

## PROCEEDING

# IL-18 ; a cytokine translates a stress into medical science

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**Abstract :** Psychological/physical stresses have been reported to exacerbate auto-immune and inflammatory diseases. To clarify a mechanism by which non-inflammatory stresses disrupt host defenses, responses to immobilization stress in mice were investigated, focusing on the role of a multifunctional cytokine, interleukin-18 (IL-18). In the adrenal cortex, the stress induced IL-18 precursor proteins (pro-IL-18) via ACTH and a superoxide-mediated caspase-1 activation pathway, resulting in conversion of pro-IL-18 to the mature form which was released into plasma. Inhibitors of caspase-1, reactive oxygen species and P38 MAPK prevented stress-induced accumulation of plasma IL-18. These inhibitors also blocked stress-induced IL-6 expression. This, together with the observation that IL-6 was not induced in stressed-IL-18 deficient mice, showed that IL-6 induction by stress is dependent on IL-18. In stressed organisms, IL-18 may influence pathological and physiological processes. Controlling the caspase-1 activating pathway to suppress IL-18 levels may provide preventative means against stress-related disruption of host defenses. *J. Med. Invest.* 52 Suppl. : 236-239, November, 2005

**Keywords :** stress, interleukin (IL), adrenocorticotrophic hormone (ACTH), hypothalamus-pituitary-adrenal (HPA) axis

## BACKGROUND

Psychological/physical stresses have been known empirically to cause relapse of auto-immune and inflammatory diseases. However, molecular biological basis of the linkage between psychological/physical stressors and inflammatory diseases is not well clarified. Interleukin-18 (IL-18) was originally reported as interferon-gamma inducing factor (1), and has been demonstrated to have several biological activities, including production of Th2 cytokines (2, 3), and

induction of Fas ligand, elevation of cytolytic activity of T cells (4).

It has been reported that IL-18 mRNA is expressed in response to ACTH in the adrenal gland (5). Sugama *et al.* have reported differential IL-18 promoter usage in the adrenal gland and immune cells with showing adrenal gland specific expression of IL-18 mRNA by ACTH (6). They have shown pro-IL-18 protein accumulation in the adrenal gland but not bioactive matured IL-18. It is unclear whether psychological/physical stresses are responsible for high levels of mature IL-18 in plasma. In this study, the expression of IL-18 in immobilization-stressed mice was investigated, focusing on the processing of pro-IL-18 and the release of the mature form into plasma. In addition, involvement of IL-18 in the regulation of another stress-related cytokine, IL-6(7,8), was examined.

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**METHODS**

Responses to immobilization stress in mice were investigated focusing on the role of a multifunctional cytokine, interleukin-18 (IL-18) .

For the immobilization stress, mice were placed in a restrainer for 3 or hours and cytokines levels were analyzed. Forms of IL-18 proteins in the adrenal gland and plasma were investigated by Western Immunoblot and immunoprecipitation. The adrenal glands were formalin-fixed, paraffin-embedded, cut to 4 μm sections, and were studied with immunohistochemistry for caspase-1 and IL-18. Stress effect on renal dysfunction of model mice for inflammatory lupus-like nephritis was studied. Plasma levels of IL-18 in patients with mental disorders before and after a newly designed psychotherapy were preliminarily studied.

**RESULTS**

Stress elevated IL-18 levels both in plasma and in the adrenal gland (Figure 1A). IL-18 proteins in plasma were 18 KD bioactive form and 24 KD precursor proteins were in the adrenal gland (Figure1B). Inhibition of ACTH suppressed both levels and ACTH induced pro-IL-18 accumulation in the adrenal gland but not the IL-18 rise in plasma (data not shown) . Accumulation of IL-18 in plasma was suppressed by inhibition of caspase-1, which is a putative converting

enzyme for pro-IL-18 into mature form (Figure 2A and B). The caspase-1 activity in the adrenal gland (Figure 3A) and IL-18 rise (Figure 3B) were suppressed by superoxide dismutase (SOD), suggesting that caspase-1 pathway to cause IL-18 secretion is mediated by superoxide anions. Stress-induction of interleukin-6, another stress-related cytokine, was suppressed by those inhibitors (Figure 4A) and in IL-18 knock-out mice (Figure 4 B) , showing that IL-6 rise by stress is mediated by stress-induced IL-18. Preliminary studies showed that a stress progresses the renal dysfunction in lupus-like nephritis model mice and that elevated levels of IL-18 in plasma in psychiatric patients were down-regulated after the psychotherapy.

**DISCUSSION**

In the present study, it is indicated that a stress activates the hypothalamus-pituitary-adrenal axis to induce ACTH, which induces pro-IL-18 in the adrenal cortex. At the same time, stress generates superoxide anions to activate caspase-1. Caspase-1, thus activated, converts pro-IL-18 in the adrenal cortex into the mature form, which is secreted into plasma. IL-18 and IL-6, which are elevated systemically, may lead disruption of host-defenses possibly resulting in the exacerbation, relapse, and potentiation of inflammatory and auto-immune diseases. IL-18 in plasma may be available as indicator for stress impacts on body. Controlling the caspase-1 pathway to suppress IL-

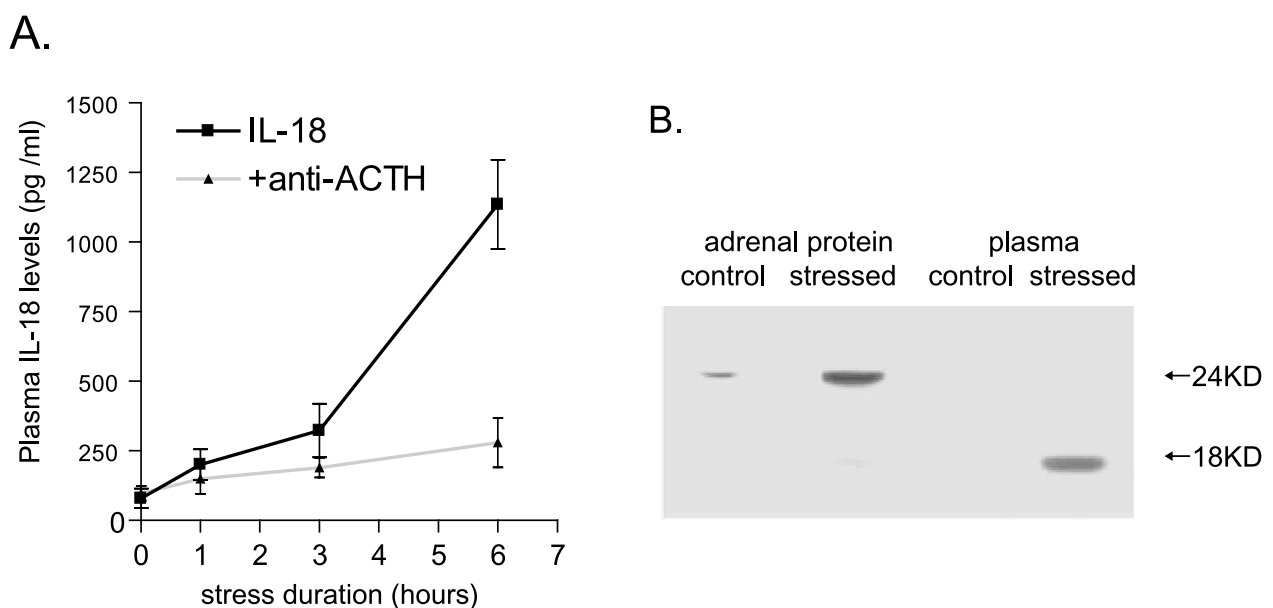


Figure 1. IL-18 level were up-regulated in plasma by immobilization stress. (A) effect of immobilization stress on IL-18 levels in plasma. Anti-ACTH antiserum inhibited the up-regulations. (B) Immunoblotting for IL-18 proteins in the adrenal gland and in plasma. (Data are presented in means ±S.D. n = 12)

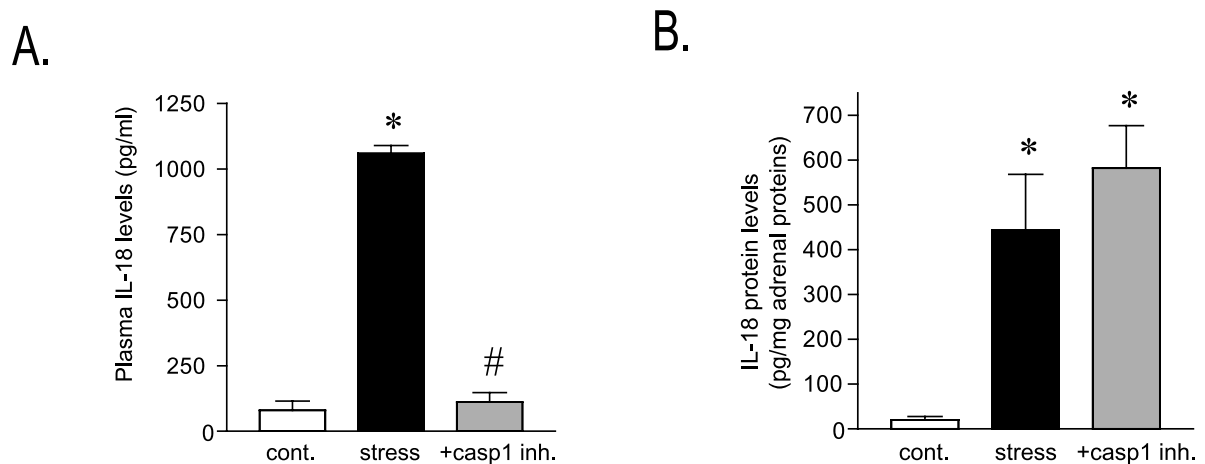


Figure 2. The IL-18 rise in plasma is mediated by caspase-1.

Effect of caspase-1 inhibitor, YVAD-CHO, on IL-18 levels in plasma (A) and in the adrenal gland (B) after stress. (Data are presented in means  $\pm$  S.D. \*;  $p < 0.001$ , to control, #;  $p < 0.01$  to stressed.  $n = 6-9$ )

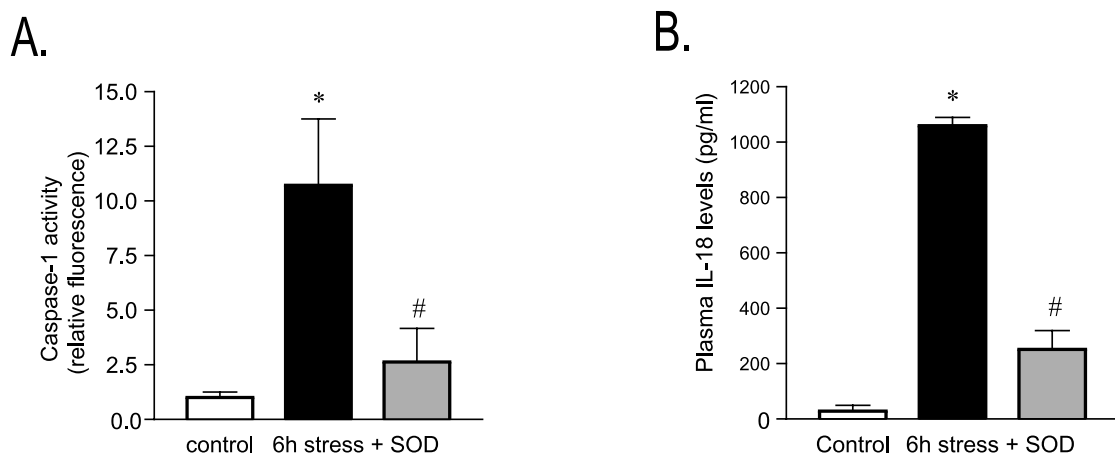


Figure 3. Superoxide mediates the stress-induced IL-18 up-regulation in plasma.

(A) Effect of superoxide dismutase (SOD) on caspase-1 activity in the adrenal gland. (B) Effect of SOD on IL-18 levels in plasma after the stress. (Data are presented in means  $\pm$  S.D. \*;  $p < 0.001$ , to control, #;  $p < 0.01$  to stressed.  $n = 6-9$ )

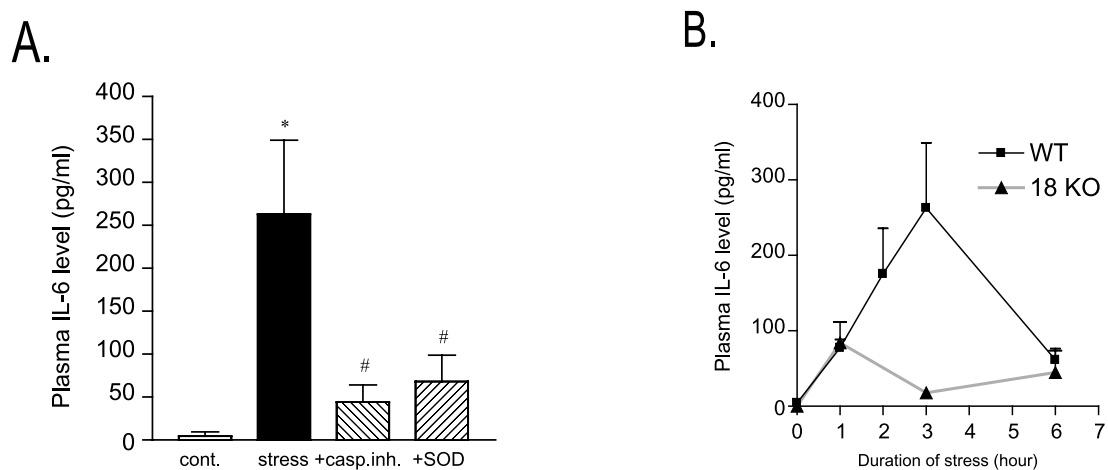


Figure 4. Stress-induction of interleukin-6 is IL-18 dependent.

(A) Effect of caspase-1 inhibitor or SOD on IL-6 levels after stress. (B) Stress-induction of IL-6 in IL-18 deficient mice. (Data are presented in means  $\pm$  S.D. \*;  $p < 0.001$ , to control, #;  $p < 0.01$  to stressed.  $n = 6$ )

18 levels may provide preventative means against stress-related disruption of host defenses.

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