Flow and Composition of Afferent Mammary Gland Lymph

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ABSTRACT

Afferent mammary lymphatic flow was characterized in conscious lactating cows during milking and prior to, during, and after intramammary infusion of endotoxins. Lymph flow (13 to 45 ml/h) was pulsatile with monophasic and multiphasic episodes. Flow resulted from 62 to 67 episodes per h. Episodes varied from 1 to 53 s in duration. Maximum instantaneous flow ranged from 163 to 245 µl/s. Flow did not increase consistently during milking. Lymph flow increased (5.5- to 8-fold) during endotoxin-induced mastitis. Flow rates were elevated for up to 48 h after infusion of endotoxin. Compositional comparisons between afferent mammary lymph and blood plasma showed distinct differences. Lymph contained 7, 6, and 10 times less protein, albumin, and globulin, respectively, than did plasma. Glucose concentrations were equivalent. Lymph had 17 times less cholesterol and about one-half as much triglyceride and calcium as did plasma. γ-Glutamyl-transaminate and aspartate transaminate were substantially higher in plasma than in lymph. Afferent mammary lymph has unique compositional characteristics. The lymph ducts contained an intrinsic mechanism for lymph movement. Moreover, this mechanism was altered by inflammation. The techniques herein provide a better understanding of the mammary lymphatic system.

INTRODUCTION

Rhythmic contractions of lymphatics have been described in various species of animals, including rats, guinea pigs, squirrels, sheep, and humans (1, 2, 3, 9, 10). These movements are important for propulsion of lymph through the lymphatic system (4). Only a few reports (8, 9) describe spontaneous flow of lymph in efferent lymphatic vessels (lymphatic ducts exiting the supramammary lymph node) of ruminants. No reports characterize afferent mammary lymphatic flow in conscious lactating cows. Very little is known about the physiological processes that control mammary lymphatic flow. No information exists on the composition of afferent lymph versus blood plasma from lactating cows.

The objectives of our work were 1) to develop a technique to measure afferent mammary gland lymphatic flow in conscious lactating cows, 2) to characterize the flow dynamics of lymph through individual afferent mammary gland lymph vessels, and 3) to characterize partially the composition of afferent mammary lymphatic fluid and to compare it with that of blood plasma.

MATERIALS AND METHODS

Cows

Four lactating, multiparous nonpregnant Holstein cows and two lactating, multiparous nonpregnant Swedish Red cows, producing from 30 to 32 kg/d of milk, were used.

Milking

Cows were milked twice daily at 12-h intervals. Swedish Red cows were milked by an
Alfa-Laval Duo Vac (Tumba, Sweden); the vacuum level, ratio, and rate were set at 50 kPa, 70:30, and 60 cycles/min, respectively. Holstein cows were milked using Delaval clusters (Alfa-Laval, Inc.), pneumatic pulsators, and stainless steel milk buckets. The milking vacuum and pulsation rate were as just described. However, the ratio was 60:40.

Surgery and Cannulations
Cows were not milked prior to surgery. They were premedicated with an intramuscular injection of acepromazine (325 mg/kg of BW), placed in a dextrolateral position, and intubated using 10% lidocaine aerosol. General anesthesia was induced with an intravenous injection of thiopentone and maintained with halothane plus nitrous oxide inhalation (7). Cows were placed in a semidorsal position with the left hind leg slightly abducted to expose the lateral surface of the left hind quarter of the udder. An incision was made in the lateral wall of the udder, dorsal to the teat and medial to the thigh, and carried through the lateral suspensory ligament, directly outside the mammary parenchyma. Afferent lymph ducts were isolated by blunt dissection between the suspensory ligament and mammary parenchyma. Patent blue dye (.25%, wt/vol) dissolved in .85% sterile saline was injected into the parenchymal tissue adjacent to the incision to identify afferent lymphatic ducts of Swedish Red cows. The dye was distributed inside the lymph ducts within 10 min after injection.

Two types of cannula were placed within lymph vessels. Swedish Red cows were cannulated with 30-cm polyethylene catheters (1.5 mm i.d., 2.5 mm o.d.) treated with 7% tridodecy-l-methylammonium chloride-heparin to achieve thromboresistance (5). Lymph ducts of Holstein cows were cannulated with 30-cm polyurethane (.07 mm i.d., .095 o.d.) catheters (Braintree Scientific, Braintree, MA). These catheters were nonthrombocytic. Therefore, pretreatment with heparin was not necessary. Afferent lymphatic ducts ranged from .05 to 1.5 mm in diameter. The size of the vessels and ease of isolation were directly related to the size of the udder and the amount of milk produced. The lymphatic walls were very compliant, which enabled good fit between the vessel and cannula. However, care had to be taken because vessels sometimes collapsed with excessive manipulation.

Just after lymph cannulation, a 2-mm precalibrated, Transit-Time ultrasonic blood flow probe (2R628; Transonic Systems, Ithaca, NY) was placed on the same duct distal to the cannula on five cows (one Swedish Red and four Holstein) and on an adjacent duct, in the same quarter as the cannulated one, in one Swedish Red cow. Flow probes were secured to the surrounding connective tissue, as outlined by Gorewit et al. (7). Cannulas were placed upstream to the downward flow of lymph. Cows were fitted with indwelling polyethylene cannulas in the right jugular vein during surgery, as described by Gorewit (5).

Lymphatic flow rates were verified manually for accuracy using a siliconized graduated cylinder. Lymphatic fluid volume was measured and more easily obtained, over 1-min intervals, during endotoxin treatment when flow frequency increased.

Data Acquisition Program
The Asystant Plus computer program (Asyst Software Technologies, Inc., Stanford, CT) was used for data acquisition. Flow data were recorded in the strip chart mode. The figures presented in this paper were created by Asystant Plus.

Treatments
Cows were allowed 1 wk for postsurgical recuperation. Lymph flow then was measured before, during, and after milking and before and after experimentally induced mastitis, using Salmonella typhimurium SH 4809 and Escherichia coli 055:B5 endotoxin (Sigma Chemical Co., St. Louis, MO). Endotoxins were prepared as previously described by Gorewit (6) and Saad and Ostensson (13). One Swedish Red cow received 10 \( \mu \)g of S. typhimurium endotoxin and the other 50 \( \mu \)g of Salmonella endotoxin through the teat meatus. Holstein cows were treated with 25 \( \mu \)g of E. coli endotoxin as described. Endotoxins were infused into the quarters containing the corresponding cannula and flow probe.

Analysis of Metabolites
Blood plasma and lymph samples were taken 1 h prior to endotoxin infusion for all
AFFERENT MAMMARY GLAND LYMPH

Cows and analyzed for various metabolites. Samples were analyzed at the New York State College of Veterinary Medicine, Department of Pathology, Cornell University (Ithaca, NY). All samples were analyzed in an autoanalyzer (DACOS; Coulter Electronics, Hialeah, FL) using standard techniques recommended by the manufacturer.

RESULTS AND DISCUSSION

Lymph Flow Dynamics

Normal resting lymph flow in conscious cows was pulsatile with monophasic and multiphasic episodes (repeated pulses without a return to zero). A typical resting flow pattern is shown in Figure 1. Total flow, ranging from 13 to 45 ml/h, resulted from 62 to 67 pulsatile episodes per h. The duration of episodes varied from 1 to 53 s. Intervals without flow, between episodes, varied from 1 to 182 s. Individual instantaneous peak flow (an episode) ranged from 163 to 245 μl. Flow did not increase consistently during milking. However, flow increased in four of the six cows during hand massage of the udder during machine stripping.

Lascelles and Morris (11) measured lymphatic flow in the efferent mammary lymph duct of conscious lactating and dry ewes. Lymph flowed at a rate of 20 to 40 ml/h in early lactation ewes and 2 to 6 ml/h in dry ewes. The lymph flow rate in the afferent vessels of lactating cows was close to the range for efferent flow in lactating ewes.

Hall et al. (8) used pressure transducers to measure lymph movement in conscious sheep and reported that lymph flow in various afferent and efferent vessels of lymph nodes, lymphatic trunks, and lymph ducts was intrinsic and pulsatile. Flow was intermittent with a characteristic rhythm that was unrelated to muscle movements and respiration. Pulsatile pressures ranged from 1 to 25 mmHg; pulse frequencies ranged from 1 to 30/min. The pulse frequencies of mammary afferent lymphatics from lactating cows did not depend on muscle movements and respiration. The magnitude of pressure and pulsation rate increased as lymph flow rate increased in the sheep (8).

During endotoxin-induced mastitis, lymph flow increased abruptly within 30 min of infusion of endotoxin, regardless of type and dosage. Flow increased, on average, more than 8-fold (232 ml/h, 10 μg of endotoxin; 371 ml/h, 50 μg of endotoxin) for the Swedish Red cows. Holstein cows showed, on average, a 5.5-fold increase (35 to 192 ml/h). Intervals without flow were substantially decreased and less variable. Flow rates were elevated above resting rates for up to 48 h after infusion of endotoxin. Flow increases appeared to be directly related to the dose of endotoxin given. Figure 2 shows a typical lymphatic flow pattern 60 min after administration of 10 μg of Salmonella endotoxin.

Endotoxin is expected to increase interstitial fluid concentrations after infusion into the

Figure 1. A representative profile of afferent mammary gland lymphatic flow. The spontaneous nature of the flow can be seen. One or more episodes occur approximately every 60 s.

Figure 2. A representative profile of afferent mammary gland lymphatic flow 60 min after intramammary infusion of 10 μg of Salmonella typhimurium endotoxin. Pulse flow frequency decreases. Episodes occur approximately every 25 s or less.

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Comparison Between Composition of Afferent Mammary Lymph and Blood Plasma

Differences were distinct among several metabolites analyzed in afferent lymph and blood plasma of cows (Table 1). Lymph contained 7, 6, and 10 times less protein, albumin, and globulin, respectively, than for plasma. Albumin:globulin ratios were higher for afferent lymph than for blood plasma. Lymph contained half as much calcium as did plasma. Glucose concentrations in afferent mammary lymph were similar to those in plasma, showing that glucose concentrations remain stable between the interstitial spaces of mammary tissue and blood plasma of lactating cows.

Correlation Between Measured and Observed Flow Rates

Figure 3 shows a scatter plot of measured flow (derived by flow meter) versus real flow from graduated cylinder collections of lymph fluid. Data were collected from one Swedish Red cow for 30 consecutive min, 1 h after infusion of Salmonella endotoxin (10 μg). A significant correlation ($R^2 = .99$) existed between real and measured flow, confirming the accuracy of the Transit-Time probe flow measurements. We were unable to verify the accuracy of the flow meter during normal undisturbed lymphatic flow because peak flow was spontaneous and duration was short (Figure 1). After exposure of cows to endotoxins, the frequency of spontaneous flow was reduced dramatically (Figure 2). This reduction allowed for almost continuous flow measurements, which could then be more easily synchronized with collection of lymph into graduated cylinders.

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Lymph had approximately 17 times less cholesterol and about half as much triglyceride as blood plasma (Table 1). Cholesterol is the principal sterol of mammals and is found in all body tissues. Concentrations of free cholesterol are high in the central nervous system and in fat (4). Our data suggest that the mammary glands of lactating cows do not secrete significant amounts of cholesterol.

Triglycerides are normally synthesized in the mammary gland from fatty acids extracted from blood and from those originating from de novo synthesis within the mammary gland.
TABLE 1. Mean concentrations and standard errors of mammary lymph and blood plasma samples.

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1(n = 6) Four Holstein and two Swedish Red breeds.
2Albumin to globulin ratio.

(11). Our work is the first to document concentrations of triglycerides in afferent mammary lymphatic fluid of lactating cows.

CONCLUSIONS

This paper describes the first measurement of real-time lymph flow in the afferent mammary lymph ducts of conscious lactating cows at rest, during milking, and after endotoxin-induced mastitis. Lymph flow was pulsatile and spontaneous at rest and followed an intrinsic rhythm. The act of milking itself did not seem to influence lymph flow. After endotoxin treatment, the intrinsic pulsatile flow was increased.

Differences were distinct between the metabolite composition of afferent lymph and that of blood plasma.

The cannulation and flow techniques described herein will enable dairy scientists to investigate biological factors that control mammary gland function. Furthermore, researchers will also be able to calculate more accurately substrate and metabolite fluxes across the mammary gland after the contribution of the lymphatic system has been determined.

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REFERENCES


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