

Effects of in Vitro and in Vivo Administration of Recombinant Bovine Interferon- γ on Bovine Neutrophil Responses to *Brucella abortus*

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ABSTRACT

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The effects of in vitro and in vivo treatment of bovine polymorphonuclear leukocytes with recombinant bovine interferon- γ on in vitro bovine polymorphonuclear leukocyte functions and the survival of *Brucella abortus* were determined. Activation of neutrophils in vitro with interferon- γ resulted in enhanced production of O_2^- and myeloperoxidase- H_2O_2 -halide activity by neutrophils in the presence of *B. abortus*. The improved iodination responses were correlated with an enhanced ability to perform iodination in the presence of 5'-guanosine monophosphate and adenine which have previously been shown to contribute to inhibition of neutrophil myeloperoxidase- H_2O_2 -halide activity by *B. abortus*. The ability of opsonized *B. abortus* to survive in the presence of neutrophils activated in vitro or in vivo was partially decreased by ~10% of control when compared to survival rates within control phagocytes. These results suggest that activation of neutrophils with recombinant interferon- γ partially enhances their oxidative metabolic responses, resulting in a slightly enhanced ability to kill virulent *B. abortus*.

INTRODUCTION

Brucella abortus, the etiologic agent of brucellosis, is a facultative intracellular bacterium capable of surviving within phagocytic cells. The mechanisms used by the organism to survive within polymorphonuclear leukocytes (PMNs) are not fully understood. Previous studies have indicated that both smooth and rough strains of *B. abortus* are efficiently ingested by bovine and human neutrophils (Robertson et al., 1979; Riley and Robertson). This work also indicated that the products of the myeloperoxidase- H_2O_2 -halide (MPO- H_2O_2 -hal-

ide) antibacterial system of the neutrophil are capable of killing *B. abortus*. Other studies (Morris, 1977; Kreutzer et al., 1979) indicate that ingestion of non-opsonized *B. abortus* and organisms treated with heat-inactivated homologous normal bovine serum does not stimulate a respiratory burst by PMNs. Recent experiments (Harmon and Adams, 1987) have indicated that opsonized *B. abortus* are capable of eliciting an oxidative response by bovine mammary gland macrophages. Similar experiments involving bovine neutrophils have confirmed that ingestion of *B. abortus* opsonized with specific antibody stimulates O_2^- production by bovine PMNs (Canning et al., 1988). Thus, a lack of stimulation of a respiratory burst by neutrophils upon ingestion of the bacteria does not appear to contribute to the intracellular survival of antibody-opsonized *B. abortus*.

Virulent *B. abortus* possess two components (fractions 3b and 10) which inhibit the (MPO- H_2O_2 -halide) antibacterial reaction of PMNs (Canning et al., 1985). Further studies have indicated that specific suppression of primary granule degranulation by the bacteria is responsible for the decreased MPO- H_2O_2 -halide activity of the phagocytes (Bertram et al., 1986). Fraction 3b has been identified as GMP and fraction 10 as adenine (Canning et al., 1986). These results were in agreement with those of other workers (Chiang et al., 1986) which indicated purine nucleotides, nucleosides and bases were capable of suppressing iodination responses of bovine PMNs.

Previous studies indicate that protection against a variety of facultative intracellular bacteria including *B. abortus* is primarily dependent upon cell-mediated immune responses (Campbell, 1976; Cheers and Pagram, 1979). Numerous lymphocyte-derived products have been shown to modulate the responses of the cellular immune system to infection. Considerable amounts of data have accumulated which detail the immunoregulatory activities of interferon- γ (IFN- γ). In vitro activities attributed to this lymphokine include induction of major histocompatibility antigens on cell surfaces (Wallach et al., 1982; Basham and Merigan, 1983), activation of macrophage-mediated cytotoxicity (Pace et al., 1985), and activation of oxidative and microbicidal activities of peritoneal and alveolar macrophages (Nathan et al., 1983; McCabe et al., 1984; Hoover et al., 1985; Murray et al., 1985). In vivo administration of recombinant IFN- γ enhances the anti-toxoplasma activity of murine peritoneal macrophages (Black et al., 1987). Both respiratory burst and lysosomal enzyme release activities of human neutrophils were shown to be enhanced in the presence of purified human IFN- γ (Kowanko and Ferrante, 1987). It has been shown (Steinbeck et al., 1986) that bovine PMNs exposed to recombinant bovine IFN- γ exhibited suppressed random migration under agarose while their ability to ingest *Staphylococcus aureus*, produce superoxide anion and perform myeloperoxidase-mediated iodination was not affected. Both antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-indepen-

dent neutrophil cytotoxicity (AINC) activities were enhanced in the presence of IFN- γ .

The purpose of the present study was to determine the effects of in vitro and in vivo activation of PMNs with IFN- γ on the interactions of bovine PMNs with *B. abortus*.

MATERIALS AND METHODS

Preparation of bacteria

The procedures used to grow *B. abortus* have been described in detail previously (Canning et al., 1985). Smooth virulent strain 2308 of *B. abortus* was grown on potato agar slants (Difco, Detroit, MI). Following incubation for 48 h at 37°C, the cells were harvested and washed three times with saline (0.85% NaCl). Following washing, the bacteria were resuspended in Earle's balanced salt solution (EBSS, Grand Island Biologics Co., Grand Island, NY) at a concentration of either 1×10^{10} or 1×10^7 cells/ml.

Nucleotide and base preparations

Authentic samples of GMP and adenine were purchased from Sigma Chemical Co., St. Louis, MO. For the PMN function assays, a solution containing 0.1 mg/ml each of GMP and adenine was prepared in EBSS.

In vitro and in vivo activation of PMNs

Eight apparently healthy adult Holstein-Friesian cows free of detectable anti-*Brucella* antibodies were used as a source of neutrophils for the in vitro activation studies. Bovine PMNs were isolated as previously described (Roth and Kaerberle, 1981) and resuspended in RPMI 1640 (Grand Island Biologic Co., Grand Island, NY). In vitro activation of neutrophils with IFN- γ was performed using procedures described previously (Steinbeck et al., 1986). Recombinant bovine IFN- γ containing <10ng/ml of endotoxin was obtained from Ciba-Geigy Limited, Basel, Switzerland (Lot no. 3229/38). A solution containing 2×10^{-8} g/ml of IFN- γ was prepared in RPMI 1640. This material used at a final concentration of 1×10^{-8} g/ml (22 U/ml) has been shown to provide maximal activation of bovine neutrophils (Steinbeck et al., 1986). Standardized PMNs (1×10^8 cells/ml for migration, cytochrome c reduction, or iodination assays or 2×10^7 cells/ml for bactericidal assays) were mixed with an equal volume of IFN- γ solution and the cells were incubated for 2 h at 37°C in a humidified atmosphere containing 5% CO₂. Control preparations of neutrophils were incubated with an equal volume of RPMI 1640. Both treated and control PMNs were then used in the functional assays without washing.

Fifteen apparently healthy adult Holstein-Friesian and Brown Swiss steers were randomly divided into two treatment groups and one control group each containing five animals for use in the in vivo activation studies. There were no differences in in vitro neutrophil function parameters between groups prior to the initiation of the experiment. The stock solution of IFN- γ was diluted in phosphate-buffered saline solution (PBS, 0.15 M, pH 7.2) prior to use. Animals in each treatment group were given a single subcutaneous injection of 5.0 ml of PBS containing 0.0, 0.1, or 0.5 mg (0, 2.2×10^5 , or 1.1×10^6 Units) of recombinant IFN- γ . Bovine PMNs were isolated, as referenced above, 24, 48, and 72 h after treatment. The cells were resuspended in PBS at a concentration of either 5×10^7 cells/ml for migration and iodination assays or 1×10^7 cells/ml for bactericidal assays.

PMN function assays

Random migration under agarose

This assay was used to determine if PMNs exposed to IFN- γ were activated by the treatment as evidenced by a decrease in migration ability. Agar used in the assay consisted of RPMI 1640 containing 1.0% agarose (Bethesda Research Laboratories, Bethesda, MD), 10% fetal bovine serum, and 0.1% gentamycin sulfate (Schering Corp., Kenilworth, NJ). Eight ml of warm agar (48°C) was added to graduated radial immunodiffusion plates (Miles Laboratories, Elkhart, IN) and the plates were stored at 4°C prior to use. To evaluate random migration, 12.0 μ l of either control or IFN- γ -activated PMNs were added to wells (3.0 mm in diameter) in the agar. The migration plates were incubated for 5 h in a humidified 5% CO₂ atmosphere at 37°C. Following incubation, the agar surface was flooded with an 8% solution of glutaraldehyde for an additional 30 min to fix the PMNs to the plate. The agar was removed and the cells adhering to the plate were stained with modified Wrights stain (Diff-Quick, Harleco, Gibbstown, NJ). The area of migration was determined by direct observation using an inverted tissue culture microscope and the area of the center well was corrected for by subtraction. The results were expressed as the mean percent of control.

Cytochrome c reduction

This test was used to determine the amount of O₂⁻ produced by control and in vitro-activated PMNs during the oxidative metabolic burst. The assay was performed in 96-well plates with membrane filters attached to the bottom of the wells (Millititer™ Model SV, 0.45 μ m pore size, Millipore Co., Bedford, MA) as previously described (Canning et al., 1986). Samples were evaluated in duplicate and the average of duplicate values was used for calculation. The filters of the wells were prewetted with a solution of 0.5% bovine serum albumin prior to the addition of reaction reagents. The standard reaction mixture

contained 0.075 ml of cytochrome c solution (stock solution = 538 μM in EBSS), 0.025 ml of PMNs in PBS (1.25×10^6 PMNs), 0.05 ml of EBSS, and 0.025 ml of preopsonized zymosan (7.5 mg/ml in EBSS). Measurement of O_2^- production by resting PMNs was performed similarly, except the preopsonized zymosan was replaced with 0.025 ml of EBSS. To determine the effects of *B. abortus* on production of O_2^- by opsonized zymosan-stimulated PMNs, 0.05 ml of *B. abortus* (5×10^8 bacteria/well) was substituted for the EBSS in the reaction mixture. The ability of superoxide dismutase to inhibit the reduction of cytochrome c by opsonized zymosan-stimulated PMNs was evaluated by adding 0.01 ml of superoxide dismutase (0.1 mg/ml) to the reaction mixture. Control and principal wells contained the same final volume of fluid. The reaction was started with the addition of either control or IFN- γ -activated PMNs. Following incubation at 37°C for 30 min with agitation, the culture filtrates were collected into a conventional 96-well flat-bottomed plate. The entrapped PMNs were washed twice by filtration with 0.075 ml of EBSS which was also collected. The plates were agitated briefly, and the optical density of the solutions at 550 nm was determined using a micro ELISA spectrophotometer (Model MR 600, Dynatech Corp., Alexandria, VA). The nmoles of O_2^- produced by 10^6 PMNs in 30 min were determined using the extinction coefficient of $2.1 \times 10^4 \text{ cm}^{-1} \text{ M}^{-1}$.

Iodination assay

The iodination assay (a measure of MPO- H_2O_2 -halide activity by PMNs) was performed as previously described (Roth and Kaerberle, 1981) in 12 \times 75 mm tubes in duplicate and the average of duplicate values was used for calculations. The standard reaction mixture contained 0.05 ml of PMNs (2.5×10^6 cells), 0.1 μCi of [^{125}I] in 0.025 ml of EBSS (carrier free, Amersham Corp., Arlington Heights, IL), 0.05 ml of NaI in EBSS (20 nmoles), and 0.3 ml of EBSS. Various concentrations of opsonized zymosan (0.05 ml) were used to stimulate iodination activity by control or activated PMNs. Iodination by resting cells was determined similarly, except preopsonized zymosan was omitted from the reaction mixture. To determine the effects of the GMP and adenine mixture or *B. abortus* on the MPO- H_2O_2 -halide activity of opsonized zymosan-stimulated PMNs, 0.05 ml of the purine mixture, or 0.05 ml of the bacterial suspension (5×10^8 bacteria/tube), or 0.05 ml of EBSS as a control was added to the standard reaction mixture. Following preincubation of the tubes at 37°C for 10 min, the reaction was started with the addition of either control or IFN- γ -activated PMNs. The tubes were incubated for 20 min at 37°C with tumbling before the amount of trichloroacetic acid-precipitable radioactivity was determined. The results were expressed as nmoles of NaI/ 10^7 PMNs h^{-1} .

Bacterial killing assay

This assay was used to measure the total number of bacteria killed by an aliquot of control or IFN- γ -activated PMNs over a period of time. The test was

performed in 96-well microtiter plates. Fifty μl of *B. abortus* (1×10^7 cells/ml) were opsonized with 25 μl of a subagglutinating dilution of bovine anti-*B. abortus* sera. Following incubation for 10 min at 37°C , 50 μl (1×10^7 cells/ml) of either control or IFN- γ -activated PMNs were added to each well resulting in a 1:1 ratio of bacteria:PMNs. This ratio of bacteria to PMNs was chosen to insure that the majority of the bacteria were ingested by the phagocytes. Control wells contained either bacteria mixed with sera and RPMI-1640 or bacteria mixed with sera and RPMI 1640 containing 1×10^{-8} g/ml of IFN- γ . Control and principal wells contained the same volume of fluid. The reaction mixtures were incubated at 37°C with agitation and 5- μl aliquots were removed at 0, 30, 60, and 120 min intervals for the *in vitro* studies or 30 and 90 min intervals for the *in vivo* studies. The aliquots were diluted in 0.15 M phosphate-buffered deionized water (pH 7.2) to lyse the PMNs and 100 μl of each dilution was plated on duplicate tryptose agar plates. Following incubation for 72 h, plates containing between 30 and 300 colonies were examined to determine the number of CFU.

Statistical analysis

To determine the effects of different bacterial preparations or the purine mixture on each PMN function, the mean values obtained when a bacterial preparation or purine mixture was added to PMNs was compared with the mean values for control (EBSS-treated) PMNs from the same animal. The effects of *in vitro* recombinant IFN- γ treatment on PMN function were determined by comparing the mean values obtained when PMNs were treated with IFN- γ with the mean values for control (RPMI 1640) PMNs from the same animal. Similarly, the effects of *in vivo* recombinant IFN- γ treatment on PMN function were determined by comparing the mean value obtained with PMNs from IFN- γ -treated animals with the mean value for PMNs from control animals. Either an analysis of variance procedure (blocked by day) or a Student's *t*-test were used to determine significance of the differences in PMN function.

RESULTS

Effects of in vitro activation of PMNs with IFN- γ

Random migration

Exposure of PMNs to IFN- γ for 2 h had no detrimental effect on the viability of the phagocytes (mean viability post treatment was 97%). *In vitro* activation of PMNs with recombinant bovine IFN- γ significantly ($P < 0.01$) suppressed the ability of neutrophils to migrate under agarose to $41.8 \pm 4.8\%$ of control.

Superoxide anion production

Results of the O_2^- production studies are shown in Fig. 1. Significant ($P < 0.01$) production of O_2^- by neutrophils was stimulated by opsonized zymosan, and the mixture of *B. abortus* with opsonized zymosan. Opsonized zymosan-stimulated production of O_2^- by PMNs was not significantly ($P < 0.05$) affected in the presence of *B. abortus*. The ability of opsonized zymosan-stimulated PMNs to reduce cytochrome c was suppressed by $> 95\%$ in the presence of superoxide dismutase.

The ability of both resting and opsonized zymosan-stimulated PMNs to produce O_2^- was enhanced in the presence of $IFN-\gamma$. The (mean \pm SEM) value for O_2^- production by resting PMNs increased from 1.9 ± 0.3 nmol $O_2^-/10^6$ PMNs per 30 min to 2.9 ± 0.3 nmol $O_2^-/10^6$ PMNs per 30 min. Opsonized zymosan-stimulated production of O_2^- increased from 13.0 ± 0.9 nmol $O_2^-/10^6$ PMNs per 30 min to 17.4 ± 1.0 nmol $O_2^-/10^6$ PMNs per 30 min. A similar enhancement of opsonized zymosan-stimulated production of O_2^- in the presence of *B. abortus* was observed with $IFN-\gamma$ pretreatment of PMNs.

Iodination

Results of the iodination assays designed to determine the optimum concentration of opsonized zymosan to use for stimulation of control and in vitro-activated PMNs are shown in Fig. 2. The concentration of opsonized zymosan which provided the greatest sensitivity for the detection of either enhancement or suppression of iodination activity was approximately 8.0 mg/ml (0.4 mg/tube). This concentration of opsonized zymosan was used for all subsequent experimentation.

Results of the iodination assays involving RPMI-1640-treated and in vitro-activated PMNs are shown in Fig. 3. Significant ($P < 0.01$) increases in iodination activity occurred when PMNs were stimulated with opsonized zymosan

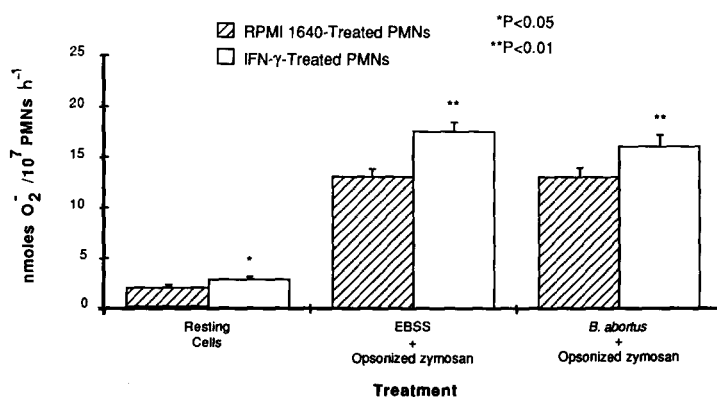


Fig. 1. Superoxide anion production by control and $IFN-\gamma$ -treated PMNs (mean \pm SEM, $n = 6$). Asterisks denote a statistically different response between $IFN-\gamma$ -treated and control cells.

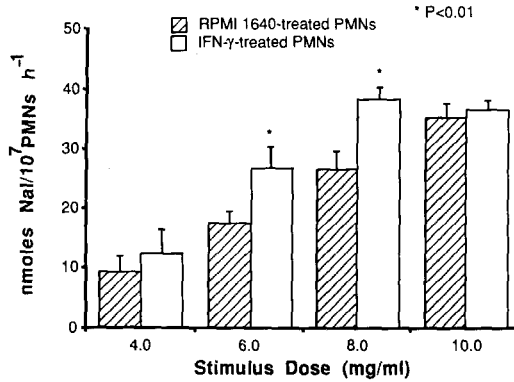


Fig. 2. Dose-related effect of opsonized zymosan stimulation on iodination responses of control and in vitro IFN- γ -treated PMNs (mean \pm SEM, $n=5$). Asterisks denote a statistically different response between IFN- γ -treated and control cells.

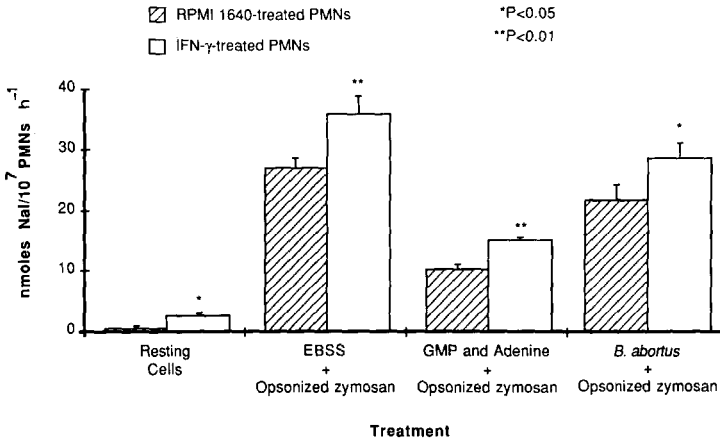


Fig. 3. Iodination responses of control and IFN- γ -treated PMNs (mean \pm SEM, $n=6$). Asterisks denote a statistically different response between IFN- γ -treated and control cells.

(27.4 ± 1.8 nmol NaI/ 10^7 PMNs h^{-1}). Opsonized zymosan-stimulated iodination was significantly ($P < 0.01$) suppressed to 10.3 ± 0.9 nmoles NaI/ 10^7 PMNs h^{-1} and 21.6 ± 2.6 nmoles NaI/ 10^7 PMNs h^{-1} in the presence of GMP and adenine or live *B. abortus* respectively.

Pretreatment of PMNs with IFN- γ for 2.0 h significantly ($P < 0.01$) increased the ability of opsonized zymosan-stimulated neutrophils to iodinate proteins when compared to RPMI-1640-treated cells (35.9 ± 2.9 nmoles NaI/ 10^7 PMNs h^{-1} vs. 27.4 ± 1.8 nmol NaI/ 10^7 PMNs h^{-1} respectively). The ability of PMNs to perform opsonized zymosan-stimulated iodination in the presence of GMP and adenine was also significantly ($P < 0.01$) increased from 10.3 ± 0.9 nmol NaI/ 10^7 PMNs h^{-1} to 15.1 ± 0.5 nmol NaI/ 10^7 PMNs h^{-1} by

IFN- γ pretreatment of PMNs. Similarly, opsonized zymosan-stimulated iodination in the presence of *B. abortus* was increased from 21.6 ± 2.6 nmol NaI/ 10^7 PMNs h $^{-1}$ to 28.7 ± 2.8 nmol NaI/ 10^7 PMNs h $^{-1}$ by IFN- γ pretreatment of PMNs.

Bactericidal activity

Results of the studies to determine the effects of in vitro administration of IFN- γ on the brucellacidal activity of PMNs are shown in Fig. 4. Exposure of *B. abortus* to immune sera for up to 2 h did not affect the survival of the bacteria (data not shown). Treatment of *B. abortus* with IFN- γ alone had no effect on the viability of the bacteria. Pretreatment of PMNs with IFN- γ for 2 h resulted in a slightly increased ability of neutrophils to kill *B. abortus*. Approximately 0.47 ± 0.04 log $_{10}$ of the bacteria were killed following 2 h of incubation with normal PMNs. Neutrophils activated with IFN- γ were capable of killing 0.64 ± 0.05 log $_{10}$ of exposed *B. abortus*, a statistically significant ($P < 0.01$) greater number than normal PMNs. The greatest difference in killing potential between normal and IFN- γ -treated PMNs occurred within the first 30 min following the addition of bacteria. Normal PMNs killed 0.12 ± 0.02 log $_{10}$ of *B. abortus* during the first 30 min of exposure, while IFN- γ -treated PMNs killed 0.28 ± 0.03 log $_{10}$ of the organisms in the same amount of time.

Effects of in vivo activation of PMNs with IFN- γ

Random migration

In vivo treatment of animals with recombinant bovine IFN- γ suppressed the ability of neutrophils to migrate under agarose in a dose- and time-dependent manner when compared with PMNs from non-treated animals. Treatment of animals with 0.5 mg of IFN- γ significantly ($P < 0.01$) inhibited the ability of PMNs to migrate to $43.1 \pm 10.0\%$ of control 24 h following the administration of IFN- γ . The inhibition of migration was no longer detectable at either 48 or 72 h post-treatment. Neutrophils from animals treated with 0.1 mg of IFN- γ did not exhibit any significant ($P > 0.05$) reduction in migration capability at any of the times evaluated.

Iodination

Results of the iodination assays involving PMNs from normal and IFN- γ -treated animals are shown in Fig. 5. Neutrophils from control animals had a mean (\pm SEM) iodination value of 24.9 ± 3.6 nmol NaI/ 10^7 PMNs h $^{-1}$ when stimulated with opsonized zymosan. Opsonized zymosan-stimulated iodination by PMNs from control animals was significantly ($P < 0.05$) suppressed in the presence of GMP and adenine or live *B. abortus* to 16.4 ± 2.3 and 16.4 ± 2.0 nmol NaI/ 10^7 PMNs h $^{-1}$ respectively.

Interferon- γ treatment of animals significantly ($P < 0.01$) enhanced the io-

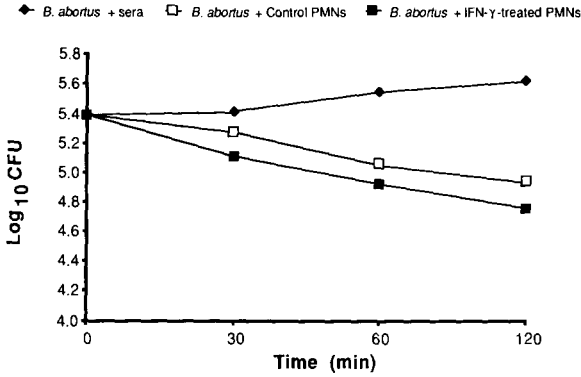


Fig. 4. Bactericidal activity of control and IFN- γ -treated PMNs for *B. abortus* during a 2-h incubation period. Points on the graph represent the means of six different determinations. Standard errors of the means did not exceed 0.05 log₁₀ CFU.

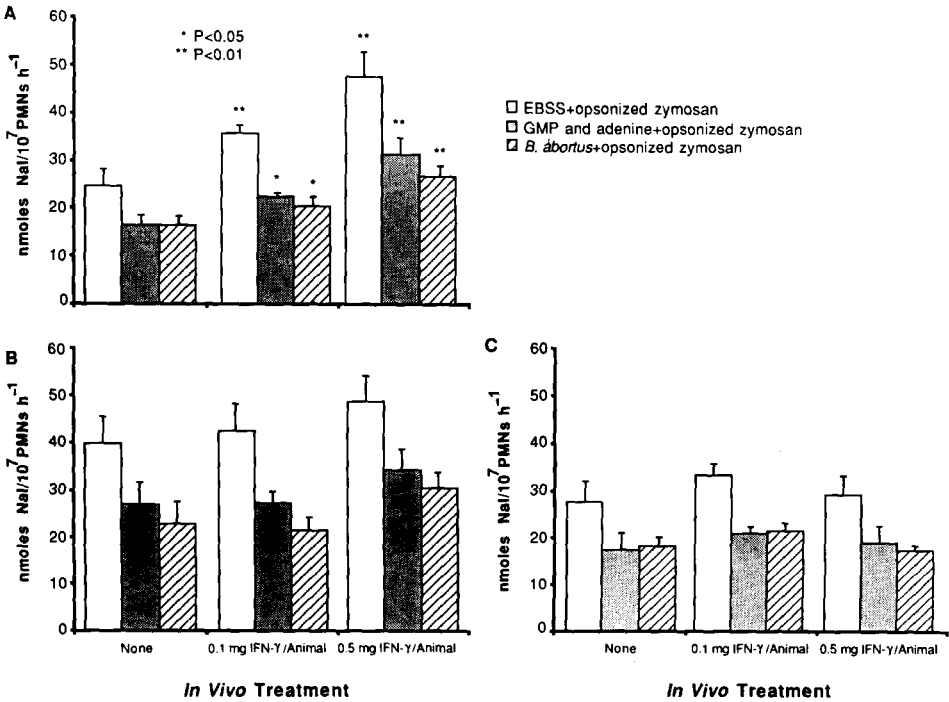


Fig. 5. Iodination responses of PMNs from IFN- γ -treated and control animals (mean \pm SEM, n = 5). Asterisks denote a statistically different response from similarly treated PMNs from control animals. Graphs A, B, and C represent 24, 48, and 72 h post-treatment respectively.

dination activity of EBSS-treated PMNs in a dose- and time-dependent manner. The ability of PMNs from animals given either the low or high dose of IFN- γ to perform opsonized zymosan-stimulated iodination in the presence of GMP and adenine was significantly ($P < 0.01$) increased from 16.4 ± 2.3 nmol NaI/ 10^7 PMNs h^{-1} to 22.3 ± 1.0 or 31.3 ± 3.9 nmol NaI/ 10^7 PMNs h^{-1} respectively 24 h after IFN- γ treatment. Both the low and high doses of IFN- γ enhanced opsonized zymosan-stimulated iodination in the presence of *B. abortus* from 16.4 ± 2.0 nmol NaI/ 10^7 PMNs h^{-1} to 20.7 ± 1.8 or 27.0 ± 2.1 nmol NaI/ 10^7 PMNs h^{-1} respectively within 24 h of IFN- γ treatment. However, both the purine mixture and *B. abortus* were still capable of inhibiting iodination by the IFN- γ activated PMNs. Neither dose of IFN- γ resulted in enhanced iodination activity at 48 or 72 h post-treatment.

Bactericidal activity

Results of the bactericidal assays involving PMNs from normal and IFN- γ -treated animals (Fig. 6) were similar to those for the vitro activation studies.

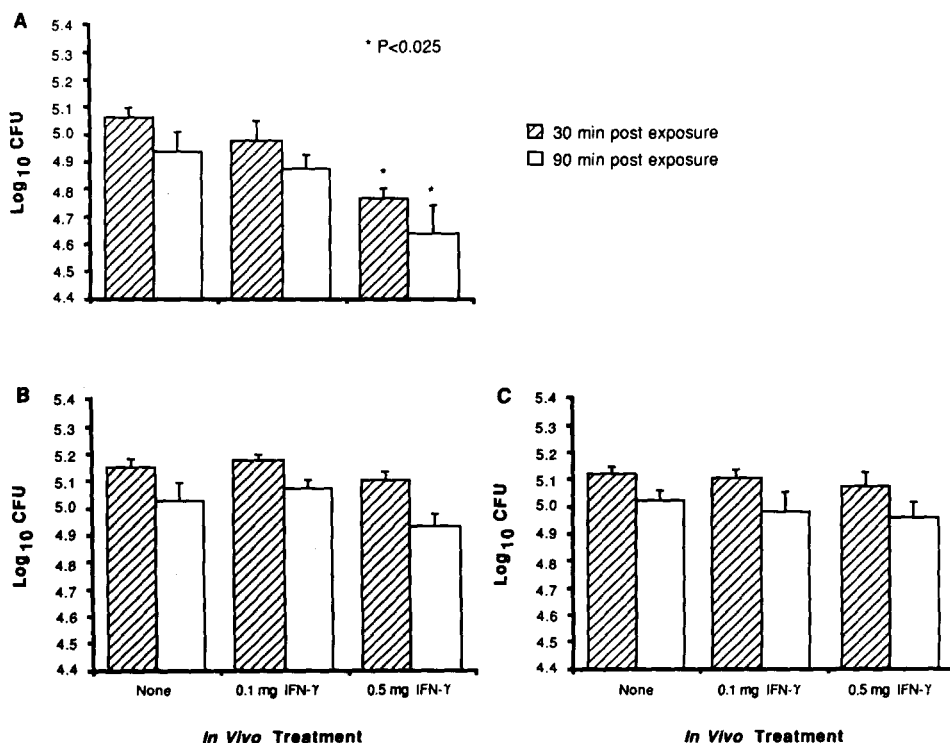


Fig. 6. Bactericidal activity of PMNs from IFN- γ -treated and control animals (mean \pm SEM, $n = 5$) for *B. abortus* during a 90-min incubation period. Graphs A, B, and C represent 24, 48, and 72 h post-treatment respectively. Asterisks denote a statistically different response from PMNs obtained from control animals.

Oposonized bacteria incubated with PMNs from control animals were killed in a time-dependent manner with approximately $5.07 \pm 0.04 \log_{10}$ of the organisms surviving after 30 min of exposure and $4.94 \pm 0.08 \log_{10}$ of the bacteria surviving at 90 min.

Treatment of animals with 0.1 mg of IFN- γ did not significantly ($P > 0.05$) affect the ability of PMNs to kill *B. abortus* at any of the time intervals tested. Neutrophils from animals treated with 0.5 mg of IFN- γ exhibited a statistically significant ($P < 0.025$) enhancement of brucellacidal activity 24 h following treatment when compared with PMNs from control animals. Phagocytes from animals treated with 0.5 mg of IFN- γ were capable of killing $0.84 \pm 0.11 \log_{10}$ of exposed *B. abortus* within 90 min while PMNs from control animals killed $0.54 \pm 0.08 \log_{10}$ of the bacteria. The greatest difference in killing potential between PMNs from control and IFN- γ -treated animals occurred within the first 30 min following the addition of bacteria. Neutrophils from control animals killed $0.41 \pm 0.04 \log_{10}$ of *B. abortus* during the first 30 min of exposure, while PMNs from animals treated with 0.5 mg of IFN- γ killed $0.71 \pm 0.04 \log_{10}$ of the organisms in the same amount of time. There were no significant ($P > 0.05$) differences in the killing potentials of PMNs from control or IFN- γ -treated animals at 48 or 72 h post-treatment.

DISCUSSION

Activation of PMNs either in vitro or in vivo with recombinant bovine IFN- γ resulted in the activation of these cells as evidenced by the markedly suppressed ability of neutrophils to migrate under agarose as reported previously (Steinbeck et al., 1986).

Both resting and opsonized zymosan-stimulated PMNs produced greater amounts of O_2^- in the presence of IFN- γ . These results differ from those reported previously in which IFN- γ -activation of PMNs failed to increase production of O_2^- (Steinbeck et al., 1986). However, a bovine lymphokine preparation containing interferon activity has previously been shown to enhance O_2^- production by opsonized zymosan-stimulated bovine neutrophils (Lukacs et al., 1985). The reasons for this discrepancy are unclear, but it is possible that variations in experimental protocols, INF- γ preparations or differences between animals might be responsible.

Brucella abortus survival within neutrophils has been associated with the expression of PMN suppressive purines on the bacterial surface which inhibit MPO- H_2O_2 -halide activity (Canning et al., 1986). Results of the experiments designed to determine the effects of recombinant IFN- γ on the MPO- H_2O_2 -halide activity of PMNs indicated that in vitro activation of PMNs with IFN- γ significantly enhanced the iodination responses of neutrophils when they were stimulated with concentrations of opsonized zymosan which produced less than maximal iodination. When a concentration of opsonized zymosan

was used which stimulated maximal iodination activity (10 mg/ml), the IFN- γ treatment did not enhance iodination in either the present or a previous study (Steinbeck et al., 1986). The iodination assay used in the current experimentation involved stimulation of iodination responses with a dose of opsonized zymosan which stimulated approximately 80% of maximal iodination activity. This level of stimulation provided the optimal sensitivity for the detection of enhanced iodination activity due to IFN- γ -induced PMN activation while maintaining the sensitivity needed for detecting suppression by either purines or live *B. abortus*.

Results of the iodination experiments involving in vivo activation of PMNs indicated IFN- γ significantly increased the iodination responses of either resting or opsonized zymosan-stimulated neutrophils within the first 24 h following treatment (Fig. 5).

Both in vitro and in vivo activation of PMNs with IFN- γ resulted in an improved iodination response by phagocytes in the presence of GMP and adenine or *B. abortus*. Neutrophils from animals treated with either the high or low dose of IFN- γ and assayed after 24 h produced similar levels of iodination activity in the presence of GMP and adenine or *B. abortus* as control preparations of PMNs from non-treated animals. The magnitude of the iodination-enhancing effect of IFN- γ was directly proportional to the dose administered. The duration of the enhancement of iodination by IFN- γ activation of PMNs was short lived (less than 48 h).

Bactericidal assays involving normal PMNs indicated *B. abortus* was capable of resisting killing by PMNs. Although a substantial number of opsonized *B. abortus* were killed by neutrophils, a residual population (approximately 35–40% of the original inoculum) of *B. abortus* survived exposure to PMNs for 90–120 min.

If suppression of the MPO-H₂O₂-halide activity of PMNs plays a significant role in the survival of *B. abortus*, it seems plausible that reversal of this inhibition would result in an increase in the brucellacidal activity of PMNs. Results of the studies to determine effects of IFN- γ on the brucellacidal activity of PMNs indicated pretreatment of PMNs with IFN- γ for 2 h resulted in a modest, but statistically significant ($P < 0.01$), enhancement of the ability of PMNs to kill *B. abortus*. The enhancement of killing was most notable during the first 30 min following the addition of the bacteria. During this time interval, the IFN- γ -treated PMNs killed approximately twice the amount of *B. abortus* as did the normal PMNs. The differences in the killing potential between normal and activated PMNs decreased at 60 and 120 min post-exposure.

Recombinant IFN- γ treatment of animals also resulted in slightly improved brucellacidal activity of PMNs in a dose- and dependent manner. The low dose of IFN- γ did not measurably affect the bactericidal potential of PMNs. Neutrophils from animals treated with 0.5 mg of IFN- γ exhibited statistically significant ($P < 0.025$) increases in their ability to kill *B. abortus* within 24 h of

treatment. The magnitude of the enhancement of killing was greatest during the first 30 min following the addition of the PMNs to the reaction mixture, similar to the *in vitro* activation studies. Neutrophils from animals treated with 0.5 mg of IFN- γ killed approximately 25% more bacteria during this time interval than did PMNs from control animals. The differences in killing potential between PMNs from control or IFN- γ -treated animals decreased to approximately 10% at 90 min post-exposure. These results suggest that IFN- γ -activation of PMNs may change the kinetics of the antibacterial activity of neutrophils by increasing the early antibacterial capacity of the phagocyte, resulting in an overall enhanced bactericidal potential. Neither the low nor the high dose of IFN- γ enhanced the brucellacidal activity of PMNs beyond 24 h post-treatment.

The results of these studies suggest that activation of PMNs with INF- γ partially enhances both oxygen radical production and iodination responses, resulting in a statistically significant increase in their ability to kill *B. abortus* when compared with normal neutrophils. However, the small magnitude of the difference and survival of a significant number of bacteria at 90–120 min post-exposure forces one to question the biological significance of the increased killing potential.

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