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Growth Hormone & IGF Research 13 (2003) 282–286
www.elsevier.com/locate/ghir

Growth hormone enhances proinflammatory cytokine production by monocytes in whole blood

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Received 27 January 2003; received in revised form 6 May 2003; accepted 8 May 2003

Abstract

Growth hormone (GH) has been used as anabolic therapy to treat catabolic patients. In a recent study, however, administration of high doses of GH to critically ill adults was associated with an increase in morbidity and mortality. Preponderance of septic shock and uncontrolled infections as causes of death in these patients suggests an immuno-modulatory effect of GH. Our hypothesis was that GH treatment may modulate the production of proinflammatory cytokines, which are implicated in sepsis. In our study, human monocytes in whole blood were activated with lipopolysaccharide (LPS) (1–100 ng/ml) purified from a clinical isolate of group B *Neisseria meningitidis* in the presence of a high dose of GH (100 ng/ml). The subsequent proinflammatory cytokine response was analysed by intracellular cytokine staining and flow cytometry. Our results show that GH enhances IL1-α, IL-6 and TNF-α production by LPS activated monocytes in whole blood. The modulation of cytokines by GH may be responsible for the adverse consequences of GH in critically ill patients.

Keywords: Growth hormone; Cytokines; Monocytes; Whole blood

1. Introduction

Growth hormone (GH) has been used as anti-catabolic therapy to treat several different patient groups [1–8]. In a recent study, however, administration of high doses of GH to critically ill adults receiving prolonged intensive care was associated with an increase in mortality and morbidity [9]. The deleterious effects of GH are probably multifactorial and complex, but the preponderance of septic shock and uncontrolled infections as causes of death in these patients suggests that a modulation of immune function may have been involved. In healthy individuals, most of the cells in the immune system, including monocytes, express GH receptors [10]. Activation of proinflammatory cytokine production by monocytes is a crucial step in host defence in response to pathogens, but if exaggerated or inappropriate, may also result in severe tissue injury and septic shock [11]. The precise influence of GH on the immune system, modulating either through autocrine, paracrine or endocrine pathways still requires elucidation.

We studied the immunomodulatory capacity of GH in human monocytes in whole blood at single-cell level by flow cytometry. Human monocytes in whole blood were activated with meningococcal lipopolysaccharide (LPS) in the presence of a high dose of GH. Our data clearly show that GH is able to modulate the proinflammatory cytokine response by increasing IL-1α, IL-6 and TNF-α production by monocytes in response to LPS.

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doi:10.1016/S1096-6374(03)00034-0

2. Materials and methods

2.1. Stimulation of monocytes

Heparinised venous blood was diluted 1:2 in RPMI 1640. Monocytes were stimulated with purified meningococcal LPS (kind gift from Dr. Svein Andersen, EJIVR, Compton, UK) at 1, 10 or 100 ng/ml in the presence or absence of 100 ng/ml (0.0003 IU/ml) of human GH (Genotropin, kind gift from Pharmacia & Upjohn). Control monocyte preparations (no LPS) with and without GH were included. The dose of GH was chosen to reflect concentrations found in patients treated with high dose GH [9]. After 4 h in culture, protein transport inhibitor Brefeldin A (10 μg/ml, Sigma, Poole, UK) was added for additional 2 h.

2.2. Intracellular cytokine staining

In order to identify monocytes, 5 μl FITC-conjugated CD14 antibody or isotype control (Dako, Denmark) were added for 30 min. Erythrocytes were then lysed with FACS lysing buffer (Becton–Dickinson, San Jose, USA), and washed in PBS containing 0.5% bovine serum albumin and 0.02% sodium azide. The cells were then fixed in 4% paraformaldehyde for 15 min, washed with PBS and permeabilised in 50 μl Leucoperm (Serotec, Oxford, England) together with 8 μl PE-conjugated monoclonal antibodies to TNF-α, IL-6 or IL-1α (Becton–Dickinson) and isotype control for 30 min in the dark at RT. Finally, the cells were washed and fixed in Cellfix (Becton–Dickinson) and analysed by FACScalibur using Cellquest software (Becton–Dickinson). In each acquisition, at least 2000 events were collected.

2.3. Statistics

Our hypothesis was that the presence of GH with LPS modulates cytokine production. In detail this corresponds to three hypotheses. (1) Cytokine response is greater with GH than without, (2) the GH effect is greater with LPS present than absent, irrespective of dose, and (3) the GH effect with LPS present is independent of LPS dose. All three hypotheses were tested using repeated measures ANOVA. All tests were two-tailed with P values <0.05 considered as significant.

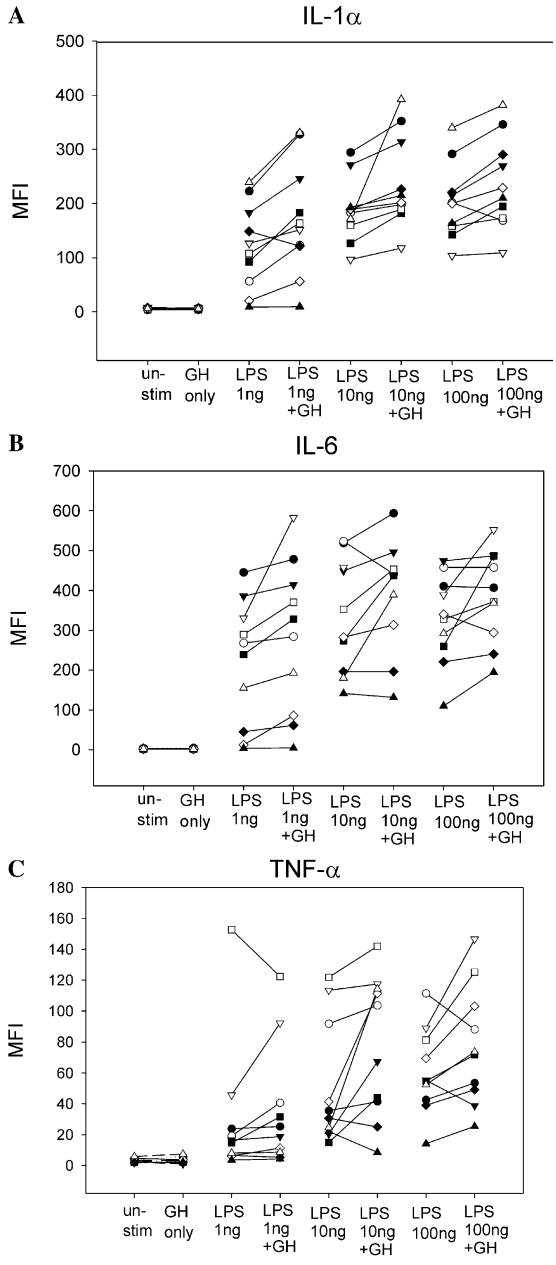

Fig. 1. Effect of growth hormone on cytokine production by monocytes activated with purified meningococcal LPS. Blood was cultured for 4 h with LPS (1–100 ng/ml) in the presence or absence of growth hormone, and then for additional 2 h in the presence of Brefeldin A. The cells were surface stained for CD14, permeabilised and stained intracellularly for IL1-α (A), IL-6 (B), TNF-α (C). The results were determined as median fluorescence intensity (MFI) of CD14 gated cells from ten separate experiments among six different blood donors. GH, growth hormone.

3. Results

3.1. Cytokine production by monocytes in the presence of growth hormone

After 6 h of stimulation by LPS and GH, the production of IL-1α, IL-6 and TNF-α by monocytes was analysed by intracellular staining (Fig. 1). The presence of GH increased the production of IL-1α (Fig. 1A), IL-6 (Fig. 1B) and TNF-α (Fig. 1C) in response to LPS, but the GH effect did not depend on the concentration of LPS. No cytokines were produced by unstimulated cells or by GH treatment alone.

Table 1 shows the corresponding P values obtained from analysis of variance. For all three cytokines it shows significant effects for GH compared to no GH

Table 1
Significance levels of three hypotheses about the effect of GH on cytokine production analysed by ANOVA

Hypothesis	IL-1α	IL-6	TNF-α
GH affects response	<0.0001	0.0003	0.002
GH effect greater with LPS present	0.004	0.03	0.07
GH effect depends on LPS dose	0.5	0.9	0.2

($P < 0.002$), but an insignificant concentration effect of LPS with GH ($P \geqslant 0.5$). A significant effect of GH with LPS compared to GH without LPS was found for IL-1α and IL-6 ($P \leqslant 0.05$). The modulatory effect of GH on TNF-α production in response to LPS was not statistically significant ($P = 0.07$). In addition, the overall levels of TNF-α, in particular at 1 ng/ml of LPS, were significantly lower than IL-1α and IL-6 as judged by the median fluorescence intensity (MFI).

3.2. Effect of brefeldin A on cytokine production by monocytes

Cytokines were detected intracellularly by blocking cytokine secretion with Brefeldin A. However, the results described above were only observed when Brefeldin A was added at least 4 h after the whole blood was stimulated with LPS and GH. When Brefeldin A was added simultaneously with LPS and GH no enhancement in cytokines was observed.

4. Discussion

In this study, we have shown that GH can enhance monocyte derived cytokine production induced by meningococcal LPS. The effects were most marked for the cytokines IL1-α and IL-6 and to a lesser extent TNF-α. No significant effect of LPS dose could be detected. GH itself did not induce cytokine production in this whole blood model.

Several studies have demonstrated that GH is able to modulate cytokine responses, but the data have been variable and inconclusive [12–17]. The most recent study failed to show an effect of GH on cytokine production by peripheral mononuclear cells (PBMC) in response to LPS [18]. Different experimental methodologies may have led to these conflicting results. Zarkesh-Esfahani et al. used only 0.01 ng/ml of LPS to stimulate the cells. Our study used much higher concentrations of LPS (1–100 ng/ml) in order to induce high levels of cytokines [19]. These levels correspond to those reported in gram negative sepsis in vivo [20,21] and severity of the meningococcal disease correlates with the concentration of LPS in the plasma [22]. Interestingly, a recent study has indicated a far higher bacterial load in patients than previously estimated in menigococcal sepsis [23]. In this study it was shown that the bacterial load in patients with severe disease reached as high as 10^8 organisms/ml of blood. Quantification of the LPS content in *N. meningitidis* based on spectrophotometric analysis shows that LPS at 100 ng/ml is equivalent to about 10^8 bacteria and would therefore represent the load of LPS in bacteria present in the severest of cases.

Previous studies have mainly used separated cells (PBMCs) or cell lines. Our model allowed for the study of monocytes within fresh whole blood. As the cells in whole blood do not need any further purification or isolation, artifactual stimulation of cells is avoided. In addition, most groups use ELISA methods to measure cytokines, whereas we have used intracellular cytokine staining by flow cytometry. Importantly, in our study, we observed that GH influenced cytokine production in the first 6 h of stimulation, whereas other studies have reported much longer time points. The timing of the assay is therefore of paramount importance, if changes in cytokine production are to be observed.

Not only did the modulatory effects of GH require analysis at early time points, but the timing of Brefeldin A was also found to be critical. When Brefeldin A was added to the cultures simultaneously with LPS and GH, no increase in the cytokine production could be detected, suggesting that protein secretion is necessary for GH action. We offer two possible explanations for this: (1) Brefeldin A, a protein secretion inhibitor, may interfere with GH receptor cycling mechanisms and therefore influence the availability of the receptors on the cell surface [24]. (2) Blocking the protein secretion by Brefeldin A may also interfere with a GH, mediated release of a unidentified mediator, thus preventing its effect on cytokine production.

The indirect effects of GH are mediated through insulin-like growth factor-I (IGF-I), which is frequently reduced in patients with sepsis. In the study by Takala et al., expected increases in serum IGF-I concentrations in response to GH were less frequently observed in non-survivors suggesting a continuing dissociation between GH and IGF-I, a feature more often observed in the acute phase of critical illness, rather than the prolonged chronic phase [25]. Proinflammatory cytokines including TNF-α, IL1-α and IL-6 can reduce the levels and activity of IGF-I [26–28]. In the light of our findings, the increased proinflammatory cytokine production by monocytes in response to GH could provide a mechanism by which IGF-I induction is reduced in critically ill patients. Interestingly, a recent study by Hansen et al. showed that GH increased

serum levels of mannose binding lectin (MBL), an innate immune defense plasma protein, which can bind to microbial surfaces activating the complement. This immunomodulatory effect of GH was shown to be IGF-I independent and the effect of GH on MBL and other acute binding proteins may be mediated through changes in proinflammatory cytokines [29,30].

In this report we have shown that monocytes activated by LPS in the presence of GH lead to increased proinflammatory cytokine production. The high mortality seen in septic animals [14] and critically ill patients [9] treated with GH could be related to the immunostimulatory effects of GH on monocyte cytokine production during sepsis. The immunomodulatory role of GH in the clinical scenario should therefore continue to be addressed.

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