Immunoenhancement through Growth Hormone Treatment

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Abstract Growth hormone (GH) and insulin-like growth factor I (IGF-I) have immunostimulatory effects in addition to their anabolic action. In this review the immunoenhancing effects of exogenous anabolic hormones will be described according to our findings in a bacterial peritonitis model. Pretreatment with GH (0.48 or 4.8 mg/kg/day) or IGF-I (24 mg/kg/day) increased the survival time in a murine model of Escherichia coli (E. coli) peritonitis. In addition, the numbers of peritoneal exudative cells in the GH-and IGF-I-treated mice were greater than those of the controls after bacterial challenge. Moreover, pretreatment with GH or IGF-I improved clearance of E. coli from the peritoneal cavity. GH and IGF-I increased CD11b and CD32/16 expression on peritoneal exudative neutrophils. These hormones directly enhanced the E. coli killing activity of murine peritoneal exudative cells in vitro. In this E. coli peritonitis model, GH and IGF-I both primed exudative phagocytes for enhanced production of cytokines, tumor necrosis factor (TNF), interleukin-1 (IL-1) and IL-6. Pretreatment with either GH or IGF-I also enhanced the capacity of plasma to support the in vitro bactericidal activity of peritoneal exudative cells. This suggests that GH and IGF-I improve host defense against infection.

Key words: growth hormone, insulin-like growth factor I, immunity, sepsis, phagocytosis

Introduction

Postoperative infections, such as peritonitis, wound infection and pneumonia, are the most common postoperative complications observed in our department. The over-all incidence of postoperative infection is about 15 % and the mortality rate for postoperative diffuse peritonitis is 62 %. Abdominal sepsis thus is particularly difficult to manage. Unfortunately, conventional treatment has not proven to be particularly successful in reducing the morbidity and mortality associated with severe surgical sepsis.

Both growth hormone (GH) and insulin-like growth factor I (IGF-I) have potent anabolic action(1). In addition, recent investigations have revealed that these hormones
have immunostimulatory effects (2, 3). We have reported enhancement of cellular immunity, with postoperative GH treatment, in patients who had undergone colon surgery (4). Moreover, recent studies have shown that both GH and IGF-I can modulate the functions of myeloid cells (5, 6). In this article the immunoenhancing effects of exogenous GH are discussed, according to our findings in a bacterial peritonitis model.

Effect of GH on Survival in a Peritonitis Model

It is known that GH enhances resistance to experimental Salmonella typhimurium infection in hypophysectomized rats (7, 8). However, Salmonella typhimurium infection is rarely seen in surgical patients. Opportunistic pathogens, such as Escherichia coli (E. coli), are more common in surgical wards. We investigated the effects of GH and IGF-I on survival in a murine model of E. coli peritonitis (9). The hormones were given subcutaneously every 8 hours for 6 days. The mice were then challenged intraperitoneally with 10⁸ cfu of E. coli. The survival times of the two GH-pretreated groups (0.48 mg/kg/day or 4.8 mg/kg/day) and the IGF-I-pretreated group (24 mg/kg/day) were significantly longer than those of the saline control group.

Effects of GH on Bacterial Clearance

Polymorphonuclear neutrophils (PMNs) and macrophages play major roles in host defense in the E. coli peritonitis model (10). We also investigated the effects of GH and IGF-I on peritoneal exudative cell (PEC) numbers, tissue viable bacterial counts and cytokine production, in the same murine model (9). Pretreatment with GH and IGF-I increased the number of peritoneal resident cells harvested before bacterial challenge, as compared with saline treatment. In addition, the numbers of PEC in both the GH and the IGF-I groups were significantly greater than in the control group after bacterial challenge. The numbers of bacteria in peritoneal lavage fluids, from both the GH and the IGF-I group, were lower than in the control group at 4 and 6 hours after bacterial inoculation. Thus both GH and IGF-I increase bacterial clearance from the peritoneal cavity.

There was a significant negative correlation between PEC and bacterial counts in peritoneal fluids after bacterial challenge. This observation suggests that the better clearance of inoculated bacteria in hormone-treated animals may be partially dependent on the increase in PECs. The first host response to invading bacteria is the proliferation and migration of phagocytes to the site of infection. It has been demonstrated that GH enhances granulopoiesis (11) and increases peripheral leukocyte counts (12, 13). PMNs and monocytes then kill bacteria by oxidative and non-oxidative processes. Secretion of superoxide anions from human PMNs has been shown to be augmented by GH and IGF-I (14). Moreover, PMNs can be primed by GH for respiratory burst (15).

Effects of GH on Cytokine Production in vitro and in vivo

The PEC cytokine productions are interesting. We harvested PECs 4 hours after
bacterial challenge, then stimulated these cells with lipopolysaccharide (LPS) in vitro and cultured them for 24 hours. The production of IL-1 was greater in GH-and IGF-I-treated animals than in the control group. IL-6 production by PECs was also enhanced by GH. Edwards et al. reported that GH increases TNF synthesis by LPS-stimulated peritoneal macrophages from hypophysectomized rats (16). Cytokines, such as TNF, IL-1 and IL-6, activate neutrophil adherence, degranulation, and superoxide production. Therefore, the enhancement of in vitro cytokine production by PEC, in both the GH and the IGF-I group, may improve the killing of E. coli through increased production of reactive oxygen intermediates.

In contrast to the enhanced in vitro cytokine production by PEC, in GH and IGF-I treated animals, plasma cytokine levels in our murine peritonitis model were significantly lower in the GH and IGF-I groups than in the control group (9). Similarly, both plasma IL-1 and IL-6 levels were significantly lower in the hormone-treated groups than in the non-treated control group. Excessive production of systemic cytokines appears to induce multiple organ failure in sepsis. These hormones may therefore prevent the development of multiple organ failure through inhibition of increase in systemic cytokine production.

**GH and Leukocyte Surface Expression of CD11b and CD32/16**

Neutrophil adhesion to the endothelium is a critical step in the neutrophil response to bacterial stimuli. In addition, PMN phagocytosis is mediated by Fcγ receptors and complement receptors. CD11b is a member of the integrin family of adhesion molecules on PMNs. CD11b participates in leukocyte adhesion to the endothelium, complement binding and phagocytosis. CD32/16 complexes are low-affinity Fcγ receptors which are important for degranulation, respiratory burst and phagocytosis.

We harvested PECs and blood from mice challenged intraperitoneally with E. coli after 6-days pretreatment with GH. GH increased the percentage of the peritoneal exudative neutrophil population exhibiting high levels of CD11b expression, as analyzed by flow cytometry. In addition, GH increased the mean fluorescence intensity of the peritoneal exudative neutrophils exhibiting high levels of CD32/16 expression (unpublished data). Thus GH has the capacity to enhance CD11b and CD32/16 expression on peritoneal exudative neutrophils. IGF-I also increases mature human PMN complement receptor expression (17). This may lead to enhanced recruitment, phagocytosis and bacterial killing of exudative neutrophils in bacterial peritonitis.

**Indirect and Direct Effects of GH on in vitro Bactericidal Activity**

Phagocytes can effectively ingest and eliminate bacteria with the assistance of opsonins. We have demonstrated that pretreatment with GH or IGF-I increases plasma capacity to support the in vitro bactericidal activity of murine PECs (18). In addition to the enhanced plasma bactericidal activity associated with these hormones, we investigated the direct effects of GH and IGF-I on the in vitro bactericidal activity of PEC from mice. E. coli
and pooled normal plasma were added to PEC precultured with various concentrations of GH or IGF-I. These hormones decreased the viable bacterial counts significantly after mixed incubation. These results suggest that GH and IGF-I directly enhance the in vitro bactericidal activity of murine PEC.

**Effect of GH on PMN Death in vitro**

Whether these anabolic hormones influence the fate of PMNs after incubation with bacteria is not well understood. Our recent study has revealed that pretreatment of human PMNs with GH or IGF-I decreased the percentage of PMNs with hypodiploid deoxyribonucleic acid (DNA), as analyzed by flow cytometry. This result suggests that both hormones may slow the rate of PMN cell death. Further study is needed to define the role of GH and IGF-I in the fate of PMNs.

**Conclusions**

GH and IGF-I, especially GH, promote the proliferation, maturation, mobilization, and migration of neutrophils. These hormones also enhance the activities of phagocytosis, killing and elimination of bacteria by increasing Fcγ receptor expression and opsonic activity. In addition, local production of cytokines is enhanced. Moreover, these hormones prevent PMN cell death after the invasion of bacteria. Administration of GH and IGF-I thus effectively improves host defense, through immunomodulation, against bacterial infection. These benefits of GH and IGF-I in critical surgical illness will be examined in the near future.

**References**


