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The Inflammatory Response to Social Defeat is Increased in Older Mice

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Abstract

Previous research indicates that repeated social defeat of mice causes increased lymphocyte trafficking to the spleen, elevated proinflammatory cytokine production, and induced glucocorticoid insensitivity in splenocytes. Social defeat also causes increases in anxiety-like behavior. This study investigated whether repeated social defeat results in similar immunoregulatory and behavioral changes in older mice as those seen previously in young adult mice. The data revealed that, regardless of age, defeated mice had significantly more splenic CD11b+ Gr-1+ monocytes and neutrophils than controls. Supernatants harvested from cultured splenocytes from older mice contained comparatively higher IL-6 and TNF- α than supernatants from younger animals. In addition, those same cells derived from older defeated mice were hypersensitive to lipopolysaccharide (LPS) and insensitive to glucocorticoids in vitro. As seen previously in young adult mice, social defeat caused an increase in anxiety-like behavior in the open field test, but had no effect on learned helplessness in the forced swim test. These data indicated that repeated social defeat results in a proinflammatory state that is exacerbated in older mice. The implications of these data are noteworthy, given the strong role of inflammation in many age-related diseases.

Keywords

Aging; Social Defeat; Social stress; CD-1; Mouse; Bullying; Immunosenescence; Glucocorticoid; Corticosterone; Anxiety; Depression; Steroid insensitivity; IL-6; TNF

Introduction

A search of the extant literature reveals that advanced age is associated with the deterioration of many biological systems, a phenomenon typically referred to as senescence. In the brain, senescence is characterized by cognitive deficits; these deficits often manifest as senile dementias. Similarly, the immune system is subject to age-dependent functional deficits referred to as immunosenescence; older individuals respond poorly to immune challenges. As a result, opportunistic infections that are easily defended in the younger adult can be life threatening to the older adult [1,2].

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Among the contributing factors to the progression of immunosenescence is decreased cytokine regulation. In healthy young adults absent of apparent disease, serum concentrations of proinflammatory cytokines are generally undetectable. However, with advancing age, these cytokines can reach high concentrations and can have untoward effects on the overall health of an individual. For example, high serum concentrations of the proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) are predictive of atherosclerosis in the aged adult population [2,3]. These cytokines are associated with increased mortality due to a myriad of causes including coronary artery disease [4–6]. Similarly, there is an age-dependent loss of proinflammatory cytokine regulation in rodents. Elevated TNF- α and IL-6 mRNA gene expression has been detected in the coronary arteries of older Fisher 344 rats [7], and cells from the lymph nodes and spleens of older mice produce comparatively more IL-6 than cells from younger adults [8]. These age-related cytokine changes are not limited to the periphery. Recently, Godbout and colleagues showed that older mice have higher levels of the proinflammatory cytokines in their brains when the animals were challenged peripherally with LPS [9,10]. In fact, the brains of older mice expressed twice as much mRNA for IL-1 β and IL-6 as compared to the brains of younger adult mice [9]. This noted elevation in brain IL-1 β and IL-6 contributed to neuroinflammation. In sum, older individuals have higher basal pro-inflammatory cytokine expression and are hyper-responsive to mitogenic stimulation. Taken together, these observations indicate that there is a progressive loss of cytokine regulation with advancing age that is characteristic of immunosenescence.

In addition to aging, psychological stress also disrupts immune regulation and can result in immunological deficits. Recent findings from our laboratory indicate that a social stressor (social disruption, SDR) results in dysregulation of proinflammatory cytokine responses in young adult mice [10–12]. In contrast to many experimental stressors, which are generally immunosuppressive, SDR causes an increase in proinflammatory cytokine production, hyper-responsiveness to mitogenic stimulation, and glucocorticoid insensitivity. Similar to the effects of immunosenescence, this hyperinflammatory response is characterized by an increase in IL-6 concentrations in serum and increased production of IL-6 and TNF- α in response to LPS-stimulation [12,13]. These effects can have a potentially negative influence on the health of the young adult animals. For example, young adult mice subjected to SDR and injected with LPS were more likely to die from endotoxic shock than were control animals [14]. The increased production of pro-inflammatory cytokines was shown to be the mechanism for this increased susceptibility to endotoxic shock. Thus, the stress of repeated social defeat (i.e., SDR) induces inflammatory changes in young mice that parallel those inflammatory changes linked to immunosenescence.

In addition to the immunological changes, repeated social defeat also causes behavioral changes in young adult animals. For example, mice subjected to six cycles of social disruption stress spent less time in the center of the open field test and more time in the dark portion of the light/dark preference test, indicating an increase in anxiety-like behavior [15]. Like social defeat, aging also causes an increase in anxiety-like and depressive-like behaviors in mice [16,17].

In light of these parallel observations between the effects of aging and that of social defeat, it was hypothesized that these effects would interact, causing negative health outcomes. In fact, studies have already shown that older mice are more susceptible to influenza-induced mortality than younger mice, and mortality increased when older mice were subjected to repeated restraint stress [18]. However, the increased mortality of the older animal subjected to restraint stress was attributed to a severely depressed immune response to viral challenge. More specifically, restraint stress suppressed the expression of cytokines necessary for the activation of anti-viral immune responses. Therefore, because of the apparent increase in pro-inflammatory cytokines due to the stress of social defeat, the objective of the present study was

somewhat contrary. In sum, these experiments were designed to test whether social defeat of older (14-month-old) animals resulted in hyperinflammatory immune changes and behavioral effects similar to those seen in young adult (2-month-old) animals. In addition, the data were analyzed to determine whether aging enhanced the effects of social defeat.

Methods

Animals

Male CD-1 mice (n=40) were ordered from Charles River at 6–8 weeks of age. Younger adult mice were 2 months old, and older adult mice were 14 ± 1 months of age at the beginning of the experiments. Mice were housed in our facility and aged for one year prior to the experiment to generate the cohort of 14-month-old animals. All mice were initially group-housed, and then randomly assigned by age group into Younger Defeat (n=6), Younger Control (n=10), Older Defeat (n=15), or Older Control (n=9) treatment group, singly housed, and left undisturbed for at least 2 weeks before testing began. The mice were singly housed for this experiment, in order to reduce the incidence of spontaneous fighting within the cages. Previous observations from our lab indicated that the older mice were more likely than the younger mice to engage in such fighting within the home cage. Because fighting is itself an independent variable in the present study, the mice were singly housed to avoid this potential confound. All mice were kept in an American Association for Accreditation of Laboratory Animals Care (AAALAC) accredited facility at the Ohio State University and provided standard lab diet and tap water *ad libitum*. The Institutional Laboratory Animal Care and Use Committee (ILACUC) at the Ohio State University approved all experimental protocols.

Social Defeat

The social defeat paradigm was described previously [19]. An aggressive intruder mouse was introduced to the home cage of each resident mouse for 30 minutes for six daily sessions, starting at the beginning of the nighttime active cycle (1600 EST). The defeated resident mice were the subjects of this study. The aggressor mice were singly housed for several weeks prior to the experiment to induce territoriality and agonistic behavior toward unfamiliar male mice. The aggressor typically started to attack the resident mice within 5 minutes of being introduced into the cage. Behavior was observed to ensure that the resident mice were attacked and defeated by the aggressor. Defeated mice did not initiate attacks but exhibited defensive behaviors, such as fleeing, freezing, and upright submissive postures in response to the aggressor [12,19–21]. In cases in which the aggressor did not attack the resident mouse, or the resident attacked and defeated the aggressor, the aggressor was replaced. After each 30-min defeat session, the aggressor was removed and returned to its home cage. Each mouse was defeated in its home cage each night, although a different aggressor was used during each defeat cycle to prevent habituation (Avitsur et al. 2001; Quan et al., 2003; Stark et al. 2001). Control mice were left undisturbed in their home cages during defeat sessions.

Glucocorticoid Sensitivity Assay

The glucocorticoid sensitivity assay was performed as described previously [12,19]. Briefly, at sacrifice, spleens were harvested and weighed. A single cell suspension was prepared by homogenizing the spleens in a stomacher (Biomaster #80, Seaward, London, England), and red blood cells were lysed with a hypotonic salt solution (0.16M NH_4Cl , 10mM KHCO_3 , and 0.13 mM EDTA). Triplicate samples of splenocytes were cultured at 2.5×10^5 cells per well in flat-bottom 96-well tissue culture plates in complete RPMI (containing 10% heat-inactivated fetal bovine serum, 0.075% sodium bicarbonate, 10mM Hepes buffer, 100 U/ml penicillin G, 100 $\mu\text{g}/\text{ml}$ streptomycin sulfate, 1.5 nM L-glutamine, and 0.0035% 2-mercaptoethanol). Cultures were stimulated with 0.40 $\mu\text{g}/\text{ml}$ LPS and increasing concentrations of corticosterone (dose range 0.005 – 0.05 μM) for 48 h at 37°C and 5% CO_2 . Cell viability was measured with

a tetrazolium substrate solution (Cell Titer 96 non-radioactive proliferation kit, Promega), and read at 490 nm on an ELISA plate reader [12]. Viability for each sample was expressed as the mean optical density (OD) of each LPS-stimulated sample, minus OD of non-stimulated wells treated with the same corticosterone concentration. For comparison purposes, data were converted into a corticosterone resistance index, which represents the viable cells in each corticosterone treatment group, as a percentage of non corticosterone treated cultures from the same age and defeat group [19,20].

Cytokine Assays

Pro-Inflammatory cytokines (IL-6 and TNF- α) from splenocytes culture supernatants were quantified by a standard sandwich ELISA as described previously [12,23]. Briefly, for IL-6 determination, 96-well ELISA plates were coated with 50 μ l/well of 2 μ g/ml rat anti-mouse IL-6 antibody (BD Pharmingen, San Diego, CA) overnight at 4°C and then washed 2X with PBS-Tween wash buffer. Non-specific binding was blocked with 200 μ l/well of PBS/10% FBS for 2 hours at room temperature. After two washings, 50 μ l of samples or standards were added, and plates were incubated overnight at 4°C. After 4 washes, 100 μ l/well of biotinylated anti-mouse capture antibody (BD Pharmingen) was added and incubated for 45 min at room temperature. Plates were washed 6X with wash buffer, and 100 μ l/well of 1:1000 avidin-peroxidase (BD Pharmingen) was added and incubated for 30 min at room temperature. Plates were washed 8X with wash buffer, and 100 μ l/well of 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt substrate (Sigma, St. Louis, MO) plus 10% H₂O₂ was added and developed in the dark for 30 minutes. Plates were then read at 405 nm on an ELISA plate reader. The IL-6 concentrations for each sample were calculated using a polynomial curve generated from known standards. The ELISA for TNF- α was similar with the exception of using 3,3',5,5' tetramethylbenidine (TMB) as the substrate and 1M H₃PO₄ as a stop solution; TNF- α plates were read at 450 nm.

Flow Cytometry

Cell suspensions containing 2.5×10^5 cells were incubated at 4°C for 45 minutes with FITC-conjugated Gr-1/Ly-6G (clone RB6-8C5), PE-conjugated anti-mouse Pan-NK (clone DX5), PerCP-conjugated anti-mouse CD45R/B220 (clone RA3-6B2), and APC-conjugated anti-mouse CD11b/Mac-1 (clone M1/70). A second panel was run in parallel, using FITC-conjugated anti-mouse CD3e (clone 145-2C11), PE-conjugated anti-mouse CD8a (clone 53-6.7), and PerCP-conjugated anti-mouse CD4 (clone RM4-5). All monoclonal antibodies were purchased from BD Pharmingen (San Diego, CA). A standard lyse-wash procedure using PBS (Dulbecco's PBS without Ca and Mg, 2% FBS, and 0.1% NaN₃) was used to stain the cells. A total of 10,000 events from each sample were analyzed on a dual-laser flow cytometer (FACSCalibur, BD Immunocytometry Systems) using Cell Quest Pro and Attractors software. Lymphocytes, neutrophils, and monocytes/macrophages were identified by forward and side scatter characteristics and differences in antibody staining. Matched isotype controls were used to set negative staining criteria.

Serum Corticosterone Determination

Serum corticosterone was measured with a radioimmunoassay (RIA) kit (ImmuChem Double Antibody corticosterone ¹²⁵I RIA kit; MP Biomedicals, Orangeburg, NY), per the manufacturer's protocol [12,24,25]. Blood was collected from the retroorbital plexus within 3 minutes of opening the cage immediately following the first cycle of defeat (i.e., at 1830 EST). A baseline sample was collected from each animal at the same time of day but one week before the start of the experiment. Blood samples were immediately put on ice, allowed to clot for one hour, the clots were removed, and the samples were centrifuged at 4000 $\times$ g at 4°C for 20 minutes. Serum was stored at -70°C until analysis. Blood samples were collected from each

animal in this study, and a random selection of samples from each treatment group was assayed in duplicate. The minimum sensitivity of the assay was 23.5 ng/ml.

Behavior testing

Behavior was assessed on the morning following the sixth defeat session. Anxiety-like behavior was measured in the open field test, and depressive-like behavior was measured in the forced swim test. The open field test apparatus consisted of a $30 \times 30 \times 25$ cm square Plexiglas box with a solid floor with a grid drawn on the floor, dividing the open field into 36 identical squares. This test was lighted by overhead room lighting. The open field test is designed to take advantage of a rodent's natural tendencies to explore the environment while avoiding open spaces. Mice expressing anxiety-like behavior (hypervigilance) tend to spend less time in the center of the open field and also tend to locomote near the walls of the apparatus (thigmotaxis). These effects can be reversed by anxiolytic compounds [26–30]. For this experiment, the dependant variables were: total number of line crosses, and total time spent in the center/perimeter of the open field. The test apparatus was cleaned with water-dampened cloths between subjects. For the Porsolt forced swim test, individual mice were placed in a glass cylinder (43 cm high, 22 cm diameter) filled with room-temperature water to a depth of 15 cm. The rim of the cylinder was high enough that the mouse could not climb or jump out, and the water was deep enough that the mouse could not touch the bottom of the cylinder with its tail [31–34]. This test was recorded in the dark (< 3 lux), using infrared lighting. Struggling behavior was measured as time spent in one of three mutually exclusive states: actively swimming around the apparatus, climbing the walls of the tank (forepaws break the surface of the water), or floating (which included minor movements to keep the head above water). The water was replaced between subjects. Both tests were video taped for 5 minutes. These tapes were later digitized and coded by a trained ethologist using the Observer program (Noldus Information Technologies, the Netherlands).

Statistics

Spleen size, cytokines, and cell population data were compared by 2×2 between measures (age vs. defeat treatment) ANOVA. Repeated measures 2×2 (age vs. defeat treatment) ANOVA tests were used to assess statistical significance for the GC insensitivity, behavioral measures, and serum corticosterone. Behavioral data were compared using one-way between-groups ANOVA. Fisher's PLSD tests were used for follow-up comparisons of pairwise differences. All results are presented as mean $\pm$ SE. Statistical significance was set at $p < 0.05$.

Results

The first set of experiments was designed to confirm that the 14-month-old animals in this study showed classical signs of immunosenescence before they were subjected to the stress of social defeat. Whereas the typical mouse aging study uses animals greater than 20 months of age, the social stress model used in these studies precluded the use of such old mice for multiple reasons. Most important was our concern for their ability to survive the stress of repeated aggressive interactions with an intruder. Thus, the mice chosen for the experiment were 14 months old. Even so, the data revealed that the 14-month-old CD-1 mice used in the following studies showed multiple signs of immunosenescence, including thymic involution and increased proinflammatory cytokine production. Mean relative thymus mass, expressed as thymus mass/body mass, from older mice was significantly lower than in the younger mice ($F(1,25) = 112.65$; $p < 0.0001$; Fig 1A). The proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α) secreted by spleen cells stimulated with LPS were measured via ELISA and compared between groups. As would be predicted in an aged mouse, production of both IL-6 ($F(1,17) = 4.40$; $p < 0.05$; Fig 2) and TNF- α ($F(1,17) = 8.64$; $p < 0.01$) was increased in older mice, compared with younger adult mice.

In addition to confirming that the 14-month-old animal showed signs of immunosenescence, it was important to verify that our model of social defeat was a stressor for the older animals. One of the core stress responses typically noted after exposure to a stressor is activation of the hypothalamic-pituitary-adrenal axis resulting in increased circulating glucocorticoids. In rodents, the predominant glucocorticoid is corticosterone. As compared to age-matched control animals, both younger and older mice, subjected to social defeat, had significantly elevated levels of plasma corticosterone immediately after social defeat ($F(1,26) = 42.81$; $p < 0.0001$; Fig 3). There was no apparent age effect with regard to the corticosterone response to social defeat ($p > 0.10$).

Previous studies showed that SDR of young mice causes splenomegaly [12,14,19]. Therefore, we tested whether aging exacerbated this effect. Although non-stressed old mice had a lower spleen/body mass than non-stressed younger adult animals ($F(1,17) = 7.29$; $p < 0.05$; Fig 1B), social defeat of either age group caused an increase in relative spleen mass (splenomegaly) ($F(1,36) = 16.00$; $p < 0.01$). Previously, we also reported that splenomegaly was associated with increased accumulation of neutrophils, macrophages and B cells in the spleen [35]. Flow cytometric analysis of the cells in the spleens from both younger and older mice revealed that total cell numbers were higher in both defeated groups regardless of age ($F(1,36) = 24.39$; $p < 0.0001$; Table 1). The stress-induced increase in cell number was predominantly due to an accumulation of granulocytes ($F(1,36) = 9.30$; $p < 0.01$) and monocytes ($F(1,36) = 10.49$; $p < 0.01$). Social defeat had no effect on T cell populations in younger adult mice. However, in older mice, there was a significant increase in CD8+ cells ($F(1,22) = 6.54$; $p < 0.05$) and a marginally significant increase in CD4+ T cells ($F(1,22) = 3.92$; $p < 0.06$). Although not statistically significant, there was a trend suggesting an interaction between aging and defeat in granulocyte ($F(1,36) = 3.58$; $p < 0.07$) and monocyte ($F(1,36) = 2.77$; $p < 0.10$) populations in the spleen.

Previous studies also showed that SDR increased proinflammatory cytokine production from cultured splenocytes. Both IL-6 and TNF- α were higher in supernatants derived from splenocytes of defeated younger mice as compared to those from home cage (non-defeated) younger controls [10,11,13]. Again, the data from this current study agreed with these previous findings in younger mice -- defeat increased production of both IL-6 ($F(1,16) = 8.73$; $p < 0.01$; Fig 2) and TNF- α ($F(1,16) = 8.84$; $p < 0.01$). Similarly, social defeat of older mice significantly increased TNF- α production ($F(1,22) = 9.34$; $p < 0.01$) and increased IL-6, a trend toward statistical significance ($F(1,22) = 3.46$; $p < 0.08$). There was no statistically significant interaction between aging and stress.

Of particular interest to us has been the observation that SDR results in the development of functional glucocorticoid insensitivity in splenocytes from younger mice. Specifically, LPS-stimulated CD11b+ monocytes from SDR animals remain viable, even when co-cultured with increasing concentrations of corticosterone. Therefore, the effects of aging on the development of stress-induced GC insensitivity were assessed in the present study. The data showed that aging, by itself, had no effect on GC sensitivity. Cells obtained from either older or younger non-stressed control animals were equally sensitive to corticosterone culture ($F(1,17) = 0.72$; $p > 0.40$; Fig 4). However, there was an interaction between defeat and age treatments ($F(1,36) = 7.22$; $p < 0.01$). Follow-up comparisons revealed that this interaction was driven primarily by an increased in cell viability in the presence of high concentrations of corticosterone (older defeated vs. all other groups: $p < 0.01$ at 5.0 μ M, $p < 0.001$ at 0.5 μ M concentration). There was a main effect of aging ($F(1,36) = 4.66$; $p < 0.05$), due primarily to the high GC insensitivity in the older, defeated group ($F(1,22) = 35.56$; $p < 0.0001$). Cell viability (i.e., glucocorticoid insensitivity) was high in both defeated groups, compared to non-defeated controls ($F(1,38) = 42.96$; $p < 0.0001$). There was also a main effect of aging, with higher GC insensitivity in older mice compared with younger mice. Follow up comparisons confirmed higher GC insensitivity

in older defeated mice than in all other treatment groups [younger defeated mice ($p < 0.01$) and younger and old control groups ($p < 0.0001$)].

As in younger adult mice, social defeat resulted in increased anxiety-like behavior in the older mice, although the effects were not as pronounced. Time spent in the center of the open field test was decreased in defeated mice ($F(3, 36) = 3.36$; $p < 0.05$; Fig 5A), and follow-up comparisons revealed a significant decrease in older, defeated mice ($p < 0.05$). These mice also entered the center of the open field fewer times than non-defeated mice ($F(3, 36) = 2.86$; $p < 0.05$; Fig 5B). However, locomotion was also significantly reduced by social defeat ($F(3, 36) = 3.37$; $p < 0.05$; Fig 5C). Depressive-like behavior, reported here as immobility in the forced swim test, was unaffected by social defeat ($F(3, 36) = 1.80$; Fig 5D). Similarly, latency to become immobile in the forced swim test was not affected by social defeat ($F(3, 36) = 0.34$; Fig 5E).

Discussion

Social stress is a common and salient stressor experienced by humans living in developed areas of the world. Many experimental models of stressors have been developed to study the effects of stress on many regulatory systems, most notably the immune system. The social defeat paradigm used in the present study was designed to specifically address the concern that previously developed stressors lacked a social component. As reported herein and in previous studies [12,14,36], social defeat has unique physiologic and immunological effects that are not characteristic of other stressors. Whereas most experimental stressors, such as restraint, result in activation of the hypothalamic-pituitary-adrenal axis (i.e., elevated corticosterone) and an associated suppression of inflammation, social defeat stress contrastingly results in higher production of TNF- α and IL-6 from LPS-stimulated splenocytes [23]. Furthermore, unlike restraint stress, social defeat stress results in the development of functional glucocorticoid resistance in splenic CD11b+ macrophages [10,11]. In other words, social defeat results in higher pro-inflammatory cytokine secretion that is resistant to the innate feedback control mechanisms that are typically engaged to limit inflammatory cytokine production [37]. Therefore, because of the link between excessive inflammation and age-related diseases, we tested whether the effects of social defeat would be exacerbated in older individuals.

Although mice over 20 months of age are typically used in aging research, mice aged 14 months were chosen for this series of experiments for several reasons. First, there was a concern that older, geriatric mice (e.g. 20 months) would be unable to defend themselves or would not engage the aggressor mouse during the social defeat paradigm used in this study. Second, there was a strong desire to study the direct effects of aging, not age-related disease. Thus, mice aged 14 months were old enough to show some early signs of immunosenescence (e.g. thymic involution) [20], yet young enough to present low levels of apparent age-related disease. Mice as young as 10 months show an age-dependent increase in mortality when infected with a sub-lethal dose of influenza virus [18]. The third reason for using 14-month-old mice for the older group was that we did not wish to bias our sample by using only those mice that survived to a very old age. In any animal population, only a few individuals survive to very late life, and these individuals consist of a small, very healthy subset of the population [38,39]. Indeed, age-related alteration of NK activity and IL-2 and TNF- α release observed in “old” mice disappear in “very old” mice [40]. Thus, by using adult animals that are aged, but not geriatric, our results are indicative of age-dependent trends that are applicable to all members of the population and avoided selecting for a subpopulation of extraordinarily healthy animals. Regardless, because social defeat is the underlying theme to this experimental model of social stress, older mice would be more likely to be quickly defeated by the aggressive intruder. Thus, it is highly likely that the observed effects of social defeat would be even more pronounced in even older, albeit not geriatric mice.

In sum, both younger adult and older adult mice were subjected to social defeat – they were repeatedly defeated by an aggressive conspecific for six consecutive days. Confirming previous reports [10,13], splenocytes from defeated mice produced higher levels of the proinflammatory cytokines IL-6 and TNF- α . In addition, social defeat resulted in a marked insensitivity to the anti-proliferative effects of corticosterone when splenocytes were co-stimulated with LPS. Aging exacerbated these stress effects. Most evident was a substantially marked decrease in sensitivity to corticosterone in the older animals. Even though the only cell that has, thus far, been shown to become insensitive to glucocorticoids after social defeat is the CD11b+ macrophage [12], it should translate that if the macrophage is insensitive to the anti-inflammatory effects of GC, proinflammatory cytokine production should increase. In fact, that is just what happens as we age.

Quan and colleagues [22] reported that, in the cell that was insensitive to GC signaling, the CD11b+ cell, the expression of the glucocorticoid receptor (GR) protein was not affected by social defeat. However, the activation or translocation of the GR to the nucleus was impaired in socially defeated mice, and thus, its anti-inflammatory properties were muted. For example, exogenous corticosterone was unable to reduce activation of the NF- κ B complex in cells from animals subjected to social defeat; in turn, this accentuated the production of proinflammatory cytokines. Although age-specific effects of social defeat on the GR were not examined in the present study, clear parallels exist in the development of GC insensitivity and increased IL-6 and TNF- α production, which implies that the same mechanisms are likely active in older animals as previously identified in younger adult mice. Still, the development of glucocorticoid insensitivity should not suggest that the ‘inflammatory’ effect of social defeat is dependent on corticosterone signaling or the lack thereof. To the contrary, the hyper-inflammatory response appears not to be regulated by the influence of the GR. In other words, the lack of GC signaling does not directly increase inflammatory gene transcription. Instead, the lack of GC signaling has an indirect effect by simply removing a repressive influence on pro-inflammatory cytokine gene transcription if it is initiated through one of the typical activation pathways (e.g., NF- κ B).

Previous research from various other labs has found that aging increases [41–44], decreases [45–47], or has no effect on proinflammatory cytokine production [48]. There are some methodological differences in these studies, although it appears that isolated macrophages and monocytes from older individuals tend to down-regulate TNF- α and IL-6 production. On the other hand, unfractionated splenocytes from older mice appear to increase production of TNF- α and IL-6 [42]. In the present study, whole spleen cell populations were studied for cytokine release. The observed increases in IL-6 and TNF production by splenocytes from socially defeat mice were likely due to activated macrophages, which presumably received proinflammatory signals from other splenocytes including T cells in the cell culture.

Along with this change in cytokine regulation, advancing age brings increased risk from a variety of diseases. These diseases range from those of infectious origin such as influenza virus, to those of an inflammatory nature such as coronary artery disease. Although we can vaccinate against those threats of the infectious nature and presumably lessen morbidity and mortality in the elderly population, those diseases of an inflammatory nature are more insidious and difficult to ameliorate. In fact, chronically elevated systemic inflammation can have a substantial impact on health for older individuals. For example, with regard to heart disease, which is the leading cause of mortality in the population over the age of 65 years [49], circulating levels of IL-6 and TNF- α predict the development of atherosclerosis [4–6]. Conversely, blocking proinflammatory cytokines can lessen disease symptomology. For example, long-term TNF- α antagonist treatment alleviated symptoms of rheumatoid arthritis, possibly via restored HPA axis function [50].

Altered cytokine regulation of inflammation is not limited to the peripheral immune system. There is now a solid link between excessive and uncontrolled inflammation in the brain and the development of clinical symptoms of Alzheimer's disease [51]. In addition, increased production of proinflammatory cytokines IL-1 and TNF- α predicts the development of Alzheimer's disease in late adulthood [52]. Thus, if inflammation contributes to the development of many of the diseases of aging such as coronary artery disease, arthritis, and Alzheimer's disease, then it is reasonable to assume that conditions that exacerbate inflammation may also worsen those diseases.

In other words, the inability to modulate activation of inflammatory mediators would exacerbate diseases associated with aging. The stress of social defeat weakens one of those balancing systems; it decreases an animal's sensitivity to the natural anti-inflammatory effects of corticosterone. The loss of sensitivity to corticosterone is further magnified in older animals, in which inflammatory conditions are more likely to contribute to disease. In conclusion, the findings delineated herein suggest that the stress of social defeat, which is exacerbated by aging, would contribute to the development of age-associated disease.

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Figure 1.

Aging and stress affect thymus and spleen mass. Male CD-1 mice were subjected to repeated social defeat for 6 days. (a) Mice aged 14 months show thymic involution, a typical feature of immunosenescence. Repeated social defeat caused a reduction in relative thymus mass (thymus mass/body mass) in younger mice (2 months old), but not older mice (14 months old). (b) Relative spleen mass (spleen mass/body mass) is lower in older mice than younger adult mice. Repeated social defeat causes an increase in both younger and older mice. * $p < 0.05$ vs. controls; ** $p < 0.05$ vs. younger adult mice.

Figure 2.

Aging and social defeat cause increased proinflammatory cytokine production in cultured splenocytes. Spleen cells were harvested from younger adult and older adult mice, stimulated with LPS, and incubated for 18 hours. Cytokines were quantified by ELISA. * $p < 0.05$ vs. younger adult mice; ** $p < 0.05$ vs. age-matched controls.

Figure 3.

Serum corticosterone concentrations from younger adult (aged 2 months) and older adult (14 month) male mice subjected to repeated social defeat. Samples were obtained via retro orbital plexus immediately after defeat session (approximately 1830 EST) and quantified from serum via radioimmune assay. * $p < 0.05$ vs. baseline.

Figure 4.

Social defeat causes insensitivity to the glucocorticoid (GC) hormone corticosterone. Spleen cells were harvested from younger adult and older adult mice, costimulated with LPS and corticosterone and incubated for 48 hours. GC insensitivity is defined as high cell viability in the presence of increased concentrations of corticosterone. Both defeated groups (closed circle & square) demonstrate GC insensitivity, as compared with control groups (open circle & square). Cell viability was higher in older, defeated mice (closed square) than any other group. * $p < 0.05$ vs age-matched controls; ** $p < 0.05$ vs. all groups.

Figure 5.

Defeat increased anxiety-like behavior in older mice. Younger and older adult mice were subjected to six days of repeated social defeat, and then assessed in the open field test. Data from younger adult mice area presented here, but were not analyzed due to abnormally high anxiety-like behavior. (a) Defeated mice spent less time in the center of the open field. (b) Defeated mice entered the center of the field fewer times than controls. (c) Locomotion was reduced in defeated mice. (d, e) The Porsolt forced swim test indicated that defeat did not affect time immobile or latency to become immobile, two tests of depressive-like behavior. * $p < 0.05$ vs. age-matched controls.

Table 1

Social defeat alters splenic cellularity in both younger and older adult mice. Splenic cell populations were phenotyped by flow cytometry and expressed as mean cell count with standard error in italics.

	Younger Control	Younger Defeat	Older Control	Older Defeat
Total cells	$1.21 \times 10^8 \pm 5.43 \times 10^7$	$2.02 \times 10^{8*} \pm 4.23 \times 10^7$	$1.21 \times 10^8 \pm 1.15 \times 10^7$	$2.81 \times 10^{8*} \pm 2.22 \times 10^7$
Granulocytes	$4.06 \times 10^6 \pm 2.05 \times 10^6$	$1.93 \times 10^{7*} \pm 7.75 \times 10^6$	$4.42 \times 10^6 \pm 6.47 \times 10^5$	$6.97 \times 10^{7*} \pm 1.61 \times 10^7$
Monocytes	$1.80 \times 10^6 \pm 2.64 \times 10^5$	$8.02 \times 10^{6*} \pm 2.89 \times 10^6$	$2.90 \times 10^6 \pm 5.43 \times 10^5$	$2.23 \times 10^{7*} \pm 4.77 \times 10^6$
Lymphocytes	$1.13 \times 10^8 \pm 1.65 \times 10^7$	$1.66 \times 10^{8*} \pm 2.99 \times 10^7$	$1.11 \times 10^8 \pm 1.03 \times 10^7$	$1.66 \times 10^{8*} \pm 1.84 \times 10^7$
T cells	$3.56 \times 10^7 \pm 3.58 \times 10^6$	$4.56 \times 10^7 \pm 6.90 \times 10^6$	$3.72 \times 10^7 \pm 4.43 \times 10^6$	$6.36 \times 10^{7*} \pm 6.37 \times 10^6$
CD4+ T cells	$2.50 \times 10^7 \pm 2.44 \times 10^6$	$3.01 \times 10^7 \pm 4.15 \times 10^6$	$2.44 \times 10^7 \pm 2.80 \times 10^6$	$3.57 \times 10^{7*} \pm 4.09 \times 10^6$
CD8+ T Cells	$8.52 \times 10^6 \pm 1.08 \times 10^6$	$1.02 \times 10^7 \pm 1.83 \times 10^6$	$1.08 \times 10^7 \pm 1.55 \times 10^6$	$1.96 \times 10^{7*} \pm 2.49 \times 10^6$

*
 $p < 0.05$ vs. age-matched controls.