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Effect of recombinant human cytokines on porcine neutrophil function

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ABSTRACT

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The activity of four recombinant human cytokines on porcine neutrophils was evaluated Porcine neutrophils were treated with varying doses of recombinant human tumor necrosis factor-alpha (rHu-TNF) interferon-gamma (rHu-IFN), interleukin-8 (rHu-IL-8) or granulocyte-macrophage colony-stimulating factor (rHu-GM-CSF) The function of treated neutrophils was compared with that of non-treated controls in the following assays antibody-independent neutrophil cytotoxicity (AINC), antibody-dependent cell-mediated cytotoxicity (ADCC) iodination *Staphylococcus aureus* ingestion cytochrome C reduction random migration, and chemotaxis Treatment with rHu-TNF produced significant ($P<0.05$) depression of neutrophil random migration (2.5 25 and 250 ng ml^{-1} rHu-TNF) and iodination (250 ng ml^{-1}) and a near significant ($P=0.08$) depression in ADCC (250 ng ml^{-1}) Treatment with 25 000 U ml^{-1} of rHu-IFN caused a significant increase in AINC At lower doses of rHu-IFN, there was a trend ($0.05<P\leq 0.08$) toward depression of AINC (250 U ml^{-1}) and ADCC (25 U ml^{-1}) and enhancement of iodination (250 U ml^{-1}) Treatment with 50 ng ml^{-1} of rHu-IL-8 caused a near significant increase ($P=0.06$) in AINC There were no significant differences noted when porcine neutrophils were treated with rHu-GM-CSF (2.5–2500 U ml^{-1}) No synergism was noted between rHu-TNF and rHu-IFN

ABBREVIATIONS

ADCC, antibody-dependent neutrophil cytotoxicity AINC, antibody-dependent neutrophil cytotoxicity, IFN, interferon gamma, IL-8, interleukin-8, LAK, lymphokine-activated killer, rHu-GM-CSF recombinant human granulocyte-macrophage colony stimulating factor, rHu-TNF recombinant human tumor necrosis factor-alpha

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INTRODUCTION

Research over the past decade has identified numerous cytokines (protein factors produced and released by specific cell types in response to inflammatory and infectious stimuli) that are capable of modulating host immune responses (reviewed by Balkwill and Burke, 1989) Such immunomodulatory cytokines have clinical potential, especially as vaccine adjuvants and as adjuncts to conventional therapy for infectious and neoplastic diseases Much progress has been made toward the understanding of cytokines and their roles in immune regulation, especially in human models However, research on cytokine activation of immune responses in domestic food animals has been somewhat limited by the lack of commercially available homologous host-origin cytokines Identification of commercially available human cytokines that have activity in other animal species would facilitate the characterization of immune regulation in those species There are published reports of recombinant human cytokine activity in cells from several species of domestic animals, including canine lymphokine-activated killer (LAK) cells (Raskin et al , 1991), bovine neutrophils (Sample and Czuprynski, 1991), and porcine mononuclear cells (Fong and Doyle, 1986) In this study, we evaluated the effect of recombinant human tumor necrosis factor-alpha, interferon-gamma, interleukin-8, and granulocyte-macrophage colony-stimulating factor on porcine neutrophil function

MATERIALS AND METHODS

Animals

Crossbred pigs, 3–5 months of age, were maintained as blood donors for this study Blood was collected from the vena cava of randomly selected pigs into syringes pre-loaded with 7 5% EDTA in 0 85% saline (1 30 v/v)

Cytokines

Recombinant human tumor necrosis factor alpha (rHu-TNFα), recombinant human interferon-gamma (rHu-IFNγ), and recombinant human granulocyte-macrophage colony-stimulating factor (rHu-GM-CSF) were obtained from Boehringer Mannheim, (Indianapolis, IN) Recombinant human interleukin-8 (rHu-IL-8) was obtained from Genzyme, (Cambridge, MA) Working dilutions of cytokines were made in M199 medium without phenol red immediately prior to use Cytokine concentrations were expressed as ng ml^{-1} or U ml^{-1} depending on the unit of measurement used by the manufacturer The specific activity of our rHu-TNFα, specified by the manufacturer, was greater than 2×10^{7} U ml^{-1}

Neutrophil isolation and cytokine treatment

Neutrophils were isolated from whole blood by a combination of hypotonic RBC lysis and ficoll-diatrizoate density centrifugation as described previously (Coe et al, 1992) and adjusted to 10^8 cells ml^{-1} in M199 medium without phenol red Each individual cytokine was tested with neutrophils from five to seven pigs except combinations of rHu-TNFα and rHu-IFNγ, which were tested with neutrophils from four pigs, and the 25 000 U ml^{-1} dose of rHu-IFNγ, which was tested with neutrophils from two pigs Neutrophils were aliquoted so that non-treated cells and cells treated with each of three to four consecutive ten-fold dilutions of a given cytokine (Table 1) were tested in each pig The cells were mixed with an appropriate dilution of cytokine or M199 medium alone (non-treated controls) to achieve a final concentration of 5×10^7 cells ml^{-1} All neutrophils were then incubated at 37°C per 5% CO_2 for 2 h prior to use in functional assays

Assays of neutrophil function

Assays of antibody-dependent cell-mediated cytotoxicity (ADCC), iodination, *Staphylococcus aureus* ingestion, cytochrome C reduction of stimulated and resting neutrophils, random migration, and chemotaxis were performed as described previously (Coe et al, 1992) Assays of antibody-independent neutrophil cytotoxicity (AINC) were performed as described by Lukacs et al (1985)

TABLE 1

Recombinant human cytokines tested for activity on porcine neutrophils

Cytokine	Dosages Tested
Tumor necrosis factor-α (rHu-TNFα)	0 25 2 5 25 and 250 ng ml^{-1}
Interleukin-8 (rHu-IL-8)	5 50, and 500 ng ml^{-1}
Interferon-γ (rHu-IFNγ)	25 250 2500 and 25 000 U ml^{-1}
Granulocyte-macrophage colony-stimulating factor (rHu-GM-CSF)	2 5, 25, 250 and 2500 U ml^{-1}
Combination of rHu-TNFα and rHu-IFNγ	250 ng ml^{-1} TNF + 2500 U ml^{-1} IFN 25 ng ml^{-1} TNF + 250 U ml^{-1} IFN

Statistical analysis

An analysis of variance was performed for each assay The data were blocked by date of the assay to minimize the effect of the daily variation inherent in these assays *P*-values of 0 05 or less were considered significant

RESULTS

When compared with non-treated controls, porcine neutrophils treated with rHu-TNFα exhibited a dose-dependent depression of random migration, ADCC, and iodination (Fig 1) Random migration was significantly depressed ($P<0$ 005) when the neutrophils were treated with 250, 25, or 2 5 ng ml^{-1} rHu-TNFα To establish an extinction point for the depression of migration, neutrophils incubated with 0 25 ng ml^{-1} rHu-TNFα were assayed for random migration Although neutrophil migration was still depressed at this dose of rHu-TNFα, the difference was no longer statistically significant Iodination was significantly depressed when neutrophils were exposed to 250 ng ml^{-1} rHu-TNFα The depression of ADCC, while not statistically significant ($P=0$ 08), was evident at the 250 ng ml^{-1} dose

Treatment with rHu-IFNγ (25 000 U ml^{-1}) elicited a significant increase in AINC of porcine neutrophils (Fig 2) Iodination was slightly increased in treated neutrophils, an effect that was nearly significant ($P=0$ 08) at 250 U ml^{-1} rHu-IFNγ The increase observed at 25 000 U ml^{-1} rHu-IFNγ, though

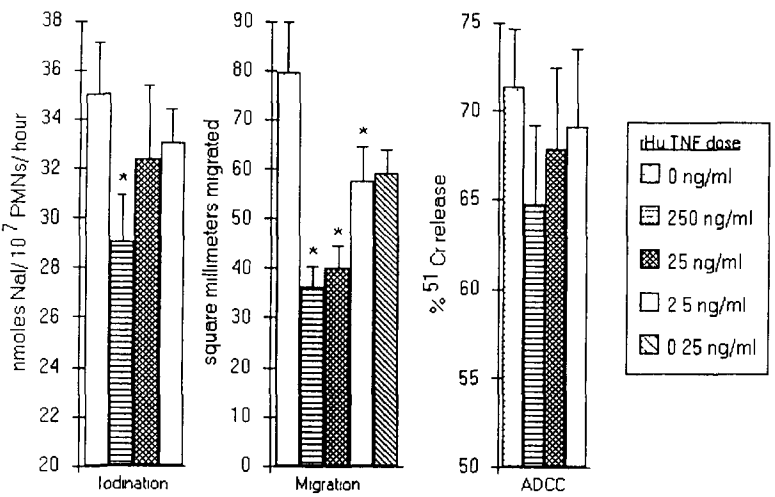

Fig 1 Effect of recombinant human tumor necrosis factor α (rHu-TNFα) on porcine neutrophil iodination, random migration, and antibody-dependent cell-mediated cytotoxicity (ADCC) Bars represent mean values +/− SEM from five to seven pigs *$P<0$ 05 when compared with non-treated controls Iodination and ADCC not evaluated at 0 25 ng ml^{-1} rHu-TNFα

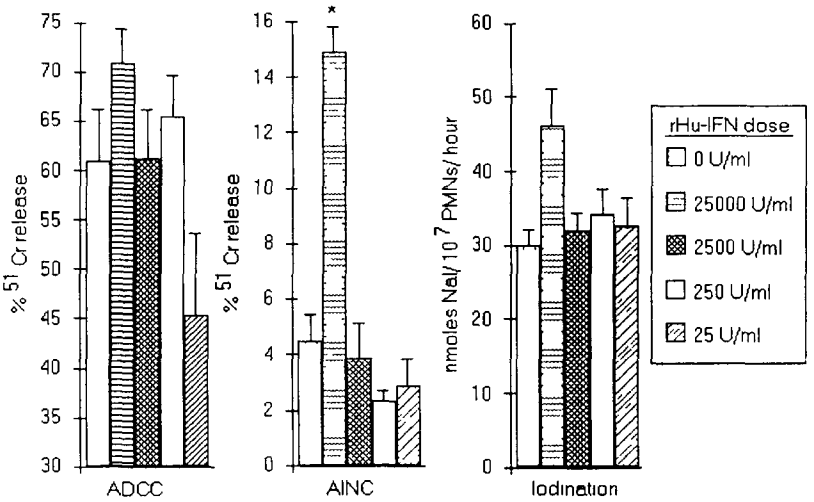

Fig 2 Effect of recombinant human interferon γ (rHu-IFN$_\gamma$) on porcine neutrophil iodination antibody-dependent cell-mediated cytotoxicity (ADCC), and antibody-independent neutrophil cytotoxicity (AINC) Bars represent mean values +/− SEM from five to seven pigs except at the 25 000 U ml^{-1} dose which represents the mean of two pigs *$P<0$ 05 when compared with non-treated controls

arithmetically larger than the increase at 250 U ml^{-1}, was not statistically significant ($P=0$ 12) ADCC was decreased to a near significant level ($P=0$ 07) when neutrophils were treated with 25 U ml^{-1} rHu-IFNγ

No significant changes in function were noted when porcine neutrophils were treated with rHu-IL-8 AINC was slightly enhanced in treated cells, an effect that was most pronounced at the 50 ng ml^{-1} dose level ($P=0$ 06)

No significant or near significant differences in neutrophil function were observed when porcine neutrophils were treated with rHu-GM-CSF Also, no significant interaction between rHu-TNFα and rHu-IFNγ was noted when porcine neutrophils were treated with combinations of both cytokines (results not shown)

DISCUSSION

Cytokine-induced cell activation is ideally studied using homologous-origin cytokines, but only a few porcine cytokines have been characterized The genes for porcine IL-1α, IFN-α, and IFN-γ have been cloned, but these cytokines are not available commercially (summarized by Blecha, 1991) Because numerous recombinant human cytokines are commercially available, the identification of human cytokines that could be used in porcine models of cytokine-induced cell activation is desirable

There is evidence that certain human and porcine cytokines are cross-re-

active Porcine and human gamma interferons share up to 59% nucleotide homology (Charley et al , 1987), they interact antagonistically in human cell culture (Filipic et al , 1991) Porcine interleukin-1α, interleukin-2, and interleukin-6 share 82%, 72%, and 62% homology, respectively, with human IL-1α, IL-2, and IL-6 (Maliszewski et al , 1990, Richards and Saklatvala, 1991, Goodall et al , 1991) The structure of tumor necrosis factor (TNF) apparently is conserved among animal species because monoclonal antibodies against human TNF are reactive against TNF from dogs, pigs, and monkeys (Moller et al , 1990) However, the nucleotide sequence of porcine TNF has not yet been reported, so the extent of homology with human TNF is unknown The nucleotide sequences for porcine IL-8 and GM-CSF also have not been reported

Human cytokines are active upon cells from a number of domestic animal species Human IL-2 stimulates bovine (Majury and Shewen, 1991) and porcine (Bhagyam et al , 1988) lymphocytes, porcine natural killer cells (Hennessy et al , 1990), and canine LAK cells (Raskin et al , 1991) Bovine neutrophils are stimulated by human IL-1α and TNFα (Sample and Czuprynski, 1991) In this study, we showed that recombinant human TNFα, IFNγ, and IL-8 affected the function of porcine neutrophils

In human neutrophils, treatment with human tumor necrosis factor causes reduced neutrophil migration and chemotaxis and increased phagocytosis, superoxide production, iodination, and ADCC (Shalaby et al , 1985, Klebanoff et al , 1986, reviewed by Steinbeck and Roth, 1989) Functional alterations are observed after incubating neutrophils with TNFα for 20–30 min (phagocytosis, migration, chemotaxis) to 1 h (ADCC, superoxide production) and with TNFα concentrations of less than 100 U ml^{-1} Treatment of porcine neutrophils with rHu-TNFα elicited a similar reduction in random migration No significant changes in chemotaxis, phagocytosis, or superoxide production (as measured by cytochrome C reduction) were noted in our study, but this may be due to reduced sensitivity of porcine neutrophils to heterologous TNFα or differences in incubation periods rather than any inherent differences in the response of porcine neutrophils to TNFα Treatment of porcine neutrophils with rHu-TNFα caused a mild dose-dependent depression in iodination and a near significant decrease in ADCC, which contrasts with the responses observed in human neutrophils The porcine responses more closely correlated with the decreased iodination and unaffected ADCC observed by Chiang et al (1991) in bovine neutrophils treated with recombinant bovine TNFα (50 ng ml^{-1}, 2 5 h incubation)

Porcine neutrophils exhibited enhanced AINC when treated with high doses of rHu-IFNγ This increase must be interpreted with caution owing to the small number of pigs tested at the 25 000 U ml^{-1} dose, but increased AINC was also noted in bovine neutrophils treated for 2 5 h with 0 5 ng ml^{-1} recombinant bovine IFNγ (Steinbeck et al , 1986) No similar assay data for human

neutrophils could be found in the literature, however, treatment with IFNγ (1 U ml^{-1}, 2 h incubation) caused increased ADCC in human neutrophils (Shalaby et al., 1985) Treatment with rHu-IFNγ also increases superoxide anion production and phagocytosis of human neutrophils and decreases random migration and chemotaxis (reviewed by Steinbeck and Roth, 1989) Thus, it appears that rHu-IFNγ is practically inactive on porcine neutrophils when compared with the effects elicited in the homologous host species

Interleukin-8 is a potent human neutrophil activator Treatment with IL-8 increases human neutrophil superoxide anion production (Peveri et al., 1988, Thelen et al., 1988), stimulates degranulation (Peveri et al., 1988), and enhances chemotaxis (Rot, 1991) Recombinant human IL-8 also induces migration and chemotaxis of neutrophils from a number of domestic animal species, including dogs, goats, and chickens (Rot, 1991) The optimum doses of rHu-IL-8 for these heterologous species range from 1 to 10 μg ml^{-1}, which are approximately 10–100 times more than the optimal dose for human PMNs (150 ng ml^{-1}) In our study, rHu-IL-8 activity on porcine neutrophils was limited to an enhancement of AINC when given at 50 ng ml^{-1} We did not observe significant changes in random migration or chemotaxis, but the highest dose of rHu-IL-8 that we tested was 500 ng ml^{-1} Also, the neutrophils in our study were pre-incubated with rHu-IL-8, whereas in the study by Rot, IL-8 was used as a chemoattractant in a Boyden chamber chemotaxis assay Our study does agree with that of Rot in that porcine neutrophils were relatively insensitive to rHu-IL-8

Recombinant human granulocyte-macrophage colony-stimulating factor has widespread effects on human neutrophil function, including enhancements of superoxide anion production, phagocytosis, iodination, and ADCC (Lopez et al., 1986) Enhancement was observed with 2 ng ml^{-1} rHu-GM-CSF after 2 h pre-incubation (ADCC) or no pre-incubation (iodination, phagocytosis, superoxide anion) with rHu-GM-CSF Similar enhancements have been reported in bovine neutrophils treated with 5–10 ng ml^{-1} bovine GM-CSF for 12 h (Reddy et al., 1990) rHu-GM-CSF activity has also been reported with monkey neutrophils (Welte et al., 1987), but treatment with rHu-GM-CSF had no effect on the porcine neutrophils in our study

Tumor necrosis factors and interferon-gamma act synergistically to alter neutrophil function in humans (Shalaby et al., 1985) and cattle (Chiang et al., 1991) We investigated possible synergism between rHu-TNFα and rHu-IFNγ in porcine neutrophils by incubating cells with mixtures of both cytokines, no synergistic activity was noted at any dilution tested

In summary, certain recombinant human cytokines (TNFα, IFNγ, IL-8) had activity on porcine neutrophils The alterations in porcine neutrophil function were limited in comparison with those induced by the same cytokine in homologous host neutrophils, and higher concentrations of cytokine were usually required to effect the changes However, in the absence of commer-

cially available porcine-origin cytokines, such recombinant human cytokines may be useful in porcine neutrophil studies

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