percentage of time that each animal spent in passive physical contact and grooming with other monkeys. Those animals above the distribution median in the percentage of time spent in physical contact and grooming then were categorized as "high affiliative" monkeys, while the rest were categorized as "low affiliative" animals. The same procedure was used with respect to the average rate of performance of aggressive behavior (subsuming all aggressive motor patterns from Table I). Here, animals were divided at the median into "high aggressive" and "low aggressive" monkeys. These behavioral dimensions were largely orthogonal, so that approximately equal numbers of individuals fell into each of the four possible groupings.

Following division of animals into high and low aggressive (contact and noncontact) and affiliative (passive body contact, grooming) categories, initial analyses of variance (ANOVAs) were performed to demonstrate that the rates of performance of aggression and affiliation were independent; subsequent ANOVAs were used to evaluate the influence of affiliation and aggressiveness on NK activity and on lymphocyte proliferation in response to PHA and ConA. The change from control values in ^{3}H incorporation in lymphocytes was used as the index of immune response, as preliminary analyses (with ANOVAs) revealed no differences in control values among the behaviorally defined subsets. In addition to these analyses, a subsidiary analysis was run with respect to dominance status (i.e., dominant vs. subordinate). All statistical manipulations were done using the BMDP statistical package on a VAX computer, and all tests of significance were two-tailed. The level of significance for all statistical tests was 0.05. Where necessary, standard data transformations were used to meet the assumptions for parametric analyses. For ANOVAs yielding significant interaction terms, post hoc pairwise comparisons were made using Tukey's HSD procedure; Scheffe's test, recommended for complex, but not pairwise comparisons, was used for contrasts involving more than two means [Kirk, 1968].

RESULTS

Behavioral Characterizations

The purpose of the first set of analyses was to demonstrate the independence of aggressiveness and affiliation. Animals above and below the median for both rate of aggression (contact and noncontact) and percentage of time spent in passive body contact and grooming were evaluated by means of 2 × 2 ($\text{Aggression}_{lo,hi}$ × $\text{Affiliation}_{lo,hi}$) ANOVA, with rate of aggression over the entire experiment as the dependent measure (Figure 1a). As might be expected, there was a significant

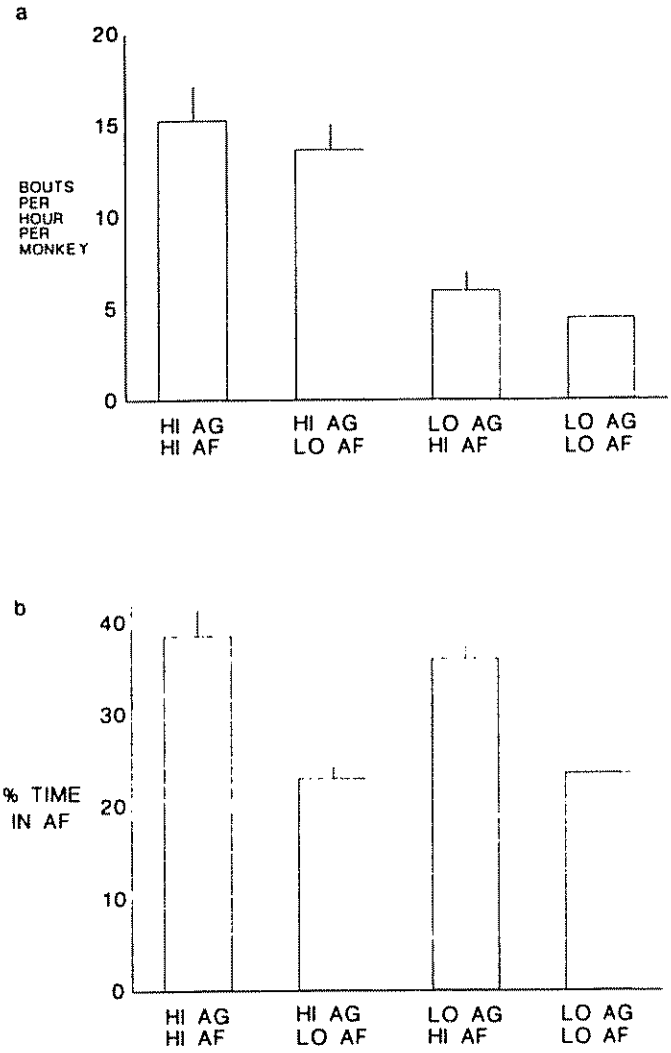

Fig. 1 **a:** The rate of aggression (+SEM) observed among high and low affiliative animals when arranged into high and low aggressive subjects; the results demonstrate that aggressiveness and affiliativeness are unassociated with respect to rate of performance of aggression **b:** The rate of affiliation (+SEM) among high and low affiliative animals arranged into high and low aggressive subsets; the results demonstrate that aggressiveness and affiliativeness are unassociated with respect to the percentage of time engaged in affiliative states

main effect for Aggression ($F_{1,24} = 53.1$, $P < 0.01$). However, the Affiliation main effect was not significant, nor was there a significant interaction between Aggression and Affiliation (P's > 0.10). Hence, high affiliative animals displayed as much aggression as did low affiliative animals. Conversely, a 2 × 2 (Affiliation$_{lo, hi}$ × Aggression$_{lo,hi}$) ANOVA using the percentage of time in passive physical contact and grooming over the entire experiment as the dependent measure (Fig. 1b) revealed a significant main effect for Affiliation ($F_{1,14} = 55.8$, $P < 0.01$). Here, there was no significant main effect for Aggression and no significant interaction between Aggression and Affiliation (P's > 0.10). Thus, highly aggressive animals

could not be differentiated from their low aggressive counterparts in terms of affiliation.

Subsidiary analyses were performed in which the dependent measures were the rate of performance of aggression and the percentage of time spent in affiliation *during the month in which the immune samples were obtained*. The purpose here was to demonstrate that behavioral characteristics derived from the entire experiment also represented the actions displayed during the period in which blood samples were collected for immunologic testing. Importantly, results identical to those presented above were obtained from these latter ANOVAs (Rate of Aggression during month 26: main effect for aggression, $F_{1,24} = 10.22$, $P < 0.01$; all other P's > 0.10; Percentage of time spent in affiliation during month 26: main effect for affiliation, $F_{1,24} = 12.85$, $P < 0.01$; all other P's > 0.10).

Regarding social status, there were a number of significant differences in the behavior of dominant and subordinate animals. These included differences in overall rates of aggression and submission, with dominant animals engaging in more aggression (13.6 [SEM = 1.3] episodes/hr vs. 5.9 [0.9] episodes/hr, $t_{26} = 4.86$, $P < 0.01$) and less submission (8.7 [1.3] episodes/hr vs. 20.1 [1.3] episodes/hr, $t_{26} = 6.23$, $P < 0.01$) than their subordinate counterparts. There was one difference between dominant and subordinate animals in affiliative behavior, namely, dominant animals were groomed for a larger percentage of time than subordinates (8.9 [0.7] % *vs.* 5.2 [0.7] %, $t_{26} = 3.7$, $P < 0.01$).

Immunologic Responses

Affiliation/Aggression

Lymphocyte proliferation. A series of 2 × 2 (Affiliation$_{lo,hi}$ × Aggression$_{lo,hi}$) ANOVAs using the change from control values in ^{3}H thymidine incorporation in lymphocytes as the index of immune response was performed. Square root transformations were first applied to these data. Further, a separate ANOVA was run at the lowest mitogenic concentration (1 μg/ml) due to lost samples (ConA: 3 animals; PHA: 2 animals); repeated measures analyses were used for data from the 5 and 10 μg/ml concentrations, as all samples were usable in these determinations.

The results with respect to ConA stimulation are shown in Figure 2a–c. The analysis at 1 μg/ml revealed significant main effects for Affiliation ($F_{1,21} = 11.45$, $P < 0.01$) and Aggression ($F_{1,21} = 5.92$, $P < 0.03$), as well as a significant Affiliation × Aggression interaction ($F_{1,21} = 6.51$, $P < 0.02$). Inspection of Figure 2a (untransformed values) indicates that lymphocyte responses to ConA were relatively enhanced in Hi Affiliative/Lo Aggressive animals, in comparison to the three remaining groups; post hoc comparisons by Scheffe's test (at $P < 0.05$) showed this contrast to be significant. At higher mitogenic concentrations there was no significant main effect of aggression or affiliation, nor were the Aggression-by-Affiliation or Aggression-by-Affiliation-by-Mitogenic Concentration interaction terms significant (all P's > 0.05). In order to further elucidate the Affiliation × Aggression interaction, the correlations between affiliation and lymphocyte proliferation (at 1 μg/ml) were calculated at each level of aggression (high, low). Consistent with the ANOVA result, this correlation was significant, but only among the low aggression animals ($r = 0.56$, $P < 0.05$); the correlation between lymphocyte proliferation and affiliation was not significant among high aggression monkeys ($r = 0.22$).

Figure 3a–c depicts the results of parallel analyses done with respect to PHA. While at 1 μg/ml there were no significant main effects for either Affiliation ($F_{1,22} = 3.84$, $P = 0.06$) or Aggression ($F_{1,21} = 2.48$, $P > 0.10$), the Affiliation × Aggression interaction term was again significant ($F_{1,22} = 5.91$, $P < 0.03$). The mean

values depicted in Figure 3a indicate that, similar to the response to ConA stimulation, lymphocyte proliferation induced by PHA was relatively enhanced among Hi Affiliative/Lo Aggressive animals in comparison to the three other groups; however, this difference did not reach statistical significance on post hoc comparison ($P = 0.07$). There were no significant main or interaction effects involving Affiliation or Aggression at PHA concentrations of 5 or 10 μg/ml (all F's < 1.0, all P's > 0.20). And, while there was a significant interaction between Affiliation and mitogen concentration ($F_{1,24} = 4.42$, $P < 0.05$), post hoc contrasts again revealed no significant differences between high and low affiliative animals at concentrations of either 5 or 10 μg/ml (P's > 0.05). Correlation analysis at the 1 μg/ml concentration revealed, similar to the outcome with respect to ConA, a significant relationship between affiliation and lymphocyte proliferation, but only among low aggression animals ($r = 0.46$, $P = 0.05$); the correlation among high aggression animals was nonexistent ($r = 0.01$).

NK cell activity. In evaluating in vitro NK activity, we examined the percent specific lysis at various target/effector cell ratios by means of a 2 × 2 × 4 (Affiliation$_{lo,hi}$ × Aggression$_{lo,hi}$ × Target-Effector Ratio$_{1:12.5:25:50:100}$) ANOVA (Figure 4; SEMS ranged from 1.2 to 1.7). This analysis revealed no significant main effects for Affiliation or Aggression, nor was the Affiliation-by-Aggression interaction term significant (all F's < 1.5, all P's > 0.25). However, there was a significant Affiliation × Ratio interaction ($F_{3,69} = 4.87$, $P < 0.005$). Inspection of Figure 4 indicates that divergence of groups was most appreciable at the 1:100 level; a post hoc analysis at this target effector ratio confirmed that the NK cells of Hi Affiliative animals lysed more target cells than those of their Lo Affiliative counterparts ($P < 0.05$). As expected, there was also a significant effect of target/effector ratio, with a higher percentage of target cells lysed (killed) at higher concentrations of effector cells ($F_{3,69} = 162$, $P < 0.01$). Finally, there was a significant Affiliation × Aggression × Ratio interaction ($F_{3,69} = 3.30$, $P < 0.04$), and again, group differences were most apparent at the 1:100 target effector ratio. Post hoc comparisons at this ratio revealed a significant difference in target cell lysis only between High Aggressive-High Affiliative and Low Aggressive-Low Affiliative groups.

Status

Lymphocyte proliferation. The hypothesis that social status modulates responses to mitogenic stimulation was evaluated with the use of a t test at the 1 μg/ml concentration, and a 2 × 2 (Status$_{lo,hi}$ Concentration 5, 10 mg/ml) repeated measures ANOVA (Table II). These analyses revealed no effect of status at the 1 μg/ml concentration for either PHA or ConA, and no main effect of dominance or interaction of dominance with mitogenic concentration at the 5 and 10 μg/ml levels (all P's NS).

NK cell activity. A 1 × 4 ANOVA (Status$_{lo,hi}$ × Target Effector Ratio$_{1:12.5:25:50:100}$) was used to determine if status influenced NK cell activity. Similar to the outcome with respect to lymphocyte proliferation, this analysis revealed no significant effects, either for Status, or for the Status × Ratio interaction (Table II).

Correlations Among Measures

Finally, correlational analysis revealed significant associations between responses to PHA and ConA stimulation at the same mitogenic concentrations (all P's < 0.01; see Table III). Although affiliation was related to both NK activity (at the 1:100 target/effector ratio) and lymphocyte proliferation (at the 1 μg/ml con-

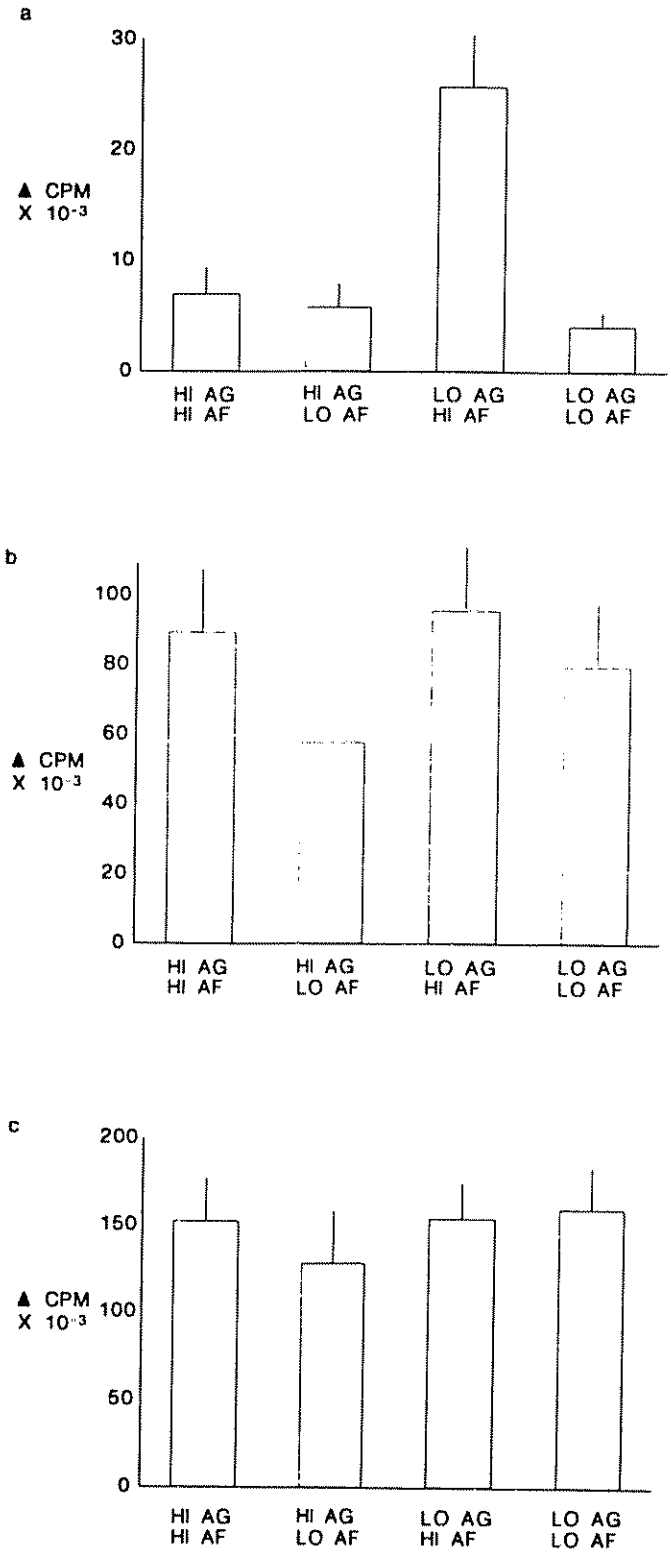
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Fig 2

centration for PHA and ConA), NK activity at the 1:100 ratio was not associated with lymphocyte responses to either ConA or PHA (all P's > 0.05; Table III). Additionally, Table III reveals that the majority of correlations within each response parameter (i.e., different concentrations for ConA and PHA; target/effect or ratio for NK cell activity) were significant (9 of 12 r's > 0.45, P's < 0.01).

DISCUSSION

The data reported above indicate that behavioral differences among individuals, as demonstrated by repeated observations over a period of 26 months, were associated with differences in three indices of cell-mediated immune function among monkeys living in small groups and subjected to periodic social disruption. In particular, NK cell activity and lymphocyte proliferation in response to mitogenic stimulation by ConA and PHA were greater among highly affiliative adult males, relative to monkeys spending less time in affiliative contact with other animals. While the positive association between affiliation and lymphocyte proliferation was limited to animals which were also low in aggressiveness, aggressiveness did not similarly modulate the affiliation-NK cell relationship. Finally, it is worth noting that affiliation was a common element in the relationship of behavior to both lymphocyte and NK cell activity, despite the absence of a significant correlation between these immunologic indices.

It is interesting that only the lowest concentrations of ConA and PHA were associated with enhanced mitogenic activity for the low aggression, high affiliative monkeys. One μg/ml of nonspecific mitogen is considered a sub-optimum concentration and may be capable of eliciting differences between monkeys that are masked when optimum and supra-optimum concentrations of mitogen are used. Thus, high mitogenic concentrations may obscure, or overwhelm, subtle differences induced by behavior. We expect that either neuropeptides, neurohormones, or hormonal concentrations differ in the plasma of the high affiliation low aggression and high affiliation high aggression monkeys. Many of these substances bind to receptors on lymphocyte surfaces and are known to influence mitogen responses (e.g., catecholamines, dynorphin, vasopressin, prolactin, somatostatin) or NK cell activity (glucocorticoids, ACTH) [Payan et al., 1987]. As such, second messengers may be changed in concentration in the lymphocytes of some of the animals. However, nonspecific mitogen is also a ligand which binds to receptors on lymphocyte surfaces and activates second messengers. The effect of low concentrations of hormones binding to ligands may be detected when low concentrations of nonspecific mitogen are used whereas higher concentrations of nonspecific mitogen (optimum concentration) would tend to obscure the differences.

The action of immune modulators varies depending on their concentration, target cell, and immune function studied [Khansari et al., 1990]. Therefore, it is not surprising that no significant correlations were found between NK activity and altered mitogenic activity. Previous studies in rats have indicated that stress-induced immune alteration is mediated by various mechanisms. For example, suppression of splenic NK activity is mediated by a pathway that can be inhibited by the opiate antagonist, naltrexone [Cunnick et al., 1988], while suppression of

Fig. 2. **a:** Lymphocyte proliferation (+SEM) in response to Concanavalin A at 1 μg/ml among animals high or low aggressiveness and high and low in affiliation (CPM = counts per minute). **b:** Lymphocyte proliferation (+SEM) in response to Concanavalin A at 5 μg/ml among animals high or low aggressiveness and high and low in affiliation. **c:** Lymphocyte proliferation (+SEM) in response to Concanavalin A at 10 μg/ml among animals high or low in aggressiveness and high and low in affiliation.

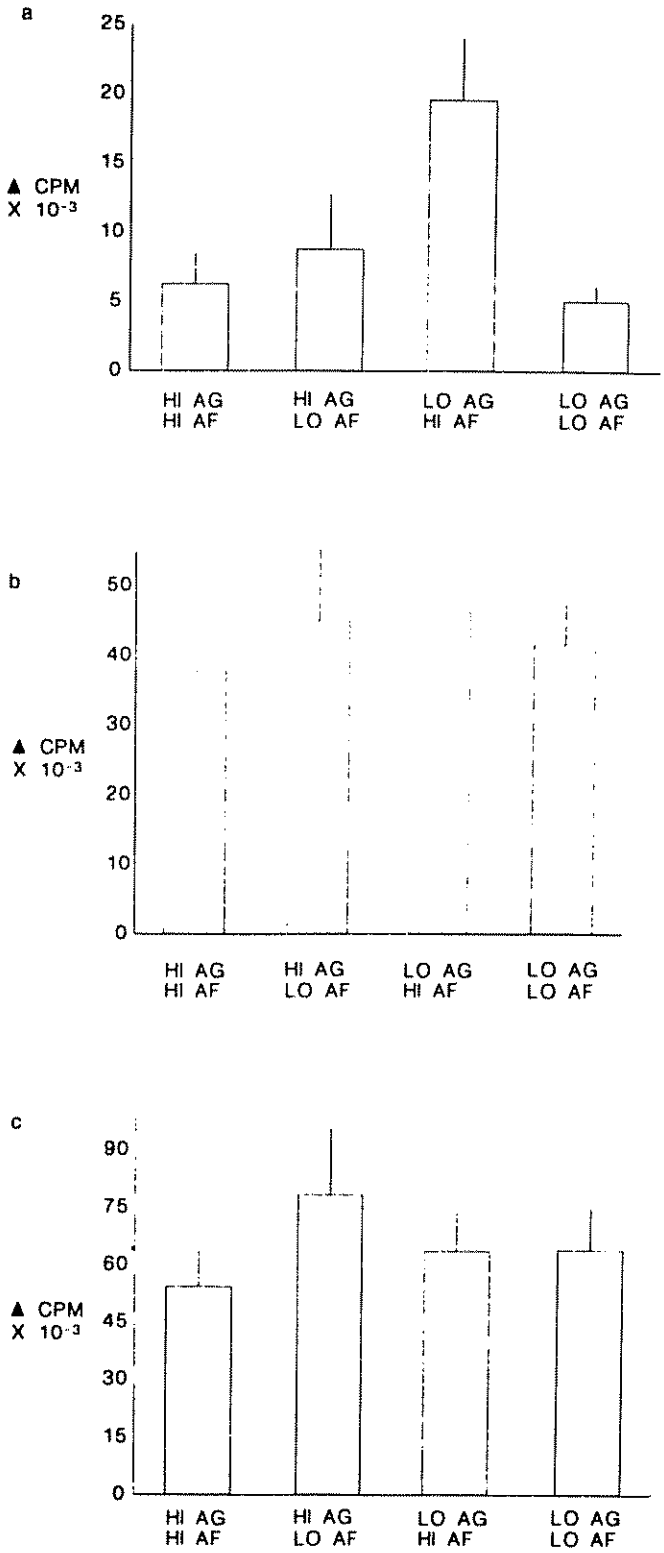
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Fig 3

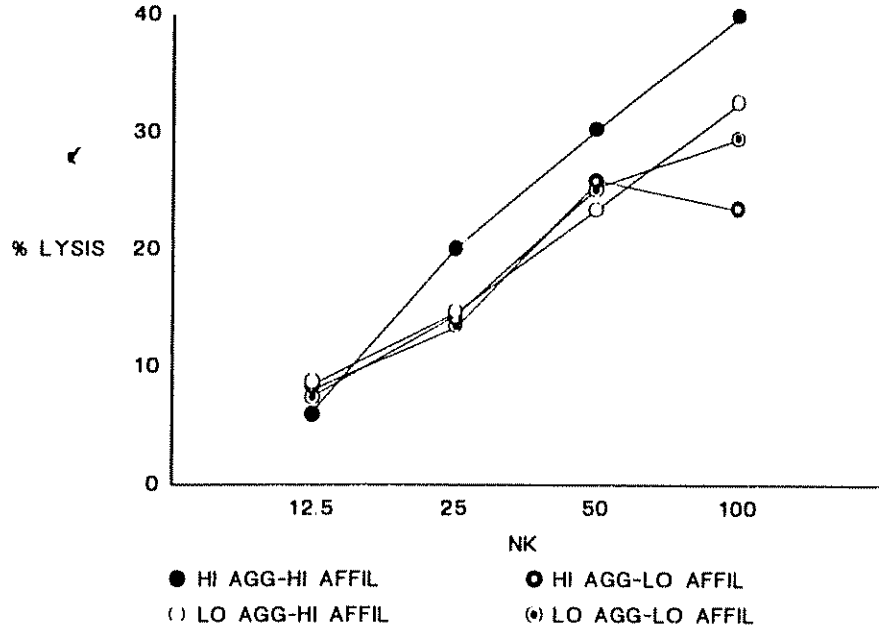

Fig. 4 Natural killer cell activity (% lysis) at target to effector ratios of 1:12.5, 25, 50, and 100 among animals high or low in aggressiveness and high or low in affiliation

splenic lymphocyte responsiveness to T-cell mitogens can be inhibited by the beta-adrenergic receptor antagonist, propranolol [Cunnick et al., in press].

The observation that nonspecific blastogenesis and NK activity were greatest among highly affiliative (and for T-cell mitogenesis, low aggressive) individuals was not unexpected in view of previous findings relating loneliness, bereavement, and lack of social support among human beings to relatively impaired or suppressed immune responses [Bartrop et al., 1977; Schleifer et al., 1984; Linn et al., 1984; Kiecolt-Glaser et al., 1984b]; similarly suppressed responses in either B- or T-cell function have been observed among monkeys subjected to peer-peer or mother-infant separation [Coe et al., 1988a,b; Reite et al., 1981; Laudenslager et al., 1982; Payan et al., 1987].

Notably, in the present experiment, overt behavioral performance was of relatively greater importance than dominance rank in modulating immune function. Hence, social dominance was not associated with the measured indices of immunologic competence, despite the relative stability of status in the current experiment. This lack of an association is also interesting in view of the many reports that dominance status in primates is associated with adrenal responsiveness or size [Shively & Kaplan, 1984; Chamove & Bowman, 1978; Golub et al., 1979; Sassenrath, 1970], heart rate [Cherkovitch & Tatoyan,, 1973; Kaplan et al., 1990],

Fig. 3 a: Lymphocyte proliferation (+SEM) in response to Phytohemagglutinin at 1 μg ml among animals high or low aggressiveness and high and low in affiliation (CPM = counts per minute). b: Lymphocyte proliferation (+SEM) in response to Phytohemagglutinin at 5 μg ml among animals high or low aggressiveness and high and low in affiliation. c: Lymphocyte proliferation (+SEM) in response to Phytohemagglutinin at 10 μg/ml among animals high or low aggressiveness and high and low in affiliation

TABLE II. Lymphocyte Proliferation and NK Cell Activity (counts per minute ± SEM) Among Dominant and Subordinate Monkeys*

Test	Dominant's response	Subordinate's response	*P*
ConA 1μg/ml	10692 (2807)	10395 (3699)	NS
ConA 5μg/ml	71148 (12395)	89494 (12302)	NS
ConA 10μg/ml	136056 (19275)	160788 (17213)	NS
PHA 1μg/ml	10117 (3121)	8995 (2105)	NS
PHA 5μg/ml	36712 (6543)	48160 (4553)	NS
PHA 10μg/ml	57875 (10842)	71676 (6938)	NS
NK 1:12.5	7.23 (1.0)	7.71 (1.1)	NS
NK 1:25	15.53 (2.2)	15.82 (1.9)	NS
NK 1:50	24.69 (2.5)	27.64 (2.6)	NS
NK 1:100	31.58 (2.9)	31.57 (2.7)	NS

*ConA and PHA at 1μg/ml evaluated with *t* test; ConA and PHA at 5 and 10 μg/ml and NK at all concentrations evaluated with repeated measures ANOVAs

TABLE III. Correlations Among Measures of Immune Function

	ConA			PHA			NK 1:			
	1	5	10	1	5	10	12.5	25	50	100
Con 1	—	45*	25	69***	32	13	−.03	−.19	.21	.04
ConA 5		—	89***	43*	74***	60***	.29	.35	.44*	.42*
ConA 10			—	.35	81***	78***	.14	.17	.27	.25
PHA 1				—	53**	.31	−.07	−.16	−.10	.01
PHA 5					—	91***	.36	.26	.34	.21
PHA 10						—	.31	.16	.21	.08
NK 1:12.5							—	.54**	.50**	.18
NK 1:25								—	.92***	.76***
NK 1:50									—	.82***
NK 1:100										

Critical Values (two-tailed): .05 = .38; * = .01 = .45; ** = .001 = .55

atherosclerosis [Kaplan et al., 1987; Kaplan et al., 1982], and reproductive function or activity [Wilson, 1981; Adams et al., 1985]. Among rodents, there have been numerous observations that social status is associated with physiologic condition [Christian, 1978; Christian, 1980]; one such study even contains a report of enhanced antibody responses to antigenic challenge among submissive mice [Fauman, 1987]. The outcome of the current experiment should probably not be interpreted, however, as indicating that social status does not influence immune function under any circumstances; indeed, it is possible that abrupt changes in social status could be related to immunologic alterations. This is suggested, in part, by the positive association observed between serum testosterone concentrations and social status, which is similarly present among male monkeys during periods of contested, but not stable, social ranks [Clarke et al., 1986; Gordon et al., 1979; Bernstein et al., 1978; Bernstein et al., 1979].

More generally, the results of the present experiment suggest that the cynomolgus macaque is a good model for investigating the influence of psychosocial influences on immune function in a group setting. However, it should be recognized that the current results are limited in a number of ways. First, the data were

collected at only a single point in time, at the end of a chronically imposed psychosocial manipulation. Hence, it is uncertain whether the behavior-immune response association described here was present initially, or emerged over the course of the study. Moreover, inasmuch as all animals were subjected to a disrupted social condition, the potential role of the social environment in modulating behavior-immune relationships is unclear. Perhaps most importantly, the current study, like most psychoimmunologic investigations, concerned immune system modulation rather than the broader issues of immunity to disease or susceptibility to infection. While it is generally assumed that impairments in T- or B-cell responsiveness reflect an increase in underlying susceptibility to disease or infection, such assumptions have usually not been tested explicitly. Hence, although there are numerous epidemiologic studies which indicate that social support, for example, is positively associated with longevity [Cohen, 1988; House et al., 1988] and can reduce or ameliorate stress-induced psychological and physical symptomatology [Cohen & Wills, 1985; Kessler & Mcleod, 1985], there is yet little direct evidence that these relationships are immunologically mediated. Prospective evaluations under a variety of social conditions would aid in addressing some of the limitations outlined here; and clearly, such investigations would be enhanced by employing challenges utilizing infectious agents.

CONCLUSIONS

1. Adult male cynomolgus monkeys, when housed in repeatedly disrupted groups of five, engage in aggression and affiliation independently; hence, animals may be high or low in aggressiveness regardless of the degree to which they affiliate with other group members.

2. Natural killer cell activity and lymphocyte proliferation in response to mitogenic stimulation by ConA and PHA are greater among highly affiliative adult males in relation to monkeys spending less time in affiliative contacts with other animals; the positive association between affiliation and lymphocyte proliferation is limited to those animals which are also low in aggressiveness.

3. Dominance status does not appear to influence cellular immune function in the context of repeatedly disrupted small groups.

4. The associations between affiliative/aggressive behavior and lymphocyte proliferation in response to stimulation by ConA and PHA are most clearly observed at sub-optimal (low) mitogenic concentrations.

5. Cynomolgus monkeys in small, periodically disrupted groups are suitable models for investigating psychoimmunological phenomena.

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