Inhibition of interleukin-1 beta release from cultured human peripheral blood mononuclear cells by prednisolone

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Summary. Prednisolone, a water-soluble glucocorticoid hormone, suppressed the secretion of interleukin-1 beta from human peripheral blood mononuclear cells in culture. The prednisolone-induced suppression of the monokine release was dose-related and the half maximal response was observed at 0.1 nM.
Key words. Prednisolone; interleukin-1; immunoneuroendocrinology; immunoregulatory feedback circuitry.

Increasing evidence suggests that there exists bidirectional communication between the immune and neuroendocrine systems. A variety of classical hormones, including steroids, peptides and proteins, can influence immune responses, and receptors for a number of these hormones have been located in lymphocytes [1, 2]. In turn, factors secreted from cells of the immune system may affect endocrine function. We have recently reported [3–5] that interleukin-1 (IL-1), a polypeptide hormone produced by activated monocytes/macrophages, stimulated the release of hypothalamic corticotropin-releasing factor (CRF), causing the activation of the hypothalamic-pituitary-adrenal (H-P-A) axis. This observation was later confirmed by two other independent research groups [6, 7].

Glucocorticoid hormones, the final products of the H-P-A axis, have a broad range of inhibitory effects on the various functions of immunocompetent cells involved in the regulation of the immune system [8]. Due to these pharmacological actions, glucocorticoid hormones have been clinically used for the treatment of various autoimmune diseases.

On the basis of these results, it has been hypothesized that there might exist an immunoregulatory negative feedback circuit between IL-1 and corticosteroids [4–7, 9]. In fact, it has been shown that glucocorticoid hormones inhibit the production of IL-1 activity, as determined by IL-1 bioassay, in rats [10] and mice [11]. However, major species differences have been recognized in the effects of glucocorticoids on lymphoid cells and tissues; those of the mouse, rat, hamster and rabbit are found to be steroid sensitive, whereas those of the guinea pig, monkey and man are shown to be steroid resistant [8]. Furthermore, the IL-1 bioassay used by those investigators is known to be influenced by other lymphokines and monokines including IL-2 [12, 13]. The carry-over of glucocorticoids from original cultures could also influence the IL-1 bioassay.

In the present study, therefore, we directly addressed the question of whether or not glucocorticoid hormones inhibited the production of IL-1 beta in man, using human peripheral blood mononuclear cells (PBMNC) in culture and a recently-developed specific radioimmunoassay (RIA) system for IL-1 beta. We report here that glucocorticoid hormones inhibit the release of IL-1 beta from cultured human PBMNC in a dose-related manner.

Materials and methods. Chemicals: Lipopolysaccharide (LPS, from *E-coli*, serotype 055:B5) and prednisolone sodium succinate, a water-soluble synthetic glucocorticoid hormone, were purchased from Sigma Chemical Co. (USA) and from Shionogi & Co. (Japan), respectively.

Preparation of PBMNC culture: Human PBMNC cultures were prepared as described previously [14]. In brief, PBMNC were obtained from the heparinized peripheral venous blood of normal donors by density gradient centrifugation with Ficoll-Conray (Pharmacia, Sweden). The isolated cells were washed three times and resuspended in RPMI-1640 culture medium (GIBCO, USA) containing 10% heat-inactivated fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin and 100 µg/ml streptomycin (complete medium). LPS was used at 25 µg/ml as a stimulant of IL-1 beta production. Water-soluble prednisolone was added at various concentrations ranging from 1×10^{-12} M to 1×10^{-7} M. Mononuclear cells suspended in complete medium were plated at a final density of 1×10^6/ml in 24-well culture plates (Costar, USA) in 1-ml volumes, and then incubated in the presence or absence of LPS and prednisolone at 37 °C in a humidified atmosphere of 95% air/5% CO_2 for 48 h. After the 2-day culture, supernatants were recovered by centrifugation at 400 × g for 10 min and frozen at −80 °C until used for IL-1 determination.

RIA for IL-1 beta: The IL-1 beta levels in culture supernatants were determined by a commercially-available RIA kit for the monokine (Cystron, USA). The antiserum used in this assay system was shown to be specific for IL-1 beta, and no cross-reactivity was apparent with other monokines such as IL-1 alpha, IL-2 and tumor necrosis factor. The presence of prednisolone did not influence the measurement of IL-1 beta levels by the RIA system employed in the present study (data not shown). The lowest concentration of IL-1 beta reproducibly detectable from zero level (95% confidence) was 50 pg/ml.

Results and discussion. The IL-1 beta levels in supernatants cultured in the absence of LPS for 48 h were consistently undetectable, i.e. less than 50 pg/ml. The addition of 25 µg/ml LPS, a potent stimulator of endogenous IL-1, raised the IL-1 beta levels in the supernatants to 357.4 ± 9.5 pg/ml (mean ± SEM of 6 replicate cultures). Figure 1 illustrates the effect of prednisolone on the LPS-induced IL-1 beta release. The secretion of IL-1 beta was inhibited by the glucocorticoid hormone in a dose-related manner. The maximal suppression of IL-1 beta release was observed at 10 nM prednisolone. The half maximal inhibition of the monokine secretion occurred at a dose of 0.1 nM.

There are two distinct forms of IL-1, IL-1 alpha and IL-1 beta [15]. Both are 17-kD polypeptides that differ in their amino acid sequences. Human peripheral blood monocytes produce both IL-1 beta and IL-1 alpha in a ratio of 9:1 or greater [15]. Although it had been reported that the two forms of IL-1 exerted similar immunological responses despite the distant homology in their primary structures, we recently found that only IL-1 beta, but not IL-1 alpha, activated the H-P-A axis, suggesting that the two species of IL-1 might have different spectra of biological activities [16]. In the present study, therefore, the effect of prednisolone on the release of IL-1 beta was exclusively examined.

Ever since it was discovered that IL-1 stimulated the release of hypothalamic CRF, it has been hypothesized that there might be an immunoregulatory negative feedback circuit between IL-1 and the H-P-A axis [4–7, 9]. It was previously reported that glucocorticoid hormones inhibited the production of IL-1 activity, as determined by IL-1 bioassay, in rats [10] and mice [11]. However, it remained to be clarified whether or not the glucocorticoid-induced suppression of IL-1 release would be observed in man, who is known to be steroid resistant. The present study clearly demonstrated that glucocorticoid hormones inhibited the release of IL-1 beta from cultured human PBMNC as well, in a dose-related

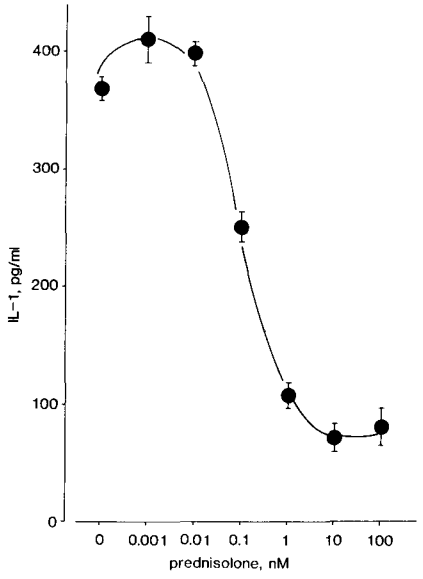

Figure 1. The effect of prednisolone on the LPS-induced IL-1 beta release from human peripheral mononuclear cells in culture. All incubations were carried out for 48 h in the presence of LPS and varying concentrations of prednisolone. The IL-1 beta levels in culture supernatants were determined by RIA. Each point represents mean ±SEM of 4 replicate cultures.

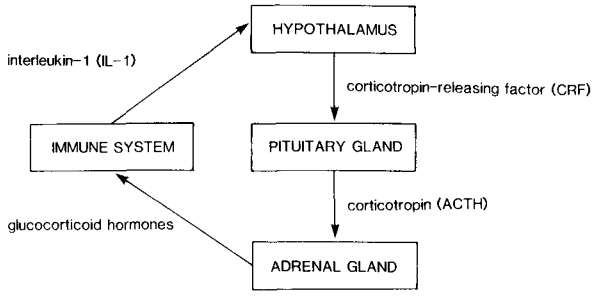

Figure 2. The hypothetical model for an immunoregulatory negative feedback circuit between the immune and neuroendocrine systems, in which IL-1 acts as an afferent and glucocorticoid as an efferent hormonal signal.

manner. It was found that the maximal suppression of IL-1 release occurred at a dose of 10 nM of prednisolone and the half maximal inhibition at 0.1 nM (fig. 1). Increased plasma glucocorticoid levels have been detected during immune responses[17] and infections[18]. Moreover, the injection of IL-1 causes an increase in free plasma corticosteroid levels to more than 150 nM[9], sufficient to suppress the release of IL-1. These results suggest that the glucocorticoid-mediated inhibition of IL-1 release is likely to take place in vivo, providing further evidence for the hypothesis that there may exist an immunoregulatory feedback circuit between the immune and neuroendocrine systems (fig. 2). A better understanding of the interactions between these two systems would help to explain the pathophysiology of diseases having immune and neuroendocrine components. Along this line, a possible pathophysiological role for this immuno-neuroendocrine feedback loop in the development of autoimmune disease has been more recently reported in the Obese strain of chickens, an animal model with Hashimoto-like spontaneous autoimmune thyroiditis[19].

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