INFECTION AND IMMUNITY, June 1992, p. 2514–2521
0019-9567/92/062514-08$02.00/0

Vol. 60, No. 6

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

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Received 2 December 1991/Accepted 27 March 1992

Purified and recombinant forms of growth hormone (GH) as well as of recombinant rat gamma interferon (IFN-γ) enhance the survival of rats deprived of endogenous pituitary GH secretion by hypophysectomy (HX rats) and infected with virulent *Salmonella typhimurium*. Macrophages obtained from rats with intact pituitaries (pituitary-intact rats) or HX rats that were treated in vivo with either GH or the closely related hormone prolactin released elevated ($P < 0.05$) levels of superoxide anion (O_2^-) after in vitro opsonized-zymosan stimulation compared with those from placebo-treated animals. These levels of O_2^- release were similar in magnitude to those of macrophages from rats treated in vivo with IFN-γ. In time course in vivo macrophage activation studies, both IFN-γ and GH significantly increased O_2^- secretion within 24 h, with maximal secretion occurring at day 3. Macrophages obtained from pituitary-intact and HX rats injected in vivo with GH also released elevated ($P < 0.05$) levels of hydrogen peroxide (H_2O_2) and displayed enhanced ($P < 0.01$) phagocytic activity toward opsonized *Listeria monocytogenes* in vitro. The mechanism of action of GH in vivo is likely to be a direct one because resident peritoneal macrophages from rats could be primed in vitro for enhanced secretion of O_2^- following triggering of these cells with opsonized zymosan. These data show that in vivo administration of two closely related pituitary hormones, GH and prolactin, can effectively prime macrophages, which is consistent with the hypothesis that GH mediates resistance to *S. typhimurium* by a direct stimulatory action on macrophages.

Macrophages can be triggered by substances such as opsonized particles and activators of protein kinase C to secrete reactive oxygen intermediates. These substances are key mediators of the inflammatory, microbicidal, and tumoricidal activities of macrophages (41). Macrophages from animals that have been infected with bacterial organisms show an enhanced production of microbicidal oxygen metabolites upon stimulation, a phenomenon known as priming (1). Activated macrophages are characterized by augmented secretion of reactive oxygen intermediates, so it is likely that priming is an important part of the process leading to development of activated macrophages (2). Gamma interferon (IFN-γ) is one well-characterized macrophage-activating factor that enhances in vitro and in vivo respiratory bursts of macrophages (34, 39) and increases host resistance to *Salmonella typhimurium* (23, 25, 32). IFN-γ increases the rate of transcription of the 91-kDa subunit of cytochrome b_{558} (22, 42), which is found in specific granules of phagocytic cells and is translocated to the phagosomal membrane during cellular activation (24). This protein is an essential component of superoxide-generating system of phagocytic cells that leads to the one-electron reduction of oxygen to superoxide anion (O_2^-).

Growth hormone (GH) and prolactin (PRL) are highly conserved members of the somatolactogenic gene family (43). Lipopolysaccharide as well as lipopolysaccharide-induced macrophage proteins such as interleukin 1 (IL-1) and tumor necrosis factor alpha alter the synthesis and release of a number of pituitary hormones, including GH and PRL (for reviews, see references 11 and 51). Receptors for both of these hormones have been cloned and recently designated as members of the newly defined cytokine receptor superfamily (10), which also includes receptors for IL-2, IL-3, IL-4, IL-6, granulocyte macrophage–colony-stimulating factor, and erythropoietin. Both GH and PRL appear to be synthesized by leukocytes as well as by the pituitary gland, specific receptors for both hormones have been identified on leukocytes, and both hormones affect a number of activities of T, B, natural killer, and myeloid cells (for reviews, see references 4, 27, and 56).

In order to compare the in vivo biological actions of GH and PRL to those of IFN-γ, we have conducted a series of experiments in which these compounds were administered in vivo to both rats with intact pituitaries (pituitary-intact rats) and hypophysectomized rats (HX rats). These experiments show that both native and recombinant GHs effectively enhance the survival of HX rats challenged with virulent *S. typhimurium*. Peritoneal macrophages from both normal and

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HX rats treated in vivo with IFN-γ, PRL, or different preparations of pituitary-derived or recombinant-derived GH are primed to release elevated levels of O_2^- and hydrogen peroxide (H_2O_2). These experiments demonstrate that GH administration in vivo results in many of the macrophage-activating microbicidal properties of the classically defined macrophage-activating factor, IFN-γ. The data also show that the enhanced resistance of both GH- and IFN-γ-treated rats to an experimental infection with *S. typhimurium* is correlated to the ability of macrophages from these animals to generate toxic oxygen metabolites.

MATERIALS AND METHODS

Animals. Specific-pathogen-free 6-week-old albino female Sprague-Dawley rats (Johnson Laboratories, Bridgeview, Ill.) that had been hypophysectomized at least 15 days earlier were used for either the in vitro studies with peritoneal macrophages or the in vivo macrophage activation experiments. Upon receipt, both normal (pituitary-intact) and HX rats were fed autoclaved laboratory chow (Purina, St. Louis, Mo.) ad libitum. Pituitary-intact rats were allowed sterile tap water ad libitum, and the HX rats were provided with a 0.15% salt solution supplemented with nominal concentrations of plasma cations (NaCl, 25.5 mM; KCl, 0.81 mM; $CaCl_2$, 0.34 mM; $MgCl_2$, 0.2 mM). HX animals weighed approximately 85 to 90 g at the start of the studies. They were maintained on a 12-h light-dark cycle at 68 to 70°F (20.0 to 21.1°C).

Peritoneal macrophage harvest and culture. Peritoneal macrophages were obtained according to previously published methodologies (12, 13, 21). Briefly, for in vitro and in vivo macrophage activation studies, pituitary-intact or HX rats were sacrificed by CO_2 suffocation and macrophages were lavaged from the peritoneal cavity with 60 ml of RPMI 1640 medium containing 100 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 24 mM $NaHCO_3$ (pH 7.4) (Sigma, St. Louis, Mo.). All cell culture reagents were determined to be free of endotoxin by a chromogenic limulus amoebocyte lysate assay (M. A. Bioproducts, Walkersville, Md.). Macrophages from four rats, all from the same treatment group, were pooled and washed twice in RPMI 1640 medium (400 × *g*, 15 min, 4°C). These cells were plated in 24-well Nunc (Intermed) tissue culture plates at a concentration of 2×10^6 to 5×10^6 cells per ml in 2 ml of RPMI 1640 medium supplemented with 10% low-endotoxin (<25 pg/ml) fetal bovine serum (HyClone, Logan, Utah).

After a 4-h incubation period, during which time the macrophages were allowed to adhere to plastic, the medium was aspirated and 1 ml of RPMI 1640 medium supplemented with 5% fetal bovine serum was added with or without recombinant rat IFN-γ (rRaIFN-γ) (specific activity = 4×10^6 U/mg of protein when tested on rat RATEC cells; Amgen, Thousand Oaks, Calif.), dissolved in sterile deionized distilled H_2O, or native, pituitary-derived rat growth hormone (nRaGH) (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK]) which was dissolved in 0.15 M NaCl–0.03 M $NaHCO_3$, pH 9.5. Peritoneal macrophages, which were >95% nonspecific α-naphthyl butyrate esterase positive, had >90% viability by trypan blue staining at the time of lavage. Macrophage cultures were incubated for 18 h (37°C, 5% CO_2), after which the supernatants were removed and wells were gently washed twice with Hank's balanced salt solution (HBSS) (M. A. Bioproducts) warmed to 37°C without Ca^{2+}, Mg^{2+}, or phenol red.

Superoxide anion (O_2^-) and H_2O_2 determination. Production of O_2^- was measured as previously described (12, 13, 21). In brief, following the 18-h culture, an assay mixture containing cytochrome *c* (Sigma) and opsonized zymosan (Op-zym) (Los Alamos Diagnostics, Los Alamos, N.M.) in HBSS with or without 50 μM superoxide dismutase (SOD) (ICN Immunobiologicals, Lisle, Ill.) was added to each culture of macrophages. Superoxide anion concentration was determined by using the molar extinction coefficient of $E_{550} = 21.0 \times 10^3\ M^{-1}\ cm^{-1}$. After the supernatant was removed, wells were washed twice with HBSS (37°C) and the protein content was determined by the method of Lowry et al. (31) by using bovine serum albumin as the standard.

The H_2O_2-generating activity of macrophages was determined by the peroxidase-dependent oxidation of phenol red (47, 48). A_{600} was determined on a Bio-Tek microtiter plate reader (Bio-Tek Instruments, Inc., Burlington, Vt.) with a 10-nm bandwidth filter. H_2O_2 content was determined by using its molar extinction coefficient of $E_{600} = 19.8 \times 10^3\ M^{-1}\ cm^{-1}$ (46). Protein content was determined as described above, and results were expressed as nanomoles of H_2O_2 per milligram of protein per 4 h.

Biological activity of native and recombinant Pituitary hormones. The biopotency of nRaGH; native, pituitary-derived rat prolactin (nRaPRL); or recombinant porcine GH (rPoGH) was measured on the basis of body weight gain and percent weight gain in normal or HX (Johnson Laboratories) female rats according to the method of Parlow et al. (45). Normal controls were from the same litter. All rats were kept in quarantine for another week before initiation of experiments to allow acclimation to the laboratory. Complete hypophysectomy was confirmed by both gross and histological examination, as previously described (28; examinations of 4-μm sections that were stained with hematoxylin and eosin). Peritoneal macrophages were collected from control and treated rats on day 10 (24 h after the last injection). In the time course studies of in vivo macrophage activation by placebo (Parlow's buffer), rRaIFN-γ, native pituitary-derived porcine GH (nPoGH), heat-inactivated nPoGH (70°C for 1 h), or porcine serum albumin (PSA) (Sigma), macrophages were harvested at several time periods and O_2^- release was measured 18 h later.

Infection experiments with *S. typhimurium*. Infection experiments with *S. typhimurium* were adapted to rats from a previously described mouse model (14). HX rats were challenged intraperitoneally with 0.6 to 0.8×10^6 *S. typhimurium* bacteria (strain 84-4728, a field isolate kindly supplied by B. O. Blackburn, USDA Infectious Disease Center, Ames, Iowa). *S. typhimurium*, previously passaged through young mice to enhance its virulence, were cultivated in Trypticase soy agar broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 8 to 12 h to a cell density of 5×10^9 cells per ml (A_{600} = 1.2 to 1.5). Infecting dose values for HX or normal rats were determined by classical methods (49).

Measurement of in vitro phagocytosis by activated macrophages. The phagocytosis assay was conducted by the method described by Zanetti et al. (60). Briefly, 10^7 CFU of a virulent rat *Listeria monocytogenes* (kindly supplied by David Hinrichs, VA Research Hospital, Portland, Oreg.) was opsonized by incubating bacteria with 10% fresh rat serum in a water bath at 37°C for 20 min, with agitation. Following centrifugation at 7,000 × *g* for 15 min, the bacteria were resuspended in phosphate-buffered saline, pH 7.4. One hundred microliters of this preparation was added to peritoneal macrophages obtained from HX or normal rats treated in vivo with placebo, rRaIFN-γ, or nRaGH and cultured

overnight in four-chamber Labtek slides (Miles Scientific, Grovepark, Ill.). Incubation of bacteria and macrophages was at 37°C, 5% CO_2 for 60 min to permit phagocytosis. After incubation, the microscope slides were washed twice with sterile HBSS (37°C), and the cells were stained with acridine orange (140 μg/ml in HBSS) for 45 s (37°C) and then briefly washed with HBSS to remove excess stain. To quench extracellular bacteria-associated fluorescence, the cells were incubated with crystal violet (500 μg/ml in HBSS) for 45 s (37°C) and then briefly washed with HBSS. Coverslips were applied to the slides and the edges were sealed with nail polish. The cell preparations were immediately examined with a Zeiss Photomicroscope at a magnification of ×630 with excitation filter BP 450-490, beam split mirror FT 510, and cutoff filter LP 520. Intracellular bacteria within each macrophage displaying orange or green fluorescence were quantitated. In each sample, six separate fields were examined, with each field consisting of 25 cells.

Statistics. A general linear model procedure was performed with the data by using SAS (50), and differences between means were assessed by using either a paired Student *t* test or Duncan's New Multiple Range Test for multiple treatment comparisons. Host resistance data for *S. typhimurium* were analyzed by using a contingency chi-square analysis test (50).

RESULTS

Recombinant IFN-γ and GH protect HX rats from lethal *S. typhimurium* infection. HX rats were injected for 12 days with rRaIFN-γ, native pituitary-derived GH from two different species, and rPoGH. Survival curves through 14 days postinfection are shown in Fig. 1. Chi-square analysis was performed as preplanned comparisons on day 7 after infection. By this time, all rats treated with buffer had died (survivor/total = 0/36 [0%]), whereas rRaIFN-γ (20/36 [56%]) and pituitary-derived GH of both porcine (10/24 [42%]) and bovine (9/24 [38%]) origin significantly enhanced ($P < 0.05$) survival rates. This beneficial effect of nPoGH could be directly attributed to GH because a recombinant form of this molecule (rPoGH) also significantly increased ($P < 0.05$) the survival rate at day 7 (8/12 [67%]). These data confirm our recent results that porcine GH increases host resistance to *S. typhimurium* (16) and extend these findings to bovine GH.

Rat macrophages are primed by in vivo injection of rRaIFN-γ, nRaGH, nRaPRL, or rPoGH. The next series of experiments was designed to address the question of whether macrophages from rats with intact pituitaries, and therefore normal levels of circulating GH, could be activated in vivo by injections of rat GH. We also wanted to learn whether the closely related hormone, PRL, was capable of priming macrophages from pituitary-intact and HX rats when injected in vivo. As a positive control, we tested HX rats injected with rRaIFN-γ or rPoGH, both of which have been previously shown to prime rat resident peritoneal macrophages in vivo (12). Data in Table 1 confirm that macrophages from HX rats treated for 9 days with rRaIFN-γ (500 U per rat per day) or rPoGH (48 μg per rat per day) in vivo release augmented amounts ($P < 0.05$) of O_2^- in vitro compared with those released by macrophages from placebo-treated rats. These data also extend earlier findings by showing that in vivo treatment with both nRaGH (48 μg per rat per day) and nRaPRL (48 μg per rat per day) significantly ($P < 0.05$) increases the release of O_2^- by peritoneal macrophages following stimulation with Op-zym in vitro. As

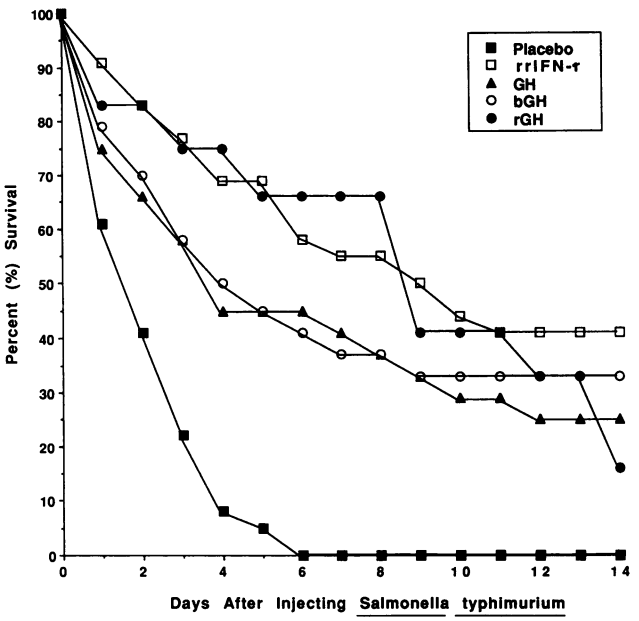

FIG. 1. GH and IFN-γ increase survival of rats following challenge with *S. typhimurium*. HX rats were injected intraperitoneally with 0.75×10^6 *S. typhimurium* organisms on day 0 following subcutaneous treatment for 6 days with 200 μl of buffer (placebo; 0.15 M NaCl and 0.03 M $NaHCO_3$, pH 9.5; $n = 36$); 500 U of recombinant rat IFN-γ ($n = 36$); or 48 μg of pituitary-derived porcine GH (GH; $N = 24$), pituitary-derived bovine GH (bGH; $n = 24$), or recombinant porcine GH (rGH; $n = 12$). These treatments were continued for another 6 days postinfection, and mortality was recorded at 12-h intervals. Statistical analysis by chi-square test at day 7 is presented in the text.

expected, both the native and recombinant forms of GH were biologically active, as determined by body growth. Neither rRaIFN-γ nor nRaPRL augmented body growth in HX rats.

To determine whether similar results could be obtained with rats with intact pituitary glands, normal rats were treated for 9 days with rRaIFN-γ (500 U per rat per day) or nRaGH, nRaPRL, or rPoGH (48 μg per rat per day). In contrast to results with HX rats, none of the hormone treatments resulted in activation of macrophages, as assessed by the release of less than 60 nmol of O_2^- per mg of protein per 4 h when triggered with Op-zym in vitro. Furthermore, the lower doses of GH were not biologically active as assessed by animal growth. Injection of 500 U of IFN-γ increased O_2^- secretion fivefold, although this increase was not statistically significant. However, macrophages from pituitary-intact rats could be significantly activated when the injected amount of rRaIFN-γ was increased 5-fold and the dose of each pituitary hormone was increased 10-fold (Table 1). The O_2^- levels released from macrophages of normal rats treated with these higher concentrations of each hormone were all significantly higher ($P < 0.05$) than those from rats treated with placebo and the lower dose of rRaIFN-γ, nRaGH, nRaPRL, or rPoGH. The higher dose of nRaGH and nRaPRL was also biologically active, as determined by significantly increased growth rates compared with those of placebo-treated rats, and this trend was similar for rPoGH. These data are the first to show that nRaGH as well as another member of the somatolactogenic gene family,

TABLE 1. Peritoneal macrophages from normal or HX rats treated in vivo with rRaIFN-γ, nRaGH, nRaPRL, or rPoGH release superoxide anion (O_2^-) in vitro after Op-zym stimulation[a]

Rat type and in vivo treatment	n	Superoxide anion (nmol of O_2^- mg of protein/4 h (mean ± SEM)	Wt gain (g) (mean ± SEM)	% wt gain (mean ± SEM)
HX rats				
Placebo (Parlow's buffer)	6	−32.1 ± 21.9[e]	2.3 ± 0.63[h]	2.3 ± 0.67[h]
rRaIFN-γ (500 U)	6	280.1 ± 25.9[a,b]	2.8 ± 0.52[h]	3.03 ± 0.6[h]
Rat GH (48 μg)	6	274.8 ± 24.3[a,b]	28.72 ± 0.70[f]	29.81 ± 0.79[d,e,f]
Rat prolatin (48 μg)	6	276.4 ± 32.5[a,b]	7.23 ± 0.42[h]	7.67 ± 0.47[h]
rPoGH (48 μg)	6	208.2 ± 32.9[b,c]	15.5 ± 1.8[g]	16.2 ± 1.9[g]
Pituitary-intact rats				
Placebo (Parlow's buffer)	6	24.9 ± 17.3[d,e]	48.7 ± 4.2[c,d,e]	32.6 ± 3.4[c,d,e]
rRAIFN-γ (500 U)	3	122.3 ± 29.8[c,d]	41.7 ± 3.9[e]	25.6 ± 2.3[f]
rRaIFN-γ (2,500 U)	3	231.3 ± 80.3[a,b]	49.7 ± 2.0[c,d]	35.2 ± 1.1[c,d]
Rat GH (48 μg)	3	9.2 ± 19.3[d,e]	48.4 ± 0.5[c,d,e]	29.5 ± 0.5[d,e,f]
Rat GH (500 μg)	3	345.1 ± 42.2[a]	61.2 ± 0.6[a]	43.4 ± 0.1[a]
Rat prolactin (48 μg)	3	21.0 ± 59.6[d,e]	52.1 ± 1.1[b,c]	31.8 ± 1.0[c,d,e,f]
Rat prolactin (500 μg)	3	307.2 ± 22.8[a,b]	57.9 ± 4.1[a,b]	41.2 ± 2.7[a,b]
rPoGH (48 μg)	3	60.1 ± 56.4[d,e]	44.2 ± 3.3[d,e]	26.6 ± 2.1[e,f]
rPoGH (500 μg	3	262.5 ± 20.8[a,b]	51.3 ± 4.7[b,c,d]	36.5 ± 2.8[b,c]

[a] Randomized incomplete block one-way analysis of variance was used to analyze results of O_2^- production induced by Op-zym that was inhibitable by SOD ($F = 11.0$; 18, 41 df; $P < 0.001$); daily weight gain (grams) ($F = 65.3$; 18, 41, df; $P < 0.001$) and percent weight gain treatments ($F = 42.3$; 18, 41 df; $P < 0.001$) values within a column with different superscripts are different ($P < 0.05$), as assessed by Duncan's New Multiple Range Test.

nRaPRL, are able to prime macrophages from normal rats when injected at biologically active doses in vivo.

Time course studies of peritoneal macrophage activation in HX rats treated in vivo with rRaIFN-γ or nPoGH. To assess the time course of macrophage activation when either IFN-γ or GH is injected in vivo, several groups of noninfected HX rats were treated for 9 days with buffer, rRaIFN-γ (500 U per rat per day), nPoGH (native porcine somatotropin; 48 μg per rat per day), PSA (48 μg per rat per day), or heat-inactivated nPoGH (48 μg per rat per day), a process which destroys over 60% of the bioactivity of GH (15). On days 0, 1, 3, 6, 9, and 12, peritoneal macrophages were obtained from the treated rats, and 18 h later the in vitro Op-zym-stimulated O_2^- levels were measured. On day 0, no significant differences could be detected among the five treatment groups (Fig. 2). Twenty-four hours later, macrophages obtained from rats injected with either rRaIFN-γ or nPoGH had minor but statistically significant increases in the secretion of O_2^- compared with macrophages from rats treated with placebo, heat-inactivated nPoGH, or PSA. By day 3, injections of both rRaIFN-γ and nPoGH maximally and significantly ($P < 0.05$) primed macrophages compared with any of the other treatments. Secretion of O_2^- in response to Op-zym remained significantly elevated ($P < 0.05$) for the following 9 days compared with results for either the placebo or PSA treatment group. It is also important that heat inactivation of nPoGH significantly attenuated priming induced by this molecule on each day of the experiment.

In vivo administration of GH increases secretion of H_2O_2. Although H_2O_2 is not a free radical, it can spontaneously lead to the formation of singlet oxygen and the hydroxyl radical. In the presence of myeloperoxidase, H_2O_2 readily forms hypochlorous acid. Therefore, we determined whether in vivo treatment with GH for 9 days would augment the ability of macrophages to secrete H_2O_2 as well as O_2^-. As shown in Table 2, nRaGH (48 μg/day) increased ($P < 0.05$) the secretion of H_2O_2 (to 238 ± 19 nmol/mg of protein per 4 h) compared with that of placebo-treated HX rats (42 ± 14 nmol/mg of protein per 4 h). Similar results were obtained when 10-fold-higher concentrations of nRaGH were injected into pituitary-intact rats.

Enhanced in vitro phagocytosis by macrophages activated in vivo by rRaIFN-γ or GH. Macrophages from normal or HX rats treated in vivo for 9 days with rRaIFN-γ or nRaGH (at concentrations shown to induce body growth, as described above) had a significantly enhanced ($P < 0.05$) ability to phagocytize opsonized *L. monocytogenes* in vitro (Table 3). On average, each macrophage from these two groups ingested seven more bacteria than macrophages from rats on the placebo treatment.

Rat peritoneal macrophages can be directly primed in vitro by nRaGH. Although these in vivo experiments clearly establish that nRaGH primes peritoneal macrophages to release elevated levels of O_2^-, it is unknown whether nRaGH can act like rRaIFN-γ to directly stimulate O_2^- secretion by rat macrophages in vitro. Therefore, it was important to learn whether GH priming of rat macrophages to release O_2^- could be induced directly by incubating macrophages with GH in vitro. In three separate experiments (Table 4), resident peritoneal macrophages from HX rats incubated in vitro with nRaGH for 18 h released three- to sixfold larger amounts ($P < 0.05$) of O_2^- upon Op-zym stimulation than macrophages stimulated with Op-zym alone. These levels of O_2^- were comparable to the in vitro priming effects of rRaIFN-γ. All responses were blocked by SOD, for which O_2^- is the only known substrate.

DISCUSSION

In this study, a number of experiments were conducted to evaluate the in vivo roles of IFN-γ and GH in activating macrophages. It is concluded that both agents increase the resistance of rats to lethal infection with *S. typhimurium* and that this effect is directly related to the ability of both IFN-γ

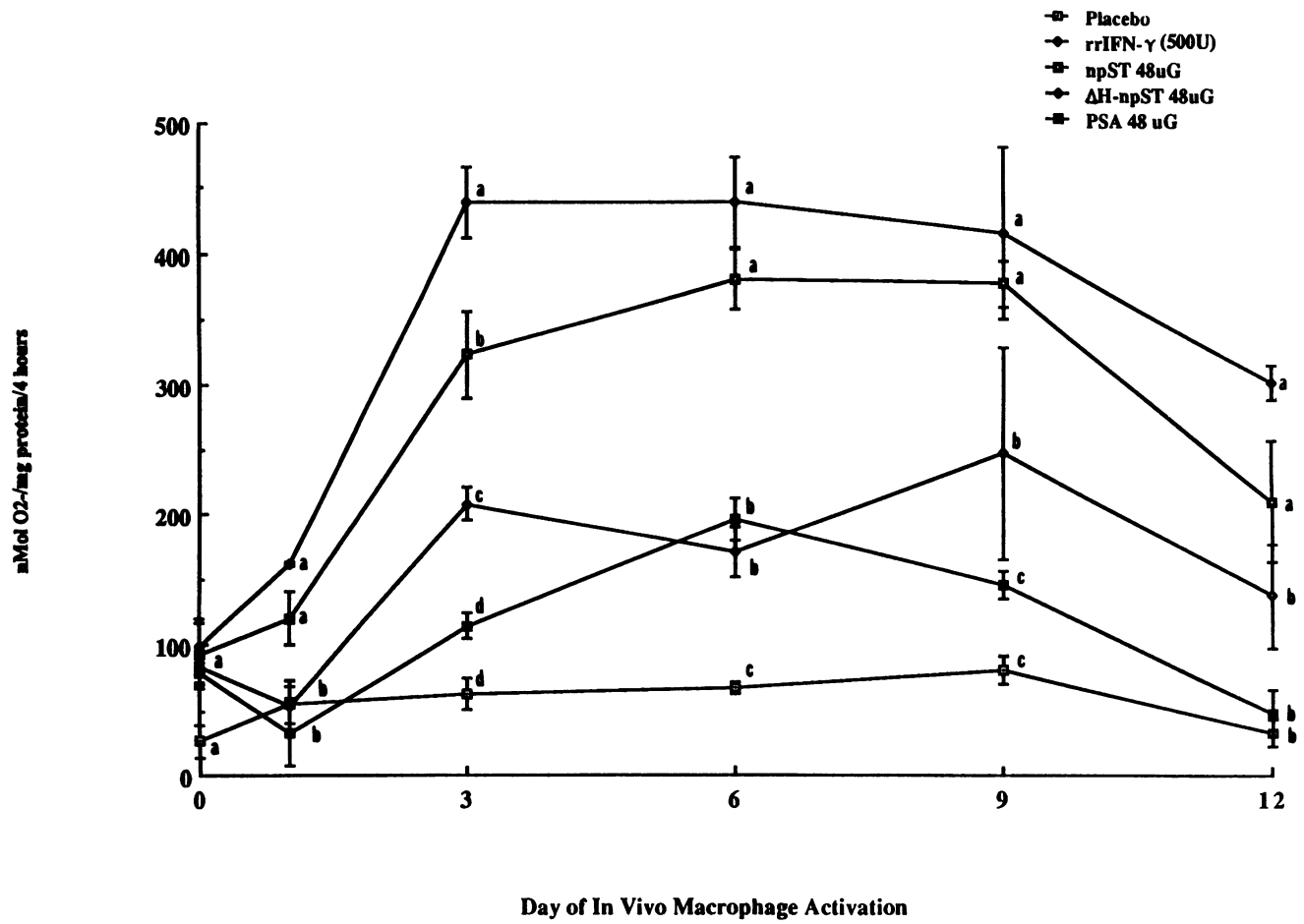

FIG. 2. Time course of peritoneal macrophage activation in HX rats treated in vivo with IFN-γ or GH. One-way analysis of variance was used to analyze results of in vivo macrophage O_2^- release at each time period that was induced by Op-zym and that was inhibitable by SOD. Results are as follows. Day 0, $F = 0.9$; 6, 8 df; $P = 0.55$. Day 1, $F = 8.76$; 6, 8 df; $P < 0.004$). Day 3, $F = 35.6$; 6, 8 df; $P < 0.001$. Day 6, $F = 30.7$; 6, 8 df; $P < 0.001$. Day 9, $F = 6.5$; 6, 8 df; $P < 0.001$. Day 12, $F = 10.6$; 6, 8 df; $P < 0.002$. Animals ($n = 3$ per day per treatment) were treated for 9 consecutive days with rRaIFN-γ (rrIFN-γ; 500 U per rat per day), nPoGH (npST; 48 μg per rat per day), or PSA (48 μg per rat per day). Means within each time point having different letters are significantly different at $P < 0.05$ by Duncan's New Multiple Range Test. Placebo (200 μl per rat per day) or heat-inactivated nPoGH (ΔH-npST; 48 μg per rat per day) served as controls in this experiment.

and GH to activate macrophages. These conclusions are based on the findings that (i) biologically active GHs of both bovine and porcine origin can protect rats from lethal infection with *S. typhimurium* as well as IFN-γ can; (ii) IFN-γ and GH as well as the closely related hormone PRL all prime peritoneal macrophages to release augmented amounts of O_2^- when these substances are injected in vivo; (iii) maximal macrophage priming by either IFN-γ or GH occurs within 3 days; (iv) GH injections also activate macrophages as assessed by both phagocytosis and the secretion of H_2O_2; and (v) GH can directly prime rat macrophages in vitro, which is consistent with a direct action of GH on macrophages.

TABLE 2. Peritoneal macrophages from HX or normal rats treated in vivo with nRaGH release H_2O_2[a]

Rat type and in vivo treatment	*n*	nmol of H_2O_2/mg of protein/4 h (mean ± SEM)
HX rats		
Placebo (Parlow's buffer)	3	42.2 ± 14.2[b]
Rat GH (48 μg)	3	238.1 ± 18.6[a]
Pituitary-intact rats		
Placebo (Parlow's buffer)	3	32.5 ± 14.9[b]
Rat GH (500 μg)	3	242.0 ± 27.5[a]

[a] One-way analysis of variance was ued to analyze H_2O_2 production induced by Op-zym that was inhibitable by catalase ($F = 35.9$; 3, 8 df; $P < 0.05$). Values with different superscripts are different ($P < 0.05$) by Duncan's New Multiple Range Test.

We recently demonstrated that the pituitary gland is required for rats to effectively resist lethal infection with virulent *S. typhimurium* (16). Since macrophages are critical for host defense against *S. typhimurium*, those studies also assessed the in vitro bactericidal capability of macrophages from both pituitary-intact and normal rats that had been injected in vivo with either IFN-γ or GH. Those experiments demonstrated that rat macrophages activated in vivo with either GH or IFN-γ had a significant 4- to 10-fold increase in their capability to kill *S. typhimurium* in vitro. More importantly, the bactericidal activity of macrophages was significantly blocked by including either SOD or catalase in the

TABLE 3. Peritoneal macrophages from HX or normal rats treated in vivo with rRaIFN-γ or nRaGH have enhanced phagocytic activity toward opsonized *L. monocytogenes*

Rat type and in vivo treatment	*n*	No. of *L. monocytogenes* organisms/macrophage (mean ± SEM)[a]
Hx rats		
Placebo (Parlow's buffer)	3	12.0 ± 1.7[b]
rRaIFN-γ (500 U)	3	19.3 ± 1.5[a]
nRaGH (48 μg)	3	19.3 ± 2.1[a]
Pituitary-intact rats		
Placebo (Parlow's buffer)	3	11.7 ± 1.2[b]
rRaIFN-γ (2,500 U)	3	19.3 ± 1.5[a]
nRaGH (500 μg)	3	16.7 ± 3.5[a]

[a] One-way analysis of variance was used to analyze results of in vitro phagocytosis ($F = 6.9$; 7, 10 df; $P < 0.001$). Values with different superscripts are different at $P < 0.05$ by Duncan's New Multiple Range Test.

incubation medium. These results suggested, but did not prove, that the bactericidal activity of rat macrophages depended on the ability of IFN-γ and GH to stimulate the secretion of toxic oxygen products. Experiments in the present report strongly support this interpretation because in vivo administration of IFN-γ or various forms of biologically active GH increased host resistance to *S. typhimurium* while also enhancing the ability of macrophages from these animals to secrete O_2^- and H_2O_2 and to phagocytize opsonized microorganisms. The mechanism of action of both substances seems to be direct because both IFN-γ and GH primed rat peritoneal macrophages directly in vitro for enhanced secretion of O_2^-. Therefore, these data offer strong support for the idea that both IFN-γ and GH act in vivo to increase host resistance to *S. typhimurium* by activating macrophages to an antibacterial state.

The finding that rRaIFN-γ enhances macrophage killing by respiratory burst oxygen intermediates is similar to those of several investigators (33, 35–38, 40). Lepay et al. (30) specifically showed that cell-mediated immunity in a murine listeriosis model is correlated with an influx of macrophages capable of generating reactive oxygen intermediates, and Buchmuller-Rouiller and Mauel (7) demonstrated that macrophage oxygen metabolism constitutes a necessary prerequisite for efficient bacterial killing. Furthermore, the ability of scavengers of reactive oxygen intermediates to partially block the killing of *S. typhimurium* by macrophages (16) suggests, at least for rats, the involvement of an oxygen-dependent killing mechanism (17).

Since the role of oxygen-independent macrophage killing was not addressed, the possibility that GH and PRL may also up-regulate lysosomal enzymes that are also important in killing intracellular pathogens cannot be excluded (20, 44, 52). Alternative viewpoints have also been expressed by other investigators who suggest that IFN-γ activates peritoneal macrophages for tumoricidal activity but not bactericidal activity (8); that IFN-γ cannot induce macrophage antibacterial activity against *L. monocytogenes* and *S. typhimurium* (29, 54, 55); that in vitro IFN-γ-induced macrophage *Salmonella* killing is mainly due to an oxygen-independent killing mechanism (25); and, finally, that bacterial lipopolysaccharide is needed in addition to IFN-γ to afford the host enhanced protection (32). Perhaps these disparate results are due to the use of a *S. typhimurium* strain with enhanced or decreased macrophage virulence (18, 57), the age of growth phase bacterial culture (3), the existence of resistance plasmids (53), the enhanced expression of selected *Salmonella* heat shock proteins (6), or species differences between mice and rats (26).

The newly defined family of receptors for a number of cytokines include PRL as well as GH receptors. All of these receptors contain the conserved feature of two pairs of cysteines in their extracellular domain and include receptors for IL-2, IL-3, IL-4, IL-6, granulocyte macrophage–colony-stimulating factor, and erythropoietin (10). Because of the strong homology between GH and PRL receptors, it was important to learn whether these closely related hormones would act similarly to prime macrophages in vivo for an enhanced respiratory burst. In addition to GH, PRL has also been shown to increase the resistance of rodents to lethal infection with *L. monocytogenes* (5). Very recently, PRL has been shown to prime human neutrophils for O_2^- secretion (19) and to be critically involved in the IL-2-driven proliferation of T lymphocytes (9). The priming effect of PRL in vitro is similar to that of GH as identified by us (19) and others (58, 59). Data in the present experiments clearly establish that both GH and PRL prime phagocytic cells in vivo in a manner similar to that of IFN-γ. Even though IFN-γ is much more effective than GH on a molar basis, GH does prime phagocytic cells at concentrations that augment body growth in HX rats. Although 10-fold-higher concentra-

TABLE 4. Peritoneal macrophages from HX rats treated in vitro with either rRaIFN-γ or nRaGH are primed to release superoxide anion (O_2^-) upon stimulus with Op-zym[a]

In vivo treatment	Expt 1		Expt 2		Expt 3	
	n	nmol of O_2^-/mg of protein/4 h (mean ± SEM)	*n*	nmol of O_2^-/mg of protein/4 h (mean ± SEM)	*n*	nmol of O_2^-/mg of protein/4 h (mean ± SEM)
No stimulation	2	80.6 ± 76.4[b]	5	−19.4 ± 20.1[b]	5	40.5 ± 17.0[d,e]
Stimulation with:						
Op-zym	2	124.7 ± 85.1[b]	5	46.4 ± 29.2[b]	5	81.0 ± 11.4[c,d]
Op-zym + SOD	2	62.4 ± 89.1[b]	5	14.9 ± 24.0[b]	5	−21.9 ± 10.1[f]
rRaIFN-γ + Op-zym	2	953.6 ± 160.1[a]	5	183.0 ± 18.8[a]	5	312.2 ± 22.8[a]
rRaIFN-γ + Op-zym + SOD	2	36.6 ± 14.8[b]	5	37.4 ± 28.3[b]	5	120.7 ± 28.2[c]
nRaGH + Op-zym	2	751.1 ± 313.9[a]	5	184.5 ± 18.2[a]	5	272.3 ± 25.9[a]
nRaGH + Op-zym + SOD	2	40.9 ± 17.8[b]	5	26.2 ± 18.4[b]	5	174.4 ± 29.4[b]

[a] One-way analysis of variance was used in each experiment to analyze the O_2^- production induced by Op-zym that was inhibitable by SOD. Experiment 1, $F = 5.62$; 7, 6 df; $P < 0.001$. Experiment 2, $F = 9.74$; 10, 24 df; $P < 0.001$. Experiment 3, $F = 31.2$; 11, 28 df; $P < 0.001$. rRaIFN-γ was used as 25 nM and nRaGH was used at 45 nM. Values with different superscripts are different at $P < 0.05$ by Duncan's New Multiple Range Test.

tions of hormone were required in pituitary-intact rats than in HX rats, 10-fold-higher concentrations of hormone were also required to induce growth in pituitary-intact rats. Coupled with the finding that heat inactivation inhibits the ability of GH to prime phagocytic cells in vivo (Fig. 2), it appears that the ability of GH to activate macrophages is directly related to its classic biological activity of inducing animal growth. These data therefore establish that some members of the somatolactogenic gene family are important for elevating cell-mediated immunity in vivo as assessed by host protection experiments, phagocytosis, and O_2^- and H_2O_2 secretion.

ACKNOWLEDGMENTS

We thank S. Raiti of the NIDDK for supplying native, pituitary-derived rat GH and PRL; A. F. Parlow for supplying native, pituitary-derived porcine and bovine GH; IMC Pitman-Moore, Inc., for furnishing recombinant porcine GH; L. M. Martin, J. M. Schepper, and D. Brunke for technical assistance; and D. L. Hinrichs for supplying *L. monocytogenes*.

This research was supported in part by grants from the National Institutes of Health (AG06246) and the U.S. Department of Agriculture (89-37265-4536) to Keith W. Kelley.

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