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Growth Hormone Activation of Human Monocytes for Superoxide Production but not Tumor Necrosis Factor Production, Cell Adherence, or Action against *Mycobacterium tuberculosis*

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We have previously demonstrated that growth hormone (GH) is a human macrophage-activating factor which primes monocytes for enhanced production of H_2O_2 in vitro. This report extends our observations to other monocyte functions relevant to infection. We find that GH also primes monocytes for O_2^- production, to a degree similar to the effect of gamma interferon. Neither macrophage-activating factor alone stimulates monocytes to release bioactive tumor necrosis factor. However, GH, unlike gamma interferon, does not synergize with endotoxin for enhanced tumor necrosis factor production. In further contrast, GH does not alter monocyte adherence or morphology, while phagocytosis and killing of *Mycobacterium tuberculosis* by GH-treated monocytes are also unaffected. Therefore, despite the multiplicity of the effects of GH on the immune system in vivo, its effects on human monocytes in vitro appear to be limited to priming for the release of reactive oxygen intermediates.

In animal models, growth hormone (GH) appears to be necessary for development of the thymus and a fully competent immune system (2). In addition, administration of GH to hypopituitary animals restores many macrophage functions relevant to infection and inflammation. The macrophages from GH-treated animals release more superoxide (O_2^-) and tumor necrosis factor alpha (TNF-α) in response to the appropriate trigger stimuli and ingest *Listeria monocytogenes* better than macrophages from untreated animals (9–11). GH has also been shown to protect hypopituitary animals from lethal *Salmonella typhimurium* infections (10). In vitro, GH-treated porcine alveolar macrophages are microbicidal for *Pasteurella multocida* (8). However, the direct in vitro priming of a human phagocyte by GH for enhanced killing of any organism has not yet been reported.

GH also primes human phagocytes for enhanced reactive oxygen intermediate (ROI) production. Polymorphonuclear leukocytes of dwarfs show depressed respiratory burst activity which is restored by GH administration (33). In vitro, the polymorphonuclear leukocytes of normal donors can be primed not only by GH but also by prolactin (PRL) and insulin-like growth factor 1 for enhanced secretion of O_2^- (12, 13). In addition, normal human monocytes are activated for enhanced hydrogen peroxide release in response to GH or PRL (42). The reported production of GH and related factors by leukocytes suggests the possibility of these molecules acting as paracrine macrophage-activating factors (27, 44).

Killing of *Mycobacterium tuberculosis* by human monocytes or macrophages has been difficult to demonstrate in vitro. Priming human monocytes in vitro with gamma interferon (IFN-γ) enhances many functions of monocytes but it does not stimulate them to kill tubercle bacilli (7, 29–31, 37). In addition, IFN-γ mildly inhibits monocyte phagocytosis of this organism (7). Other mediators, such as vitamin D_3 (5, 31), only slightly limit the growth of *M. tuberculosis* in monocyte cultures. As the monocytes of acromegalic and hyperprolactinemic patients have been reported to kill another mycobacterium, *Mycobacterium avium*, better than those of normal donors (34), it is possible that GH could prime human monocytes to kill *M. tuberculosis* in vitro. We have therefore extended our studies of GH as a human macrophage-activating factor to examine priming for the enhanced release of O_2^- and bioactive TNF as well as for changes in adherence, phagocytosis, and killing of *M. tuberculosis.*

MATERIALS AND METHODS

Cell culture reagents and mediators. Unless otherwise specified, all reagents and mediators were obtained from Sigma (Poole, Dorset, United Kingdom). Mediators were stored in aliquots at −70°C unless otherwise indicated. RPMI 1640 medium, salt solutions, and fetal calf serum were purchased from ICN Flow (High Wycombe, Bucks, United Kingdom). Pooled human serum was obtained by defibrinating 20 ml of venous blood from each of 50 normal, healthy, nonclinical, nonlaboratory volunteers. Tissue culture plates (96 well; Becton Dickinson/Falcon, Oxford, United Kingdom) were coated with human plasma fibronectin (Fn) (Sigma) according to the manufacturer's recommendations in lipopolysaccharide (LPS)-free water (Phoenix Pharmaceuticals, Gloucester, United Kingdom). Recombinant human GH was a gift from Eli Lilly (Windlesham, Surrey, United Kingdom) and was reconstituted in ammonium bicarbonate buffer containing 0.1% bovine serum albumin, diluted in a glycine-bicarbonate buffer, freeze-dried, and stored at −70°C as described previously (42); aliquots were reconstituted in water and used as required. Recombinant human IFN-γ was purchased from BCL/Boehringer Mannheim (Lewes, E. Sussex, United Kingdom) as a 10^5-U/ml solution, and frozen aliquots were used as required. Polymyxin B (1 mg/ml; Gibco BRL/Life Sciences, Paisley, Scotland) was stored at −20°C. LPS (*Escherichia coli* 0011:B4; Sigma) was stored in solutions of 1 mg/ml in phosphate-buffered saline at −20°C. Thawed aliquots were diluted and vortexed for 3 min before use. The LPS content of all other reagents (as determined by the Coatest chromogenic *Limulus* amoebocyte lystate assay; KabiVitrum, Molndal, Sweden) was <5 pg per ml of endotoxin at the final concentration in culture, except that for fetal calf serum, which contained more than 100 pg of LPS per ml.

Separation, culture, and priming of human monocytes. Monocytes were separated and cultured as described previously (42). Briefly, 100 ml of defibrinated blood was dextran sedimented and the resulting leukocyte-plasma fraction was centrifuged over Nycodenz monocytes (Nycomed, Birmingham, United King-

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dom). Monocytes at the interface were washed and adhered to Fn-coated wells of a 96-well tissue culture plate. Nonadherent cells were removed, and the remaining monolayer was cultured in 200 μl of medium (RPMI 1640 containing 2% [vol/vol] pooled human serum and 2 mM L-glutamine). One hundred fifty microliters of medium per well was replaced with fresh medium, with or without mediators, every 24 h until the day before assay. The supernatant from the first 24 h of stimulation (between 24 and 48 h of culture) was saved at −70°C and later assayed for TNF.

Reduction of cytochrome *c* by superoxide. The microplate assay of Pick and Mizel (28) was employed with minor modifications. All reagents were purchased from Sigma, dissolved in Hanks balanced salt solution (except phorbol myristate acetate, which was dissolved in dimethyl sulfoxide), and stored in aliquots at −70°C. Monocytes were treated with mediators daily up to 48 h, and then cytochrome *c* reduction was assayed at 72 h. Immediately before the assay, cells were washed three times in Hanks balanced salt solution, always leaving a residual 50 μl of the solution. Cytochrome *c* solution containing 10 nM phorbol myristate acetate was added and the A_{550} was measured at time points up to 60 min. The amount of DNA per well was then estimated by using the fluorescent DNA-binding compound, bis-benzamide, as described previously (18, 42), except that DNA standards were diluted in the cytochrome *c* solution and assayed in parallel. Results were then normalized to nanomoles of O_2^- per 10^6 cells and expressed as percentages of the control to account for constitutive interdonor variation.

Assay of bioactive TNF. Monocyte cultures or human monocyte-like cell line THP-1 (40) cultures were first allowed to equilibrate in medium for 24 h at 37°C and then incubated in fresh medium for 24 h with either a test mediator or with LPS (positive control), with LPS plus polymyxin B (10 μg/ml), or in medium alone (background control). Supernatants were then collected and stored at −70°C until the assay. Freshly trypsinized L929 cells were allowed to adhere overnight at 37°C with 3×10^4 cells per well in microtiter wells and then incubated in TNF-containing medium (RPMI 1640 plus 5% fetal calf serum) in the presence of 1 μg of actinomycin D (Sigma) per ml for 20 to 24 h and assayed as described previously (19).

Monocyte killing of *M. tuberculosis*. The bacterium *M. tuberculosis* H37rv was prepared as described previously (41). Briefly, mouse-passaged stocks were thawed from liquid nitrogen and grown to log phase in Middlebrook 7H10 broth (Difco, East Molesley, Sussex, United Kingdom) plus glycerol and without Tween and aliquots were frozen at −70°C. Immediately before infection, an aliquot was thawed, washed, sonicated, and centrifuged to remove clumps, and then the resultant, predominantly single-cell suspension was diluted in RPMI 1640 to the desired working concentration.

Two antibacterial protocols were sequentially employed. The first procedure (6) involved culturing monocytes with or without mediators on glass coverslips for 24 h before infection. After 6 h of infection, the coverslips were transferred to fresh medium with or without mediators and incubated for 6 days and then were assayed for bacterial viability as described previously (6), except that extracellular bacteria were also assayed (41). In the second protocol, monocytes were plated on Fn-coated 96-well tissue culture plates as described above for the ROI assay. The monocytes were cultured in RPMI 1640 containing 2% pooled human serum and supplied with GH (0.3 to 3.0 μg/ml) on days 0, 3, 4, and 5 (by changing 150 of 200 μl of medium in the culture well) and were infected on day 6 and assayed on days 7 and 8. On day 6, all but 50 μl of medium was removed from the wells and 50 μl of RPMI 1640 containing *M. tuberculosis* (5×10^5 bacteria per well; bacterium-to-macrophage ratio, approximately 5 to 10:1) was added. The low ratio and small volume encouraged complete bacterial uptake. No attempt was made to dissociate extracellular and intracellular fractions in subsequent estimates of the fate of the viable bacteria in the wells because of the risk of dislodging cells that were potentially rendered differentially adherent by GH treatment; monocyte viability was greater than 90% in both treatment groups. No antibiotics were used to kill extracellular bacteria because of the risk of effects on intracellular bacteria in activated cells (25). Twenty-four to 48 h later, enough time to allow for uptake and killing of *M. tuberculosis* while minimizing the opportunity for death or growth of extracellular bacilli, the contents of each well were scraped with a 3- to 4-mm-diameter polytetrafluorethylene scraper and were transferred, with washings, to a tube containing 0.25% sodium dodecyl sulfate. This first dilution tube was sonicated three times for 3 s, and serial 10-fold dilutions were made with water. Dilutions were plated on 7H11 agar (Difco) and incubated for 3 weeks at 37°C before colonies were counted. Duplicate 96-well plates were prepared in parallel with this bacterial viability assay to assess relative numbers of total cell-associated bacteria by acid-fast staining (see below).

Phagocytosis and Ziehl-Neelsen staining. The contents of wells from plates which had been infected for 24 h were removed, and the cells were washed and stained to enumerate the total bacteria per cell. This was done by gently agitating and removing the medium and any suspended cells from a well to a 1.5-ml tube (Sarstedt). The wells were gently washed several times in medium. The washings were pooled, and the residual cells in the wells were resuspended in 50 μl of a Tris-based buffer (17) by incubating the plates at 37°C for 1 h with intermittent agitation. The contents were gently removed, and the wells were rinsed several times. Greater than 95% of the cells were recovered by this method. Everything removed from each well was pooled in a single tube and centrifuged at $100 \times g$ for 5 min. The pellet was resuspended in 1 ml of buffer and centrifuged again. The pellet was resuspended in a total of 15 μl of buffer, and 5 μl of cells was put onto each of three glass slides. Slides were then formalized, heat fixed, and Ziehl-Neelsen stained by an adaptation of the protocol of Allison and Hart (1). All samples were viewed with an oil-immersion microscope objective (magnification, 100×), and the number of acid-fast bacilli per macrophage was counted in 200 to 700 cells from four sets of random fields per well. Counting large numbers of cells per condition rendered negligible any random inaccuracies introduced by small numbers of cell-adherent, noninternalized bacteria which may not have been washed away prior to staining. This protocol, using infection with small sonicated inoculum for a short time period, also prevented clumps of bacteria from interfering with counting. The results were expressed both as percentages of cells infected and the numbers of bacteria per cell.

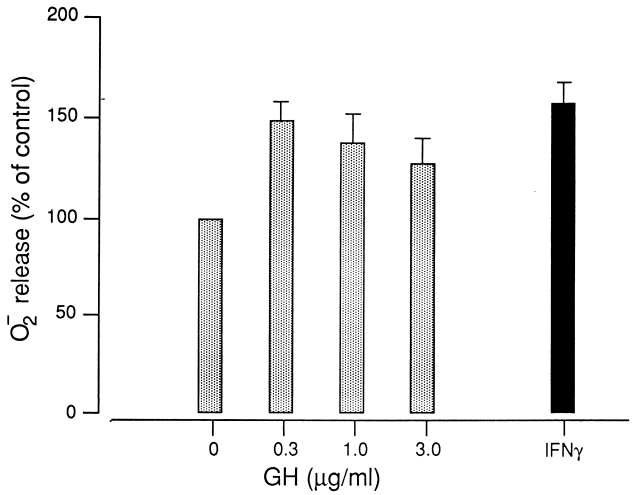

FIG. 1. O_2^- production by monocytes treated with either medium alone, GH, or IFN-γ (100 U/ml) for 72 h and then triggered with phorbol myristate acetate. The mean O_2^- production (in nanomoles per 10^6 cells per hour) in triplicate wells treated with the indicated mediators is expressed as a percentage of the mean from triplicate control wells receiving medium alone. Results shown are the means ± standard errors of the means for four experiments. GH treatment significantly enhanced O_2^- release ($P < 0.01$ by two-way analysis of variance). Control cells averaged 8.37 nmol per 10^6 cells per h.

RESULTS

GH primed human monocytes for elevated O_2^- production in response to phorbol myristate acetate. However GH did not affect monocyte adherence and morphology, secretion of bioactive TNF, or the uptake or killing of *M. tuberculosis*.

Enhanced superoxide production. Pretreatment with GH for 72 h activated monocytes for O_2^- production to a level similar to that obtained with IFN-γ (Fig. 1). A lower level of statistically significant enhancement was also obtained after 48 h of pretreatment. GH alone in cell-free wells did not reduce cytochrome *c*, and superoxide dismutase (300 U) completely abrogated O_2^- produced by GH-treated cells (not shown).

Secretion of TNF. Human monocytes that had been cultured for 24 h and then stimulated with LPS (30 to 100 ng/ml) for a further 24 h produced between 10 and 2,000 U of bioactive TNF per ml (Fig. 2) (supernatants collected at other time points between 4 and 72 h poststimulation did not contain greater levels of bioactivity). However, no other mediator stimulated the release of TNF which could be detected in this system. Mediators tested at a wide range of concentrations included GH (Fig. 2), IFN-γ (100 U/ml; 11 donors tested), insulin-like growth factor 1 (0.1 to 1,000 ng/ml; 5 donors tested), PRL (0.1 to 10 μg/ml; 2 donors tested), thyroid-stimulating hormone (0.01 to 100 μg/ml; 3 donors tested), and thyroid hormones (1 to 1,000 ng/ml; 3 donors tested) (data not shown). Unlike IFN-γ (23, 32), GH did not synergize with LPS at suboptimal concentrations (Fig. 2). The THP-1 cell line also

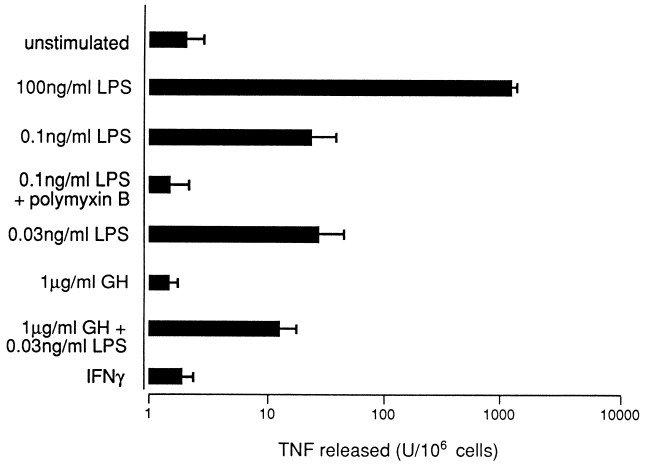

FIG. 2. GH treatment does not affect basal or LPS-stimulated TNF release by monocytes in vitro. Mediators were added to 1-day-old monocytes, and their supernatants were harvested 24 h later. The TNF bioactivities of the supernatants were assayed by their cytotoxicities for L929 cells in the presence of actinomycin D. Results shown are the means ± standard errors of the means for triplicate wells from a representative of seven donors.

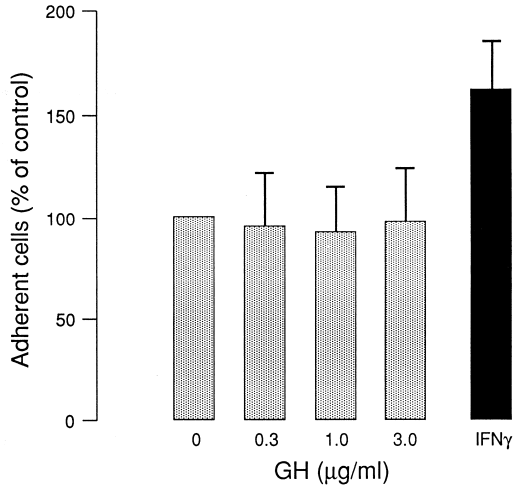

FIG. 3. Effect of IFN-γ but not GH on monocyte adherence to Fn. Fn-adherent cells were treated with fresh mediators daily for 72 h and washed, and the number of cells remaining per well was determined with the DNA-binding fluorochrome, bis-benzimide. The mean number of cells remaining adherent in triplicate wells treated with the indicated mediators was divided by the mean number of cells remaining in triplicate wells receiving medium alone. Results shown are the means ± standard errors of the means for 10 donors, and the differences between treatments are statistically significant by two-way analysis of variance for IFN-γ-treated cells ($P < 0.001$) but not for GH-treated cells ($P > 0.50$).

produced TNF in response to LPS but not to GH (data not shown).

Changes in morphology and adherence induced by monocyte-stimulating factors. Monocytes treated with IFN-γ for 24 to 48 h displayed increases in size, in spreading and extension of pseudopods on Fn, and in the number of perinuclear granules; LPS treatment induced similar changes, but cells were less spread, more granular, and more inclined to cluster (not shown). In contrast, no notable changes in morphology were caused by up to 7 days of GH treatment: monocytes remained rounded but irregular as if cultured in medium alone. Morphological appearance was reflected in the ability to remain adherent to Fn after 3 to 4 days of culture: adherence was enhanced by IFN-γ and unaffected by GH (Fig. 3).

Binding and killing of *M. tuberculosis*. In two separate protocols, one from the only published report of killing of *M. tuberculosis* by human monocytes (6) and the other which closely approximated the conditions used for the ROI assay, untreated and GH-treated cells were equally unsuccessful at limiting the growth of tubercle bacilli.

By the first protocol there was no difference in the number of cell-associated bacteria between untreated and GH-treated wells (Fig. 4), either after 6 h of phagocytosis (day 0) or after 6 days of culture. Numbers of bacteria that were not cell-associated (i.e., those in the supernatants and rinses) were unaffected by GH treatment (not shown). However, this system allowed for a number of possible artifacts (41). For example apparent cell-associated bacterial counts could be altered by possible GH effects on monocyte viability and adherence, although careful monitoring of the monolayers by phase contrast microscopy revealed no difference between the treated and untreated wells in the moderate loss of viability inflicted by infection (not shown). Additionally, *M. tuberculosis* survives poorly extracellularly in tissue culture medium containing human serum (30), and it would also be difficult to detect whether GH-treated monocytes were coordinately phagocytosing and killing more bacteria. The culture conditions may also have been unable to facilitate GH-mediated priming, which is sensitive to both the adhesive substrate and density of the cellular monolayer (42).

The second method was designed to avoid or minimize potential artifacts and to mimic conditions that allowed optimum priming with GH for ROI release. By this second protocol, which detected changes in both colony-forming units and acid-fast bacilli in wells treated with other combinations of cytokines (43), GH treatment again had no effect on the number of viable *M. tuberculosis* per well or on the number of total cell-associated bacteria (Fig. 5).

DISCUSSION

Although GH and IFN-γ both prime monocytes for enhanced ROI production, they elicit different spectra of effects on other monocyte functions.

GH-mediated priming of human monocytes in vitro for O_2^- release (Fig. 1) requires the same dose of GH (0.1 to 10.0

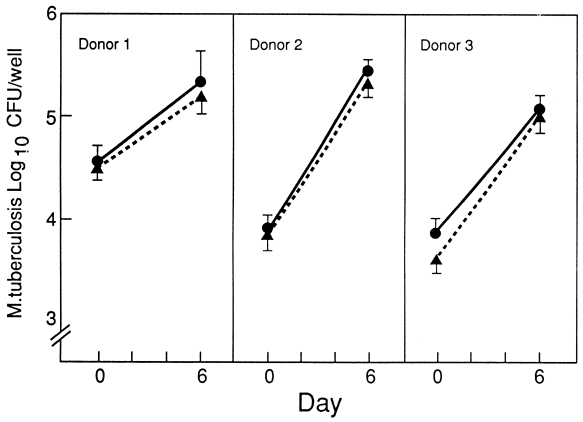

FIG. 4. Mycobacterial growth associated with glass-adherent monocytes from three donors. Each point represents the mean number of colony-forming units ± standard errors of the means for triplicate wells from either the day of infection or 6 days later. ●, untreated cells; ▲, GH (1.0 μg/ml)-treated cells.

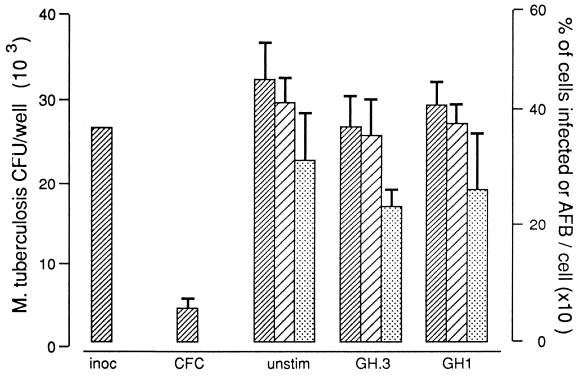

FIG. 5. Handling of *M. tuberculosis* by Fn-adherent, GH-treated monocytes. Fn-adherent monocytes were incubated in medium alone (unstim) or in 0.3 or 1.0 μg of GH per ml (GH.3 and GH1, respectively) for 6 days. Wells containing monocytes and those containing none (CFC [cell-free control]) were then given an inoculum of bacteria (inoc), and the total number of colony-forming units (▨) per well was assayed 24 h later. The number of acid-fast bacilli (AFB) per cell (▨) and the percentage of cells infected (▨) were assayed in parallel. Data shown are the means ± standard errors of the means for triplicate wells from one representative of four experiments (for colony-forming units) or of two experiments (for acid-fast bacilli per cell and percentage of cells infected). There were no significant differences ($P > 0.05$) between unstimulated and GH-treated wells.

μg/ml) as that required by porcine or rat macrophages (9) or human polymorphonuclear lymphocytes (13), and the magnitude of O_2^- released by monocytes is also similar; in all of these systems GH was at least as effective as IFN-γ. However, GH treatment is only able to prime monocytes to release approximately half as much H_2O_2 as IFN-γ-treated cells release (42). The reasons for the difference in effectiveness of these two mediators on different ROI species are not known.

Many cytokines may elicit at least some of their macrophage-activating effects via autocrine production of TNF-α (3, 32), which is arguably the main monokine stimulating macrophage and/or monocyte antimicrobial activity (14, 20). However, GH alone did not prime monocytes (Fig. 2) or the THP-1 monocyte-like cell line (not shown) for TNF production, which confirms earlier work by Edwards et al. (11) in which in vitro treatment of rat peritoneal exudate cells did not affect TNF-α release. Nevertheless, as these supernatants were assayed for bioactivity only, there is a possibility that GH does in fact stimulate TNF production but also coordinately stimulates factors (e.g., soluble receptors) which may inhibit TNF activity. In vitro GH treatment of peripheral blood mononuclear cells from AIDS patients is reported to increase TNF-α secretion (16). Paradoxically, in vivo effects appear to be opposite to those found in vitro: the administration of GH to hypophysectomized rats increased the subsequent release of TNF-α by their peritoneal exudate cells in vitro (11), and GH administration to AIDS patients has been reported to inhibit cachexia (15).

It is probable that TNF-α production requires (38) and that human immunodeficiency virus infection induces (26, 39) increased NF-κB activity in monocyte-like cells. Furthermore, IFN-γ synergizes with microbial products or other cytokines for enhanced NF-κB expression (22) and TNF-α secretion (21, 32). We therefore investigated the possibility that GH also potentiates TNF production. Coculture in GH did not affect the production of TNF bioactivity by monocytes treated with suboptimal doses of LPS (Fig. 2). Perhaps further manipulation of in vitro conditions will eventually reveal a protocol wherein GH synergizes with other factors for TNF production in vitro. For example, IFN-γ not only synergizes with other factors in coculture but also primes monocytes when applied 3 days prior to LPS exposure (32); IFN-γ synergizes with other factors in addition to LPS (21), etc. However, it is equally possible that GH does not directly stimulate TNF-α release but rather elicits the release of another paracrine agent in vivo which primes monocytes; e.g., a closely related cytokine, PRL, primes murine macrophages in vivo by, at least in part, increased IFN-γ release (4).

The absence of putative cofactors available in vivo but not in vitro may also partially account for the ineffectiveness of in vitro GH treatment in stimulating killing of *M. tuberculosis* by human monocytes (Fig. 4 and 5). In many earlier studies, thyroxine was necessary for full reconstitution of immunocompromised parameters by GH (2), and it may be that thyroid hormones or macrophage-activating steroids such as vitamin D_3 or retinoic acid are among the missing cofactors required for GH-mediated induction of these macrophage functions in vitro. It is nevertheless possible that even without such additional factors GH or PRL can stimulate human monocytes to kill organisms which may be more susceptible to ROI than *M. tuberculosis.*

It is likely that at least some of the differences between GH-activated and IFN-γ-activated monocytes can be related to differential effects on integrins. Phagocytosis of *M. tuberculosis* is believed to be mediated by complement receptors CR1, CR3, and CR4 (35); the last two are β_2-integrins deactivated by IFN-γ (36, 45). IFN-γ has also been reported to alter the adherence of monocytes to Fn (24). Identification of the differences in signalling pathways used by GH compared with those used by IFN-γ may help distinguish components which increase ROI production from those involved in decreased phagocytosis and enhanced *M. tuberculosis* growth in IFN-γ-activated cells (7) and may eventually allow selective manipulation of macrophage function in the treatment of disease.

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REFERENCES

1. **Allison, A. C., and P. D. Hart.** 1968. Potentiation by silica of the growth of Mycobacterium tuberculosis in macrophage cultures. Br. J. Exp. Pathol. **49:**465–476.
2. **Berczi, I.** 1986. The effects of growth hormone and related hormones on the immune system, p. 133–160. *In* I. Berczi (ed.), Pituitary function and immunity. CRC Press, Boca Raton, Fla.
3. **Bermudez, L. E. M., L. S. Young, and S. Gupta.** 1990. 1,25 dihydroxyvitamin D_3-dependent inhibition of growth or killing of *Mycobacterium avium* complex in human macrophages is mediated by TNF and GM-CSF. Cell. Immunol. **127:**432–441.
4. **Bernton, E. W., M. S. Meltzer, and J. W. Holaday.** 1988. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. Science **239:**401–404.
5. **Crowle, A. J., E. J. Ross, and M. H. May.** 1987. Inhibition by 1,25$(OH)_2$-vitamin D_3 of the multiplication of virulent tubercule bacilli in cultured human macrophages. Infect. Immun. **55:**2945–2950.
6. **Denis, M.** 1991. Killing of *Mycobacterium tuberculosis* within human monocytes: activation by cytokines and calcitriol. Clin. Exp. Immunol. **84:**200–206.
7. **Douvas, G. S., D. L. Looker, A. E. Vatter, and A. J. Crowle.** 1985. Gamma interferon activates human macrophages to become tumoricidal and leishmanicidal but enhances replication of macrophage-associated mycobacteria. Infect. Immun. **50:**1–8.
8. **Edwards, C. K., III, S. Arkins, L. M. Yunger, A. Blum, R. Dantzer, and K. W. Kelley.** 1992. The macrophage-activating properties of growth hormone. Cell. Mol. Neurobiol. **12:**499–509.
9. **Edwards, C. K., III, S. M. Ghiasuddin, J. M. Schepper, L. M. Yunger, and K. W. Kelley.** 1988. A newly defined property of somatotropin: priming of macrophages for production of superoxide anion. Science **239:**769–771.
10. **Edwards, C. K., III, S. M. Ghiasuddin, L. M. Yunger, R. M. Lorence, S. Arkins, R. Dantzer, and K. W. Kelley.** 1992. In vivo administration of recombinant growth hormone or gamma interferon activates macrophages:

enhanced resistance to experimental *Salmonella typhimurium* infection is correlated with generation of reactive oxygen intermediates. Infect. Immun. **60:**2514–2521.
11. **Edwards, C. K., III, R. M. Lorence, D. M. Dunham, S. Arkins, L. M. Yunger, J. A. Greager, R. J. Walter, R. Dantzer, and K. W. Kelley.** 1991. Hypophysectomy inhibits the synthesis of tumor necrosis factor α by rat macrophages: partial restoration by exogenous growth hormone or interferon γ. Endocrinology **128:**989–996.
12. **Fu, Y.-K., S. Arkins, G. Fuh, B. C. Cunningham, J. A. Wells, S. Fong, M. J. Cronin, R. Dantzer, and K. W. Kelley.** 1992. Growth hormone augments superoxide anion secretion of human neutrophils by binding to the prolactin receptor. J. Clin. Invest. **89:**451–457.
13. **Fu, Y.-K., S. Arkins, B. S. Wang, and K. W. Kelley.** 1991. A novel role of hormone and insulin-like growth factor-I. Priming neutrophils for superoxide anion secretion. J. Immunol. **146:**1602–1608.
14. **Grau, G. E., S. K. Parida, P. Pointaire, P. R. Barnes, and R. L. Modlin.** 1992. TNF and mycobacteria, p. 329–340. *In* B. Beutler (ed.), Tumor necrosis factors: the molecules and their emerging role in medicine. Raven Press Ltd., New York.
15. **Krentz, A. J., F. T. Koster, D. M. Crist, K. Finn, L. Z. Johnson, P. J. Boyle, and D. S. Schade.** 1993. Anthropometric, metabolic, and immunological effects of recombinant human growth hormone in AIDS and AIDS-related complex. J. Acquired Immune Defic. Syndr. **6:**245–51.
16. **Laurence, J., B. Grimison, and A. Gonenne.** 1992. Effect of recombinant human growth hormone on acute and chronic human immunodeficiency virus infection in vitro. Blood **79:**467–472.
17. **Lesniak, M. A., J. Roth, P. Gorden, and J. R. Gavin III.** 1973. Human growth hormone radio receptor assay using cultured human lymphocytes. Nature (London) **241:**20–22.
18. **Lowrie, D. B., and M. Fahmy.** 1987. Antimicrobial and hydrogen peroxide assays for MAFs, p. 227–258. *In* M. J. Clemens, A. G. Morris, and A. J. M. Gearing (ed.), Lymphokines and interferons: a practical approach. IRL Press, Oxford.
19. **Matthews, N., and M. L. Neale.** 1987. Cytotoxicity assays for tumour necrosis factor and lymphotoxin, p. 221–225. *In* M. J. Clemens, A. G. Morris, and A. J. M. Gearing, (ed.), Lymphokines and interferons: a practical approach. IRL Press, Oxford.
20. **Nacy, C. A., A. I. Meierovics, M. Belosevic, and S. J. Green.** 1991. TNFα: central regulatory cytokine in the induction of macrophage antimicrobial activities. Pathobiology **59:**182–184.
21. **Narumi, S., J. H. Finke, and T. A. Hamilton.** 1990. Interferon γ and interleukin 2 synergise to induce selective monokine expression in murine peritoneal macrophages. J. Biol. Chem. **265:**7036–7041.
22. **Narumi, S., J. M. Tebo, J. H. Finke, and T. A. Hamilton.** 1992. IFNγ and IL-2 cooperatively activate NFκB in murine peritoneal macrophages. J. Immunol. **149:**529–534.
23. **Nedwin, G. E., L. P. Svedersky, T. S. Bringman, M. A. Palladino, Jr., and D. V. Goeddel.** 1985. Effect of interleukin 2, interferon-gamma, and mitogens on the production of tumor necrosis factors alpha and beta. J. Immunol. **135:**2492–2497.
24. **Owen, C. A., E. J. Campbell, S. L. Hill, and R. A. Stockley.** 1992. Increased adherence of monocytes to fibronectin in bronchiectasis. Am. Rev. Respir. Dis. **145:**626–631.
25. **Patterson, R. J., and G. P. Youmans.** 1970. Multiplication of *Mycobacterium tuberculosis* within normal and "immune" mouse macrophages cultivated with and without streptomycin. Infect. Immun. **1:**30–40.
26. **Paya, C. V., R. M. Ten, C. Bessia, J. Alcami, R. T. Hay, and J.-L. Virelizier.** 1992. NF-κB-dependent induction of the NF-κB p50 subunit gene promoter underlies self-perpetuation of human immunodeficiency virus transcription in monocytic cells. Proc. Natl. Acad. Sci. USA **89:**7826–7830.
27. **Pellegrini, I., J.-J. Lebrun, S. Ali, and P. A. Kelly.** 1992. Expression of prolactin and its receptor in human lymphoid cells. Mol. Endocrinol. **6:**1023–1031.
28. **Pick, E., and D. Mizel.** 1981. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. J. Immunol. Methods **46:** 211–226.
29. **Robertson, A. K., and P. W. Andrew.** 1991. Interferon gamma fails to activate human monocyte-derived macrophages to kill or inhibit the replication of a non-pathogenic mycobacterial species. Microb. Pathog. **11:**283–288.
30. **Rook, G. A. W., J. Steele, M. Ainsworth, and B. R. Champion.** 1986. Activation of macrophages to inhibit proliferation of *Mycobacterium tuberculosis*: comparison of the effects of recombinant gamma-interferon on human monocytes and murine peritoneal macrophages. Immunology **59:**333–338.
31. **Rook, G. A. W., J. Steele, L. Fraher, S. Barker, R. Karmali, J. O'Riordan, and J. Stanford.** 1986. Vitamin D_3, gamma interferon, and control of proliferation of *Mycobacterium tuberculosis* by human monocytes. Immunology **57:**159–163.
32. **Rook, G. A. W., J. Taverne, C. Leveton, and J. Steele.** 1987. The role of gamma-interferon, vitamin D_3 metabolites and tumor necrosis factor in the pathogenesis of tuberculosis. Immunology **62:**229–234.
33. **Rovensky, J., J. Ferencikova, M. Vigas, and P. Lukac.** 1985. Effect of growth hormone on the activity of some lysosomal enzymes in neutrophilic polymorphonuclear leukocytes of hypopituitary dwarfs. Int. J. Tissue React. **7:**153–159.
34. **Sabharwal, P., B. Zwilling, R. Glaser, and W. B. Malarky.** 1992. Cellular immunity in patients with acromegaly and prolactinomas. Prog. Neuroendocrinimmunol. **5:**120–125.
35. **Schlesinger, L. S., C. G. Bellinger-Kawahara, N. R. Payne, and M. A. Horwitz.** 1990. Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement component C3. J. Immunol. **144:**2771–2780.
36. **Schlesinger, L. S., and M. A. Horwitz.** 1991. Phagocytosis of *Mycobacterium leprae* by human monocyte-derived macrophages is mediated by complement receptors CR1 (CD35), CR3 (CD11b/CD18), and CR4 (CD11c/CD18) and IFNγ activation inhibits complement receptor function and phagocytosis of this bacterium. J. Immunol. **147:**1983–1994.
37. **Steele, J., K. C. Flint, A. L. Pozniak, B. Hudspith, M. M. Johnson, and G. A. W. Rook.** 1986. Inhibition of virulent *Mycobacterium tuberculosis* by murine peritoneal macrophages and human alveolar lavage cells: the effects of lymphokines and recombinant gamma interferon. Tubercle **67:**289–294.
38. **Sung, S.-S. J., J. A. Walter, J. Hudson, and J. M. Gimble.** 1991. Tumor necrosis factor-α mRNA accumulation in human myelomonocytic cell lines. Role of transcriptional regulation by DNA sequence motifs and mRNA stabilization. J. Immunol. **147:**2047–2054.
39. **Suzan, M., D. Salaun, C. Neuveut, B. Spire, I. Hirsch, P. Le Bouteiller, G. Querat, and J. Sire.** 1991. Induction of NF-KB during monocyte differentiation by HIV type 1 infection. J. Immunol. **146:**377–383.
40. **Tsuchiya, S., M. Yamabe, Y. Yamaguchi, Y. Kobayashi, and T. Konno.** 1980. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int. J. Cancer **26:**171–176.
41. **Warwick-Davies, J., J. Dhillon, L. O'Brien, P. W. Andrew, and D. B. Lowrie.** 1994. Apparent killing of *Mycobacterium tuberculosis* by cytokine-activated human monocytes can be an artefact of a cytotoxic effect on the monocytes. Clin. Exp. Immunol. **96:**214–217.
42. **Warwick-Davies, J., D. B. Lowrie, and P. J. Cole.** 1995. Growth hormone is a human macrophage activating factor. Priming for enhanced release of H_2O_2. J. Immunol. **154:**1909–1918.
43. **Warwick-Davies, J., D. B. Lowrie, and P. J. Cole.** Selective deactivation of monocyte functions by TGFβ. J. Immunol., in press.
44. **Weigent, D. A., J. R. Baxter, W. E. Wear, L. R. Smith, K. L. Bost, and J. E. Blalock.** 1988. Production of immunoreactive growth hormone by mononuclear leukocytes. FASEB J. **2:**2812–2818.
45. **Wright, S. D., P. A. Detmers, M. T. C. Jong, and B. C. Meyer.** 1986. Interferon-γ depresses binding of ligand by C3b and C3bi receptors on cultured human monocytes, an effect reversed by fibronectin. J. Exp. Med. **163:**1245–1259.

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