

Research Paper:

Effects of Social Stresses on Immune Response in Female and Male Mice  

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ABSTRACT

Background: Social stress is a factor involved in the etiology of many diseases. Also, gender is another factor which predisposes individuals to certain disease. Results from animal and human studies suggest that socially-stressed men are more vulnerable to catching diseases compared to socially-stressed women.

Materials and Methods: The role of chronic social stress and gender were examined in the present study through implementing food deprivation, food intake inequality, and unstable social status (cage-mate change every three days) for a period of 14 days on 96 male and female mice. Then, vital activity of peritoneal macrophages and spleen's lymphocytes were measured using MTT test as well as the concentration of Tumor Necrosis Factor-alpha (TNF- α) by ELISA technique.

Results: Our results showed that cell viability of peritoneal macrophages and spleen's lymphocytes as well as serum concentration of TNF- α in all female and male stressed animals increased compared to the controls ($P < 0.05$). Moreover, gender differences in immune function were also apparent; these changes were more prominent in female mice.

Conclusion: The results suggest that social factors have significant effects on immunity and should be considered in the studies of gender differences for evaluating possible effective mechanisms.

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Introduction

Despite the undeniable role of genetic and biological factors on health status, the social and economic conditions can also be influential. Social inequalities result in unequal and unjust health outcomes [1]. Poverty and social inequality are intrinsically stressful and have deteriorating effects on health and well-being [2, 3]. It is believed that both natural and laboratory-induced stressors have profound direct and indirect influences on immune responses [4]. Such stresses along with social and psychological disturbances suppress immune responses and leave body unprotected against diseases and infections [5]. On the other hand, poverty may exacerbate immunopathology following prolonged activation and dysregulation of the immune system and causes altered production of proinflammatory cytokines including tumor necrosis factor-alpha (TNF- α) [4].

Over the years, laboratory and human studies focused on biological factors that could cause gender differences in immune responses. In this regard, the roles of social poverty and inequalities have also been amply investigated [6, 7]. Moreover, in many species, male immune system is more susceptible to diseases and infections caused by psychosocial adversities [8].

The biological basis of social inequality and injustice on health seems to be quite complex. Previous findings from our laboratory have demonstrated that animals also possess the capability of sensing social discrimination via a biopsychological and neurosocial phenomenon [9, 10]. Thus, in the present study we aimed to investigate the impact of social stresses on the immune response. In this regard, we measured serum TNF- α concentrations as well as the vital activity of peritoneal macrophages and spleen's lymphocytes. Then, we sought to establish the role of various social factors that may cause different immune responses due to gender differences.

Materials and Methods

Animal model and experimental protocol

Experimental subjects included adult male and female inbred mice of the BALB/c strain (8–10 weeks old, n=96) purchased from the Pasteur Institute (Karaj, Iran). In this study, we performed experimental protocol according to our previous studies. The animals of the same gender were divided into 6 experimental groups including [6, 11, 12]: 1. Control; 2. FD+See:

This group experienced Food Deprivation (FD) and inequality (See) by sensing the other animals' feeding; 3. FD+Isolate: This group experienced Food Deprivation (FD) without any food inequality (Isolated); 4. FD+See+CC: This group experienced Food Deprivation (FD), inequality (See), and Cage-mate Change (CC); 5. FD+Isolate+CC: This group experienced Food Deprivation (FD) as well as Cage-mate Change (CC) without any food inequality (Isolated); and 6. CC: This group only experienced Cage-mate Change (CC).

At the end of experimental protocol the subjects were weighted again (i.e. day 15 of the study), then anesthetized and their cardiac blood was collected to investigate changes of proinflammatory cytokines [12]. Finally the animals were killed by high dose of anesthetic agent.

Immunological assay

Immediately after blood collection, the samples were centrifuged at 3000 rpm for 10 minutes and their serum was taken to measure immunoreactive TNF- α using ELISA (quantitative enzyme-linked immunosorbent) kit (R&D Systems Europe, Abingdon, UK). Standard sensitivity threshold of this assay for TNF- α in the serum was 1.5 pg/mL according to the manufacturer's instruction.

Macrophage cell isolation, Preparation of splenic lymphocytes and their culturing

Isolation of macrophage cells and preparation of splenic lymphocytes as well as their culture was performed according to a previous study in Shahed University [13].

MTT Test

Twenty-four hours after culturing macrophages and 48 h after culturing lymphocytes, we performed MTT test according to a previous study in Shahed University [13, 14].

Statistical analysis

Statistical analyses were performed using the Sigma Stat software (SystatSoftware, Inc., Point Richmond, CA, USA) in which 1-way ANOVA test followed by the post hoc Tukey–Kramer test was used for multiple comparisons. A level of $P < 0.05$ was used as statistical differences. All data in the text and figures are presented as Mean \pm SEM.

Results

Comparison of serum levels of TNF-α between experimental subjects

As shown in Figure 1, food deprivation, food inequality, and cage-mate change significantly increased TNF-α levels in the serum of experimental subjects both in females and males as compared to those in the control group (P<0.05) and this increase was more obvious in both males and females which experienced all three stresses compared to other less stressed animals. However, this difference was not considerable between some of these groups. Moreover, concentration of TNF-α was more in the serum of female mice as compared to that of male mice.

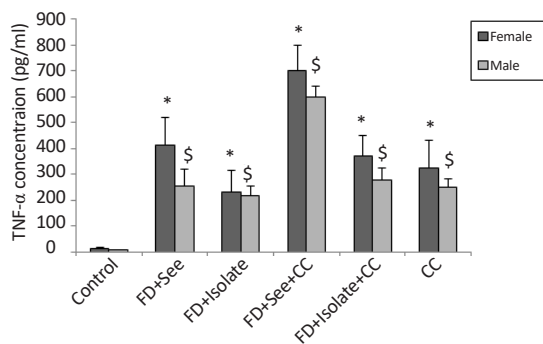
The effect of social stressors on viability of lymphocytes and peritoneal macrophage (MTT Test)

The Lipopolysaccharides (LPS) stimulator significantly increased the vital activity of macrophages (MTT test) in the control group. Implementation of food deprivation, inequality, and cage-mate change alone or with each other stressors for 14 days in males and females did not affect viability of non-stimulated macrophages, but at the presence of LPS stimulator (Figure 2), increased viability of macrophages was observed in all stressed groups (P<0.001). Moreover, there was a significant difference (P<0.05) between isolated animals and the subjects which experienced food deprivation and unequal-

ity simultaneously. The animals which experienced all three stresses had higher vital activity of macrophages (P<0.05). In females, the higher optical density was observed in FD+CC+See and then in FD+See animals, as compared to those of the controls (there was a significant difference (P=0.002) between these two groups). Moreover, no difference was observed between FD+Isolated, CC, and FD+CC+isolated female groups. In male animals which experienced all three stresses, this activity was more than that in the other stressed subjects (P=0.007). Comparison of males and females revealed that vital activity of macrophages in males was as low as that in females in all experimental groups (P<0.05).

Vital activity of lymphocytes in the control group both in female and male subjects significantly increased in the presence of Concanavalin A (ConA) stimulator. Implementation of food deprivation, inequality and cage-mate change merely or coincided with each other for 14 days did not affect viability of non-stimulated lymphocytes, but comparison of stressed animals and control group (Figure 3) showed that vital activity of lymphocytes significantly increased in the presence of ConA stimulator in these groups (P<0.05) and this increase was more considerable in animals which experienced all three stresses both in males and females compared to that of other less stressed groups (P<0.05).

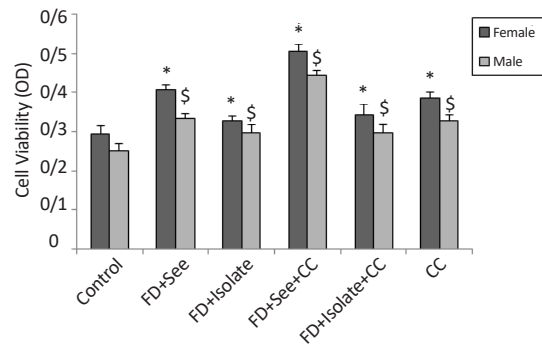
As shown in Figure 3, a greater vital activity of lymphocytes in female controls relative to control males



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Figure 1. Evaluation of food deprivation and inequality as well as social instability effects on serum concentrations of TNF-α

*P<0.05: Significant difference in stressed and controls of female animals. \$P<0.05: Significant difference in stressed and controls of male animals. Food deprivation (See) group (FD+See), Food deprivation (Isolated) group (FD+Isolate), Food deprivation plus cage-mate change (See) group (FD+See+CC), Food deprivation plus cage-mate change (Isolated) group (FD+Isolate+CC), and Cage-mate Change group (CC).



IMMUNOREGULATION

Figure 2. Evaluation of food deprivation and inequality as well as social instability effects on vital activity of peritoneal macrophages (MTT test)

*P<0.05: Significant difference in stressed and controls of female animals. \$P<0.05: Significant difference in stressed and controls of male animals. Food Deprivation (See) group (FD+See), Food Deprivation (Isolated) group (FD+Isolate), Food Deprivation plus Cage-mate change (See) group (FD+See+CC), Food Deprivation plus cage-mate change (Isolated) group (FD+Isolate+CC), and Cage-mate Change group (CC).

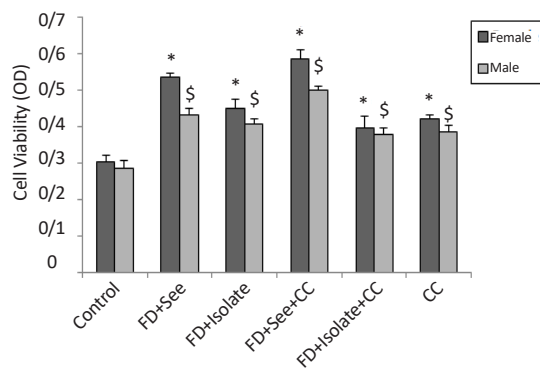


Figure 3. Evaluation of food deprivation and inequality as well as social instability effects on vital activity of lymphocytes (MTT test)

* $P < 0.05$: Significant difference in stressed and controls of female animals. \$ $P < 0.05$: Significant difference in stressed and controls of male animals. Food Deprivation (See) group (FD+See), Food Deprivation (Isolated) group (FD+Isolate), Food deprivation plus cage-mate change (See) group (FD+See+CC), Food Deprivation plus cage-mate change (Isolated) group (FD+Isolate+CC), and Cage-mate Change group (CC).

was observed, too ($P = 0.041$). Moreover, female subjects which just experienced unstable situation had a higher vital activity of lymphocytes in comparison with that in similar male group ($P = 0.03$). Food deprived male animals which experienced inequitable situation with or without cage-mate change had lower vital activity of lymphocytes as compared with that in similar female group (FD+See and FD+CC+See animals: $P < 0.001$); however, isolated male and female subjects which underwent food poverty with or without cage-mate change were not statistically different (FD+Isolated animals: $P = 0.42$, FD+CC+Isolated animals: $P = 0.12$).

Discussion

In the current study, we aimed to investigate the differences of immune responses of male and female animals in response to social stresses. To achieve our main goals, a model of chronic social stress was implemented on adult male and female mice to investigate the differences of macrophages and lymphocytes vital activity as well as proinflammatory cytokines' concentrations in association with social stresses and gender. The results obtained from macrophages and lymphocytes MTT showed that their vital activity increased in all stressed subjects, as compared to controls, and this increase was not the same in these experimental groups. Both female and male mice which were socially stressed had elevations in serum concentration of TNF- α , as compared to the control groups. On the other hand, immune response of female

mice was repressed more than that of the males after implementation of chronic social stress.

Profound effects of stressful events on immune system have widely been studied; however, most of these studies are related to physical stresses and not to social stresses [15]. It is believed that stress suppresses immune response and increases susceptibility to different diseases. However, immunity suppression caused by stress does not happen all the times and it seems that stress has bidirectional effects on immune system [16]. To our knowledge, it is unknown how different social stresses affect immune function. Chronic exposure to stressful conditions can be pathogenic and can suppress immune response, especially innate immunity [17].

Klein et al. (1992) have shown that in spite of increased level of corticosterone, there was no change in the response of lymphocytes and this reveals that increased level of corticosterone does not necessarily suppress immunity [18]. Some other studies also showed that social stresses have caused splenomegaly and changed lymphocytes function, as stress increases glucocorticoid resistance which in turn increases proinflammatory cytokines levels [19]. As mentioned, food deprivation and inequality as well as unstable social status increases vital activity of macrophages and lymphocytes in both females and males. Moreover, increase in the concentration of TNF- α was more obvious in all stressed groups than in controls. Glucocorticoids have anti-inflammatory effects. It is shown that social stresses suppress these effects and chronic exposure to these stresses decreases sensitivity of immune cells and causes inflammation, i.e. splenocytes of stressed mice had higher vital activity at the presence of ConA and produced higher amounts of proinflammatory cytokines [20, 21].

Our previous study reveals that social stress paradigm increases serum concentration of corticosterone compared to the controls (data was not published). Accordingly, it seems that implementation of these social stresses increases vital activity lymphocytes and macrophages due to increased resistance of glucocorticoids [17]. In this regard, increased level of proinflammatory cytokines in serum may increase glucocorticoids resistance which also may impede corticosterone to decrease these cytokine [22]. Thus, implementation of these social stressors may result in simultaneous increase in the serum levels of proinflammatory cytokines and corticosterone [23].

It is believed that gender differences play a prominent role in the incidence of different diseases. Although most of the research studies have shown females' lower sus-

ceptibility to infectious disease [8], some studies do not confirm this notion [24]. Gender differences in immune response can be related to differences in female and male sexual hormones. These hormones can directly affect lymphocytes and macrophages or indirectly affect HPA (Hypothalamic-pituitary-adrenal) axis [25].

In the present study, we observed that female mice displayed significantly more suppressed immune response compared with male mice following food deprivation and inequality. Thus females are more sensitive to adverse effects of social inequality. The reasons for these discrepancies are not clear, but the potential importance of gonadal hormones may be accounted for gender differences in immune response. Anyway, further studies are needed to investigate about these discrepancies between males and females. Consequently, it can be suggested that deprivation and social inequality as well as unstable social status affect immune system and may involve in the activation of HPA axis in mice. Moreover, the present study demonstrated that females are more prone to adverse effects of social stresses.

Ethical Considerations

Compliance with ethical guidelines

The described experimental protocols and procedures in the current study were approved by Institutional Animal Care and Use Committee (IACUC) in Shahed University of Medical Sciences in Iran and complied with the Guide for the Care of Use of Laboratory Animals' published by the US National Institute of Health. Moreover, we followed the Guidelines on Ethical Standards to reduce animal suffering and to use only the necessary number of animals to reach reliable data.

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Conflict of interest

The authors declare no conflict of interest. Authors' contributions is as follow: conception, design, data acquisition, interpretation and critical revision preformed by Marjan Aghajani; conception, design, data analysis, and manuscript drafting by Mohammad Reza Vaez Mahdavi; data analysis and drafting by Mohsen Khalili Najafabadi and Tooba Ghazanfar; and data collection and coordination by Ehsan Kazemi Moghaddam. All the authors read and approved the final manuscript.

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