Survival of Transfused CD18-Positive Granulocytes and Their Chemiluminescent Response in a Heifer with Leukocyte Adhesion Deficiency

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ABSTRACT. Granulocyte transfusion (GT) was performed in an 8-month-old heifer with leukocyte adhesion deficiency (BLAD) to monitor the changes in transfused CD18-positive neutrophils and associated neutrophil chemiluminescent (CL) response in β2-integrin-deficient host. The CD18-positive neutrophils were detected in blood from the BLAD heifer during the first 3 hr after 2.6 × 10⁹ cells were infused by GT, and disappeared by 5 hr after GT. The CL response of neutrophils was increased 1.7 to 2.8-fold in the BLAD heifer during the first 3 hr after GT, thereafter CL response decreased gradually from 2 to 5 hr after GT. — KEY WORDS: BLAD, CD18-positive cell, granulocyte transfusion.

Granulocyte transfusion (GT) has been proposed as a means of supplying neutrophils to human patients showing neutropenia and newborns with sepsis [2–4]. Little is known concerning the efficacy of GT in diseased animals with impaired granulocyte function. Cattle with bovine leukocyte adhesion deficiency (BLAD) appear to be an appropriate animal model for the elucidation of the dynamics and functions of transfused granulocytes in a β2-integrin-deficient host. It is of interest to evaluate the survival and response of transfused CD18-positive neutrophils in a BLAD heifer. The purpose of this study was to monitor the effects of the GT performed in a heifer with BLAD on the presence of CD18-positive neutrophils in peripheral blood and associated chemiluminescent (CL) response for characterization of transfused CD18-positive neutrophils.

Affected heifer: An 8 month-old Holstein heifer diagnosed as BLAD by DNA-polymerase chain reaction test [5] was used. The body weight of the BLAD heifer was 277 kg.

Blood donor: A clinically healthy Holstein cow, 5 year-old, was used as a blood donor. To increase the number of neutrophils in peripheral blood, dihydroheptaprenol (DHP, C₁₅H₂₉O; Eisai Co., Tokyo) was administered subcutaneously at a dosage of 1.0 mg/kg of body weight at 24 hr before the collection of neutrophils [6]. Two liters of peripheral blood were collected from the jugular vein into plastic bottles containing heparin (20 IU/ml). In the DHP treated blood sample, neutrophils were increased in number approximately 3-fold compared to the pre-treated value (1,392/µl).

Isolation of neutrophils from donor cow: Blood was centrifuged at 1,600 g for 20 min at 20°C. The leukocyte-rich layer was recovered and the red blood cells were treated with hypotonic lysis as previously described [5]. The leukocytes suspended in phosphate-buffered saline solution (PBS) were layered onto 20 ml of Ficoll-Conray solution in each 50 ml conical tubes (Falcon 2098) and were centrifuged at 500 g for 30 min at 20°C. The resultant cells from the bottom of the tubes were resuspended in 0.9% saline solution to a concentration of 2.6 × 10⁹/ml. The relative values of neutrophils, mononuclear cells and eosinophils were 77–83, 5–12 and 8–12%, respectively, and more than 96% of the cells were viable when assessed by trypan blue dye exclusion.

Clinical and hematological findings: Rectal temperature, heart and respiratory rates of the BLAD heifer were monitored from 3 days before to 4 days after intravenous administration when 2.6 × 10⁹ cells. No obvious changes of clinical findings of the BLAD heifer were observed during the GT (data not shown).

Flow cytometric analysis of CD18: Neutrophils (3 × 10⁶ cells) were reacted with FITC-conjugated anti-CD18 monoclonal antibody (Mab, Dako MHM23) as described [5]. CD18-positive cells were analyzed with a flow cytometer (Coulter Epics Elite), and the percentage of neutrophils expressing CD18 was calculated by measurement of fluorescent intensity [5]. The relationship between the number of CD18-positive cells and fluorescent intensity related to binding of FITC-anti-CD18 Mab was evaluated by adding normal neutrophils to suspensions of CD18-deficient neutrophils. When normal neutrophils were added to CD18-deficient neutrophil suspensions in the range from 0.5 to 5%, the fluorescent intensities due to the binding of FITC-anti-CD18 Mab to normal neutrophils were linearly increased (Fig. 1A).

The CL response of transfused granulocytes in a BLAD heifer during the first 3 hr after GT, and disappeared by 5 hr after GT (Fig. 1B). Approximately 0.5% of neutrophils were positive in blood at 1 hr after GT, as measured from the fluorescent intensity due to the FITC-anti-CD18 Mab binding.

CL response: CL response was employed to monitor the activity of respiratory burst of neutrophils, and luminol-dependent CL assay was performed according to a described method [5]. Five hundred microliters of neutrophils (2 × 10⁶ cells/ml) in HBSS were incubated at 37°C for 5 min in a luminometer (Aloka BLR 102, Tokyo), and 20 µl of
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luminol to yield the final concentration of 0.1 mM. This mixture was equilibrated for 5 min, then 20 µl of opsonized zymosan solution (OPZ, 10 mg/ml) were added. The peak response (peak CL, counts per minutes [cpm]) was read from the recorder. CL response of neutrophils was measured to monitor the response of transfused normal neutrophils in the BLAD heifer (Fig. 1C). CL response of transfused neutrophils in the BLAD heifer was increased 2.8 fold at 1 hr after GT as compared with pre-treated values, thereafter the response decreased gradually from 2 to 5 hr after GT, and finally returned to the pre-treated values.

The functional characteristics of leukocytes from cattle with BLAD are attributable to the deficient CD18 expression [6]. It is clear that CD18-deficient leukocytes are impaired for adherence to their ligands and migration into inflamed tissues [6]. We transfused normal neutrophils into CD18-deficient heifer and monitored for CD18-positive neutrophils in peripheral blood. After 2.6 x 10⁸ neutrophils were infused into the BLAD heifer, the prevalence of CD18-positive cells was estimated to be 0.5% in the peripheral blood based on the relationship between the number of CD18-positive neutrophils and their fluorescent intensities. The CD18-positive neutrophils were present in blood up to 3 hr, suggesting that CD18-positive neutrophils adhered to epithelium and migrated into the inflammed tissues, as previously observed in normal newborn rats by GT [1], or were phagocytosed by mononuclear phagocytes. The period of less than 5 hr is considered to be a retained time, and this finding was similar to that previously reported [1]. The procedure for GT was an experimental trial with practical concerns such as granulocyte isolation from blood and the short life span of neutrophils in blood. The efficacy of GT into cattle with impaired granulocyte functions remains to be clarified in our study.

In summary, CD18-positive neutrophils in blood and associated increased CL response were found in blood during the first 3 hr after GT in the BLAD heifer.

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REFERENCES