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Brief Note

Hydrocortisone Sodium Succinate Suppressed Production of Interleukin-10 by Human Peripheral Blood Mononuclear Cells: Clinical Significance

Hideo Kohka[a], Hiromi Iwagaki[a]*, Tadashi Yoshino[b], Kenta Kobashi[a], Shinnya Saito[a], Hiroshi Isozaki[a], Norihisa Takakura[a] and Noriaki Tanaka[a]

[a]First Department of Surgery and [b]Second Department of Pathology, Okayama University Medical School, Okayama 700-8558, Japan

Corticoids are well known for their immunosuppressive properties. Interleukin-10 (IL-10) is an intrinsic antiinflammatory peptide in immune diseases, originally identified as cytokine synthesis inhibitory factor. We examined the effect of hydrocortisone sodium succinate (HSS) on the production of IL-10 by human peripheral blood mononuclear cells (PBMCs). PBMCs from healthy volunteers and cancer-burden patients were preincubated separately with or without HSS for 1 h, then stimulated with 5 μg/ml lipopolysaccharide (LPS). Production of IL-10 by human PBMCs was detected with LPS stimulation and its production was higher in cancer-burden patients than in normal volunteers, although this was not statistically significant. HSS suppressed production of IL-10 by LPS-stimulated PBMCs in a dose-dependent manner both in normal volunteers and in cancer-burden patients. These results indicate that, in addition to their antiinflammatory properties, corticoids act to restore the immunosuppressive states even in cancer-burden states.

Key words: steroid, interleukin-10, cancer-burden state

Helper T cells are divided into at least two subpopulations on the basis of the pattern of the cytokines they produce. Th1 cells secrete interleukin-2 (IL-2) and interferon-γ (IFN-γ) which mediate predominantly the delayed-type-hypersensitivity reaction, whereas Th2 cells produce IL-4 and IL-5 which assist B cell responses (1). IL-10 was first described as a cytokine produced by Th2 clones which inhibits IFN-γ production by Th1 cells (2). Further reports indicate that IL-10 is produced by other cell types, such as B cells (3) and monocytes (4).

It has been reported that IL-10 strongly inhibits the production of many cytokines, such as IL-1α, IL-1β, IL-6, IL-8, tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and IFN-γ by activated monocytes (4), macrophages (5), and T cells (2) at the transcriptional level. These cytokines are known to induce inflammatory or immunological responses. Ding *et al.* reported that IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression (6). In addition, Willems *et al.* reported that IL-10 inhibits B7 and intercellular adhesion molecule-1 expression in human monocytes (7).

Glucocorticoids are potent immunosuppressive and anti-inflammatory agents and are widely used for the treatment of inflammatory diseases. They are known to inhibit the production and gene expression of many cytokines including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, TNF-α, TNF-β, IFN-γ and GM-CSF (8-17). However, macrophage colony-stimulating factor (M-CSF) was not suppressed by glucocorticoids. The effect of hydrocortisone sodium succinate (HSS) on IL-10 production has not yet been determined. In this report, we examine whether HSS suppresses production of IL-10 by human PBMCs.

*To whom correspondence should be addressed.

Materials and Methods

Isolation and culture of PBMCs. Human PBMCs were isolated from buffy coat of three healthy volunteers (1 male and 2 females, aged 30 to 64 years) and three cancer-burden patients (2 males and 1 female, aged 56 to 69 years, one case of esophageal cancer with bone metastasis, one of rectal cancer with multiple liver metastasis and one of unresectable hepatocellular cancer) by centrifugation on a density gradient of Ficoll-Paque (Pharmacia, Uppsala, Sweden), then washed three times in RPMI 1640 medium (Nissui Co. Ltd., Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 20 μg/ml of kanamycin and 100 μg/ml of streptomycin and penicillin (Sigma Chemical Co., St. Louis, MO, USA). PBMCs were suspended at a final concentration of 5×10^5 cells /ml in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum. Cells were preincubated in plastic dishes with or without HSS (Pharmacia & Upjohn, Tokyo, Japan), and then incubated for 0–72 h with 5 mg/ml lipopolysaccharide (LPS, Sigma, St. Louis, MO) at 37°C in a humidified atmosphere of 5% CO_2 in air.

Assay for IL-10 production. PBMCs were preincubated for 1 h in plastic dishes with or without HSS and then incubated for 0–72 h with or without 5 μg/ml LPS at 37°C in a humidified atmosphere of 5% CO_2 in air. After culture, the cell suspensions were centrifuged and the cell-free supernatant fractions were assayed for IL-10 protein by an enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Quantikine, R & D Systems, Inc., Minneapolis, MN, USA). All examined samples were analyzed in triplicate.

Statistical analysis. Data are expressed as mean ± SE. For mean comparisons, one-way analysis of variance (ANOVA) was used. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Fig. 1 represents the kinetics of IL-10 production by PBMCs in response to LPS treatment both in normal volunteers and in cancer-burden patients. LPS-induced IL-10 production by PBMCs was detected at 12 h, increased time-dependently, and reached a maximum level at 24–48 h. The maximum level of IL-10 was higher in cancer-burden patients than in normal volunteers, although statistically not significant. HSS treatment at a concentration of 1 mg/ml suppressed IL-10 production at every incubation time both in normal volunteers and in cancer-burden patients. In cancer-burden patients, the production of IL-10 was almost completely suppressed by HSS. Fig. 2 shows the dose effect of HSS on IL-10 production by PBMCs at 48 h. HSS suppressed IL-10 production in a dose-dependent manner both in normal volunteers and in cancer-burden patients.

Recently, IL-10 has been shown to be elevated in the plasma of cancer-bearing patients (18–20). Researchers have reported that the baseline concentration of IL-10 is significantly higher in cancer patients than in healthy subjects. After radical surgery, the IL-10 levels significantly dropped in cancer patients, whereas in subjects undergoing palliative operation, the concentration remained elevated (18). The origin of circulating IL-10 may be the tumor microenvironment and IL-10 may play an important role in tumor-induced immunosuppression. Several studies have shown that cancer cell lines stimulate peripheral monocytes to produce markedly increased levels of IL-10, acting as a tumor-protecting mechanism by impairing the activation of anti-tumor cytokines (19, 20). The present research demonstrates that the production of IL-10 by PBMCs tends to be higher in cancer-burden patients than in normal volunteers, and these results were consistent with the recent reports described above.

Glucocorticoids have been considered for palliative therapy of terminal cancer since the early 1960s (21). Several reports have been published which indicate that repeated injection of corticosteroid up to 600 mg/day over periods as long as 42 weeks produces consistent improvements in appetite and well-being (22, 23). Recent clinical trials have also suggested that extended administration of corticosteroids to terminal cancer patients has a beneficial effect on quality of life (24, 25). The present research clearly demonstrates that HSS suppresses LPS-induced IL-10 production by PBMCs both in normal volunteers and in cancer-burden patients. As described above, HSS has been widely used as palliative therapy for terminal cancer patients, and has yielded significant improvement in appetite, nausea, vomiting, alertness and fatigue. Therefore, the mechanism by which glucocorticoids improve the quality of life in cancer-burden patients is, at least in part, its suppressive effect of IL-10 production as well as other inflammatory cytokines such as TNF-α and IL-6.

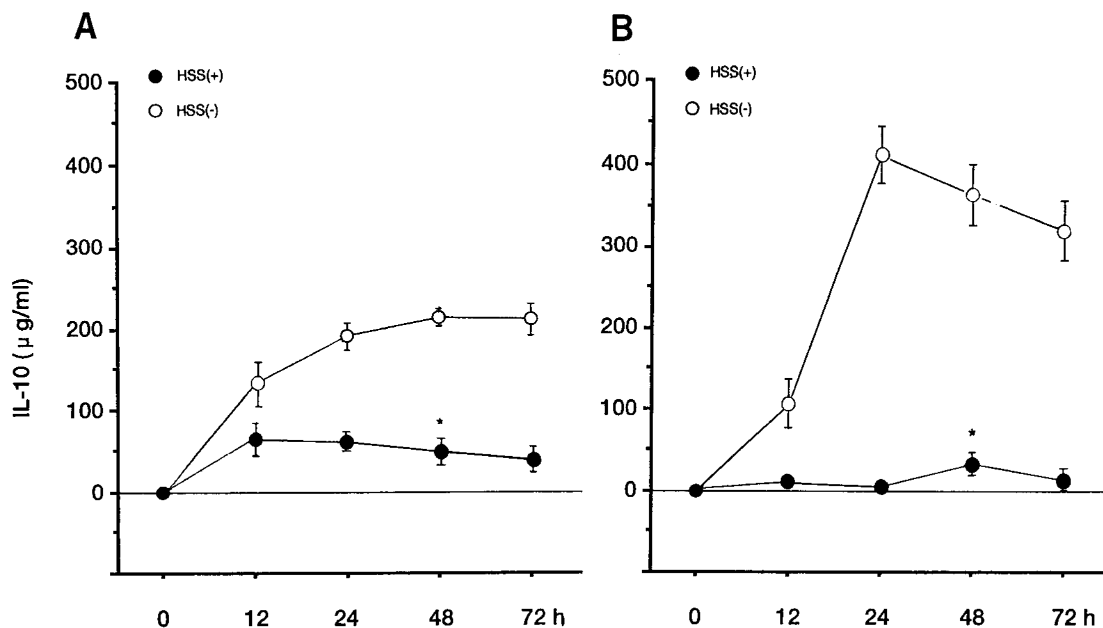

Fig. 1 The kinetics of interleukin-10 (IL-10) expression in response to hydrocortisone sodium succinate (HSS) treatment. IL-10 was produced by peripheral blood mononuclear cells (PBMCs) treated with lipopolysaccharide (LPS). PBMCs (5×10^5 cells/ml) from three healthy volunteers (**A**) and three cancer-burden patients (**B**) were incubated with LPS (5 μg/ml) and/or HSS (1 mg/ml) for 0–72 h. After the treatment, supernatant levels of IL-10 were detected by an enzyme-linked immunosorbent assay (ELISA) kit. The mean $\pm$ SEM of three experiments is shown. The amount of IL-10 was apparently suppressed by HSS at all times both in healthy volunteers and cancer-burden patients. $^*P < 0.01$ at 48 h, HSS (−).

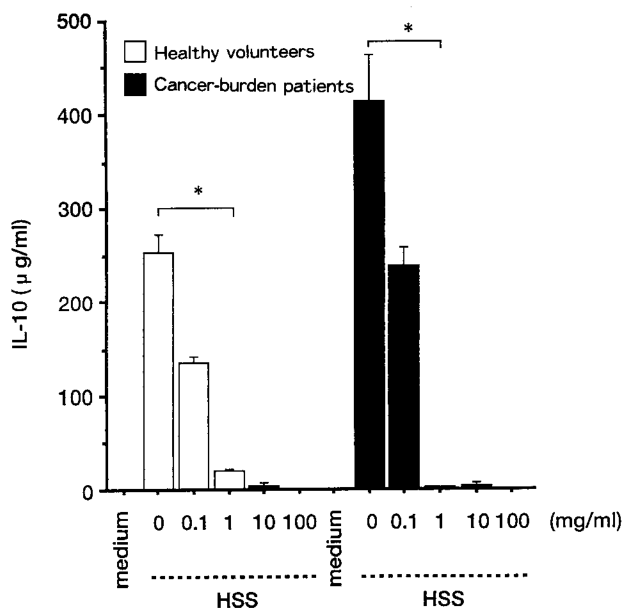

Fig. 2 The dose-dependency of interleukin-10 (IL-10) expression in response to hydrocortine sodium succinate (HSS) treatment. IL-10 was produced by peripheral blood mononuclear cells (PBMCs) treated with lipopolysaccharide (LPS). PBMCs (5×10^5 cells/ml) from three healthy volunteers and three cancer-burden patients were incubated with LPS (5 μg/ml) and/or HSS (0–100 mg/ml) for 48 h. After the treatment, supernatant levels of IL-10 were detected by an ELISA kit. The mean $\pm$ SEM of three experiments is shown. As a control, supernatant levels of IL-10 in fresh culture media (in the text) were determined. The amount of IL-10 producted was suppressed dose-dependently by HSS both in healthy volunteers and in cancer-burden patients. $^*P < 0.01$ at HSS (−).

Recent reports have shown that corticosteroids may exert some of their antiinflammatory activity through stimulation of IL-10 production *in vivo* (26-28). However, our data demonstrate that as with other cytokines, HSS inhibits synthesis of IL-10. The effects of corticosteroids on IL-10 are complex, involving numerous positive and negative regulatory influences. It has been estimated that from 50% to 75% of the IL-10 secretion induced by LPS occurs as a result of induction of TNF-α, as shown by the ability of anti-TNF-α antibodies to partially abrogate LPS-induced IL-10 secretion (29). Inhibition of IL-10 secretion by corticosteroids may therefore reflect inhibition of TNF-α production by corticosteroids. However, there are likely to be additional direct effects of corticosteroids on IL-10 transcription as suggested by the presence of consensus AP-1 and NFκB sequences in the IL-10 promoter, the activities of which would be inhibited by corticosteroids (30), in addition to effects mediated through a consensus glucocorticoid response element (31). Inhibition of IL-10 production by corticosteroids would be expected to exert antiinflammatory effects on humoral immune responses and to activate cytotoxic T cells (32).

The present research demonstrates that HSS significantly suppresses the production of IL-10, which is an immunosuppressive cytokine. Corticosteroids, therefore, both suppress and/or enhance immune reactions in a specific and coordinated manner. This biphasic role of corticosteroids is part of a highly specific interaction between the immune and endocrine systems. If regulatory loops exist between these two systems, it must be anticipated that, if one system is altered, changes will be introduced in the other. Thus, the corticosteroid-immune axis appears to be crucial in controlling immunological responses, including the direction and magnitude of immune reactions. If optimally balanced, corticosteroid-dependent functions will contribute to a resolution of cancer cachexia, infection, trauma or other immunologically related stresses. However, disruption or malfunction of the dynamic interactions of the immune and endocrine loops may result in a fatal outcome. An understanding of the biphasic role of corticosteroids in host defenses, with particular emphasis on the neglected aspects of their immune enhancing effects, could open new avenues for the treatment or prophylaxis of immune-mediated diseases.

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