



Effect of intravenous plasma transfusion on granulocyte and monocyte oxidative and phagocytic activity in dairy calves with failure of passive immunity



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ABSTRACT

Plasma administration has been recommended in calves older than 48 h with failure of passive immunity (FPI) to provide immunity consistent with adequate colostral ingestion. However, the protective serum immunoglobulin G (IgG) concentrations (≥ 1000 mg/dL) of plasma derived IgG only lasts up to 12 h. In addition to IgG, maternally derived colostral cells also confer immunity. The objective of the study was to determine the effect of intravenous plasma transfusion on granulocyte and monocyte oxidative and phagocytic activity in calves with FPI. Twenty-seven, one day-old, Jersey calves were assigned into 3 groups. The colostral (CL, $N = 9$) group received 3 L of colostrum once by oroesophageal tubing. Two other groups of calves received 1 L of colostrum once by oroesophageal tubing and were assigned based on their health status (sick or non-sick) at 4 days of age, as the sick-group (SG, $N = 7$) or the non-sick (NG, $N = 11$) groups. At 4 days of age, the SG and NG groups were administered plasma intravenously at 30 mL/kg. Granulocyte and monocyte oxidative and phagocytic activity was determined by flow cytometry. There was no significant difference in the granulocyte and monocyte oxidative or phagocytic activity among the 3 groups ($P > 0.05$). Plasma administration had no significant effect on the oxidative or phagocytic activity of granulocytes or monocytes. In clinical practice, plasma administration for enhancing oxidative or phagocytic activity of granulocytes or monocytes, alone, might not be justified in calves with FPI.

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1. Introduction

Failure of passive immunity (FPI) is the most common cause of immunodeficiency in newborn calves (Weaver et al., 2000). In calves, colostral component absorption occurs efficiently during the first 12 h and ceases after 24–36 h of life (Matt et al., 1982). Serum immunoglobulin G (IgG) concentrations of ≥ 1000 mg/dL after colostral ingestion are indicative of adequate transfer of immunity in calves (Besser et al., 1991). Calves with FPI have decreased weight gain and increased risk for morbidity and mortality (Furman-Fratczak et al., 2011; Windeyer et al., 2014).

Sick calves >48 h of age with FPI are managed in part with intravenous administration of bovine plasma assuming that administered plasma IgG achieves immunoprotection consistent with adequate transfer of immunity. However, recent studies indicated that the protective serum IgG concentrations (≥ 1000 mg/dL) of plasma derived IgG only lasts up to 12 h (Pipkin et al., 2015). Thus, plasma administration for the purpose of supplementing IgG alone in calves with FPI may not be justified due to the short duration of the protective levels. In addition to the passive immunity conferred by IgG, colostral derived immune

cells are known to stimulate maturation of calf's immune system when exposed to environmental pathogens (Reber et al., 2008). In septic foals, plasma administration was beneficial through increased neutrophil function (McTaggart et al., 2005). Studies on the effect of intravenous plasma administration on immune cells in cattle are lacking. We hypothesized that intravenous bovine plasma transfusion at an optimum recommended dose rate of 30 mL/kg (Murphy et al., 2014) improves phagocytic and oxidative burst activity of granulocytes and monocytes in dairy calves with FPI. Assuming that diarrhea is the most common disease condition in pre-weaned calves, with leukocytes being the major cell types recruited to sites of inflammation, the objective of this study was to determine the effect of intravenous plasma transfusion on granulocyte and monocyte activity of calves with FPI.

2. Materials and methods

2.1. Calves

The study was approved by the University of California Davis Institutional Animal Care and Use Committee (Protocol #18722). Sample size was determined assuming that calves with FPI and transfused plasma are 5 times more likely to experience mortality (Pipkin et al., 2015), power = 80% and alpha = 0.05. The required sample size was at least

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6 calves in each group. To account for a 20% dropout before 7 days of age, at least eight calves were enrolled in each group. A total of 29 Jersey bull calves was enrolled.

Calves delivered from observed births were immediately separated from their dams prior to nursing colostrum, weighed, identified using ear tags and housed in individual calf hutches. A stratified (different levels of passive immune status), non-probability (non-randomized) sampling technique was used to assign the calves into 3 groups. The first 9 calves were assigned into the control group (CL) and fed 3 L of fresh colostrum, once by oroesophageal tubing within 2 h after birth to ensure adequate passive immunity. The next 20 calves were separated into two treatment groups; sick group (SG) and the non-sick group (NG) based on their health status at 4 days of age. Assessment of calf health status prior to assignment into the NG and SG groups included daily assessment of rectal temperature, appetite, and evidence of diarrhea or coughing. Both NG and SG calves received 1 L of fresh colostrum, once by oroesophageal tubing within 2 h after birth to insure FPI. A total of 150–200 g total IgG is required for adequate transfer of colostral immunity (Chigerwe et al., 2008). Thus, based on the average colostral concentration of IgG of 60 g/L on the farm of study, feeding 1 L of colostrum insured FPI. After colostrum administration, all calves were fed 2 L of milk replacer twice daily, and 0.5 kg of commercial calf concentrate twice daily, with ad libitum access to water.

At 4 days of age, plasma administration was performed in the SG and NG groups. Bovine plasma purchased from the UC Davis Veterinary Medical Teaching Hospital Blood Transfusion Service was used. The plasma was evaluated for sterility and considered free of transmissible blood-borne pathogens. A 5 mL aliquot of the plasma was collected prior to administration for subsequent IgG concentration determination. Plasma was administered through the external jugular vein via an intravenous catheter (Angiocath, Becton Dickinson, Franklin Lakes, NJ, USA) at 30 mL/kg. Infusion of plasma was performed at 10 mL/kg/h while monitoring for transfusion reactions. Monitoring for transfusion reactions included monitoring heart rate, respiratory rate, mucous membranes color, and abnormal behavior. In the absence of an immediate transfusion reaction, the remainder of the plasma was transfused over 20–30 min. In case of a plasma transfusion reaction, transfusion was discontinued for 10 min and resumed at 5 mL/kg/h.

The timeline for blood collection in lithium heparin for oxidative and phagocytic activity of granulocyte and monocytes, and in ethylenediaminetetraacetic acid (EDTA) for complete blood count (CBC) or no anti-coagulant for serum IgG determination is summarized in Table 1. Serum was harvested from the blood samples and stored at -20°C until immunoglobulin G (IgG) determination. Daily calf monitoring procedures included assessment of rectal temperature, appetite and evidence of diarrhea or coughing. Sick calves were medically treated by a study blinded UC Davis campus veterinarian. Calves that died during the study period were submitted for necropsy at the California Animal

Health and Food Safety Laboratory in Davis, CA. Enrollment of all calves was completed within 2 weeks, in June 2015.

2.2. Serum IgG concentration and complete blood count (CBC) analysis

A CBC was performed on EDTA treated blood samples using an automated hematology analyzer (Advia 120 Hematology System, Siemens, Malvern, PA, USA). Immunoglobulin G determination was performed on serum samples by radial immunodiffusion (RID) using a commercial bovine IgG test kit with a serum IgG determination range of 196–2748 mg/dL (Bovine IgG kit, Triple J Farms, Bellingham, WA, USA) as recommended by the manufacturer, and described as in previous studies (Pipkin et al., 2015).

2.3. Granulocyte and monocyte oxidative burst activity determination

Oxidative burst activity of granulocytes and monocytes was evaluated using a commercial test kit (Phagoburst, Glycotope Biotechnology, Heidelberg, Germany) according to the manufacturer's recommendations. The Phagoburst test determined the percentage of leukocytes that oxidized the fluorogenic substrate dihydrorhodamine 123 to rhodamine 123 and their enzymatic activity (amount of rhodamine 123 per cell). Oxidative activity of the granulocytes and monocytes was stimulated with opsonized, non-labeled *Escherichia coli* (*E. coli*), phorbol 12-myristate 13-acetate (PMA), and peptide *N*-formyl-Met-Leu-Phe (fMLP). The *E. coli*, PMA and fMLP were the particulate stimulus, strong stimuli, and weak stimuli, respectively. The cell suspension was evaluated within 30 min after preparation using a flow cytometer (FACScan, Beckton Dickinson, Franklin Lakes, NJ, USA).

2.4. Granulocyte and monocyte phagocytic activity determination

Phagocytic activity of granulocyte and monocytes was determined using a commercial test kit (Phagotest, Glycotope Biotechnology, Heidelberg, Germany) according to the manufacturer's recommendations. The Phagotest test allowed quantitative determination of phagocytosis by granulocytes and monocytes by assessing the percentage of cells that ingested bacteria and their activity (number of opsonized fluorescein-labeled *E. coli* per cell). Cells were analyzed within 1 h after preparation using a flow cytometer.

2.5. Oxidative burst and phagocytic activity of granulocytes and monocytes analysis using flow cytometry

Oxidative and phagocytic activity of cells data was acquired from the flow cytometer using a commercial software (CellQuest, Beckton Dickinson, Franklin Lakes, NJ, USA) and then analyzed using a commercial software (FlowJo, FlowJo LLC, Ashland, OR, USA). Forward and side scatter, fluorescence-1 and fluorescence-2 were used. A live gate was set in the fluorescence-1 histogram to include all granulocytes and monocytes stimulated. A forward and side scatter dot plot was created with gates set for granulocytes and monocytes to determine cells that underwent phagocytosis or oxidative activity. Median fluorescence intensity detecting the number of cells ingested or oxidative species produced was calculated based on the fluorescence-1 height on the histogram.

2.6. Statistical analysis

Descriptive statistics was used to summarize the data. Data was checked for normality using the Shapiro-Wilk test. Mean \pm standard deviation (SD) and median (range) were reported for normally and non-normally distributed data, respectively. Predicted serum IgG concentrations following plasma transfusion in the SG and NG groups were estimated as previously reported (Murphy et al., 2014). White cell blood counts from the CBC before and after plasma transfusion in the SG and NG groups were compared using the Wilcoxon rank sum

Table 1
Procedure schedule for blood collection and plasma transfusion in 29 Jersey dairy calves in CL (colostrum), SG (sick) and NG (non-sick) groups.

Group	D = 0	D = 2	D = 4		D = 5	D = 7
CL	IgG	IgG	Act CBC IgG T = -1 h PT		IgG Act CBC	IgG Act
				T = 2 h		
T = 12 h						
NG and SG	IgG	IgG	IgG Act CBC	Act	IgG Act CBC	IgG Act

IgG: serum IgG determination; Act: phagocytic and oxidative burst activity; CBC: complete blood count; PT = plasma transfusion; D = 0, 2, 4, 5 and 7 represent age of calves in days at the time of blood sample collection with D = 0 representing time prior to ingestion of colostrum. T = -1, 0, 2 and 12 represent 1 h prior, 2 h after and 12 h after plasma transfusion blood collection time points in the SG and NG groups only.

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