Regulators of hepatic glucose and glycogen metabolism

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CHAPTER 5

Interferon-gamma has immunomodulatory effects with minor endocrine and metabolic effects in humans

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Abstract

To evaluate whether interferon-γ (IFN-γ) is involved in the interaction between the immune and endocrine systems in vivo, we studied six healthy subjects twice in a placebo-controlled trial: once after administration of recombinant human IFN-γ and, on another occasion, after administration of saline. The rate of appearance of glucose was determined by infusion of [6,6-²H₂]glucose and resting energy expenditure by indirect calorimetry. Human leukocyte antigen-DR gene expression on monocytes and serum neopterin increased after administration of IFN-γ (P<0.05 vs. control). IFN-γ increased serum interleukin-6 levels significantly. Levels of tumor necrosis factor-α remained below detection limits. IFN-γ increased plasma concentrations of ACTH and cortisol (P<0.05 vs. control), IFN-γ did not alter concentrations of growth hormone, (nor)epinephrine, insulin, C-peptide, glucagon, or insulin-like growth factor I. IFN-γ did not alter plasma concentrations of glucose and free fatty acids nor the rate of appearance of glucose. IFN-γ increased resting energy expenditure significantly. We conclude that IFN-γ is a minor stimulator of the endocrine and metabolic pathways. Therefore, IFN-γ by itself is probably not a major mediator in the interaction between the immune and the endocrine and metabolic systems.
**Introduction**

There is intensive interaction between the immune and endocrine systems. This interaction involves both inhibitory and stimulatory effects of hormones on the immune system and, conversely, stimulatory and inhibitory effects of the immune system on the endocrine system (7,12,18,19,35). Mediators like cytokines participate in the interaction between these two systems (3,5). In addition to effects on the immune system, tumor necrosis factor-α (IFN-α), interleukin-2 (IL-2), interferon-α (IFN-α), and IL-6 induce in humans profound endocrine and metabolic effects (4,6,25,26,30). Remarkably, despite more or less similar endocrine effects, the metabolic effects are different between the different cytokines. For instance, we and others observed that IFN-α, IFN-α, and IL-6 all increased lipolysis to a variable extent (6,25,26,30), whereas IL-2 inhibited lipolysis (4). The effect on glucose metabolism was also contradictory between the different cytokines, despite comparable changes in plasma concentrations of glucoregulatory hormones.

IFN-γ is a cytokine involved in different diseases such as viral infections and sepsis (20,32). However, the endocrine and metabolic effects of IFN-γ in humans *in vivo* have not been studied in any detail. Therefore, it is unclear whether IFN-γ is another cytokine involved in the interaction between the immune and endocrine systems.

To evaluate whether IFN-γ, besides immunomodulatory effects, also induces endocrine and metabolic effects, we studied the immunologic, endocrine, and metabolic effects of IFN-γ administration in healthy volunteers in a saline-controlled crossover study.

**Methods**

*Subjects:* six healthy men (age 22 ± 1 (SE) yr, weight 76.1 ± 3.5 kg, height 1.85 ± 0.03 m) participated in the study. All were in good health, did not experience any febrile disease in the month before the study, did not use any medication, and gave written informed consent. The study was approved by the Research Committee and the Medical Ethical Committee of the Academic Medical Center, Amsterdam.
Study design (Fig 1.): each subject was studied twice, with an interval of at least 4 wk. On one occasion the subjects received recombinant human (rh)IFN-γ, on the other occasion saline (control study). The order in which rhIFN-γ or saline was given was determined by balanced assignment. All volunteers consumed a weight-maintenance diet, containing at least 250 g of carbohydrates. The subjects fasted from 6:00 PM the day before the study until the end of the study. At 6:45 AM, a catheter was placed into an antecubital vein for infusion of stable isotope tracers. Another catheter was inserted retrogradely into a contralateral vein of a hand the subject inserted and kept within a thermoregulated (65°C) Plexiglas box for sampling of arterialized venous blood.

Fig 1. Study design. All subjects were studied twice, once after administration of recombinant human (rh) interferon (IFN)-γ and, on another occasion, after administration of saline. RQ, indirect calorimetry.

The catheters were kept patent by infusion of 0.65% NaCl (30 ml/h). During both studies the subjects were confined to bed. At 7:00 AM (t = -2 h) blood was sampled for determination of background enrichment, and a primed (17.6 μmol/kg), continuous (0.22 μmol/kg/min) infusion of [6,6-²H₂]glucose (Isotec, Miamisburg, OH) was started and continued until the end of each study (t = 12 h). At t = -15, -10, -5, and 0 min, blood samples for determination of isotope enrichment of glucose were drawn. Blood samples for baseline values of hormones, substrates, cytokines, and human leukocyte antigen (HLA)-DR gene expression on monocytes were drawn just before t = 0 min. At t = 0 min, rhIFN-γ (100 μg/m², Immukine,
Boehringer Ingelheim, Ingelheim/Rhein, Germany) or the same volume of saline was injected subcutaneously. At 1, 2, 4, 6, 8, 10, and 12 h after injection of rhIFN-γ or saline, blood was drawn for the measurement of isotope enrichment, hormone, substrate, and cytokine concentrations. Twenty-four hours after the injection of rhIFN-γ or saline, blood was drawn for determination of cytokine and neopterin serum levels. Blood samples taken at 4, 8, and 24 h after administration of rhIFN-γ were also analyzed for HLA-DR expression on monocytes. Blood pressure (Riva Rocci method, brachial artery), pulse rate (palpation of radial artery), and oral temperature (Terumo digital clinical thermometer C11, Terumo, Tokyo, Japan) were recorded hourly. Oxygen consumption and carbon dioxide production were determined every 2 h by indirect calorimetry, using the method of a ventilated hood (model 2900, computerized energy-measurement system, Sensor Medics, Anaheim, CA).

Assays: all measurements in each individual subject were performed in the same run, with the exception of flow cytometry analysis. All samples were tested in duplicate.

Glucose concentration and enrichment were determined according to Reinauer et al. (21), using phenyl-β-D-glucoside as an internal standard. The gas chromatography column used was a Heliflex AT-1 capillary column (30 m × 0.25 mm, film thickness (df) 0.2 μm) (Alltech, Deerfield, IL) on an HP 5890 series II gas chromatograph coupled to an HP 5989A mass spectrometer (Hewlett-Packard, Palo Alto, CA). Mass spectra were recorded at mass-to-charge ratio (m/z) 187 for glucose and m/z 189 for [6,6-²H₂]glucose. The internal standard was monitored at m/z 127 and 169.

Free fatty acids were determined by using the NEFA C kit (code no. 994-75409 E) from Wako Chemicals (Ncuss, Germany).

Plasma insulin concentration was measured by RIA (insulin RIA 100, Pharmacia Diagnostic, Uppsala, Sweden; intra-assay coefficient of variation (CV) 3-5%, interassay CV 6-9%), and C peptide was measured by RIA (RIA-coat c-peptid, Byk-Sangtec Diagnostica, Dietzenbach, Germany; intra-assay CV 4-6%, interassay CV 6-8%). Glucagon was determined by RIA (Linco Research, St. Charles, MO; detection limit 15 ng/l, intra-assay CV 3-5%, interassay CV 9-13%), insulin-like growth factor I by immunoradiometric assay after a modified acid-ethanol extraction procedure (DSL, Webster, TX; detection limit 5 nmol/l, intra-assay CV
2-4%, interassay CV 3-8%). Cortisol was measured by using a fluorescence polarization immunoassay (Abbott Laboratories, North Chicago, IL, intra-assay CV 6.4%, interassay CV 9.0%), ACTH by immunoluminometric assay (Nichols Institute, Los Angeles, CA; intra- and interassay CV 4.3 and 5.4%, respectively), and growth hormone by immunoluminometric assay (Nichols Institute; detection limit 1 mU/l, intra- and interassay CV 7.3 and 9.6%, respectively). Catecholamines were measured by an in-house HPLC method. Essentially, norepinephrine (inter- and intra-assay CV 13 and 6%, respectively) and epinephrine (inter- and intra-assay CV 14 and 7%, respectively) were selectively isolated by liquid-liquid extraction and derivatized to fluorescent components with 1,2-diphenylethlenediamine. The fluorescent derivatives were separated by reverse-phase liquid chromatography and detected by fluorescence detection (23,29).

IL-6 and TNF-α were determined by an ELISA (CLB, Amsterdam, The Netherlands), both with a detection limit of 2 pg/ml. IFN-γ was measured by using an ELISA with a detection limit of 31 pg/ml (16). Serum concentrations of neopterin were measured by RIA (IMMUtect Neopterin, Hennig, Berlin, Germany). HLA-DR expression was measured by using flow cytometry. Whole blood was lysed twice, using ammonium chloride (0.155 M) with K-EDTA, and was subsequently washed with PBS supplemented with bovine serum albumin (0.5% wt/vol), sodium azide (0.01% wt/vol), and potassium EDTA (0.5 mM; PBAP). Before and after these lysis steps, cells were fixed with paraformaldehyde, 0.5 and 2% wt/vol, respectively. Subsequently, Fc receptors were blocked by using human pooled serum (10% vol/vol) in PBAP. Then, cells were incubated with anti-HLA-DR monoclonal antibodies directly labeled with FITC (Becton-Dickinson, San Jose, CA). Irrelevant mouse monoclonal antibodies directly labeled with FITC were used as the control for background staining. After 30 min, the incubated cells were washed and suspended in PBAP. Cells were kept on ice during incubation periods. For washing procedures, cold media were used. Data acquisition was performed on a FACSScan flow cytometer (Becton-Dickinson). All data were saved. Analysis was stopped after 5,000 counts in the lymphogate had been measured. Monocytes were gated by forward-and side-scatter parameters.

Calculations and statistics: All data are presented as means ± SE. After administration of IFN-γ, the rate of appearance (Rₐ) of glucose was calculated by using Steele's equation for non-steady-state conditions adapted for stable isotopes
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The data were analyzed by analysis of variance for randomized block design and the Wilcoxon test to compare data at individual time points. P<0.05 was considered to represent statistical significance.

Results

Clinical effects of IFN-γ (Fig 2). IFN-γ caused an increase in temperature from 36.2 ± 0.2 to 36.9 ± 0.1°C (P<0.05 vs. control study) (Fig 2). Blood pressure was not different between the control and intervention studies, whereas the pulse rate increased after IFN-γ (from 59 ± 3 to 72 ± 3 beats/min) (P<0.05 vs. control study) (Fig 2).

Fig 2. Clinical effects of IFN-γ. Mean blood pressure (top), pulse rate (middle), and temperature (bottom) after rhIFN-γ administration (**) vs. saline administration (o) are shown. Values are means ± SE. bpm, Beats/min. ⭐ P<0.05 vs. corresponding value on control day.
IFN-\(\gamma\) plasma concentration (Fig 3). During the control study, IFN-\(\gamma\) levels remained below or just above the detection limit of the assay (31 pg/ml). In the intervention study, IFN-\(\gamma\) levels increased gradually to 518 ± 96 pg/ml after 6 h (P<0.05 intervention vs. control) (Fig 3). The plasma values of IFN-\(\gamma\) 24 h after IFN-\(\gamma\) injection were not different from pretreatment values. Effects of IFN-\(\gamma\) on plasma cytokine concentrations. IFN-\(\gamma\) induced a modest but significant rise in IL-6 levels, with a peak after 12 h (2 ± 1 (control) vs. 5 ± 1 pg/ml (IFN-\(\gamma\) study) (P<0.05)). On the other hand, TNF-\(\alpha\) levels were always below the detection limit of our assay (2 pg/ml).

Effects of IFN-\(\gamma\) on HLA-DR expression on monocytes and monocyte activation (Fig 3). IFN-\(\gamma\) induced a considerable change in HLA-DR expression on monocytes. After an initial decrease, mean fluorescence intensity of HLA-DR on monocytes increased from 84 ± 7 to 181 ± 34 arbitrary units at t = 24 h after IFN-\(\gamma\) administration (P<0.05 vs. t = 0 h). No significant changes were observed in the control study. Serum neopterin levels increased almost threefold after the administration of IFN-\(\gamma\) from 4.7 ± 0.7 to 11.9 ± 0.4 nmol/l after 24 h (P<0.05 vs. t = 0 h).

Endocrine effects of IFN-\(\gamma\) (Fig 4). Baseline hormone levels did not differ between the two studies. After administration of IFN-\(\gamma\), there was a modest, transient increase in ACTH and cortisol levels with a peak after 4 h (P<0.05 vs. control) (Fig 4). Insulin and C peptide gradually decreased in time during both studies (P<0.05 vs. t = 0 h), but no difference between the two study periods could be detected (Fig 5). There were no differences between the two studies in growth hormone, glucagon, epinephrine, and norepinephrine levels (Figs. 4 and 5). insulin-like growth factor I concentrations decreased significantly in time during both studies, but no IFN-\(\gamma\) effect was measurable in the intervention study.
Fig 3. Effects of subcutaneous IFN-γ administration on plasma concentration of IFN-γ and immunologic effects of IFN-γ. Plasma IFN-γ concentration (top), human leukocyte antigen (HLA)-DR expression on peripheral blood monocytes (middle), and serum neopterin concentration (bottom) after rhIFN-γ administration (•) vs. saline administration (○) are shown. Values are means ± SE. MFI, mean fluorescence intensity. ★ P<0.05 vs. corresponding value on control day.
Fig 4. Endocrine effects of IFN-γ. Plasma ACTH, cortisol, epinephrine, norepinephrine, and growth hormone concentrations (top to bottom, respectively) after rhIFN-γ administration (•) vs. saline administration (○). ★ P<0.05 vs. corresponding value on control day.
Effects of IFN-γ on substrates and energy metabolism (Fig 5). Baseline values did not differ between both study periods. Plasma glucose concentrations and $R_{a}$ glucose decreased during the control study (P<0.05 vs. t = 0 h). There was no effect of IFN-γ on plasma glucose concentration or $R_{a}$ glucose (Fig 5). Plasma free fatty acid concentrations increased during the control study from $0.52 \pm 0.08$ (baseline) to $0.97 \pm 0.20$ mmol/l (t = 12 h, P<0.05) (Fig 5). There was no effect of IFN-γ on free fatty acid concentrations (Fig 5). IFN-γ increased resting energy expenditure significantly at 6 h after IFN-γ administration by ~11% compared with the control study (1,867 ± 41 (control) vs. 2,064 ± 45 kcal/day (IFN-γ study) (P<0.05)) (Fig 5).

Discussion

In this study, the endocrine, metabolic, and immunologic effects of IFN-γ were evaluated in healthy humans. IFN-γ had clear effects on HLA-DR expression on monocytes in peripheral blood and on serum neopterin levels, both reflecting activation of monocytes and macrophages (27). IFN-γ also induced a slight but significant increase in serum IL-6. Despite these clear effects of IFN-γ on the immune system, there were only minimal effects on the endocrine and metabolic pathways. With the exception of a short-lived stimulation of the pituitary-adrenal axis, there were no endocrine effects of IFN-γ detectable. The metabolic effects of IFN-γ were limited to a small stimulation of resting energy expenditure by ~11% without any effect on glucose and fat metabolism. Therefore, we conclude that IFN-γ is not a major mediator between the immune and endocrine systems. Clinically irrelevant plasma concentrations of IFN-γ are not the explanation for the limited endocrine and metabolic effects observed in our study. The dose of IFN-γ in our study resulted in plasma levels of IFN-γ that are well within the range of those reported in several diseases. In acute falciparum malaria, IFN-γ levels were 123 ± 71 pg/ml, 215-396 pg/ml in human immunodeficiency virus infection, and 238-867 pg/ml in pneumonia (15,22,33,34). However, we cannot exclude that a higher dose of IFN-γ might have resulted in more pronounced endocrine and metabolic effects. Nonetheless, the purpose of our study was to evaluate pathophysiologically relevant, rather than pharmacological, effects of IFN-γ. IFN-γ
Fig 5. Effects of IFN-γ on glucose and fat metabolism. Plasma insulin, glucagon, glucose, and free fatty acid (FFA) concentrations and rate of appearance (Rₜₐ) of glucose (top to bottom, respectively) after rhIFN-γ administration (●) vs. saline administration (○). Values are means ± SE. There were no differences in any of the parameters.
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induced a marked increase in HLA-DR expression on monocytes in peripheral blood and a rise in neopterin serum levels, in accordance with previous in vivo and in vitro studies (1,2,14,17,27). Apparently, our IFN-γ levels were sufficient to induce immunologic effects but not to induce metabolic and endocrine alterations. It could be argued that a longer observation period could have revealed distinct influences of IFN-γ. However, metabolic alterations due to acute phase response-like reactions induced by cytokines take place after a short-term interval. A cytokine-mediated metabolic response after 12 h is not to be expected.

Our results are hard to compare with data from the literature because the endocrine effects of IFN-γ are scarcely investigated in humans and there are no data on metabolic effects. In accordance with our results, IFN-γ increased plasma cortisol levels 2-6 h after administration in two other studies in humans (8,24). The data on ACTH in human studies are contradictory. In accordance with our results, Goldstein et al. (8) also observed an ACTH peak after IFN-γ, whereas Holsboer et al. (9) did not find a ACTH peak despite an increase in plasma cortisol. In our study and the study by Goldstein et al. the cortisol peak coincided with the ACTH peak. These observations and those of Holsboer et al. suggest that the increase in plasma cortisol may not mainly be due to ACTH stimulation; it leaves open the possibility of a direct stimulating effect of IFN-γ on the adrenal gland. This assumption is supported by in vitro data (28). The effect of IFN-γ on ACTH secretion has also been studied in vitro. In rat anterior pituitary cells, homologous IFN-γ did not affect basal ACTH production but inhibited the stimulatory effect of corticotropin-releasing hormone on ACTH production (31). Therefore, it is unlikely that IFN-γ stimulates ACTH secretion directly. Alternatively, other factors can be involved. For instance, IFN-γ increased IL-6 production, which in turn stimulates ACTH secretion (26).

In contrast to IFN-γ, cytokines like TNF-α, IL-6, and IFN-α have major effects on endocrine and metabolic regulation in humans. Administration of TNF-α, IL-6, or IFN-α results in prolonged and massive stimulation of the pituitary-adrenal axis and secretion of glucagon and catecholamines without any effects on plasma insulin (6,26,30). These cytokines all stimulated lipolysis. Despite this massive and comparable endocrine response, IL-6 stimulates peripheral uptake of glucose, whereas the same response induces insulin resistance after TNF-α administration and without any influences on glucose metabolism after IFN-α. These effects of TNF-α, IL-6, and IFN-α coincide with an increase in resting energy expenditure.
The differences in endocrine and metabolic effects between IFN-γ and the other cytokines cannot be ascribed to differences in the amount of cytokine administrated. The molar amount of IFN-γ administered in the present study (6.1 nmol/m²) was higher than the amounts of TNF-α, IFN-α, and IL-6 administered in our previous studies in humans (2.8, 1.3, and 3.8 nmol/m², respectively) (6,26,30). The serum levels reached in these previous studies were in the same range as in the present IFN-γ study (IFN-γ 3.1 × 10⁻² pmol/ml vs. 2.8 and 6.2 × 10⁻³ pmol/ml for IL-6 and IFN-α, respectively). TNF-α levels were higher because of intravenous, bolus administration and are therefore not suited to this molar comparison (13).

The question arises as to whether the only metabolic effect induced by IFN-γ, an increase of ~11% in resting energy expenditure, may have clinical implications. No straightforward conclusion can be drawn, because it has been shown that an increase in resting energy expenditure by itself does not necessarily induce changes in body composition. This is exemplified by the metabolic changes found in the different stages of human immunodeficiency virus infection. In both the asymptomatic phase and in the symptomatic phase of this disease, an increase in resting energy expenditure by ~10% is found with major differences in other metabolic parameters between both disease stages (10,11). It can be concluded that the proinflammatory cytokine IFN-γ is not a stimulator of endocrine and metabolic pathways, at least in comparison with IL-6, IFN-α, and TNF-α. Therefore, IFN-γ by itself is probably not a major mediator in the interaction between the immune and the endocrine and metabolic systems. However, we cannot exclude the possibility that IFN-γ, along with other mediators released during infection, may have a synergistic effect on the endocrine and/or metabolic system.

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