Stress
The International Journal on the Biology of Stress

ISSN: 1025-3890 (Print) 1607-8888 (Online) Journal homepage: informahealthcare.com/journals/ists20

Suppression of Restraint-induced Plasma Cytokines in Mice Pretreated With LPS

E. Merlot, E. Moze, R. Dantzer & P.J. Neveu

To cite this article: E. Merlot, E. Moze, R. Dantzer & P.J. Neveu (2002) Suppression of Restraint-induced Plasma Cytokines in Mice Pretreated With LPS, Stress, 5:2, 131-135, DOI: 10.1080/10253890290027921

To link to this article: https://doi.org/10.1080/10253890290027921

Published online: 07 Jul 2009.

Stress, 2002 Vol. 5 (2), pp. 131–135

Taylor & Francis
healthsciences

Suppression of Restraint-induced Plasma Cytokines in Mice Pretreated With LPS

E. MERLOT, E. MOZE, R. DANTZER and P.J. NEVEU*

INSERM U394, "Neurobiologie Intégrative", Institut François Magendie, rue Camille Saint-Saëns, 33077 Bordeaux, France

(Received 28 May 2001; Revised 28 August 2001; In final form 21 September 2001)

A previous exposure to an inflammatory reaction is known to increase or decrease the activation of the hypothalamic–pituitary–adrenal (HPA) axis induced by a psychological/physical stress. Beside HPA activation, the non- specific responses to these two kinds of stresses involve the immune system including the production of cytokines. Therefore, they could interfere in cytokine production. In order to test this hypothesis, female C3H mice were first injected i.p. with 5 μg of lipopolysaccharide (LPS) or not (C). Eight days later, half of them were submitted to a 4 h-restraint (R) applied during the nocturnal part of the dark–light cycle and sacrificed immediately after (groups LPS-R and C-R), while the non restrained mice stayed in their home cages (groups LPS-C and C-C). Restraint induced an increase in corticosterone production that was not altered by the previous administration of LPS. It had no effect on mitogen-induced lymphoproliferation. However, restraint induced an augmentation of plasma concentrations of interleukin (IL)-1 and IL-6 that was not observed in animals previously exposed to LPS. These results show that restraint, which represents a psychological stress is able to induce the production of plasma cytokines in mice. They also suggest that LPS may induce a long lasting suppression of plasma cytokines through mechanisms that remain to be elucidated.

Keywords: Lipopolysaccharide; Restraint; Desensitization; Interleukin-1β; Interleukin-6

INTRODUCTION

Physical/psychological and immune stressors induce similar non-specific responses which include the activation of the hypothalamic–pituitary–adrenal (HPA) axis, the stimulation of the sympathetic nervous system, alterations of brain metabolism and immune modifications including the production of cytokines (for review see Besedovsky and Del Rey, 1996). Therefore, it may be hypothesized that non-specific responses to a stressor may alter the responses to a subsequent stressor of a different nature. Indeed, a prior immobilization was shown to decrease the production of ACTH induced by a second physical/psychological (Marti *et al.*, 1999) or inflammatory stress (Mekaouche *et al.*, 1994). Likewise, inflammation induced by an injection of IL-1 or LPS can increase or decrease the ACTH response to a subsequent stress or to a second injection of IL-1 or LPS (Weidenfeld and Yirmiya, 1996; Schmidt *et al.*, 1995; Nagano *et al.*, 1999). These seemingly contradictory data may result from the nature of the stressors used and from the time interval between stressors. Sensitization could be observed after a mild stress applied for a short period of time and when the interval of time between stressors is rather long. By contrast, desensitization could be observed after a strong stress and when the interval of time is short (Andrès *et al.*, 1999). The mechanisms involved in the phenomena of sensitization and desensitization were mainly studied at the level of HPA axis activity. Desensitization was found to be not directly related to the plasma concentration of corticosterone, but appeared to depend on a glucocorticoid status which remains to be defined (Marti *et al.*, 1999). By contrast, sensitization was observed only in the presence of a basal level of corticosterone, suggesting that mineralo-corticoid receptors may be involved (Andrès *et al.*, 1999). Sensitization induced by IL-1 was shown to be due to a long-lasting increase in production and storage of vasopressin in the hypothalamic CRH neurons (Schmidt *et al.*, 1995).

The phenomena of sensitization–desensitization also involve the production of cytokines. The production of IL-1 induced by LPS may be increased by a previous stress (Mekaouche *et al.*, 1994) or depressed by a pretreatment with LPS (Nagano *et al.*, 1999). However, the

*Corresponding author. Tel.: +33-5-57-57-37-10. Fax: +33-5-56-98-90-29. E-mail: pierre.neveu@bordeaux.inserm.fr

ISSN 1025-3890 print/ISSN 1067-8888 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10253890290027921

mechanisms involved in the regulation of the production of cytokines remain unknown.

In the present work, we determined the influence of a pretreatment with LPS on immune reactivity and especially on the production of cytokines in response to a restraint stress.

MATERIALS AND METHODS

Animals

Female mice of the C3H/OuJiCo strain (IFFA Credo, l'Arbresle, France) were used. They were 4 weeks old on arrival and were housed in groups of five in a temperature-controlled room (24 ± 1°C) with a 12 h dark–12 h light cycle (lights on 16.30–4.30 h). They were maintained on an *ad libitum* food and water regimen.

Treatments

As the corticoid stress response (Neveu and Moya, 1997) and the neurochemical, neuroendocrine and immune responses to LPS (Delrue *et al.*, 1994) depend on lateralization, the animals were tested for their paw preference before treatment. During a 3-week period, animals were tested for paw preference according to a method previously described (Betancur *et al.*, 1991). One week after behavioral tests, half of the mice were first injected intra-peritoneally with 5 μg of lipopolysaccharide (LPS) from *Escherichia coli* (0127:B8, Sigma) dissolved in 0.5 ml of saline. Other mice did not receive any treatment (C). Eight days later, half of the mice were restrained (R). Restraint consisted of placing mice into a 50 ml aerated culture tube for 4 h at the end of the night, just before they were killed. Control mice were left in their home cage. Therefore, we used four experimental groups of 24 mice named: C-C, LPS-C, C-R, and LPS-R. In previous work (Li *et al.*, 2000), we had shown that restraint applied for a short period of time (1–2 h) during the inactive phase had no effect on plasma cytokines. We therefore used a longer duration of restraint and applied it during the active period instead of the inactive one in order to increase the potential immune effects of restraint. Mice were killed by decapitation and trunk blood was collected in EDTA-coated tubes. After centrifugation, plasma was stored at −20°C until assayed. The hypothalamus was rapidly removed from the brains and kept at −80°C until cytokine assay. Spleens were removed in sterile conditions to assess mitogen-induced lymphoproliferation.

Plasma Corticosterone

Plasma corticosterone concentration was measured following ethanol extraction by radiocompetitive binding assay using rhesus monkey transcortin, [^{3}H]corticosterone as the tracer, and dextran-coated charcoal as the adsorbent of free radioactivity (Liège *et al.*, 2000). Inter- and intra-assay coefficients of variation were 14 and 7%, respectively.

Lymphocyte Proliferation

Spleen cells were dissociated in RPMI 1640 culture medium (Gibco, Glasgow, UK) and after three washes adjusted to 2×10^6 cells/ml in medium containing 5% heat-inactivated fetal calf serum and 1% antibiotic antimycotic solution (Gibco). Triplicates of cell suspensions (2×10^5 cells/well) were cultivated in microtest-2 plates (Nunc, Roskilde, Denmark) for 4 days in a humidified atmosphere of 5% CO_2, in the absence or presence of concanavalin A (ConA) (1 or 2 μg/ml). Twenty hours before the end of culture, 0.5 μCi/well of [^{3}H]thymidine (specific activity 5 Ci/mmol, CEA, France) diluted in 50 μl was added. Cells were collected on fiberglass strips using a multiple harvester, and radioactivity determined in a liquid scintillation counter. The results were expressed as stimulation indices, calculated for each animal and each concentration of mitogen as follows: SI=mean cpm of stimulated culture/mean cpm of unstimulated culture.

IL-1 and IL-6 Determinations

IL-1 and IL-6 in the plasma and the hypothalamus were measured using ELISA kits from R&D System (Minneapolis, MN). The hypothalamus was placed in 300 μl of a protease inhibitor cocktail including: 4-(3-aminoethyl)-benzenesulfonyl fluoride hydrochlorine (AEBSF 2 mM), amino-*n*-caproic acid (100 mM), benzamidine (5 mM), Na_2EDTA (10 mM), ethylmaleimide (5 mM) and iodo acetic acid (5 mM) (Sigma, St Louis, MO). Tissues were ultrasonicated for 10 s at the setting 30. Sonicated samples were centrifuged at 10,000 rpm for 10 min. The protein content was determined by the method of Bradford (1976). The assay kit sensitivity was 3 pg/ml. The intra- and inter-assay precisions varied between 2.2 and 6.1% for IL-1 and between 3.4 and 8% for IL-6, depending on the concentration of cytokines.

Statistical Analysis

All data were analyzed by analysis of covariance (using the GLM procedure of SAS) taking the lateralization score as a covariate, and taking into account all the possible interactions. When an interaction or a variable had no effect, it was removed from the model. Because no effect of lateralization was seen for all the parameters measured, the final model only took into account the LPS injection, the restraint stress and their interaction, except in the case of IL-1. The resulting two-way ANOVAs were followed by a least square means *post-hoc* analysis. Plasma concentrations of corticosterone and IL-1 were analyzed after square root transformation, and plasma IL-6 after $\log(x+1)$ transformation. In the case of IL-1, data were

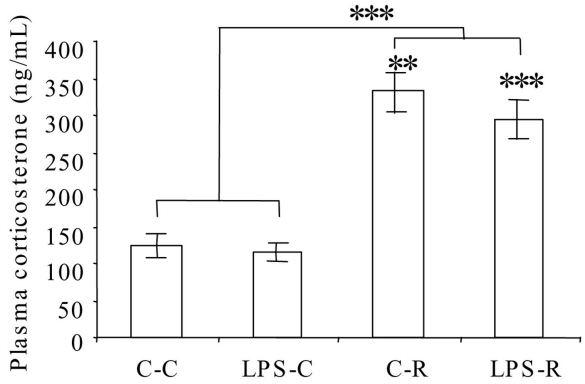

FIGURE 1 Plasma concentrations of corticosterone (ng/ml, mean ± sem) in control (C-C, $n = 24$), LPS-pretreated (LPS-C, $n = 24$), restrained (C-R, $n = 23$) and restrained and LPS-pretreated (LPS-R, $n = 22$) groups. ***$P < 0.001$.

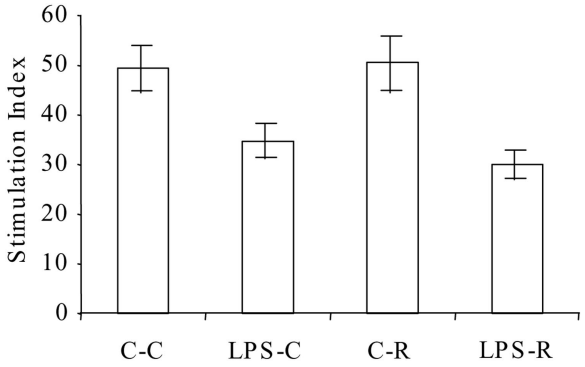

FIGURE 2 Splenocyte proliferation induced by 2 μg/ml of ConA in control ($n = 21$), LPS-pretreated ($n = 22$), restrained ($n = 23$) and restrained and LPS-pretreated ($n = 24$) groups. The results are expressed as stimulation index (mean ± sem).

then analyzed independently for left- and right-pawed groups (lateralization score <21 and >30, respectively).

RESULTS

Plasma Concentrations of Corticosterone

Restraint induced a clear increase in plasma corticosterone concentration ($F(1,92)=59.75$, $P < 0.001$, Fig. 1). However, no LPS × restraint interaction ($P > 0.1$) or LPS effect ($P > 0.1$) were found.

Concanavalin A-induced Lymphoproliferation

For lymphocyte proliferation induced by 2 μg of concanavalin A, there was no LPS × restraint interaction ($P > 0.1$) and no effect of restraint ($P > 0.1$), but an effect of LPS ($F(1,88)=4.58$, $P < 0.05$, Fig. 2). Indeed, LPS decreased lymphoproliferation ($P < 0.001$). With 1 μg ConA, a similar pattern of lymphocyte reactivity was observed, but the differences did not reach statistical significance (data not shown).

Interleukin (IL)-1 and IL-6 Determinations

For plasma IL-1, there was a restraint × LPS interaction just below statistical significance ($F(1,75)=3.76$, $P=0.06$), a LPS effect ($P < 0.05$) and a restraint effect ($P < 0.001$). Plasma concentrations of IL-1 were higher in the C-R ($P < 0.001$) and in the LPS-R groups ($P < 0.05$) than in the C-C group. These effects were due to the right-pawed group, as shown in Fig. 3, where the interaction LPS × restraint was significant ($F(1,24)=8.1$, $P < 0.01$). Furthermore, plasma concentrations of IL-1 in the LPS-R right-pawed group were lower than those observed in the C-R group ($P < 0.01$). In the left-pawed group, the interaction between LPS and restraint was not significant ($P > 0.1$). For plasma concentrations of IL-6, no restraint × LPS interaction ($P > 0.1$) or LPS effect was revealed ($P > 0.1$), but there was a restraint effect ($F(1,93)=7.92$, $P < 0.01$). The C-R group exhibited higher plasma concentrations of IL-6 than controls ($P < 0.05$).

The contents of IL-1 and IL-6 were also determined in the hypothalamus. No differences among the experimental groups were observed. IL-6 concentrations, expressed as pg/mg protein, were 11.4 ± 0.8, 12.3 ± 0.9, 12.4 ± 1.4 and 13.3 ± 0.7 in the C-C, C-R, LPS-C and LPS-R

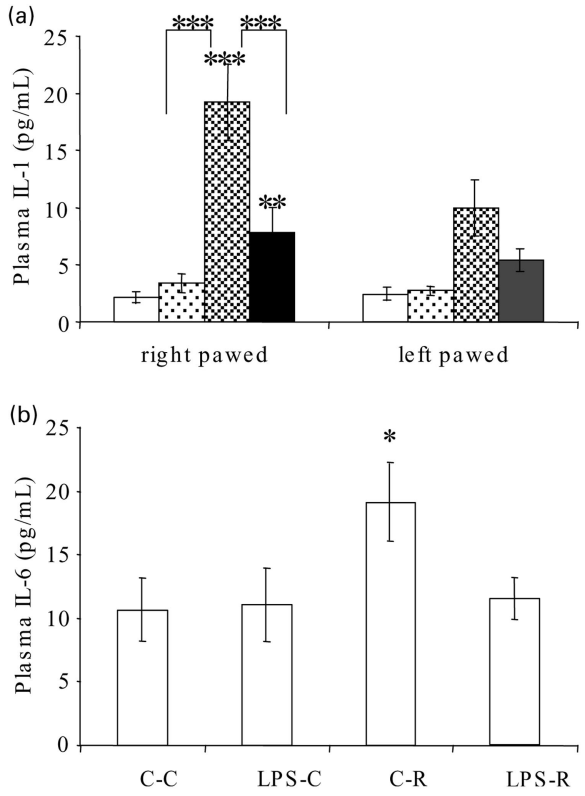

FIGURE 3 A: Plasma concentrations of IL-1 (pg/ml, mean ± sem) in control (□. $n = 19$), LPS-pretreated (▤, $n = 19$), restrained (▧, $n = 18$) and restrained and LPS-pretreated (■, $n = 20$) groups. B: Plasma concentrations of IL-6 (pg/ml, mean ± sem) in control ($n = 24$), LPS-pretreated ($n = 24$), restrained ($n = 24$) and restrained and LPS-pretreated ($n = 22$) groups. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

groups, respectively. Concentrations of IL-1 were below the detection threshold of the ELISA used.

DISCUSSION

The possible interaction between LPS treatment and restraint was studied in female C3H mice. Animals were first injected with 5 μg of LPS, a dose with early effects on the immune and neuroendocrine systems that are well known (Delrue *et al.*, 1994). Eight days after the administration of LPS, mice were submitted to a restraint stress lasting 4 h during the active phase. The main results of the present work were that: (1) restraint was able to induce the production of plasma IL-1 and IL-6; and (2) these increased levels of cytokines were reduced in restrained mice when pretreated with LPS.

Most of the interactions between immune and non-immune stimulations that have been already published concern the activity of the HPA axis and especially the plasma concentration of ACTH (Mekaouche *et al.*, 1994; Schmidt *et al.*, 1995; Weidenfeld and Yirmiya, 1996). In the present experiment, there was no interaction between administration of LPS and restraint for the production of corticosterone: plasma levels of corticosterone were increased after restraint, whether or not the animals were pretreated with LPS. Similar results were obtained when measuring plasma levels of ACTH (data not shown).

As shown in the C-R group, a 4 h-restraint stress did not modify lymphocyte proliferation. A briefer restraint (15 min) has been shown to induce a depression of ConA-induced proliferation of lymphocytes taken 45 min after the end of the stress (Neveu and Moya, 1997). A long lasting restraint (16–18 h) was also associated with a depression of mitogenesis (Sheridan *et al.*, 1991). The observation of a slight depression of mitogenesis after 15 min of restraint, but not after 1 (Neveu and Moya, 1997), 2 (Li *et al.*, 2000) or 4 h (present experiments) suggests that restraint may induce a transient depression of mitogenesis. Then, after a lag period, increased duration of restraint would lead to a long-lasting immunosuppression. The transient depression of mitogenesis may relate to recirculation of lymphocytes as previously described during stress responses (Dhabbar *et al.*, 1995). We have previously shown that the administration of the same dose of LPS induced a depression of mitogenesis 2 and 4 h later (Delrue *et al.*, 1994). The present results show that depression of lymphoproliferation persists for a long time, whereas neuroendocrine (see above) and behavioral effects (Bluthé *et al.*, 1997) of LPS have disappeared.

Even though restraint had no detectable effects on mitogen-induced lymphoproliferation, it was able to augment plasma concentrations of IL-1 and IL-6. Plasma and brain levels of cytokines have been shown to be increased after exhausting stress in rats (Minami *et al.*, 1991; Zhou *et al.*, 1993; Takaki *et al.*, 1994; Shintani *et al.*, 1995; Shizuya *et al.*, 1998). To our knowledge, the present results are the first demonstration of an increase of plasma IL-1 level during a non-immune stress in mice. Our results are in accordance with those of Nukina *et al.* (2001) who demonstrated a restraint induced increase in plasma IL-6 in mice.

In a previous study, a 1–2 h-restraint during the inactive phase did not induce an increase in plasma concentrations of IL-1 and IL-6 (Li *et al.*, 2000). This suggests that cytokine production depends on the severity of stress. During stress, it is not known whether the production of cytokines occurs primarily in the periphery or in the brain. In accordance with the hypothesis of a primary peripheral synthesis of cytokines, it has been shown that stress increases gut permeability, therefore allowing intestinal endotoxins to stimulate the production of cytokines (Yagi *et al.*, 1997). Similarly, different stressors, including restraint, increase cutaneous permeability (Denda *et al.*, 2000). Such alterations could be indirectly responsible for the production of cytokines. Consequently, peripheral cytokines could induce the production of brain cytokines. Indeed, LPS-stimulated production of peripheral cytokines has been shown to induce the synthesis of brain cytokines (Layé *et al.*, 1994). It is also possible that during stress, cytokines are first produced in the brain and thereafter stimulate the production of cytokines in the periphery. Such a possibility is supported by the fact that the intra-cerebroventricular injection of IL-1 has been shown to induce high levels of plasma IL-6 (De Simoni *et al.*, 1990; Liège *et al.*, 2000), even though the mechanisms involved are not yet fully understood. However, in the present experiment, we were unable to demonstrate an increased IL-1 and IL-6 production in the hypothalamus. Interestingly, the stress-induced increase in plasma IL-1 occurred in right-, but not in left-pawed mice. This result is in agreement with our previous data if we consider that an increase in plasma IL-1 level is a stress marker. Indeed, as compared to left-pawed mice, right-pawed animals were shown to be more sensitive to restraint (Neveu and Moya, 1997), LPS (Delrue *et al.*, 1994), or IL-1 (Neveu *et al.*, 1998) when measuring endocrine, immune, or behavioral responses.

One week after the administration of LPS, there was no increase in plasma concentrations of IL-1 and IL-6. It is well known that LPS induces the production of cytokines, especially IL-1 and IL-6, but the increase of their concentrations in plasma occurs very shortly after injection (Schöbitz *et al.*, 1994). Interestingly, IL-1 was decreased in the LPS-R group as compared to the C-R group. Such an interaction between stresses was observed by Nagano *et al.* (1999) who showed that the increased plasma levels of IL-1 induced by LPS were decreased by previous repeated injections of LPS during 8 days.

Several hypotheses may be proposed to explain the finding that LPS prevented the restraint-induced increase in plasma cytokine concentration. First, LPS could depress the synthesis of cytokines. Second, LPS and cytokines are known to induce soluble cytokine receptors and binding proteins that can inhibit or increase cytokine activity (Fernandez-Botran *et al.*, 1996). Binding of cytokines to

soluble receptors or non-receptor proteins can interfere with the detection of cytokines using ELISAs (Krakauer and Krakauer, 1999). Finally, LPS is well known to induce the production of acute phase proteins including proteases that have been supposed to play a role in the cytokine network (Fernandez-Botran *et al.*, 1996). Moreover, the production of peptidases such as DPP IV (or CD 23) has been shown to be inversely correlated to the production of IL-6 (Maes *et al.*, 2001).

In summary, this study leads to important conclusions: (1) restraint, that may be considered as a mild psychological stress, is able to increase the concentrations of plasma cytokines in mice; and (2) the increase in plasma cytokines was not observed in mice previously treated with LPS. The present data suggest that LPS may induce long-lasting mechanisms that limit the availability of cytokines and therefore limit chronic inflammatory processes.

Acknowledgements

This work was supported by the INSERM and the DGA (agreement no. 00.34.060.00.470.75.01).

References

Andrès, R., Marti, O. and Armario, A. (1999) "Direct evidence of acute stress-induced facilitation of ACTH response to subsequent stress in rats", *Am. J. Physiol.* **277**, R863–R868.

Besedovsky, H.O. and Del Rey, A. (1996) "Immune–neuro-endocrine interactions: facts and hypotheses", *Endoc. Rev.* **17**, 64–102.

Betancur, C., Neveu, P.J. and LeMoal, M. (1991) "Sex and strain differences in the degree of paw preference in mice", *Behav. Brain Res.* **45**, 97–101.

Bluthé, R.M., Michaud, B., Kelley, K.W. and Dantzer, R. (1997) "Cholecystokinin receptors do not mediate the behavioral effects of lipopolysaccharide in mice", *Physiol. Behav.* **62**, 385–389.

Bradford, M. (1976) "A rapid and sensitive method for quantitation of micrograms of protein utilizing the principle of protein–dye binding", *Annal. Biochem.* **72**, 248–254.

De Simoni, M.G., Sironi, M., De Luigi, A., Manfridi, A., Mantovani, A. and Ghezzi, P. (1990) "Intracerebroventricular injection of interleukin-1 induces high circulating levels of interleukin 6", *J. Exp. Med.* **171**, 1773–1778.

Delrue, C., Deleplanque, B., Rouge-Pont, F., Vitiello, S. and Neveu, P.J. (1994) "Brain monoaminergic, neuroendocrine, and immune responses to an immune challenge in relation to brain and behavioral lateralization", *Brain Behav. Immun.* **8**, 137–152.

Denda, M., Tsuchiya, T., Elias, P.M. and Feingold, K.R. (2000) "Stress alters cutaneous permeability barrier homeostasis", *Am. J. Physiol.* **278**, R367–R372.

Dhabbar, F.S., Miller, A.H., McEwen, B.S. and Spencer, R.L. (1995) "Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms", *J. Immunol.* **154**, 5511–5527.

Fernandez-Botran, R., Chilton, P.M. and Ma, Y. (1996) "Soluble cytokine receptors: their roles in immunoregulation, disease, and therapy", *Adv. Immunol.* **63**, 269–336.

Krakauer, T. and Krahauer, H. (1999) "A method for correcting for the variability of inhibitory effects of soluble human interleukin 1 receptor II measured by different ELISAs", *J. Immunoassay* **20**, 185–200.

Layé, S., Parnet, P., Goujon, E. and Dantzer, R. (1994) "Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice", *Mol. Brain Res.* **27**, 157–162.

Li, K.S., Liège, S., Moze, E. and Neveu, P.J. (2000) "Plasma corticosterone and immune reactivity in restrained C3H mice", *Stress* **3**, 285–298.

Liège, S., Layé, S., Li, K.S., Moze, E. and Neveu, P.J. (2000) "Interleukin-1 receptor accessory protein (IL-1RAcP) is necessary for centrally mediated neuroendocrine and immune responses to IL-1β", *J. Neuroimmunol.* **110**, 134–139.

Maes, M., Capuron, L., Ravaud, A., Gualde, N., Bosmans, E., Egyed, B., Dantzer, R. and Neveu, P.J. (2001) "Lowered serum dipeptidyl peptidase IV activity is associated with depressive symptoms and cytokine production in cancer patients receiving interleukin-2-based immunotherapy", *Neuropsychopharmacology* **24**, 130–140.

Marti, O., Andrès, R. and Armario, A. (1999) "Defective ACTH response to stress in previously stressed rats: dependence on glucocorticoid status", *Am. J. Physiol.* **277**, R869–R877.

Mekaouche, M., Givalois, L., Barbanel, G., Siaud, P., Maurel, D., Malaval, F., Bristow, A.F., Boissin, J., Assenmacher, I. and Ixart, G. (1994) "Chronic restraint enhances interleukin-1-beta release in the basal state and after an endotoxin challenge, independently of adrenocorticotropin and corticosterone release", *Neuroimmunomodulation* **1**, 292–299.

Minami, M., Kuraishi, Y., Yamaguchi, T., Nakai, S., Hirai, Y. and Satoh, M. (1991) "Immobilization stress induces interleukin-1β mRNA in the rat hypothalamus", *Neurosci. Lett.* **123**, 254–256.

Nagano, I., Takao, T., Nanamiya, W., Takemura, T., Nishiyama, M., Asaba, K., Makino, S., De Souza, E.B. and Hashimoto, K. (1999) "Differential effects of one and repeated endotoxin treatment on pituitary-adrenocortical hormones in the mouse: role of interleukin-1 and tumor necrosis factor-α", *Neuroimmunomodulation* **6**, 284–292.

Neveu, P.J. and Moya, S. (1997) "In the mouse, the corticosteroid stress response depends on lateralization", *Brain Res.* **749**, 344–346.

Neveu, P.J., Bluthé, R.-M., Liège, S., Moya, S., Michaud, B. and Dantzer, R. (1998) "Interleukin-1-induced sickness behavior depends on behavioral lateralization in mice", *Physiol. Behav.* **63**, 587–590.

Nukina, H., Sudo, N., Aiba, Y., Oyama, N., Koga, Y. and Kubo, C. (2001) "Restraint stress elevates the plasma interleukin-6 levels in germ-free mice", *J. Neuroimmunol.* **115**, 46–52.

Schmidt, E.D., Janszen, A.W.J.W., Wouterlood, F.G. and Tilders, F.J.H. (1995) "Interleukin-1-induced long lasting changes in hypothalamic corticotropin-releasing hormone (CRH)-neurons and hyperresponsiveness of the hypothalamus–pituitary–adrenal axis", *J. Neurosci.* **15**, 7417–7426.

Schöbitz, B., Reul, J.M.H.M. and Holsboer, F. (1994) "The role of the hypothalamic–pituitary–adrenocortical system during inflammatory conditions", *Crit. Rev. Neurobiol.* **8**, 263–291.

Sheridan, J.F., Feng, N., Bonneau, R.H., Allen, C.M., Hunneycutt, B.S. and Glaser, R. (1991) "Restraint stress differentially affects anti-viral cellular and humoral responses in mice", *J. Neuroimmunol.* **31**, 245–255.

Shintani, F., Nakaki, T., Kanba, S., Sato, K., Yagi, G., Shiozawa, M., Aiso, S., Kato, R. and Asai, M. (1995) "Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotropic hormone and in the release of hypothalamic monoamines in the rat", *J. Neurosci.* **15**, 1961–1970.

Shizuya, K., Komori, T., Fujiwara, R., Miyahara, S., Ohmori, M. and Nomura, J. (1997) "The influence of restraint stress on the expression of mRNA for IL-6 and the IL-6 receptor in the hypothalamus and midbrain of the rat", *Life Sci.* **61**, PL135–PL140.

Takaki, A., Huang, Q.H., Somogyvari-Vigh, A. and Arimura, A. (1994) "Immobilization stress may increase plasma interleukin-6 via central and peripheral catecholamines", *Neuroimmunomodulation* **1**, 335–342.

Weidenfeld, J. and Yirmiya, R. (1996) "Effects of bacterial endotoxin on the glucocorticoid feedback regulation of adrenal response to stress", *Neuroimmunomodulation* **3**, 352–357.

Yagi, S., Takaki, A., Ito, K., Maeda, M. and Hori, T. (1997) "Changes in IL-6 and LPS levels in the hepatoportal vein during non-inflammatory stress in rats", *Soc. Neurosci. Abstr.* **23**, 2824.

Zhou, D., Kusnekov, A.W., Shurin, M.R., De Paoli, M. and Rabin, B.S. (1993) "Exposure to physical and psychological stressors elevates plasma interleukin-6: relationship to the activation of the hypothalamic–pituitary–adrenal axis", *Endocrinology* **133**, 2523–2530.