

MAST CELL DEFICIENT W/W^v MICE LACK STRESS-INDUCED INCREASE IN SERUM IL-6 LEVELS, AS WELL AS IN PERIPHERAL CRH AND VASCULAR PERMEABILITY, A MODEL OF RHEUMATOID ARTHRITIS

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Corticotropin releasing hormone (CRH) and interleukin-6 (IL-6) are implicated in inflammatory diseases triggered by stress. Acute restraint stress increases serum IL-6 in the blood, but its source is not known. Our current study was carried out in order to determine the contribution of mast cells to stress-induced IL-6 release and to investigate skin CRH and vascular permeability in mice. W/W^v mast cell deficient and their wild type control +/+ mice were stressed in a plexiglass restraint chamber for 60 or 120 min. Serum corticosterone and IL-6 levels were measured. Other mice were injected with 99-Tc gluceptate (⁹⁹Tc) and its extravastion, indicating vascular permeability, was determined along with CRH levels in the skin and knee joints. Acute stress increased serum IL-6 in mice, but was greatly inhibited in W/W^v mast cell deficient mice. Vascular permeability to ⁹⁹Tc, as well as local CRH levels, were also increased by stress, but not in W/W^v mice. Findings from our current study suggest a link between mast cells and stress-related skin and joint inflammation and may explain initial events in psoriatic and rheumatoid arthritis.

Acute psychological stress is implicated in the pathophysiology of numerous neuroinflammatory syndromes (1). Stress activates the hypothalamic-pituitary-adrenal (HPA) axis through the release of corticotropin-releasing hormone (CRH), which regulates the stress response (1). Unlike its centrally mediated immunosuppressive actions through the release of glucocorticoids, peripherally synthesized CRH has proinflammatory effects (1, 2); some of these may be contributed by the CRH related peptide urocortin (Ucn) (3, 4).

IL-6 is a pleiotropic cytokine with multiple functions, such as stimulatory effects on lymphocytes and activation of the HPA axis independent of CRH (5). Mast cells are involved

in allergic reactions (6) but also in inflammatory conditions that are precipitated or exacerbated by stress because of their ability to secrete many proinflammatory molecules, including IL-6 (7, 8). High serum IL-6 has been reported in rheumatoid arthritis (9, 10), which is characterized by increased vascular permeability and inflammation in the joints. Moreover, mast cells have been implicated in arthritis (11-14).

Here, we report that acute stress increases serum IL-6 in mice, as well as local CRH and vascular permeability, all of which are greatly inhibited in W/W^v mast cell deficient mice.

Key words: arthritis, CRH, IL-6, mast cells, skin, stress

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MATERIALS AND METHODS

Restraint stress

W/W^v mast cell deficient mice (WBB6F1/J-W/W^v) and their wild type control +/+ mice (Jackson Laboratories, Bar Harbor, ME) were obtained 8 to 11-week-old for at least one week before use. They were allowed food and water *ad libitum* and were maintained in a 14:10 hr dark-light cycle. Mice were kept in the animal facility. Each mouse was brought into an isolated procedure room inside the animal facility, between 9-11 am (to avoid any effect of diurnal rhythms) for 30 min every day for 3 days in order to reduce the stress of handling. During the day of the experiment, each control mouse was allowed to stay in its cage for the designated period of time on a bench top at room temperature in the procedure room. At a different time, the experimental mouse was placed in a clear, plexiglass restraint chamber (Harvard Apparatus, Cambridge, MA) for the designated times. No mouse was ever in close proximity while another was stressed or dissected. At the end of the experiment, each mouse was killed by asphyxiation over CO₂ vapor and decapitated. Blood was collected for corticosterone and IL-6 measurements. These procedures were approved by the University's Animal Use Committee.

Extravasation of ⁹⁹Tc-glucaptate

Vascular permeability in peripheral tissues was measured as extravasation of intravenously administered ⁹⁹Tc-glucaptate (⁹⁹Tc) in a manner similar to that previously described for assessing the permeability of blood-brain barrier (BBB) in the rat (15). Briefly, the Technetium-D-glycero-D-gluco heptonate complex was prepared by mixing the content of one vial of the Technetium Glucaptate kit (DRAXIMAGE, Inc., Kirkland, Quebec, Canada) with 5 mCi ^{99m}Tc (Du Pont, Billerica, MA) in 10 ml of 0.9 % NaCl. While awake, each animal was injected with 75 μCi ⁹⁹Tc-glucaptate in 0.15 ml volume through the tail vein. Mice to be stressed were placed in plexiglass restrainers (Harvard Apparatus, Cambridge, MA) 10 minutes following the injection of ⁹⁹Tc. Control mice were kept in a different room until the time of perfusion. Mice were stressed and were then immediately anesthetized with an intraperitoneal injection of a mixture (0.15 ml) ketamine (80 mg/kg) and xylazine (10 mg/kg). Before perfusion, blood for serum corticosterone IL-6 measurements was drawn through a 27.5G needle inserted in the right ventricle of the heart. The circulation was then flushed with 1 ml of 0.9% NaCl (EM grade, Polysciences Inc., Warrington, PA) through a blunted needle inserted into the left ventricle of the heart. Following the perfusion, mice were decapitated, tissues were removed and were counted in Gamma Well Counter for one minute. Results are expressed as counts/g tissue.

Corticosterone measurements

Blood samples were collected from neck vessels after decapitation and were allowed to clot overnight at 2 - 8 °C before centrifuging for 20 min at approximately 2000 x g. The serum was collected and subjected to corticosterone radioimmunoassay using the Corticosterone ¹²⁵I-RIA kit (ICN, Costa Mesa, CA).

IL-6 measurements

Mouse serum and plasma samples were both assayed for IL-6 with the quantitative sandwich enzyme immunoassay technique (Quantikine M Murine, Mouse IL-6, R&D Systems Inc., Minneapolis, MN). No significant difference was found between values obtained from serum or plasma; serum samples were used thereafter.

ELISA for CRH and Ucn

CRH was measured in the supernatant or the tissue pellet after the respective tissue-sample was lysed in 0.5% Triton X-100 containing Aprotinin and 100 mM PMSF in PBS with sonication 3 times x 15 sec each at full power (Model No 161, Dimco gray Co., Dayton, OH) at 4 °C. The supernatant was then removed after centrifugation at 30,000 x g for 30 min at 4 °C and assayed for CRH and Ucn by ELISA (Phoenix Pharmaceuticals, Belmont, CA).

Statistical analysis

One way ANOVA run by SigmaStat was used to compare the differences between the stressed and unstressed group, as well as among different treatment groups. Results are presented as mean ± standard deviation. A P value of less than 0.05 was considered statistically significant.

RESULTS

Effect of acute stress on serum corticosterone levels

Serum corticosterone levels in all control animals were less than 100 ng/ml (Fig. 1A), but increased similarly by 60 and 120 min of stress in +/+ mice (325.7 ± 43.1 ng/ml and 410.0, respectively, Fig. 1A, *p* < 0.05) and in W/W^v mast cell deficient mice (328.1 ± 29.7 ng/ml and 389.9 ± 144.4, respectively, Fig. 1B, *p* < 0.05).

Effect of acute stress on IL-6 release in normal +/+ and W/W^v mice

Acute stress increased IL-6 release in +/+ mice in a time-dependent fashion from 0 pg/ml to

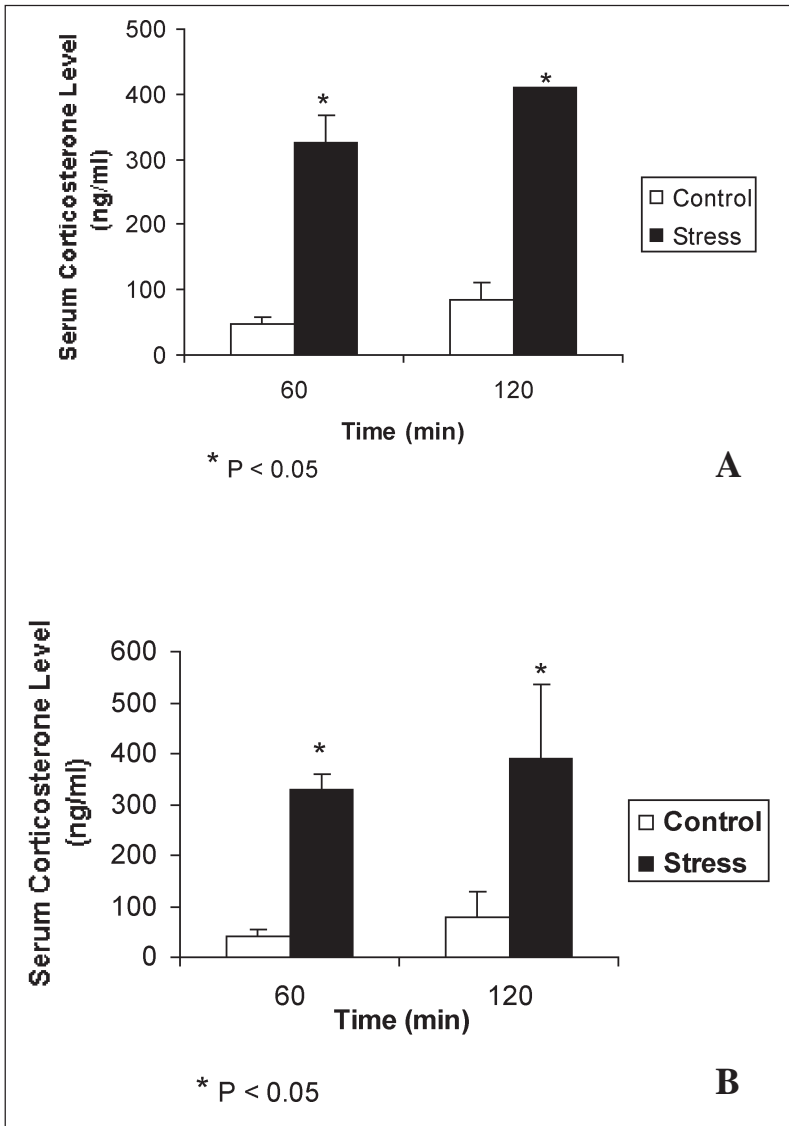


Fig. 1. Comparison of serum corticosterone levels in (A) +/+ (60 min, n=3; 120 min, n=2); and (B) W/W^v mast cell deficient (n=3).

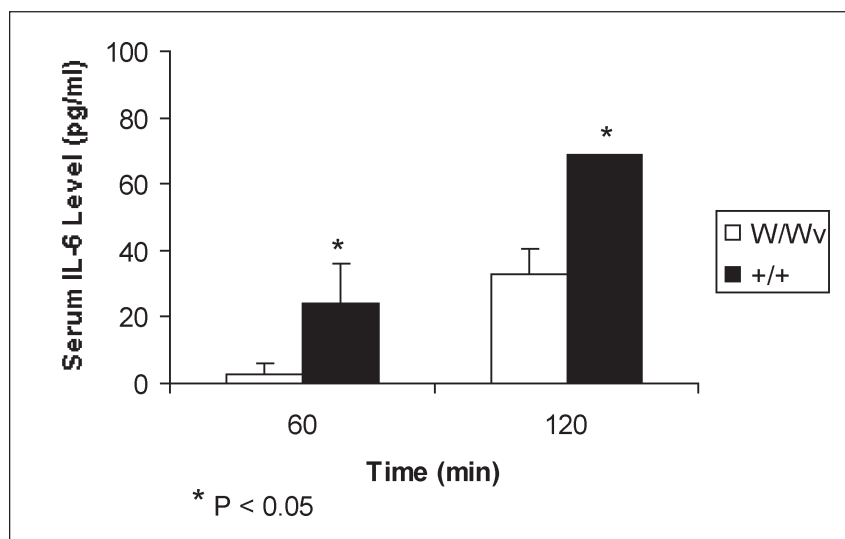


Fig. 2. Comparison of serum IL-6 levels in +/+ and W/W^v mast cell deficient mice after 60 (n=3) and after 120 of stress (+/+, n=2; W/W^v, n=3).

Tab. I. Effect of acute stress on mouse skin ⁹⁹Tc extravasation and CRH Levels

Conditions	n	⁹⁹ Tc extravasation	CRH ⁺
C57BL - control	8	588,580±84,912	0.06
C57BL+ stress	11	2,700,327±391,322	0.109#
W/W ^v + stress	5	492,606±67,937	0.008

* counts/g tissue

+ pg/ml

p<0.05 compared to either control or W/W^v mice

23.9 ± 11.9 pg/ml (p < 0.05) after 60 min, and to 68.6 pg/ml (p < 0.05) after 120 min (Fig. 1B). The average basal serum IL-6 levels in W/W^v mice were similar to those in their wild type control +/+ mice (Fig. 1B). The surprising finding was that after 60 min of restraint, serum IL-6 in W/W^v mice was only 2.8 ± 2.9 pg/ml, as compared to 23.9 ± 11.9 pg/ml in +/+ mice (Fig. 1B, p < 0.05). Although serum IL-6 in W/W^v mice increased to 32.7 ± 8.0 pg/ml after 120 min of stress, this amount was much less than that seen in +/+ mice that increased to about 70.0 pg/ml (n=2).

Vascular permeability

Restraint stress for 120 min increased vascular permeability in the skin from 588,580±84,912 count/g tissue in controls to 2,700,927±391,322 counts/g tissue (Tab. I). However, the degree of ⁹⁹Tc extravasation in W/W^v mast cell deficient mice was less than control at 492,606±67,937 counts/g tissue (Tab. I). Similar results were obtained when knee joint tissue was used instead of skin (results not shown).

Tissue CRH levels

CRH was increased in skin from 0.006 pg/ml to 0.109 pg/ml following 120 min of stress (n=11, p<0.05). Instead, the CRH level in W/W^v mice remained at control levels (0.008, n=5). Similar results were obtained when knee joint tissue was analyzed before and after stress (results not shown).

DISCUSSION

Our data show that 60 min of stress did not induce any IL-6 release in W/W^v mice, indicating that this process is mast cell-dependent. The lack of serum IL-6 increase could not be due to any

difference in the activation of the HPA axis since the serum levels of corticosterone, were equivalent between the +/+ and W/W^v mast cell deficient mice. Other studies using W/W^v mast cell deficient mice had shown that their immune system is otherwise intact, thus excluding the possibility of defective monocyte/macrophage function that may explain our results (16, 17). Mast cells are well known for their involvement in allergic and "late phase" reactions by virtue of their release of different prestored or newly synthesized mediators (18). It is also evident that mast cells also release many cytokines, including IL-6 (19). As a result, the mast cell is now considered as an important effector cell in neuroinflammatory conditions, (7) with a versatile role not suspected previously (20). Although W/W^v mice increased serum IL-6 level after 120 min of stress, this level was much lower than that observed in their wild type controls, consequently mast cells may be the predominant players at the early phase of the response to acute stress; this IL-6 release could derive from other sources, such as monocytes/ macrophages.

We had previously reported that acute restraint stress increases vascular permeability in rat dura (15) and skin (21), in the latter of which increased CRH was also documented. This is the first instance that acute stress-induced vascular permeability and CRH was shown to be absent in mast cell deficient mice.

Increasing evidence indicates that mast cells are involved in the pathophysiology of arthritis (11-14, 22-31). This premise recently became indisputable since arthritis could not develop in mast cell deficient mice (32). In fact, stress activates mast cells (8, 33) and worsens arthritis (34-36). Moreover, CRH and its structural analogue urocortin have been reported to be increased in the joints of rheumatoid arthritis patients (37-42). In this context, it is important that CRH receptors have been identified on mast cells from rheumatoid arthritis joints (38) and CRH (43), as well as urocortin (44), have been shown to activate mast cells that express mRNA for CRH receptors. Moreover, high IL-6 has been documented in the serum (9, 10, 45) and in peripheral blood mononuclear cells (46) in juvenile arthritis. Our findings, therefore, provide additional evidence for the importance of mast cell activation in skin and joint inflammation, as may occur in stress-induced psoriatic and rheumatoid arthritis.

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