Characterization of the Priming Effect by Pituitary Canine Growth Hormone on Canine Polymorphonuclear Neutrophil Granulocyte Function

THOMAS K. PETERSEN,1* C. WAYNE SMITH,2 AND ASGER L. JENSEN1

Department of Clinical Studies, Central Laboratory, The Royal Veterinary and Agricultural University, Copenhagen, Denmark,1 and Section of Leukocyte Biology, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 770302

Received 20 June 1999/Returned for modification 21 September 1999/Accepted 6 January 2000

In this report, we demonstrate that canine growth hormone (cGH) is capable of priming canine polymorphonuclear neutrophil granulocytes (PMN) in a manner resembling that of human PMN. The cGH influences important functions that are involved in the process of recruitment of PMN, i.e., shape change, chemotaxis, CD11b/CD18 expression, adhesion, and subsequent transendothelial migration. Also, intracellular O2− production was evaluated. We investigated the priming effect by incubating PMN with purified pituitary cGH at various concentrations (10 to 800 μg/liter). The capacity for shape change was significantly (P < 0.05) enhanced, whereas the chemotactic response under agarose was significantly (P < 0.05) reduced. The chemotactic migration in Boyden chambers (10-μm-thick polycarbonate filter; lower surface count technique) was significantly (P < 0.05) enhanced, presumably due to cGH-induced hyperadhesiveness to the lower surface of the filters. The adhesion in albumin-coated microtiter plates and adherence to canine pulmonary fibroblasts were significantly (P < 0.05) increased, and the increased adhesion resulted in a significant (P < 0.01) increase in transendothelial migration using canine jugular vein endothelial cells. The increase in adhesion was associated with a significant increase in CD11b/CD18 expression. Furthermore, intracellular O2− production was significantly enhanced in response to both phorbol myristate acetate (P < 0.01) and opsonized zymosan (P < 0.05). In the absence of a PMN-stimulating agent, cGH did not influence the effector functions investigated except for an increased expression of CD11b/CD18.

Polymorphonuclear neutrophil granulocytes (PMN) play an important role in organisms’ first line of defense against invading agents (25, 42). Upon activation in the bloodstream, the PMN become more adhesive, allowing receptor-mediated margination and adhesion to the vasculature (26, 40–42, 44, 51, 58). Then, adherent PMN undergo shape change and crawl on the surface of the endothelial cells, followed by transendothelial migration into the extracellular compartment (5, 40–42, 44, 51). Subsequently, PMN migrate along a chemotactic gradient toward the offending stimulus to finally kill the invading agent by phagocytosis or release of granule contents and reactive oxygen metabolites (2, 25, 42, 56).

Growth hormone (GH) has been identified as a factor involved in the regulation of PMN function by priming (17–19, 28, 37–39, 43, 52, 54), whereby the response of PMN to an activating stimulus is potentiated (7). Except for one report (37), previous work shows that GH primes human (18, 19, 28, 39, 43, 52), porcine (18, 19), and bovine (18) PMN in vitro for an enhanced respiratory burst and a reduced chemotactic migration of human PMN (17, 52, 54), which has been suggested to be due to GH-induced hyperadhesiveness (52). These findings correlate with observations for human acromegalic patients, in whom the respiratory burst is increased (38) and the chemotactic response is decreased (16). In contrast to the findings for human acromegalic patients, the chemotactic responsiveness of canine PMN in canine acromegaly has been reported to be increased (24). The aim of this study was, therefore, to further investigate the priming effect by canine GH (cGH) on canine PMN function in vitro.

MATERIALS AND METHODS

Isolation of canine PMN. Clinically healthy beagle or mongrel dogs, aged 1 to 7 years (either sex), were used for the collection of blood samples. PMN were isolated as previously described (34). Briefly, canine PMN were isolated from EDTA-anticoagulated venous blood that was sedimented in 0.15% methylcellulose. The leukocyte-rich supernatant was centrifuged over a gradient (Lymphoprep; Nycomed, Oslo, Norway) for 30 min at 400 × g. The resultant cell pellet was subjected to hypotonic lysis and washed twice in RPMI (Life Technologies, Inc., Grand Island, N.Y.). This yielded a preparation that was >98% PMN and >98% viable as determined by the eosin Y dye exclusion test (1).

Priming of canine PMN with cGH. Pituitary cGH with a purity of >95% was generously provided by A. F. Parlow (NIDDK National Hormone and Pituitary Program, Los Angeles, Calif.). A stock solution at 20 μg/liter in RPMI–0.1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) was prepared and stored at −20°C. PMN were primed by incubating cells (5 × 106 cells/ml) with various concentrations of cGH (10 to 800 μg/liter) for 30 min at 37°C. For a control, we used a corresponding buffer in which cGH was dissolved, i.e., RPMI with 0.1% BSA. Depending on the analytical assay performed, cell concentration was subsequently adjusted to a desired final concentration.

Test of canine PMN function. Each test of PMN function was performed with PMN from 10 different dogs—five males and five females. The procedures for each test are described below.

Shape change. Shape change assay was performed as previously described (46). Briefly, PMN in suspension (105 cells/ml) were stimulated with recombinant human interleukin-8 (IL-8; 10 nM; Boehringer Mannheim, Indianapolis, Ind.) for 7 min at 37°C. Immediately after this, cells were fixed with equal volumes of 2% glutaraldehyde. The morphology was assessed under a phase-contrast microscope, and a total of 100 cells in each sample were scored on a scale of 1 to 4 (50). A mean shape change was calculated by summing the product of the number of PMN (PMN,i) displaying a morphology, s, using the following equation:

\[ M = \sum_{i=1}^{4} \text{PMN}_i \times S^{100} \]
Chemotaxis assay. Chemotaxis under agarose was determined as previously described (34). Briefly, agarose medium consisting of 1.2% agarose and 0.25% gelatin in RPMI was prepared. The agarose medium was added to specially constructed chemotaxis chambers with a gelatin-coated microtiter plate at the bottom of each chamber. After the agarose was gelled, six series of three wells were cut in each gel. Immediately after priming of PMN with cGH (100 to 800 ng/liter), a 10-μl cell suspension with approximately 5 × 10⁶ cells was added to the chemotaxis wells. To one of the outer wells, RPMI 1640 was added as a control, and to the opposite outer well, the chemotactic factor (10 μl of IL-8) was added. The chambers were immediately sealed and incubated at 37°C for 135 min. Incubation was terminated by flooding the agarose with DPBS and centrifugation at 1,000 × g. Following this, filters were rinsed with DPBS and stained with Coomassie brilliant blue, and migration was estimated by the lower surface counting (LSC) technique (48) using a 100× objective. The results were expressed as chemotactic differentials, i.e., migration in the presence minus migration in the absence of chemotaxant. All experiments were performed in duplicate.

Canine PMN adhesion in albumin-coated microtiter plates. Adherence was first investigated using a fluorometric microtiter plate cellular adhesion assay as previously described (22). Briefly, cells were labeled at a concentration of 5 × 10⁶ cells/ml with the fluorescent indicator calcein AM (5 μM; Molecular Probes, Eugene, Ore.) for 30 min at 37°C and subsequently washed twice in RPMI. The labeling procedure was followed by incubating the PMN with cGH (10 to 800 ng/liter) for 45 min at 37°C. The assay was performed in 96-well plates coated with albumin (BSA). A 100-μl cell suspension was added to each well, and assays with stimulated as well as unstimulated cells were conducted. PMN were stimulated with phorbol myristate acetate (PMA; Sigma Chemical Co.; final concentration, 100 nM) immediately before the addition of PMN to the wells. Microtiter plates were incubated for 30 min at 37°C, and then the relative fluorescence was measured (excitation, 485 nm, and emission, 538 nm; Fluoromax Ascent; Labsystems, Helsinki, Finland). The microtiter plates were washed four times, and the fluorescence was measured again. The percentage of adherent PMN was calculated as [(relative fluorescence after washing procedure/relative fluorescence before washing procedure) × 100]. All measurements were performed in triplicate.

Canine PMN adhesion to canine pulmonary fibroblasts. Healthy control dogs (n = 8) were euthanatized with intravenously administered sodium pentobarbital (30 mg/kg of body weight). Peripheral lung tissue was removed aseptically, and lung fibroblasts were isolated and cultured as previously described (4). Briefly, peripheral lung tissue was cut into slices (4 mm thick) and the pleural surfaces were removed by dissection of the connective tissue. The pieces and seeded into plastic tissue culture dishes (100-mm diameter; Corning, Corning, N.Y.) containing M199 medium supplemented with 10% heat-inactivated fetal calf serum (Life Technologies), 2 mM glutamine, and antibiotics (100 U of penicillin per ml, 100 μg of streptomycin per ml, 2.5 μg of amphotericin B/ml, and 10 μg of gentamicin per ml). The lung explants were removed after 10 days of culture in 5% CO₂ at 37°C. Fibroblasts that grew out of the lung explants were grown to confluence and serially passaged by nonenzymatic harvest in Hanks balanced salt solution (HBSS; Life Technologies) containing 1% BSA and 5 mM EDTA. Fibroblasts passaged two or three times were used, and coverslips covered with canine lung fibroblasts were inserted in adhesion chambers (4, 40). Briefly, prior to the assay, the coverslips were rapidly dipped in three changes of Dulbecco’s phosphate-buffered saline plus glucose (DPBS; Life Technologies), placed in the adhesion chamber, and covered with a plain glass coverslip that was separated from the lower coverslip by a rubber O-ring. Immediately after priming of PMN (400 ng/liter), the suspension with PMN (10⁶ cells/ml) was injected into the closed compartment. Experiments were conducted at 37°C using a Nikon Eclipse TE 2000-E inverted microscope equipped with phase-contrast optics. During an initial 500-s observation period, the number of PMN that settled and came into contact with the fibroblast monolayer was observed (five fields, 40× objective) and the number of PMN per field was recorded. The adhesion chamber was then inverted for an additional 500 s, nonadherent PMN were washed off the fibroblast monolayer, and the remaining adherent number of PMN per field was determined. The percentage of adherent PMN was calculated as [adherent number of PMN/contact number of PMN] × 100]. Experiments were performed with stimulated and unstimulated PMN (100 ng/liter IL-8) in duplicate. The percentage of adherent PMN that transmigrated across the endothelial monolayer appeared refractile with a surrounding halo (40). The percentage of total migrating adherent transmigrated PMN was calculated as [(transmigrated and adherent number of PMN/contact number of PMN)] × 100]. The percentage of total transmigrated PMN [(transmigrated number of PMN/contact number of PMN) × 100]. The percentage of total transmigrated PMN of total interacting cells was calculated as [number of transmigrated PMN/number of total interacting PMN] × 100]. In experiments with stimulated endothelial cells, monolayers were exposed to 10 ng of lipopoly-saccharide (LPS) (Escherichia coli; Sigma Chemical Co.) per ml for 4 h at 37°C and rinsed by dipping in two changes of DPBS before being inserted into the adhesion chambers. All experiments were performed in duplicate.

Adhesion molecule expression. The priming effect of cGH on the surface expression of the adhesion molecule CD11b/CD18 in response to IL-8 was determined by indirect immunofluorescence (9). Following the priming procedure (400 or 800 ng of cGH per liter for 30 min at 37°C), PMN (10⁶ cells/ml) were washed twice in IL-8 (0.1 to 10 nM) in duplicate for 45 min at 37°C. After this, cells were washed once and subsequently incubated with a primary anti-cDNA1b/CD18 antibody (MY904; 20 μg/ml), a clone from the American Type Culture Collection (Manassas, Va.), for 20 min at room temperature. The samples were washed twice in DPBS and then incubated with a 1:30 dilution of fluorescein-conjugated goat anti-mouse F(ab)’ (Zymed Laboratories, Inc., San Francisco, Calif.) for 15 min at room temperature. Nonspecific fluorescence was assessed by replacing the primary antibody with a nonbinding murine isotype-matched control antibody. The samples were then washed once in DPBS and resuspended in 1% paraformaldehyde. Samples were immediately analyzed in duplicate on a Becton Dickinson FACScan (Mountain View, Calif.) cell sorter. PMN in suspension were gated based upon their light scatter characteristics, and fluorescent intensity was measured and interpreted as the surface expression of CD11b/CD18.

Respiratory burst. The respiratory burst was investigated by the nitroblue tetrazolium (NBT) reduction assay, which is based on the intracellular reduction of NBT to formazan by O₂⁻ generated by O₂⁻-dissociating oxidants. The measurement of NBT was used as previously described (35) with modifications. Briefly, cells were resuspended in HBSS and primed for 30 min at 37°C with cGH (400 ng/liter) prior to the assay. A 100-μl cell suspension (10⁶ cells/ml) was added to each well of albumin-coated 96-well microtiter plates (33). Following this, 100 μl of NBT (Sigma Chemical Co.; final concentration, 0.5 mg/ml) was added with HBSS, opsonized zymosan (OPZ; final concentration, ~10⁷ opsonized particles/ml [29]), or PMA (final concentration, 100 nM). One row of eight wells was served as reference, and in these wells the cells were preincubated with 10 μM iodoacetamide in HBSS for 10 min at 37°C. The amount of formazan accumulating in PMN after 60 min of incubation at 37°C was quantitated in an enzyme-linked immunosorbent assay reader at 550 nm (Labsystems Multiskan RC) and interpreted as the intracellularly produced O₂⁻. Each experiment was performed in quadruplicate, and results were expressed as optical densities.

Statistical analysis. Paired t test or Wilcoxon signed rank test was used for comparison of paired samples. P values below 0.05 were considered to indicate statistical significance.

RESULTS

We wished to identify and investigate the priming effect of cGH on canine PMN function in vitro. In human PMN, the priming effect has been shown to be evident after 15 min of preincubation with human GH with respect to O₂⁻ production (43, 52). In our preliminary studies, we found that 30 min of preincubation with cGH was sufficient for detecting a priming
effect on PMN function, i.e., chemotaxis under agarose and adhesion in albumin-coated microtiter plates. We primed the PMN with concentrations of cGH ranging from 10 to 800 μg/liter, which is within the pathophysiological range of the levels in serum observed in canine acromegaly (14).

In our first series of experiments, we looked at shape change, which is a locomotor process absolutely independent of adhesion (55). Compared to controls (0 μg of cGH per liter), the priming effect of cGH in response to 10 nM IL-8 was statistically significant at concentrations of 200 μg/liter and above, whereas the shape change was unaffected with cGH alone (Fig. 1).

The cGH significantly reduced the chemotactic migration using cGH at ≥100 μg/liter (Fig. 2) (P < 0.05; Wilcoxon signed rank test) toward IL-8 in the under-agarose method. No influence of the cGH on the spontaneous migration was observed (data not shown; P > 0.05; Wilcoxon signed rank test). When we investigated the chemotactic migration in Boyden chambers (using 400 μg of cGH per liter), there was an increased chemotactic migration as assessed by the LSC method (48) (Fig. 3).

![Figure 1](image1.png)

**FIG. 1.** Enhancement of shape change (mean values ± standard errors of means) of canine PMN incubated with cGH stimulated with 10 nM IL-8 (●) compared to 0 μg of cGH per liter (significance: *, P < 0.05; **, P < 0.01; paired t test; n = 10). Unstimulated shape change (●) was not influenced by cGH (P > 0.05 [n = 10] compared to 0 μg of cGH per liter).

![Figure 2](image2.png)

**FIG. 2.** Decreased chemotactic migration under agarose of canine PMN (box-whiskers plot) incubated with cGH for 30 min at 37°C toward 100 nM IL-8 compared to control (significance: *, P < 0.05; **, P < 0.01; Wilcoxon signed rank test; n = 10).

![Figure 3](image3.png)

**FIG. 3.** cGH (400 μg/liter) enhances the chemotactic migration of canine PMN toward IL-8 (10 nM) in Boyden chambers as determined by the LSC technique (*, P < 0.05; paired t test; n = 10) (mean values ± standard errors of means).

(P < 0.05; paired t test).

By investigating the priming effect of cGH on the adhesive capability of canine PMN, we found that cGH significantly enhanced the PMA-stimulated adhesion in albumin-coated microtiter plates at 100 μg/liter and above (Fig. 4). cGH alone did not stimulate adhesion significantly (Fig. 4) (P > 0.05; paired t test). In order to focus more precisely on the priming effect of cGH on adhesion, we decided to select 400 μg of cGH per liter as a concentration for which such priming could be readily observed with shape change, chemotaxis, and adhesion in albumin-coated microtiter plates. First, we investigated the adherence of PMN stimulated with PAF to a monolayer of canine pulmonary fibroblasts. Priming resulted in a significantly enhanced adhesion of 19% to the fibroblast monolayer compared to control (Fig. 5) (P < 0.01; paired t test), whereas there was no effect on the unstimulated adhesion (Fig. 5) (P > 0.05; paired t test). Second, we studied the interaction of PMN with monolayers of endothelial cells. No transmigration of PMN across unstimulated endothelial monolayers occurred, and only a low percentage of adherence was observed (Fig. 6A). There was no significant effect of cGH on the adherence to unstimulated endothelial cells (Fig. 6A) (P > 0.05; paired t test). There was a significant increase (28%) in the percentage of total PMN interacting with the LPS-stimulated endothelial monolayer (Fig. 6B) (P < 0.05; paired t test). The percentage of transmigration based on the contact number of PMN was enhanced by cGH (Fig. 6C) (P < 0.01; paired t test) (45%). Based on the total number of interacting PMN, there was no significant difference in the percentage of transmigration between primed PMN and controls (Fig. 6D) (P > 0.05; paired t test).

The adhesive process investigated above is largely dependent on the β2 integrins (4, 8, 40), and to further investigate the role of the β2 integrins, we measured the β2 integrin CD11b/CD18. As shown in Table 1, the priming resulted in a significantly enhanced CD11b/CD18 expression using cGH at 400 and 800 μg/liter alone (P < 0.05; paired t test). Priming with 400 μg/liter significantly increased the expression of CD11b/CD18 in response to 10 nM IL-8 (P < 0.05; paired t test), whereas priming with 800 μg/liter increased the expression at all concentrations of IL-8 applied (Table 1) (P < 0.05; paired t test).

The respiratory burst has often been characterized when a priming effect has been investigated (7). Intracellular O2− was determined by the NBT assay by measuring the optical density (35). The optical density reached maximum after 60 min of
incubation with both OPZ and PMA (data not shown). There was a significant increase in the $O_2$ production of PMN primed with cGH using OPZ ($P<0.01$; paired t test) and PMA ($P<0.01$; paired t test) as stimulants (Fig. 7), whereas there was no influence of cGH on the spontaneous $O_2$ production (Fig. 7) ($P>0.05$; paired t test).

**DISCUSSION**

In this study, we found that cGH was capable of priming canine PMN in vitro and thereby potentiating PMN functions. Following priming, the response toward IL-8 was potentiated as determined by the shape change assay, whereas the response toward IL-8 using the under-agarose assay was inhibited. This is explained by the fact that shape change is a locomotor process independent of adhesion whereas chemotaxis under agarose depends on adhesion (55). It has previously been demonstrated that relatively small changes in cell substratum adhesion strength, through altering integrin expression levels or integrin affinity, can translate into substantial changes in migration speed (32). Thus, we hypothesized that the decreased chemotaxis under agarose was due to cGH-induced hyperadhesiveness. We confirmed this hypothesis by observing that the adhesion in albumin-coated microtiter plates was significantly enhanced using $\geq 100$ µg of cGH per liter. Additionally, the adherence of PAF-stimulated PMN to canine pulmonary fibroblasts was significantly enhanced by using 100 µg of cGH per liter. When we studied the interaction of PMN with monolayers of canine jugular venous endothelial cells, we observed that cGH priming increased the percentage of interacting PMN, i.e., adherent and transmigrated cells. Furthermore, there was an increased percentage of transmigrated PMN based on the total number of PMN that were in initial contact with the endothelial monolayer. However, when we calculated the percentage of transmigration based on the number of interacting cells there was no difference. These findings indicate that the increased number of transmigrated PMN is a result of increased adhesion and that the transmigratory process itself is not influenced by cGH.

The increase in CD11b/CD18 expression following cGH priming explains the increase in adhesion observed in our study. PMN adhesion is largely dependent on the leukocyte β2
were stimulated with recombinant human IL-8.

Table 1: Enhancement of CD11b/CD18 expression when priming canine PMN with cGH

| IL-8 (nM) | CD11b/CD18 expression (mean fluorescence intensity)* on PMN primed with cGH (µg/liter) |
|-----------|--------------------------------------------------------------------------------------------|
| 0         | 125 ± 53 132 ± 60b 137 ± 58** |
| 0.1       | 130 ± 59 142 ± 60 146 ± 63a |
| 1.0       | 143 ± 47 158 ± 66 161 ± 63b |
| 10        | 151 ± 56 167 ± 54b 171 ± 62a |

* Values are means ± standard deviations (n = 10) for mean fluorescence intensity expressed by canine PMN primed with cGH for 30 min at 37°C. PMN were stimulated with recombinant human IL-8.

b Significantly different (P < 0.05) from control as determined by paired t test.

t integrins (i.e., CD11a/CD18, CD11b/CD18, and CD11c/CD18) when the adherence to fibroblasts (4) and endothelial cells (40, 45) is considered. Also, the adherence to albumin-coated surface is dependent on CD11b/CD18 and CD11c/CD18 (8).

Thus, the increased adhesion following cGH priming may not be attributable solely to an increased expression of CD11b/CD18 but may also involve the other β integrins (i.e., CD11a/CD18 and CD11c/CD18). Interestingly, CD11b/CD18 expression was increased when PMN were primed with cGH alone, whereas cGH had no influence on the unstimulated adhesion to the substrates. These observations indicate that cGH priming increases the expression of CD11b/CD18 but does not fully activate the β integrin on resting PMN. Further studies are, however, required to clarify the role of the β integrins in the cGH priming of PMN.

The chemotactic response has previously been characterized in a case of canine acromegaly (24) where an increased chemotactic response compared to that of healthy dogs was reported. We also found that the in vitro priming with cGH increased the chemotactic response using the Boyden chamber assay (LSC method). However, in studies of human PMN chemotaxis in Boyden chambers following GH priming in vitro and in human acromegalic patients, it was demonstrated that the chemotactic responsiveness was reduced (52, 54), a result also found in the under-agarose assay in this study and in humans (17). While the study of PMN chemotaxis in canine acromegaly was assessed in a modified Boyden chamber utilizing 10-µm-thick polycarbonate filters and quantified by the LSC method (24, 48), the studies with human PMN were performed with 140-µm-thick nitrocellulose filters and quantified by the leading front (LF) method (52, 54). A conceivable explanation for the divergence in the results obtained with human and canine PMN is that different filter types and procedures (LF and LSC) have been applied in the measurement of the chemotactic response. The polycarbonate filters applied in the LSC method are only ~10-µm thick, and the only way to score the response is to count cells that have penetrated the filter (48, 55). PMN must migrate along the upper surface of the filter to reach a pore, and they may or may not stay attached once they reach the lower surface (55); thus, the assay is adhesion dependent, and increased adhesiveness of the PMN results in an increased chemotactic score (55). By use of 140-µm-thick nitrocellulose filters (i.e., LF method), PMN migrate into the filters and do not reach the lower surface of the filters. The chemotactic migration is quantified by measuring the LF of PMN that have migrated into the filter (52, 55, 57), and it has previously been suggested that hyperadhesiveness induced by priming results in a decreased chemotactic response using this method (52).

cGH enhanced the intracellular O2 production in response to both PMA and OPZ as determined by reduction of NBT, which correlates with previous results (52) obtained for human PMN using the NBT assay, except that the magnitude by which human GH enhanced the O2 production was higher. However, in the study of human PMN, fMLP (i.e., iC3b [20, 36]) and mediates O2 production (6, 20), and the enhanced O2 production in response to the OPZ further suggests that the activity of CD11b/CD18 is increased following GH priming. Other investigators (18, 19) have also investigated the O2 production extracellularly by human PMN following GH priming, and the magnitude by which human GH enhanced the O2 production was far higher than that in our results. The difference in magnitude is presumably due to the fact that these investigators measured the extracellularly produced O2 also and to the fact that the PMN were primed for 3 to 4 h.

The observed potentiation of PMN function, i.e., shape change, adhesion, transendothelial migration, and CD11b/CD18 expression, suggests that cGH in vivo would promote the recruitment of PMN from the bloodstream toward an inflammatory focus. Furthermore, the change in chemotaxis is believed to retain the PMN at the inflammatory site (47, 53), and the increase in the generation of reactive oxidative metabolites potentiates PMN microbicidal capacity. Thus, we propose the hypothesis that administration of cGH in vivo may improve canine host defense and be of important biological relevance. This is supported by results obtained in disease models where the role of GH in host defense has been investigated. In murine disease models, GH administration in vivo enhanced host resistance to bacterial infection as a consequence of enhanced function of PMN (21–23). Furthermore, it has been documented that GH priming extends to other leukocytes, e.g., macrophages (10, 11), and in rats deprived of endogenous pituitary GH, administration of GH significantly augmented host resistance to bacterial infection due to an increased bactericidal capacity of macrophages (12, 13). Thus, GH seems to play an important role in connecting the neuroendocrine and immune systems. Our findings in vitro suggest that the role of cGH in the dog is of corresponding importance; however,
further studies are required to evaluate the priming effect in vivo.

In conclusion, cGH was identified as a priming agent of canine PMN in vitro, and cGH significantly altered canine PMN function in a manner resembling that of the priming effect of human GH on human PMN.

ACKNOWLEDGMENTS

The skillful technical assistance of Lisa Thurmon and Nelson Bennett, Section of Leukocyte Biology, and Peggy Jackson, Section of Cardiovascular Science, Baylor College of Medicine, is greatly acknowledged. We thank Helle Aaes, Leo Pharmaceutical Products, Ballerup, Denmark; Britta V. Bysted, Department of Clinical Studies, The Royal Veterinary and Agricultural University; and Lloyd H. Michael, Section of Cardiovascular Science, Baylor College of Medicine, for providing blood samples from dogs.

REFERENCES

1. Absolon, D. R. 1988. Basic methods for the study of phagocytosis. Methods Enzymol. 132:136–137.
2. Babior, B. M. 1984. Oxidants from phagocytes: agents of defense and destruction. Blood 64:959–966.
3. Bommakanti, R., K. M. Bokoch, J. O. Tolley, R. E. Schreiber, D. W. Siemans, K. N. Klotz, and A. J. Jesaitis. 1992. Reconstitution of a physical complex between the N-formyl chemotactic peptide receptor and G protein. Inhibition by pertussis toxin-catalyzed ADP ribosylation. J. Biol. Chem. 267:7575–7581.
4. Butcher, E. C. 1993. Specificity of leukocyte-endothelial interactions and diapedesis: physiologic and therapeutic implications of an active decision process. Res. Immunol. 144:659–698.
5. Cohen, H. J., P. E. Newburger, M. E. Chovaniec, J. C. Whitin, and E. R. Simons. 1981. Epoxomicin-zymosan-stimulated granulocyte-activation and activity of the superoxide-generating system and membrane potential changes. Blood 58:975–982.
6. Condliffe, A. M., E. Kitchen, and E. R. Chilvers. 1998. Neutrophil priming: pathophysiological consequences and underlying mechanisms. Clin. Sci. 94:461–471.
7. Davis, G. E. 1992. The Mac-1 and β1p50 beta 2 integrins bind denatured proteins to mediate leukocyte-cell-substrate adhesion. Exp. Cell Res. 206:282–292.
8. Dreyer, W. J., L. H. Michael, E. E. Milliman, and K. L. Berens. 1995. Neutrophil activation and adhesion molecule expression in a canine model of open heart surgery with cardiopulmonary bypass. Cardiovasc. Res. 29:775–783.
9. Edwards, C. K., III, S. M. Ghiasuddin, J. M. Schepper, L. M. Yunger, and R. M. Lorence. 1993. Specificity of leukocyte-endothelial interactions and diapedesis: physiologic and therapeutic implications of an active decision process. Res. Immunol. 144:659–698.
10. Fornari, M. C., M. P. Scolnik, M. F. Palacios, A. D. Intebi, and R. A. Diez. 1994. Growth hormone inhibits normal B-cell differentiation and neutrophil chemotaxis in vitro. Int. J. Immunopharmacol. 16:657–673.
11. Fornari, M. C., 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.
12. Fornari, M. C., S. Arkins, B. S. Wang, and K. W. Kelley. 1991. A novel role of growth hormone and insulin-like growth factor-I. Priming neutrophils for superoxide anion secretion. J. Immunol. 146:1602–1608.
13. Hoogerwerf, M., R. S. Weening, C. E. Hack, and D. Roos. 1990. Complement fragments C3b and iC3b coupled to latex induce a respiratory burst in human neutrophils. Mol. Immunol. 27:159–167.
14. Inoue, T., H. Saito, R. Fukushima, T. Inaba, M. T. Lin, K. Matsuzawa, and T. Matsuda. 1995. Growth hormone and insulin-like growth factor I enhance host defense in a murine sepsis model. Arch. Surg. 130:1115–1122.
15. Inoue, T., H. Saito, Y. Hashiguchi, K. Fukatsu, T. Inaba, M. T. Lin, I. Han, H. Furukawa, and T. Muto. 1996. Growth hormone and insulin-like growth factor I augment Escherichia coli-killing activity of murine peritoneal exudate cells. Shock 6:345–350.
16. Inoue, T., H. Saito, N. Tsuno, K. Fukatsu, M. T. Lin, T. Inaba, I. Han, S. Furukawa, S. Ieda, T. Matsuda, and T. Muto. 1998. Effects of growth hormone and insulin-like growth factor I on opsonin receptor expression on local and systemic phagocytes in a lethal peritonitis model. Crit. Care Med. 26:338–343.
17. Jensen, A. L., M. K. Thomsen, H. Aaes, M. Andreasen, and J. Sondergaard. 1993. Polymorphonuclear neutrophil granulocyte chemoattractant responsiveness in a case of canine acromegaly. Vet. Immunol. Immunopathol. 37:329–336.
18. Klebanoff, S. J., and R. A. Clark. 1978. The neutrophil: function and clinical disorders, p. 1–4. Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands.
19. Lawrence, M. B., and T. A. Springer. 1991. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell 65:853–871.
20. Majumdar, S. M., W. Rossi, T. Fujiki, W. A. Phillips, S. Disa, C. F. Queen, B. R. J. Johnston, O. M. Rosen, B. E. Corkey, and H. M. Korchak. 1991. Protein kinase C isoforms and signaling in neutrophils. Differential substrate specificities of a translocatable calcium- and phospholipid-dependent beta protein kinase C and a phospholipid-dependent protein kinase which is inhibited by long chain fatty acyl coenzyme A. J. Biol. Chem. 266:9285–9294.
21. Matsuda, T., H. Saito, T. Inoue, K. Fukatsu, I. Han, S. Furukawa, S. Ieda, and T. Muto. 1998. Growth hormone inhibits apoptosis and up-regulates reactive oxygen intermediate production by human polymorphonuclear neutrophils. J. Parenter. Enteral Nutr. 22:368–374.
22. Metcalf, J., J. I. Gallin, W. M. Nauseef, and R. K. Root. 1986. Functions related to microbialidal activity, p. 87–94. In J. Metcalf, J. I. Gallin, W. M. Nauseef, and R. K. Root (ed.), Laboratory manual of neutrophil function. Raven Press, New York, N.Y.
23. Nishihira, J., and J. T. O’Flaherty. 1985. Phorbol myristate-acetate receptors in human polymorphonuclear neutrophils. J. Immunol. 135:3439–3447.
24. Painter, R. G., K. Zahler-Bentz, and R. E. Dukes. 1987. Regulation of the affinity state of the N-formylated peptide receptor of neutrophils: role of guanine nucleotide-binding proteins and the cytoskeleton. J. Cell Biol. 105:2971–2971.
25. Palecek, S. P., J. J. Loftus, M. H. Ginsberg, D. A. Laufenburger, and A. F. Horwitz. 1997. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature 385:537–540.
26. Petersen, T. K., B. V. Bysted, and A. L. Jensen. 1999. Determination of the adhesive capability of canine polymorphonuclear neutrophil granulocytes using a flurometric microtiter plate cellular adhesion assay. Vet. Immunol. Immunopathol. 68:283–291.
27. Petersen, T. K., T. C. Jensen, and H. Aaes. 1998. Chemotaxis of canine polymorphonuclear neutrophil granulocytes using the under-agarose method applied to glass microscope slides. Comp. Haematol. Int. 8:31–36.
28. Pick, E. 1981. A rapid densitometric microassay for nitroblue tetrazolium reduction and application of the microassay to macrophages. J. Reticuloen- dothel. Soc. 30:581–593.
29. Ross, G. D., J. A. Cain, and P. J. Lachmann. 1985. Membrane complement receptor type three (CR3) has lectin-like properties analogous to bovine conglutinin as functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. J. Immunol. 134:3307–3315.
30. 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 hypertrophic dwarf dogs. Int. J. Tissue React. 7:153–159.
31. Rovensky, J., M. Vigas, J. Lokaj, P. Cunicik, P. Lukac, and A. Takac. 1987. Effect of growth hormone on the metabolic activity of phagocytes of peripheral and isolated in pulmonary dwarfs and acromegaly. Endocrinol. Exp. 12:29–34.
32. Rovensky, J., P. Cunicik, P. Lokaj, J. Lusakova, J. Barat, T. Dancz, and A. C. Adam. 1989. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhes- 
Downloaded from http://cvi.asm.org/ on April 26, 2019 by guest
human neutrophils in vitro. J. Clin. Investig. 83:2008–2017.
41. Smith, C. W., R. Rothlein, B. J. Hughes, M. M. Mariscalco, H. E. Rudloff, F. C. Schmalstieg, and D. C. Anderson. 1988. Recognition of an endothelial determinant for CD 18-dependent human neutrophil adherence and trans-endothelial migration. J. Clin. Investig. 82:1746–1756.
42. Smolen, J. E., and L. A. Boxer. 1995. Functions of neutrophils, p. 779–797. In E. Beutler, M. A. Lichtman, B. S. Coller, and T. J. Kipps (ed.), Williams hematology. McGraw-Hill, New York, N.Y.
43. Spadoni, G. L., A. Spagnoli, S. Cianfarani, D. Del Principe, A. Menichelli, S. Di Giulio, and B. Boscherini. 1991. Enhancement by growth hormone of phorbol diester-stimulated respiratory burst in human polymorphonuclear leukocytes. Acta Endocrinol. 124:589–594.
44. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76:301–314.
45. Stacker, S. A., and T. A. Springer. 1991. Leukocyte integrin P150,95 (CD11c/CD18) functions as an adhesion molecule binding to a counter-receptor on stimulated endothelium. J. Immunol. 146:648–655.
46. Stickle, J. E., D. K. Kwan, and C. W. Smith. 1985. Neutrophil function in the dog: shape change and response to a synthetic tripeptide. Am. J. Vet. Res. 46:225–228.
47. Thomsen, M. K. 1991. The role of neutrophil-activating mediators in canine health and disease (with special reference to the role of leukotrienes in inflammatory dermatoses). J. Vet. Pharmacol. Ther. 14:113–133.
48. Thomsen, M. K., and A. L. Jensen. 1991. reassessment of two Boyden chamber methods for measuring canine neutrophil migration: the leading front and the lower surface count assays. Vet. Immunol. Immunopathol. 29:197–211.
49. Thomsen, M. K., C. G. Larsen, H. K. Thomsen, D. Kirstein, T. Skak-Nielsen, I. Ahnfelt-Ronne, and K. Thstrup-Pedersen. 1991. Recombinant human interleukin-8 is a potent activator of canine neutrophil aggregation, migration, and leukotriene B4 biosynthesis. J. Investig. Dermatol. 96:260–266.
50. Tsang, Y. T., S. Neelamegham, Y. Hu, E. L. Berg, A. R. Burns, C. W. Smith, and S. I. Simon. 1997. Synergy between L-selectin signaling and chemotactic activation during neutrophil adhesion and transmigration. J. Immunol. 159:4566–4577.
51. von Andrian, U. H., and K. E. Arfors. 1993. Neutrophil-endothelial cell interactions in vivo: a chain of events characterized by distinct molecular mechanisms. Agents Actions Suppl. 41:153–164.
52. Wiedermann, C. J., M. Niedermühlbichler, D. Geissler, H. Beimpold, and H. Braunsteiner. 1991. Priming of normal human neutrophils by recombinant human growth hormone. Br. J. Haematol. 78:19–22.
53. Wiedermann, C. J., N. Reinisch, and H. Braunsteiner. 1993. Stimulation of monocyte chemotaxis by human growth hormone and its deactivation by somatostatin. Blood 82:954–960.
54. Wiedermann, C. J., N. Reinisch, M. Niedermühlbichler, and H. Braunsteiner. 1993. Inhibition of recombinant human growth hormone-induced and prolactin-induced activation of neutrophils by octreotide. Naunyn-Schmiedebergs Arch. Pharmacol. 347:336–341.
55. Wilkinson, P. C. 1996. Cell locomotion and chemotaxis: basic concepts and methodological approaches. Methods (Orlando) 10:74–81.
56. Zigmond, S. H. 1978. Chemotaxis by polymorphonuclear leukocytes. J. Cell Biol. 77:269–287.
57. Zigmond, S. H., and J. G. Hirsch. 1973. Leukocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of a cell-derived chemotactic factor. J. Exp. Med. 137:387–410.
58. Zimmerman, G. A., S. M. Prestoi, and T. M. McIntyre. 1992. Endothelial cell interactions with granulocytes: tethering and signaling molecules. Immunol. Today 13:93–100.