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Maternal separation in early life impairs tumor immunity in adulthood in the F344 rat

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Abstract

Neonatal stress alters the hypothalamic–pituitary–adrenal (HPA) axis in rodents, such that, when these animals are exposed to stress as adults they hypersecrete corticosterone. Given that glucocorticoids are immunosuppressive, we examined the impact of maternal separation on HPA axis reactivity, natural killer (NK) cytotoxicity, and tumor growth in Fischer 344 rats following chronic restraint stress in adulthood. Pups underwent a chronic stress protocol whereby they were separated from their dams for 3 h on postnatal days 1–21. In adulthood, corticosterone responses were assessed following exposure to chronic (6 days for 10 h) restraint stress. Rats allocated to the chronic stress condition were inoculated with MADB106 tumor cells on day 4 of the restraint protocol. Blood was assessed for NK cytotoxicity on the final day of the chronic restraint protocol, and tumor colonization was assessed 3 weeks thereafter. Maternal separation impaired developmental weight gain ($P < 0.05$), depressed NK cytotoxicity ($P < 0.05$), and increased tumor colonization in the presence of chronic restraint stress in adulthood ($P < 0.001$). These findings occurred independently of circulating plasma corticosterone as only adult stress exposure potentiated corticosterone responses ($P < 0.05$). Our findings indicate that maternal separation and chronic stress can impair NK cytotoxicity and hence tumor immunity, but these effects are not directly mediated by perturbations in HPA axis function.

Keywords: *Hypothalamic–pituitary–adrenal axis, maternal separation, natural killer cytotoxicity, stress, tumor metastasis, rat*

Introduction

Animal research has previously demonstrated that exposure of infant rat pups to stress during the early neonatal period disrupts physiological and behavioral functioning (Levine 1957; Plotsky and Meaney 1993; Ogawa et al. 1994; Shanks et al. 1995; Walker et al. 2004; Walker et al. 2009, 2010). The responses range from transient changes in cardiovascular function, temperature, and locomotion, to growth retardation following more severe stress. Exposure to stress during this period can also produce permanent physiological changes in regards to metabolic (McPherson et al. 2009), neuroendocrine (Shanks et al. 1995; Hodgson et al. 2001; Walker et al. 2009), and immune functioning (Walker et al. 2008; Avitsur and Sheridan 2009; Walker et al. 2010) among others and hence are considered to disrupt the long-term health of the organism leaving it more susceptible to later life disease states (Matthews and Phillips 2006). In accordance with this, we investigated the potential role of neonatal stress on later life immune function; specifically, whether prolonged maternal separation during the neonatal period increases tumor colonization in later life.

Previously, we demonstrated that neonatal exposure to a bacterial endotoxin, lipopolysaccharide (LPS), impaired tumor immunity whereby significantly more tumor metastases were observed following chronic restraint stress in adulthood (Hodgson et al. 2001). Importantly, perturbations to hypothalamic–pituitary–adrenal (HPA) axis activity and natural killer (NK) cytotoxicity were associated with these findings. Having previously established the impact of a physiological postnatal immune stressor on tumor cell proliferation and NK cytotoxicity, the present study aimed to determine whether a psychological stressor during the neonatal period, namely maternal separation, would produce similar long-term effects on tumor immunity, and whether associated changes

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in HPA axis function and NK cytotoxicity would occur. Such associated changes in these parameters were expected given that exposure to stress is well known to depresses NK cytotoxicity and potentiate tumor cell colonization (Hodgson et al. 1996; Ben-Eliyahu 1998, 2003; Ben-Eliyahu et al. 1999, 2007; Greenfeld et al. 2007). Furthermore, perinatal stress has been strongly documented to alter the neuroendocrine system, typically resulting in long-term perturbations to the HPA axis response to stress (Plotsky and Meaney 1993; Shanks et al. 1995; Walker et al. 2009, 2010). A mechanism proposed to underlie this change in HPA axis sensitivity is thought to be the result of a downregulation of glucocorticoid receptor numbers during perinatal life, which is a period of considerable plasticity in this system (Shanks et al. 2000). Prolonged maternal separation during the neonatal period has been shown to be capable of producing such changes with potentiated corticosterone responses to stress in adulthood, increased basal levels of hypothalamic corticotrophin-releasing factor mRNA levels (Plotsky and Meaney 1993), and reduced glucocorticoid receptor density in the hypothalamus, hippocampus, and frontal cortex (Meaney et al. 1996; Liu et al. 1997).

Glucocorticoids have long been known to regulate the immune system (Bailey et al. 2003). As such, early life environmental demands that activate the neonatal HPA axis are believed to program immune responsivity, at least in part, via this glucocorticoid-mediated mechanism. Early studies investigating perinatal stress-induced regulation of immune function employed prolonged maternal separation to demonstrate decreased cellular (Michaut et al. 1981; Laudenslager et al. 1985; von Hoersten et al. 1993) and humoral (Laudenslager et al. 1985) immune responses in adulthood, as well as increased susceptibility to growth of a transplanted tumor (Ader and Friedman 1965). Cell-mediated immunity plays an important role in the regulation of tumor development (Trinchieri 1989). A primary component, NK cells, provides protection against such tumor development through cytokine production, and has the ability to lyze and eliminate metastatic circulating tumor cells preventing establishment of large progressive tumors (Barlozzari et al. 1985; Benish et al. 2008). In this way, NK cells are believed to be a potential and viable candidate for immunotherapy of cancer (Cooper et al. 2001).

To determine the effects of neonatal maternal separation on resistance to tumor colonization *in vivo*, a mammary adenocarcinoma cell line, MADB106, obtained from a pulmonary metastasis of a mammary adenocarcinoma (MADB100) chemically induced in inbred Fischer 344 (F344) rat (Barlozzari et al. 1985) was utilized. The F344 rat strain has a hyperresponsive HPA axis (Numachi et al. 2000), which makes it an opportune rat model to examine the long-term effects of perinatal stress exposure, and has been widely employed to examine the relationship between stress and tumor metastasis (Ben-Eliyahu et al. 1999; Stefanski 2001; Page et al. 2005). Finally, the MADB106 cell line is syngeneic to the inbred F344 rat and highly sensitive to NK cell activity *in vivo* (Barlozzari et al. 1985). MADB106 tumor cells reliably metastasize only to the lungs following intravenous inoculation and pulmonary retention of these tumor cells where it forms well-defined surface metastases by 3–4 weeks post-inoculation (Hodgson et al. 1998). The subsequent growth of lung metastases is tightly regulated by NK cytotoxicity (Barlozzari et al. 1985; Ben-Eliyahu and Page 1992). Therefore, we hypothesized that chronic maternal separation combined with chronic restraint stress in adulthood will suppress NK cytotoxicity, and increase tumor metastasis following the inoculation of MADB106 tumor cells. Potentiated corticosterone responses to chronic restraint stress in maternally separated rats were expected to be associated with such impaired tumor immunity.

Materials and methods

Animals

Male offspring from naïve female F344 rats, obtained from the Animal Resource Centre (Perth, Western Australia) and mated in the University of Newcastle Psychology vivarium, were used. On Day 0 (the day of birth), the dam and pups were left undisturbed but allocated to either the 'maternal separation' or the 'control' (no maternal separation) condition. No significant differences were observed between litters allocated to either neonatal conditions in regard to litter size ($n \cong 8$ per litter) or male to female ratio of pups born. Rats were fed *ad libitum* (Rat and Mouse Pellets, Glen Forest, Western Australia) except as noted below and were maintained on a 12-h light–dark cycle during the study. The light phase began at 06:00 h, and the temperature was maintained at 20 ± 2°C. Whole litters were weaned on day 22, and remained group housed as litters until day 36, at which point litters were sexed and separated, and males were pair-housed (41.5 cm × 28.0 cm × 22.0 cm cages; Mascot Wire Works, Mascot, Australia). All experimentation occurred in accordance with the 2004 National Health and Medical Research Council Australian code of practice for the care and use of animals for scientific practice.

Neonatal stress protocol

Males in the 'maternal separation' condition were weighed and placed individually in clear plastic separation containers for 3 h each day on postnatal days 1–21. Separation commenced at the beginning of the dark cycle on each day. Following the separation period, pups were returned to their dams. The length of this separation period has been shown to be stressful

to neonatal pups, characterized by elevated corticosterone levels, without increasing mortality (Kuhn et al. 1990; Walker et al. 1991; Rosenfeld et al. 1992). Pups in the control condition were briefly removed from the cage and weighed on days 1–21. This procedure lasted approximately 3 min, and was similar to some of the handling procedures described in other studies (Levine et al. 1967; Hess et al. 1969; Hilakivi-Clarke et al. 1993). It must be noted that control offspring were not food and water restricted. Apart from a weekly weighing, animals were left completely undisturbed following weaning until adulthood (90 days).

Adult stress protocol

In adulthood, rats were randomly allocated into either the 'chronic restraint stress' condition or served as controls, thus creating four conditions: (1) rats maternally separated and restrained in adulthood, (2) rats maternally separated without restraint in adulthood, (3) rats nonmaternally separated and restrained in adulthood, and (4) rats nonmaternally separated without restraint in adulthood ($n \cong 8–10$ per group). Rats in the 'chronic restraint stress' condition were placed in a clear plastic restraint tube (14 × 7 cm) with ventilation holes in their home cages for 10 h per day commencing at 20:00 h for 6 days. This procedure is consistent with previously reported studies and is known to activate the HPA axis (Plotsky and Meaney 1993; Viau et al. 1993; Ogawa et al. 1994; Shanks et al. 1995). Rats in the 'chronic restraint stress' condition had no access to food and water during the period of restraint. After each period of stress, rats were removed from the restraint tubes and given normal access to food and water. Rats in the 'no stress' condition were left undisturbed during this time with normal access to food and water. Hence, the effects of the stress protocol are attributed to both food and water restriction as well as restraint. Rats were euthanized using 2–3% isoflurane inhalation and blood was obtained via cardiac puncture into heparinized syringes (Livingstone International, Rosebery, Australia) on the final day of restraint stress immediately following cessation of chronic restraint stress or no stress, except for a subgroup of rats ($n = 22$), which were assessed for tumor colonization. This subgroup of rats was left undisturbed for 3 weeks, and then euthanized with isoflurane and their lungs were obtained.

Tumor cell maintenance

The MADB106 tumor cells were maintained in 5% CO_2 at 37°C in monolayer cultures, and were grown in complete medium (RPMI 1640 media [Gibco, Australia], which was supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine [2 mM], nonessential amino acid [0.1 mM], sodium pyruvate [1 mM], and gentamicin [0.0l mg/ml]). Prior to use, cells were trypsinized (0.25%) to remove them from the flask and resuspended in phosphate buffered saline.

Inoculation of MADB106 tumor cells

Rats allocated to the 'chronic restraint stress' condition were lightly anesthetized with isoflurane on experimental day 4. MADB106 tumor cells (1×10^5 in 0.5 ml phosphate buffer saline) were injected into the tail vein and the rats were then returned to their home cages. Following recovery from anesthesia, rats were exposed to the 4th day of the chronic stress protocol. Rats in the 'no stress' condition were left undisturbed during this time with normal access to food and water, but were inoculated with MADB106 tumor cells at the same time of day as rats in the 'chronic restraint stress' condition.

Corticosterone assay

Heparinized blood samples were obtained at around 08:00 h, centrifuged at 1000*g*, and the plasma stored at −80°C until assayed. Plasma corticosterone concentrations were assessed with a rat corticosterone ^{125}I radioimmunoassay kit (ICN Biomedicals Inc., Costa Mesa, CA, USA). The reported recovery of free corticosterone is 100%, with a mean inter- and intra-assay variability of 4.4 and 6.5%, respectively.

NK cytotoxicity assay

On the final day of restraint, rats in the 'chronic restraint stress' and 'no stress' conditions were euthanized with isoflurane and blood was collected into heparinized tubes via cardiac puncture to assess NK cytotoxicity using a standard chromium release assay. Whole blood was used rather than separated leukocytes because this method minimizes the time between blood collection and assessment of cytotoxicity (Ben-Eliyahu et al. 1999). Previous studies have indicated this method to be comparable to methods using isolated leukocytes (Ben-Eliyahu and Page 1992; Ben-Eliyahu 1998; Shakhar and Ben-Eliyahu 1998; Ben-Eliyahu et al. 1999). This is a widely used protocol, previously described and proven to hold high validity across studies (Page et al. 1994; Ben-Eliyahu et al. 1996; Shakhar and Ben-Eliyahu 1998; Page and Ben-Eliyahu 2000). Briefly, this protocol assesses antitumor NK activity per ml of blood, without prior separation of peripheral blood mononuclear cells, reduces the time between blood withdrawal and cytotoxicity assessment, and minimizes interference with NK cell function (Page et al. 1994; Ben-Eliyahu et al. 1996). Exactly 1 ml of heparinized blood was washed once with phosphate buffer solution (diluted 1:4, centrifuged at 300*g* for 10 min and supernatant aspirated to original volume) and twice with complete medium. Murine YAC-1 lymphoma cells were used as the target cells, and were labeled by incubation with

200 μCi of ^{51}Cr for 1.5 h at 37°C. They were then washed twice in complete medium and their concentration adjusted to the desired concentration. For each of the 4 effector:target (E:T) ratios (1:2, 1:1, 2:1, and 4:1) used, l00 μl of washed blood was placed into each well of a microtiter plate and 150 μl of ^{51}Cr labeled YAC-1 tumor cells in complete medium was placed on the top of the blood. A concentration of 40×10^4/ml YAC-1 cells was used as the lowest E:T ratio (1:2 based on estimated NK cells/ml, this is approximately 1 NK cell: 40×10^6 YAC cells), and sequentially diluted by two to produce higher E:T ratios. Plates were centrifuged at 500*g* for 10 min and then incubated for 4 h at 37°C in 5% CO_2. Plates were then centrifuged again (10 min) and 100 μl aliquots of the supernatant were recovered from each well and the amount of radioactivity was determined using a γ counter. The spontaneous release (i.e. target without effector cells) and maximal release (i.e. target cells alone in medium containing 1% Triton-X) of radioactivity from tumor cells were measured and the percentage of specific lysis was calculated for each E:T ratio using the formula ([{0.8 × Experimental ^{51}Cr release – spontaneous ^{51}Cr release}/{maximum ^{51}Cr release – spontaneous ^{51}Cr release}] × l00). The experimental release was multiplied by 0.8 to correct for the reduction in supernatant volume caused by the presence of red blood cells in all wells apart from those used to assess the maximum and spontaneous release.

Assessment of tumor metastases

Following the removal of the lungs, the tissue was then fixed for 24 h in Bouin's solution (72% saturated picric acid solution, 23% formaldehyde [37%], 5% glacial acetic acid [Sigma-Aldrich, Sydney, Australia]) and subsequently transferred into 97% ethanol. Surface metastases were counted by an independent observer, blind to experimental treatment conditions. The number of surface metastases indicates the number of tumor cells that are successful in evading detection by NK cells as they traffic to the lungs and is considered to be an *in vivo* model of NK cytotoxicity (Barlozzari et al. 1985; Ben-Eliyahu et al. 1991; Ben-Eliyahu and Page 1992).

Data analysis

Data were analyzed using the Statistical Package for the Social Sciences for Windows, Version 17. Analyses of variance (ANOVA) were conducted for all analyses. Planned comparisons between experimental conditions were performed using Bonferroni's α correction to 0.05 and *t*-test analyses adjusted for multiple comparisons where significant interactions were observed.

Results

Effect of neonatal maternal separation on weight gain

Rats exposed to maternal separation displayed reduced weight gain compared with controls. Regression analysis of 'neonatal treatment' and 'day of life' on weight was significant ($F[2,689] = 997.2$, $P < 0.001$) whereby both factors made a significant contribution to weight ($P < 0.05$ in each case). After accounting for the effect of 'day of life', weight gain was 14.5*g* lower across development in rats exposed to maternal separation compared with their control counterparts (Figure 1).

Effect of neonatal maternal separation on corticosterone responses to stress

A two-way ANOVA, 'neonatal treatment' (2) × 'adult stress' (2) on plasma corticosterone concentrations following chronic restraint stress or no stress revealed a main effect of adult treatment. Rats exposed to chronic restraint stress in adulthood demonstrated significantly higher plasma corticosterone concentrations compared with nonrestrained controls, $F(1,26) = 9.7$, $P < 0.01$. A significant interaction between neonatal and adult treatment, however, was not observed (Figure 2).

Effect of neonatal maternal separation on NK cytotoxicity

Trend analysis revealed a similar pattern in cytotoxicity across the four E:T ratios; therefore, statistics were only performed on the highest 4:1 ratio. A two-way ANOVA, 'neonatal treatment' (2) × 'adult treatment' (2), on percent cytotoxicity indicated a significant interaction between neonatal and adult treatment conditions, $F(1,23) = 6.3$, $P < 0.05$

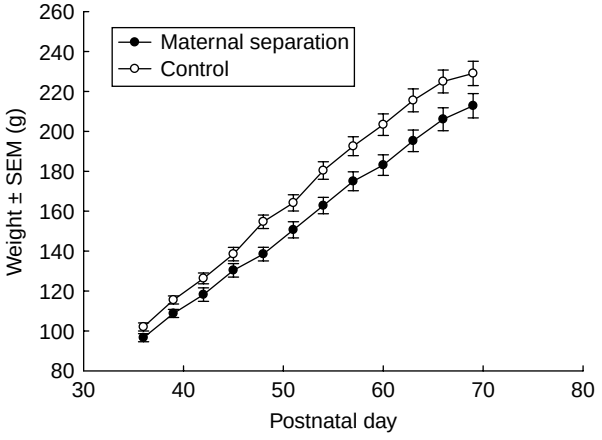

Figure 1. Mean weights (± SEM) (g) for animals in the 'maternal separation' ($n = 30$) and 'control' ($n = 28$) groups used for the assessment of corticosterone and NK cytotoxicity prior to chronic restraint stress and tumor cell treatment. Regression analysis of 'neonatal treatment' and 'day of life' on weight was significant ($F[2,689] = 997.2$, $P < 0.001$) whereby both factors made a significant contribution to weight ($P < 0.05$ in each case).

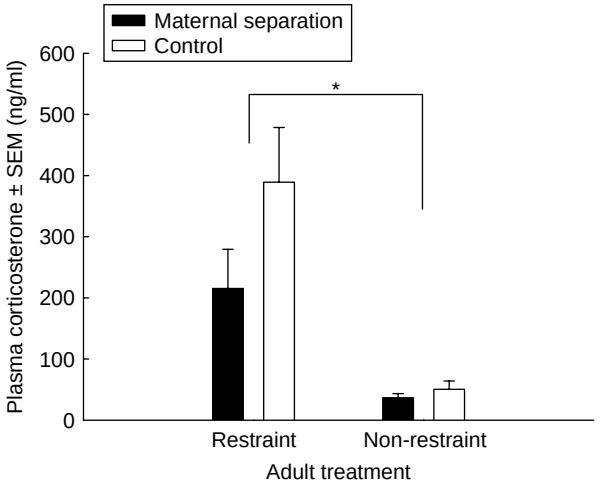

Figure 2. Mean plasma concentration of corticosterone (± SEM) for 'maternal separation' versus 'control' groups across 'restraint' versus 'non-restraint' groups ($n \cong 8$ per group) following chronic restraint stress (ANOVA: F[l,26] = 9.7, $\star P < 0.01$). These are measures at the end of 6 days of repeated restraint and tumor cell treatment.

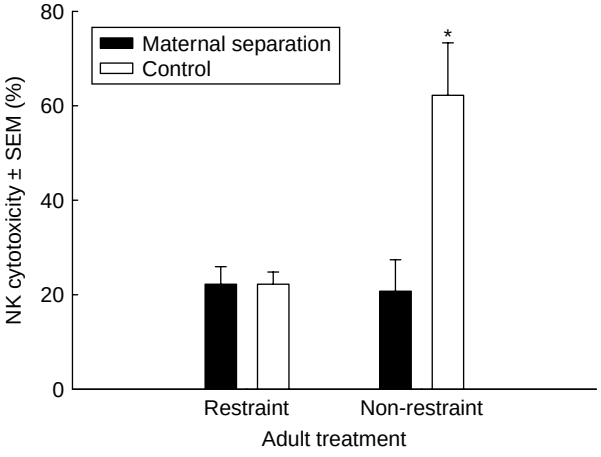

Figure 3. Mean NK cell activity (± SEM) for all animals in the 'maternal separation' versus 'control' conditions across 'restraint' versus 'non-restraint' conditions, E:T ratio 4:1 for male rats ($n \cong 7$ per group; ANOVA [$F\{1,23\} = 6.3$, $P < 0.05$]). Planned comparisons revealed that NK cytotoxicity was significantly higher in control rats subjected to neither maternal separation nor chronic restraint stress in adulthood compared with all other groups ($\star P < 0.05$, for all).

(Figure 3). Planned comparisons revealed that NK cytotoxicity was significantly higher in control rats subjected to neither maternal separation nor chronic restraint stress in adulthood compared with all other groups ($P < 0.05$, for all).

Effect of neonatal maternal separation on tumor colonization

A two-way ANOVA, 'neonatal treatment' (2) × 'adult stress' (2), on tumor number revealed a significant interaction between neonatal and adult treatment conditions, $F(1,32) = 11.7$, $P < 0.001$ (Figure 4).

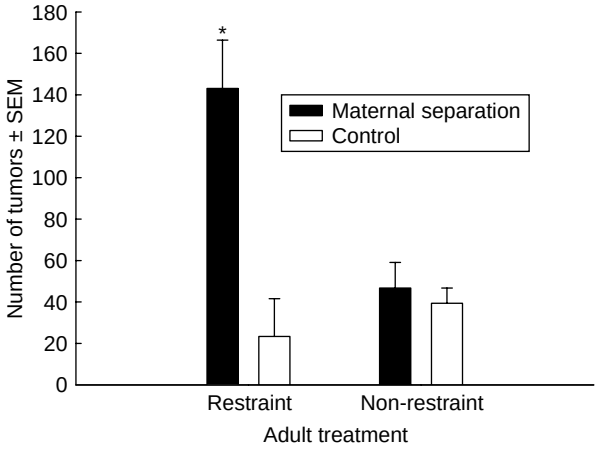

Figure 4. Mean number of lung metastases (± SEM) for 'maternal separation' versus 'control' conditions across 'restraint' versus 'non-restraint' conditions ($n = 10$ per group; ANOVA [$F\{1,32\} = 11.7$, $P < 0.001$]). Planned comparisons revealed that the rats subjected to both maternal separation and chronic restraint stress in adulthood demonstrated significantly more metastases compared with all other treatment conditions ($\star P < 0.05$, for all).

Planned comparisons revealed that the rats subjected to both maternal separation and chronic restraint stress in adulthood demonstrated significantly more metastases compared with all other treatment conditions ($P < 0.05$, for all).

Discussion

The present study demonstrates that neonatal maternal separation alters tumor cell immunity in later life, which appears to be associated with changes in NK cytotoxicity. Furthermore, the data indicate that while exposure to a secondary stressor in later life markedly increases tumor cell proliferation, this does not appear to be regulated by peripheral corticosterone concentrations.

Effect of neonatal maternal separation on weight gain

Maternal separation reduced weight gain, which is consistent with previous research (Ogawa et al. 1994; Suchecki and Tufik 1997; Biagini et al. 1998). However, failure to gain weight was more persistent in the present study and may be due to the longer duration of separation in the current study compared with previously documented findings (Suchecki and Tufik 1997; Biagini et al. 1998). Such impaired weight gain in animals exposed to perinatal stress may result from changes in feeding behaviors mediated by corticosterone as previously discussed in Hodgson et al. (2001), as well as the reduced amount of access to food and water for maternally separated animals.

Effect of neonatal maternal separation on corticosterone responses in adulthood

The present study demonstrates that maternally separated male neonates do not show the expected potentiated corticosterone response observed in other studies that have focused largely on acute stress models in adulthood (Walker et al. 2010) nor in similar models that have utilized an immunological postnatal stressor (Hodgson et al. 2001). No differences were observed between neonatal treatment groups and only later life stress exposure resulted in an increased corticosterone response. Although previous findings in our laboratory have indicated that long-term changes in HPA function are amplified in the presence of a secondary later-life stressor (Hodgson et al. 2001; Hodgson and Knott 2002; Walker et al. 2009, 2010), these studies have all utilized a bacterial mimetic as the postnatal stressor and this may highlight differences in HPA axis programing following various postnatal stressor types.

Interestingly, trend analysis indicated that maternally separated rats exposed to chronic stress in adulthood displayed attenuated corticosterone responses compared with those subjected to chronic stress in adulthood but not to maternal separation. This finding reflects previous work in our laboratory indicating that neonatal stress results in a blunted HPA axis response to chronic stress in adulthood (Walker et al. 2009). A number of recent studies using similar chronic maternal separation protocols have demonstrated potentiated corticosterone responses in later life (Mirescu et al. 2004; Veenema and Neumann 2009). The discrepancy between our findings and those previously reported is likely to reflect differences in the secondary stress exposure whereby only basal corticosterone concentrations or those in response to acute stress exposure were reported. Blunted corticosterone responses to chronic stress have been widely demonstrated (Albeck et al. 1997; Fries et al. 2005; Zalutskaya et al. 2007), and may reflect impaired negative feedback of the stress response.

Effect of neonatal maternal separation on NK cytotoxicity

Assessment of NK cytotoxicity following chronic stress revealed that only control rats that received no stress during either neonatal or adult life showed high NK cytotoxicity compared with all other groups. Rats exposed to maternal stress showed impaired NK cytotoxicity independent of chronic stress exposure in adulthood. Similarly, rats not subjected to maternal stress also showed impaired NK cytotoxicity following chronic stress in adulthood, suggestive of a floor effect whereby chronic stress exposure in either early or later life is sufficient to significantly reduce NK cytotoxicity. Notably, the patterns of NK cytotoxicity do not reflect the patterns of corticosterone levels following chronic stress, and this indicates that NK cytotoxicity is not directly mediated by plasma corticosterone after chronic stress. This is consistent with the previous literature (Page and Ben-Eliyahu 1999; Naor et al. 2009). Furthermore, it has been suggested that the sympathetic nervous system (SNS) may exert greater influence on NK cytotoxicity and tumor metastasis, given that NK cells are known to express adrenergic receptors (Ben-Eliyahu et al. 2000; Elenkov et al. 2000; Rosenne et al. 2007). Finally, it should be noted that the control values of NK cytotoxicity in rats that did not receive adult stress were higher than expected. Other studies have also reported relatively high control values for NK cytotoxicity (Hodgson et al. 2001; Benish et al. 2008). Despite the unexpected high values for control rats, the effect of maternal separation and restraint stress produced a robust suppression of NK cytotoxicity, indicative of the reliability of the effects reported.

Effect of neonatal maternal separation on tumor colonization

Maternal separation also impaired tumor immunity following chronic stress in adulthood. Rats subjected to maternal separation combined with chronic restraint stress in adulthood showed high numbers of tumors ranging from 140 to 260 on the lung surface, whereas all other control conditions displayed low numbers of tumors ranging from 25 to 125, reflective of the findings of previous studies (Hodgson et al. 2001; Hodgson and Knott 2002). It has previously been demonstrated that exposure to stress potentiates colonization of the MADB106 tumor cell (Shavit et al. 1985; Ben-Eliyahu et al. 1991), which is argued to be mediated by suppression of NK cells critically involved in the surveillance and eradication of tumor and virus-infected cells (Barlozzari et al. 1985; Ben-Eliyahu and Page 1992; Stefanski and Ben-Eliyahu 1996; Shakhar and Ben-Eliyahu 1998). Neonatal maternal separation was found to be associated with a significant increase in the number of tumor cell colonies that developed in rats inoculated with tumor cells only when animals were subjected to chronic stress in adulthood but not when they were not subjected to chronic stress. In the present and previous studies investigating early life immunological stress exposure (Hodgson et al. 2001; Hodgson and Knott 2002), the pattern of tumor colonization was not perfectly reflected in NK cytotoxicity. However, such findings are not inconsistent with predictions of the MADB106 model given that NK cytotoxicity is predictive of tumor outcome when measured within the first 24 h after tumor inoculation (Ben-Eliyahu and Page 1992). Furthermore, the discrepancy between NK cytotoxicity and tumor metastases may be resolved if marginal pulmonary NK (MP-NK) cells and lung tumor retention were assessed. Studies have shown relationships between MP-NK cells and lung

tumor retention to be stronger than that of NK cytotoxicity from blood alone (Melamed et al. 2005; Benish et al. 2008) given the action of MP-NK cells on MADB106 tumor cells has greater specificity.

Although glucocorticoids are well documented to suppress immune responses, the present study does not indicate that the suppression of NK cytotoxicity and increased tumor colonization is mediated by corticosterone. There was no direct association between high levels of corticosterone and low levels of NK cytotoxicity across groups. This may be due to findings indicating that the MADB106 tumor cell may be relatively insensitive to corticosterone modulation and more sensitive to the effects of adrenergic mechanisms (Ben-Eliyahu 1998; Shakhar and Ben-Eliyahu 1998). Although much of the research has focused on the effects of the glucocorticoids on the developing HPA axis, there is evidence to suggest that neonatal stress exposure may alter the ontogeny of the SNS as well as altering sympathetic tone. Corticotropin-releasing hormone plays a critical role in activating the HPA axis and SNS (Nijsen et al. 2000), elevating norepinephrine levels in the SNS, and stimulating the release of epinephrine from the adrenal medulla. Hence, it is possible that there are changes in sympathetic reactivity in these animals in addition to the well-documented changes in HPA axis reactivity, which may in part contribute to the impaired tumor immunity in these animals.

Conclusion

The present research suggests that neonatal stressors and exposure to stress in adulthood alter the immune system and tumor metastasis in male rats, but do not substantially alter corticosterone secretion. We expanded previous findings by investigating maternal separation as a stressor and found the effects of maternal separation to be similar to that of bacterial LPS exposure for developmental weight gain and the tumor proliferation. However, the two models differ in regard to NK cytotoxicity and plasma corticosterone responsivity. Neonatal maternal separation was found to be associated with depressed NK cytotoxicity and an increase in tumor colonization when associated with stress exposure in adulthood. The present study does not provide evidence of a causal relationship between the secretion of corticosterone and the impaired tumor immunity under stress conditions. Although a precise mechanism explaining the way in which chronic maternal separation may lead to increased susceptibility to tumor colonization has not been identified, the data reflect the growing literature supporting the impact of perinatal stress on later life health and disease. Environmental determinates have been implicated in increasing predispositions toward later life metabolic diseases (Barker and Osmond 1986; Barker 1998; Vickers et al. 2000), and behavioral and cognitive perturbations (Widom 1999; Gibb et al. 2007; Walker et al. 2009). Research has evidenced the role of the perinatal environment in altering later life immunity (Miles et al. 1996; Prescott et al. 1998; Coe et al. 2002) and pain sensitivity (Page et al. 2005) among other later life health complications. Importantly, we have added to a growing body of knowledge demonstrating neonatal stress to be a crucial factor in the susceptibility for cancer progression. Finally, the underlying physiological mechanisms that trigger immunological alterations in rats are likely to be similar to those of humans (Stefanski 2001), hence reducing perinatal stress in infants and minimizing the impact of stress in adulthood are likely to have beneficial effects on the body's ability to combat tumor in humans.

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