Phagocytosis and Killing of *Staphylococcus aureus*: Effects of Stress and Depression in Children

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While a large body of literature depicting relationships between depression or stress and immunity exists, few such studies have dealt with children, and none investigated myeloid cell-derived immunity. We investigated both phagocytosis and bactericidal activity against *Staphylococcus aureus* in children with major depressive disorder (MDD). We found that both MDD and stress influence the bactericidal but not the phagocytic activity of polymorphonuclear leukocytes. The data support the existence of psychobiologic effects in children and suggest possible mechanisms by which depression and stress may affect health.

There is much evidence that in adults, psychological states, including major depressive disorder (MDD) and bereavement, are associated with immune alterations (1, 2, 4, 5, 7, 19, 24, 32, 33, 38–40). While changes in lymphocyte numbers and function have frequently been investigated, relationships between psychologic factors in humans and myeloid-derived leukocytes (monocytes or polymorphonuclear leukocytes [PMNs]) are less well investigated. However, both enumerative and functional measures of myeloid cell immunity have been reported to be altered in adults suffering from depression.

In a study that compared adult patients with MDD to healthy, nondepressed, matched controls, Kronfol and House (27) reported increased numbers of circulating neutrophils. We also found increased numbers of granulocytes in young adults with MDD (40), and others have reported increased total leukocyte counts as well as increased numbers of neutrophils (11, 29, 30) in adults with MDD.

Abnormal neutrophil function (chemiluminescence) has also been associated with MDD (33). However, Maes and coworkers (30) found no differences in phagocytosis, chemotaxis, or superoxide release associated with adult MDD. Also, no difference in granulocyte function (cyclic AMP production) was found by Kanof and colleagues (21).

While psychoneuroimmunologic findings in human adult studies suggest altered enumerative and functional PMN measures, to our knowledge, there have been few studies concerning psychoneuroimmunological (PNI) relationships in children and adolescents, and all of these studies have investigated lymphocyte immunity. Two investigations have concerned PNI effects in adolescents (6, 44), and we previously reported one investigation of depressed children (3). These reports suggest that PNI effects can be detected in children, but to our knowledge, no previously reported PNI studies in children have focused on myeloid cells and psychosocial factors. We now report an investigation of phagocytosis and killing ability of granulocytes in children with MDD. Since factors such as comorbid psychological states, clinical course of the MDD, or prior pharmacologic treatment may confound PNI relationships (1, 2), we decided to investigate children who had had no prior episode of MDD. Further, because of the strong correlation between parental separation/divorce (PSD) and depressive disorder in our subjects, we investigated the potential influence of this chronic stressor. We hypothesized that both MDD and the chronic stress of PSD would influence myeloid immunity in children.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey. Informed consent was obtained from the parent/legal guardian, and informed assent was obtained from each child.

The 22 subjects included in this study represent the last half of the 40 children (previously reported on by Bartlett et al. [3]) who were recruited into a study of MDD and immunity in children. A granulocyte assay in our laboratory was added to the battery of immune measures utilized at that time, and the 22 children were consecutively recruited. Eleven were healthy prepubescent children with MDD who were recruited from both clinicians and school-based study teams. Also, their 11 healthy age-, gender-, socioeconomic status-, and race-matched controls, recruited from families of medical center personnel or the community, were studied on the same day and at the same time (between 8 and 10 a.m.).

Depressed subjects and controls were free of any significant or chronic medical illnesses or medications known or likely to affect immunologic function, including any history of a prior episode of MDD or any previous or current psychopharmacologic treatment. (Antidepressants have immune effects [14, 16] which may influence immunity in MDD subjects even after discontinuation [2]). Psychometric testing (WISC-R) had been done for each depressed subject prior to the study as part of their clinical care, and no subject had evidence of mental retardation. According to parental report, the controls had never had any psychiatric disorders.

All children and their parents were interviewed. Data collected included sociodemographic data and family composition data. In this interview, we asked whether the child’s family had ever experienced PSD. Only children who had lived with both biological parents and whose parents had subsequently separated and/or divorced were considered to have experienced this life event.

The Diagnostic Interview Schedule for Children (15, 41, 42) was administered to all subjects, and the diagnosis of current MDD was made by *Diagnostic and Statistical Manual III—Revised* (American Psychiatric Association, 1987) criteria. Assessment of severity of depressive symptoms in both subjects and controls was undertaken by both an interview-derived measure, the Children’s Depression Rating Scale (CDRS), and a self-report scale, the Children’s Depression Inventory (CDI). The CDRS (36) consists of 17 items and was derived from the Hamilton Depression Rating Scale. Information is obtained from both parent and child to score this measure. The CDI (25) is a 27-item self-report derived from the Beck Depression Inventory. All psychopathologic assessments were made by one of two interviewers (J.A.B. or research assistant), for whom interrater reliability for the Diagnostic Interview Schedule for Children (Kappa) and CDRS (interclass correlation) was >0.8.

Immune measurements included leukocyte counts and levels of both phagocytosis and killing of *Staphylococcus aureus* by PMNs. The total white cell count and granulocyte function measurements were minimally invasive (venipuncture only) and were carried out by laboratory personnel blind to the subjects’ status. Total leukocyte and differential counts were performed by standard techniques. Granulocyte function was assessed according to modified methods described by Leijh et al. (27a). Peripheral blood lymphocytes were separated by Ficoll-Hypaque gradient. The granulocytes were then separated from erythrocytes by...
TABLE 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depressed children</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 11)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Age</td>
<td>10.4 ± 1.3 yr</td>
<td>10.4 ± 1.4 yr</td>
</tr>
<tr>
<td>Sex</td>
<td>3 female</td>
<td>3 female</td>
</tr>
<tr>
<td>Race</td>
<td>64% white</td>
<td>64% white</td>
</tr>
<tr>
<td>CDI</td>
<td>16.1 ± 6.8</td>
<td>5.9 ± 5.3</td>
</tr>
<tr>
<td>CDRS</td>
<td>37.5 ± 4.5</td>
<td>18.1 ± 1.5</td>
</tr>
<tr>
<td>PSD</td>
<td>8+</td>
<td>2+</td>
</tr>
</tbody>
</table>

* P < 0.001.
* P < 0.01.

RESULTS

Demographic data are presented in Table 1. Three of the MDD subject/control pairs were female; seven pairs were Caucasian, non-Hispanic, two were Hispanic and two were Afro-American. By history, controls had no anxiety or no affective or other psychiatric disorder and were in good physical health. Eight of the depressed children and two of the control children had experienced PSD. Three depressed and nine control children resided in two-parent families.

The depressed children, who had met criteria for unipolar depression, had higher scores than controls (Table 1) on both measures of severity of depressive symptoms (CDI [t = 3.9, P < 0.001] and CDRS [t = 15.3, P < 0.0001]). Also, the depressed children significantly more often resided in single-parent homes secondary to PSD (t = 2.9, P < 0.008).

Characteristics of the children having experienced PSD, a life event classically considered a form of chronic stress, compared to the characteristics of those who had not experienced this event are presented in Table 2. Children with separated/divorced families were not different with respect to age, gender, or race from those who resided with both parents. They were significantly different with respect to depression, with the children with separated/divorced parents showing significantly more depressive disorder and greater severity of depressive symptoms.

Immune assay results. (i) Enumerative results. Granulocyte counts were within the normal range for both subjects and controls (Table 3). t tests revealed no significant differences between depressed subjects and controls for total leukocyte counts or numbers of granulocytes (t = 0.25, P > 0.10). There were no significant correlations for age (r = 0.04, P > 0.10), gender (t = 0.29, P > 0.10), race (r = 0.19, P > 0.10), or family history of PSD (t = 0.02, P > 0.10) and the number of granulocytes.

(ii) Phagocytosis. t tests revealed no significant differences between depressed subjects and controls for the number of bacteria phagocytized (t = 0.31, P > 0.10). A significant relationship between gender and phagocytosis (t = 2.2, P < 0.05) was found, with female gender associated with lower numbers of bacteria phagocytized. No significant effects were found for age (r = 0.23, P > 0.10), race (r = 0.29, P > 0.10), or PSD (r = 0.01, P > 0.10) and the number of bacteria phagocytized.

The regression model controlling age, gender, and race revealed no significant relationship between MDD and phagocytosis (t = 0.31, P > 0.10). Further, controlling for PSD also demonstrated no relationship between phagocytosis and MDD (t = 0.77, P > 0.10).

(iii) Bacterial killing. t tests revealed no significant differences between depressed subjects and controls for the number of bacteria killed at 1 h (t = 0.71, P > 0.10) or 2 h (t = 0.86, P > 0.10) of incubation. A significant relationship was found between gender and percent killed at 1 h (t = 3.84, df = 19.8, P < 0.001), with female gender being associated with less bacterial killing. No effects were found for age (1 h: r = 0.19, P > 0.10; 2 h: r = 0.02, P > 0.10), race (1 h: r = 0.07, P > 0.10; 2 h: r = 0.01, P > 0.10), or PSD (1 h: r = 0.33, P > 0.10; 2 h: r = 0.22, P > 0.10).

Analysis of variance for repeated measures controlling age, gender, and race revealed no significant relationship between MDD and bacterial killing (F = 0.30; df = 1, 17; P > 0.10). Analysis of variance for repeated measures controlling age, gender, race, and PSD revealed an independent effect of MDD on the percentage of bacteria killed (F = 5.26; df = 1, 16; P < 0.04) (Fig. 1). MDD predicted increased bactericidal activity. PSD was itself revealed in this model to be independently predictive of a lower percentage of killing (F = 6.62; df = 1, 16; P < 0.02) (Fig. 2).

DISCUSSION

This report is the first investigation of myeloid immunity in children with current MDD. Analyses which controlled for demographic factors and PSD revealed a significantly in-

TABLE 2. Characteristics of children with/without PSD

<table>
<thead>
<tr>
<th>Variable</th>
<th>PSD (n = 10)</th>
<th>Not PSD (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>10.4 ± 1.3 yr</td>
<td>10.5 ± 1.4 yr</td>
</tr>
<tr>
<td>Sex</td>
<td>3 female</td>
<td>3 female</td>
</tr>
<tr>
<td>Race</td>
<td>64% white</td>
<td>64% white</td>
</tr>
<tr>
<td>CDI</td>
<td>15.6 ± 4.8</td>
<td>7.2 ± 8.1</td>
</tr>
<tr>
<td>CDRS</td>
<td>34.6 ± 8.6</td>
<td>22.5 ± 8.9</td>
</tr>
<tr>
<td>MDD*</td>
<td>80%</td>
<td>17%</td>
</tr>
</tbody>
</table>

* P < 0.001.
* P < 0.01.

TABLE 3. Immune variables by groups

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Depressed children (n = 11)</th>
<th>Controls (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of WBC (10³/mm³)</td>
<td>4.7 ± 1.7</td>
<td>4.7 ± 1.4</td>
</tr>
<tr>
<td>No. of PMN (10³/mm³)</td>
<td>2.6 ± 1.4</td>
<td>2.5 ± 0.9</td>
</tr>
<tr>
<td>% PMN</td>
<td>51 ± 10</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>No. phagocytized</td>
<td>108.4 ± 17.1</td>
<td>101.0 ± 16.1</td>
</tr>
<tr>
<td>% Killed at 1 h</td>
<td>44.5 ± 10.1</td>
<td>34.3 ± 9.3</td>
</tr>
<tr>
<td>% Killed at 2 h</td>
<td>47.7 ± 9.0</td>
<td>39.4 ± 9.0</td>
</tr>
</tbody>
</table>

* All P > 0.10 between the two groups. WBC, leukocytes.
increased bactericidal activity in children with MDD. Further, an independent finding of diminished bactericidal activity associated with the chronic stress of PSD was found. These data support the theory that both MDD and psychological stress affect myeloid cell function in children. Unlike studies of adult patients with MDD, our study found no differences in granulocyte numbers associated with MDD in children.

The importance of controlling for demographic factors as well as other psychological states or factors, as suggested by Andreoli and coworkers (1, 2), is highlighted by these data. No simple relationships between group differences were found (t-tests) until these sources of variance were controlled. For example, we did find that gender, irrespective of mental status, was significantly associated with lower phagocytosis and killing activity. Others have previously reported gender differences in psychoimmunologic findings (natural killer cell activity altered in male subjects only) in adults (13). The effect of gender differences may have contributed to our inability to find a simple relationship between group differences. Indeed, in this study, no PNI relationships were found until this source of variance was controlled.

In a similar fashion, no PNI relationships were observed until both the psychiatric disorder and the stressor were entered in the same model. The opposite effects of stress and MDD and their frequent occurrence in the same subjects appear to have contributed further to diminishing our ability to detect the effects of one without controlling the other. This finding may be particularly relevant for understanding the variability of previous PNI studies (4, 5, 9), since such studies may not have excluded patients with a mixed clinical picture (e.g., comorbid anxiety disorder and depressive disorder; depressive disorder and high levels of stress) (1, 2). These data highlight the importance of controlling for the possible confounding or even opposite effects of other psychosocial factors, if possible, in any study.

Our findings of altered granulocyte function associated both with MDD and with PSD must be viewed with some caution. First, the number of subjects was small (n = 22, 11 subject/control pairs). Second, other clinical factors may have played a role. The clinical course of the depressive episode has been reported to influence psychoimmunologic relationships (2), but this factor was controlled in part, as no subject had ever previously been diagnosed with MDD and none of the children had ever been treated with pharmacologic or psychotherapeutic interventions. While all children met the criteria for MDD, total durations of the episodes varied from several months to several years. It may be that more dramatic immune effects could be detected in children with more extensive depressive histories.

While a considerable literature suggests that psychological stress influences immunity (5), we found only one study of humans which investigated psychosocial stress and myeloid cell immunity (22). However, many studies demonstrate that the function of neutrophils or granulocytes can be influenced by physiologic stress, notably in viral and/or bacterial infection (18, 35, 45). PMNs have been shown to have receptors for somatotropin, neurotensin, β-endorphins, substance P, and adrenocorticotropic. Further, hormones and neuropeptides released in stress-related conditions (both physiologic and psychologic) have been shown to alter immunity, including neutrophil function (12, 23). Stress has been shown to affect the ability of macrophages to be activated by interferon or lipopolysaccharide (34) and to affect superoxide production by PMNs (18). Quindos and coworkers (37) reported that restraint stress and fasting affect phagocytosis in rats. Further, macrophages of mice undergoing restraint stress express fewer major histocompatibility class II glycoproteins, a phenomenon affecting the immune response (49). Aversive conditioning effects on nitric oxide production of granulocytes have also been reported (10).

Findings in adults are not always the same as those in the immature of the same species. Indeed, we did not find differences between children with MDD and controls with respect to numbers of circulating granulocytes, although this difference has been reported in adults. Further, the effects described above for stress and immunity in mature animals may not apply...
to the physiologically immature. While altered granulocyte activity has been reported in physiologically stressed infants and children, no such study has, to our knowledge, been reported concerning psychological stress in children. Tolone and co-workers (47) reported diminished PMN adherence in children with acute viral infections compared to that in healthy children and healthy adults. Krause and co-workers (26) also reported decreased PMN adherence and chemotaxis in neonates “stressed” by acute, primarily noninfectious illness compared to those in both healthy neonates and adults. Following recovery, adherence and chemotaxis were improved. In contrast, neutrophil phagocytic ability was reported to be increased in stressed neonates compared to that in adults (17), but Stroobant and colleagues (46) reported diminished bactericidal activity in infants with respiratory illness or requiring ventilatory assistance compared to that in healthy adults. The present study suggests that psychological stress is associated with decreased bacterial killing, as was reported with physiological stress. However, while altered phagocytosis was reported with physiological stress, we did not find changes in phagocytosis associated with psychological stress. Whether psychological stress and physiologic stress affect the immune system in similar ways poses an interesting set of questions which require further investigation using both paradigms in the design. Also, whether the immune alterations persist when MDD is resolved with or without the continued stress poses interesting questions for further investigation.

Other factors which may have influenced our findings require comment. Additional stress-related variables such as intercurrent life stress have been shown to influence granulocyte function (22, 34). Additionally, specific depressive symptoms such as sleep, appetite disturbance, or suicidality (19, 20) may influence granulocyte function. These factors are not addressed herein.

In sum, this study supports the hypothesis that psychological factors, specifically stress and depressive disorder, influence granulocyte activity in children. The measures utilized relate to potential in vivo challenges to the immune systems of children and raise issues concerning possible health outcomes. Indeed, Cohen and Herbert (9), in a review of health outcome studies, did find that psychological states (in adults) are likely related to onset and progression of infectious disease. Further, it has been reported that psychoimmunologic alterations in PMN function can persist (8) and that the long-term impact of psychosocial factors on functional immune measures in children may ultimately have a substantial impact on health (9, 28). Future studies addressing other aspects of PMN function, and the potential for impact on health outcome are needed.

REFERENCES