

**Adrenocortical responses to tumor necrosis factor- $\alpha$  and interferon- $\gamma$  in cattle**

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## ABSRTACT

The responses of plasma cortisol and adrenocorticotrophic hormone (ACTH) were examined to intravenous injection of recombinant bovine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (INF- $\gamma$ ) in Holstein cows. INF- $\gamma$  induced dose-dependent rises in the plasma levels of both cortisol and ACTH, while TNF- $\alpha$  induced comparable plasma cortisol responses with much smaller rises in plasma ACTH. The results suggest a direct stimulatory action of TNF- $\alpha$  on cortisol secretion from the adrenal gland in cattle.

**KEY WORDS:** ACTH, cortisol, cytokines

There is a bidirectional relationship between the immune and endocrine systems. This interaction involves both stimulatory and inhibitory effects of hormones on the immune system, and conversely, those of immune cytokines on the endocrine system, and thereby forms a feedback and/or feed-forward loop to control peripheral immune activities. For example, interleukin (IL)-1 acts on the brain and activates the adrenocortical system to increase circulating glucocorticoids, which in turn, suppress the peripheral immune activities including IL-1 production. Similar adrenocortical activation is also found, more or less, for many other cytokines such as IL-2, IL-6, tumor necrosis factor (TNF) and interferon (IFN) (for a review, see 11). In addition, these cytokines induce, more or less, various pathophysiological responses such as fever, anorexia, and slow wave sleep. Such brain-mediated effects of peripheral cytokines must be important for the maintenance of homeostasis, whereas they have sometimes been considered as the side effects in the clinical application of immune cytokines in human medicine and probably also in veterinary medicine.

Although there have been lots of studies on the effects of immune cytokines on the adrenocortical activities in vivo and in vitro, a limited number of studies have been reported in domestic animals including cattle. Since current studies have suggested the possible application of recombinant cytokines for the control of infectious diseases of domestic animals<sup>10)</sup>, it seems important to clarify the effects of homologous recombinant cytokines on the brain functions including adrenocortical activity in these animal species. We<sup>9)</sup> reported previously that intravenous administration of recombinant bovine (rb)TNF-

$\alpha$  to cows increased plasma cortisol levels in an hour, suggesting a rapid activation of the adrenocortical system. In the present study, to obtain more detailed information about the effects of cytokines on the adrenocortical system in cattle, the responses of plasma cortisol and adrenocorticotrophic hormone (ACTH) were examined to intravenous injection of rbTNF- $\alpha$  and rbINF- $\gamma$  in Holstein cows. TNF- $\alpha$  and IFN- $\gamma$  were chosen because of not only their critical regulatory roles of immune cells but also the availability of large amounts of recombinant bovine proteins.

Twelve Holstein cows, weighing 330 - 550 kg, were housed in individual stalls with free access to water and a trace mineral block, and given a diet of forage-concentrate mixture twice a day. Highly purified recombinant bovine INF- $\gamma$  (rbINF- $\gamma$ ) was provided by Dr. Shigeki Inumaru, National Institute of Animal Health, Tsukuba, Japan, produced by a baculovirus gene expression system<sup>7)</sup>, and recombinant bovine TNF- $\alpha$  (rbTNF- $\alpha$ ) by Higeta Shoyu Co., Ltd., Choshi, Japan, produced by a *Bacillus brevis* host-vector system.

The cows were randomly divided into three or four groups and injected intravenously with rbINF- $\gamma$  (1.8 or 3.6  $\mu\text{g}/\text{kg}$  body weight), rbTNF- $\alpha$  (2.5 or 5.0  $\mu\text{g}/\text{kg}$  body weight), or saline as a control through a catheter fixed in the jugular vein. Before and 1 - 8 hr after the injection, blood was collected and plasma was stored at -20 C° until assay. A cross-over experiment was performed using the same cows at least one week after the first experiment. Plasma cortisol, and ACTH were assayed using RIA kits for cortisol (Amerlix Cortisol RIA kit, Amersham, Arlington Heights, IL, USA), and human ACTH (Peninsula, San Carlos, CA, USA) respectively. Data are presented as means  $\pm$  SEM and analysed by one-way ANOVA followed by Dunnett's t-test for multiple comparisons.

Potent stimulatory effects of TNF- $\alpha$  on the adrenocortical system have well been documented in rodents and humans<sup>2, 5, 8)</sup>. For example, intravenous administration of rTNF- $\alpha$  into rats induces corticotropin releasing hormone (CRH) secretion in the median eminence and elevation of plasma ACTH and glucocorticoid levels<sup>13)</sup>. Since the effects on plasma ACTH and glucocorticoids are suppressed by a CRH-antiserum, the primary site of TNF- $\alpha$  action seems to be the hypothalamic CRH-secreting neurons. We<sup>9)</sup> reported previously that rbTNF- $\alpha$  injection to cows increased plasma cortisol levels rapidly. This was confirmed in the present study, as shown in Fig.1. When rbTNF- $\alpha$  was injected intravenously at a dose of 5  $\mu$ g/kg body weight, cortisol levels were elevated remarkably to reach a peak at 2 hr, and decreased gradually to the basal levels at 8 hr. Plasma ACTH was also increased slightly, but significantly, showing a peak at 1 hr (Fig. 1). A lower dose of rbTNF- $\alpha$  (2.5  $\mu$ g/kg) gave rise to a similar cortisol response, but no significant increase in the ACTH level. Thus, TNF- $\alpha$  produced a considerable rise in plasma cortisol without corresponding increases in plasma ACTH. These results are apparently different from those in rodents and humans, where TNF- $\alpha$  induces concomitant rises in plasma ACTH and glucocorticoids<sup>11,13)</sup>. It is thus suggested that the action site of TNF- $\alpha$  in cattle may be the adrenal cortex rather than the brain CRH neurons and/or pituitary gland. This seems to be supported by an in vitro study showing the stimulatory actions of TNF- $\alpha$  on cortisol secretion from human adrenocortical cells<sup>5)</sup>. It is to be noted, however, that TNF- $\alpha$  is reported to inhibit ACTH-induced cortisol secretion from adrenal tissue and cultured cells in vitro<sup>4, 12)</sup>. It seems thus intriguing to examine whether rbTNF- $\alpha$  may directly influence cortisol secretion from bovine adrenal tissue and/or cells in vitro.

In contrast to rbTNF- $\alpha$ , rbINF- $\gamma$  injected at a dose of 3.6  $\mu\text{g}/\text{kg}$  body weight produced a rapid and large ACTH response: that is, plasma ACTH levels reached a much higher peak at 1 hr, and returned to the basal levels at 6-8 hr (Fig. 2). Plasma cortisol levels were also elevated following the rise of ACTH and reached to the maximal levels at 2 hr (Fig. 2). A lower dose of rbINF- $\gamma$  (1.8  $\mu\text{g}/\text{kg}$ ) also increased both plasma ACTH and cortisol levels, but rather slowly and to lesser extents. Thus, plasma cortisol and ACTH responses were increased in parallel with increasing doses of INF- $\gamma$ . These results are well consistent with those reported in humans and rodents<sup>2, 3)</sup>, suggesting the stimulatory effects of INF- $\gamma$  on cortisol secretion in cattle are mediated largely by activation of the CRH-ACTH axis.

In this study, we have demonstrated a quite different adrenocortical response to TNF- $\alpha$  from those to INF- $\gamma$  in cattle, and suggested a direct stimulatory action of TNF- $\alpha$  on the adrenal cortex at least in this species. Although we do not know the reason for such species difference and its pathophysiological relevance, the adrenal gland can be considered as one of the active interaction sites between the endocrine and immune systems, not only through the brain but also direct paracrine interaction<sup>6, 8)</sup>. Indeed, it has been shown in humans and rodents that the adrenal cortex not only expresses TNF- $\alpha$  receptor but also synthesizes many cytokines including TNF- $\alpha$  and INF- $\gamma$  in both parenchymal cells and resident macrophages<sup>8, 11, 12)</sup>. It is also known that the release of TNF- $\alpha$  from the adrenal gland is regulated by ACTH<sup>1)</sup>. Thus, the effects of TNF- $\alpha$  on cortisol secretion may be mediated through the direct action on the TNF receptor in cortical cells as discussed above. It may be also possible that the effects are mediated, at least in part, through a TNF- $\alpha$ -induced but yet unidentified factor derived from non-

parenchymal cells such as resident macrophages in the adrenal gland<sup>6, 8)</sup>. Collectively, further studies using bovine adrenal gland may be helpful for better understanding the molecular interaction between endocrine and immune cells.

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### **Figure legend**

Figure 1. Changes in the plasma levels of cortisol (A) and ACTH (B) after intravenous injection of TNF- $\alpha$  to Holstein cows. Either rbTNF- $\alpha$  (2.5 or 5  $\mu$ g/kg), or saline was injected into to the jugular vein, and blood samples were obtained before (Time 0) and 1 - 8 hr after the injection. Values are means  $\pm$  SEM for 6 cows. \* $p$ <0.05 vs. Saline controls.

Figure 2. Changes in the plasma levels of cortisol (A) and ACTH (B) after intravenous injection of rbINF- $\gamma$  to Holstein cows. Either rbINF- $\gamma$  (1.8 or 3.6  $\mu$ g/kg), or saline was injected into to the jugular vein, and blood samples were obtained before (Time 0) and 1 - 8 hr after the injection. Values are means  $\pm$  SEM for 6 cows. \* $p$ <0.05 vs. Saline controls



