Plasma Lactoferrin Reflects Granulocyte Activation In Vivo

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N-formyl-met-leu-phe (FMLP) causes polymorphonuclear leukocytes (PMN) to secrete and become “sticky” in vitro. We related these events to in vivo FMLP-induced neutropenia. FMLP was intravenously administered to anesthetized rabbits in doses ranging from 0.01 μg to 1.0 μg. Controls received phosphate-buffered saline (PBS). The diluent for FMLP. Blood pressure, respiratory rate, and arterial Po2 were monitored. High and intermediate doses of FMLP caused a dramatic but transient decrease in blood pressure and an increase in respiratory rate. Prior to FMLP infusion, plasma lactoferrin level was 6.4 ± 4.1 μg/ml, and the absolute granulocyte account (AGC) was 2008 ± 1229 (mean ± SD). There was a positive linear correlation between AGC and plasma lactoferrin level prior to injection of FMLP (R² = 0.74, p < 0.01). At 1 min after FMLP injection, the percent change in AGC decreased as an exponential function of dose to as low as 10% of baseline (R² = 0.86, p = 0.002) and plasma concentration of lactoferrin increased as an exponential function of dose to as high as 30 μg/ml (R² = 0.84, p = 0.006). Thus, FMLP-induced neutropenia is associated with increased levels of plasma lactoferrin, suggesting that PMN are induced to degranulate in vivo.

Chemotactic factors induce polymorphonuclear leukocytes (PMN) to degranulate, manifest increased surface membrane adhesiveness, undergo cellular aggregation, and adhere to endothelial cells in vitro. Previous studies have documented the rapid but transient decline in numbers of circulating PMN following infusion of activated complement fragments or various chemotactic synthetic n-formyl oligopeptides. The reversible adherence of activated PMN to vascular endothelium and formation of intravascular leukoaggregates has been suggested as a plausible mechanism for the observed neutropenia. In addition, cardiopulmonary dysfunction and pulmonary microvasculature leukostasis following in vivo complement activation during hemodialysis and cardiopulmonary bypass appear to reflect a similar etiology. Recent in vitro evidence suggests that release of lactoferrin from PMN-specific granules following chemotactic stimulation plays a major autoregulatory role in the promotion of PMN adhesiveness. As PMN are thought to be the exclusive source of intravascular lactoferrin, this would imply that measurable changes in plasma lactoferrin concentration may reflect its release and mediation of PMN adherence. Since degranulation is commonly considered to be one aspect of PMN activation, we employed plasma lactoferrin levels as a measurement of in vivo PMN activation. We found an inverse relationship between plasma lactoferrin levels and neutropenia induced by graded infusions n-formyl-methionyl-leucyl-phenylalanine (FMLP).

Materials and Methods

Adult male New Zealand white rabbits weighing 2 ± 0.1 kg were anesthetized by intramuscular injection of a mixture of ketamine (100 mg), acepromazine (22 mg), and atropine sulfate (5 mg), as a dose of 44 mg ketamine/kg. Polyethylene catheters were placed in the ipsilateral femoral artery and vein, and the arterial catheter flushed with heparin 1000 U/ml and connected to a pressure transducer. The venous catheter was kept patent with a slow infusion of sterile, nonpyrogenic saline.

FMLP (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.02 M sodium bicarbonate to a concentration of 1 mg/ml and diluted to final concentration in 5 ml of phosphate-buffered saline (PBS). Rabbits received infusions over 20 sec with 5 ml of PBS containing 0.01, 0.1, 0.5, or 1.0 μg of FMLP through the venous catheter. Control rabbits received 5 ml of PBS.

Samples of venous blood were serially collected in EDTA tubes over 30 min, following the injection of FMLP, and placed immediately on ice for subsequent determinations of absolute granulocyte count (AGC) and plasma concentration of lactoferrin using an antibody prepared in goats against rabbit PMN lactoferrin. Arterial blood samples for Po2, employing the pH/blood gas analyzer, were simultaneously obtained in heparinized syringes.

Total white blood cell counts were performed on a Coulter counter, and 100 cell differential counts were done on Wright-stained smears. The sample tubes were then centrifuged at 400 g for 10 min at 4°C and the upper two-thirds of the plasma removed, avoiding the cell–plasma interface.

A blood pressure monitor was used to measure systolic, diastolic, and mean blood pressures. A Hewlett-Packard ECG monitor and impedance apnea monitor were used to measure heart rate and respiratory rate, respectively. Recording was done either on a continuous chart recorder or an Apple II microcomputer.

Results

The average baseline AGC and plasma lactoferrin level prior to infusion of PBS or FMLP were 2008 ± 1229 μg/ml.
1229 cells/µl and 6.4 ± 4.1 µg/ml, respectively (mean ± SD). The baseline plasma lactoferrin concentration correlated with the AGC ($R^2 = 0.74$, $p < 0.01$), as shown in Fig. 1. Plasma lactoferrin concentration increased linearly with increasing AGC over the ranges observed.

The changes in AGC and plasma lactoferrin levels after stimulation with FMLP, expressed as a percent of initial baseline values, are shown in Figs. 2 and 3, respectively. In control rabbits, there was a gradual but significant decline in the AGC over the first 10 min following injection of heparinized buffer, which stabilized at approximately 70% of baseline levels (data not shown). As seen in Fig. 2, rabbits receiving FMLP demonstrated a rapid, dose-related fall in the AGC within 1 min following injection ($R^2 = 0.86$, $p = 0.002$). One microgram of FMLP elicited the maximum degree of neutropenia, with a fall in the AGC to less than 10% of baseline levels by 1 min.

As seen in Fig. 3, the plasma concentration of lactoferrin exhibited a dose-dependent rise in response to FMLP administration at 1 min ($R^2 = 0.84$, $p = 0.0006$) compared to the baseline value. There was a rapid initial rise in plasma lactoferrin levels in rabbits receiving 0.1 and 1.0 µg of FMLP. Plasma lactoferrin
levels rose fivefold at 1 min after stimulation with 1.0 μg FMLP and remained elevated when tested at 30 min.

Control rabbits showed no acute changes in blood pressure, respiratory rate, and arterial Po2 over the 15 min following injection of PBS. In animals, infusion of FMLP (0.1 and 1.0 μg) induced a transient tachypnea that corresponded to the onset of neutropenia. As the AGC recovered to baseline levels, the respiratory rate returned toward normal (Fig. 4). The changes in systolic blood pressure, respiratory rate, and Po2 during the initial 5 min after 1 mg FMLP administration are shown in Table 1. The fall in systolic blood pressure and Po2 was inversely related to the rise in respiratory rate at 1 min. The Po2 and systolic blood pressure returned to normal by 5 min.

**DISCUSSION**

Systemic administration of the chemotactic factors C5a or FMLP in rabbits results in a profound but transient state of neutropenia.2,3,11 In vitro, PMN degranulate and become "sticky" in response to stimulation by chemotactic factors.1 This increased adhesiveness is manifested by enhanced adherence to endothelial surfaces and generation of leukoaggregates.1,2 One of the secretory products released from PMN during degranulation is lactoferrin, a glycoprotein component of PMN-specific granules.13 Lactoferrin can induce aggregation and adherence of PMN in vitro9 and transiently promote the attachment of PMN to endothelial cells in vivo.14 Therefore, it is possible that the neutropenia observed following FMLP administration could be explained in part by in vivo activation and release of the aggregant lactoferrin, leading to subsequent PMN hyperadherence and attachment to endothelial surfaces.

As in studies performed in vitro, we observed that increasing amounts of FMLP resulted in increasing degrees of neutropenia and levels of plasma lactoferrin. Correspondingly, we also observed a temporal relationship between the onset of neutropenia and tachypnea, hypoxia, and hypotension. The relatively rapid recovery of these physiologic measurements is consistent with the observations of others employing C5a infusion that lasting damage to the lung did not seem to be caused by these two chemotactic stimuli.15

Since release of lactoferrin serves as an indicator of PMN activation in vitro,9 our results would suggest that monitoring of PMN lactoferrin levels could reflect this process in vivo. Recent observations in humans undergoing hemodialysis indicate that plasma lactoferrin levels correlate with complement activation induced by the dialysis membrane.16 In addition, the studies reported here in rabbits indicate that a close correlation likely exists between induced PMN activation in the circulation and cardiopulmonary changes.

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