NOTE  Immunology

Transient Detection of Proinflammatory Cytokines in Sera of Colostrum-Fed Newborn Calves

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ABSTRACT. To obtain basic information on the state of proinflammatory cytokines in newborn calves, we determined the kinetics of five cytokines (IL-1β, IL-6, TNF-α, IFN-γ and IL-1 receptor antagonist) in sera of newborns during the first 4 weeks of life. At birth, none of the 5 cytokines were detected in almost all serum samples, but the cytokines became detectable within 12 hr after being fed colostrum. The mean concentrations of the cytokines reached peak levels by 24 hr and then gradually decreased and became undetectable by 4 weeks after birth. Cytokine mRNA expressions in peripheral blood mononuclear cells of newborns were observed without reference to the cytokine concentrations in sera. Serum cytokines detected in newborn calves are probably colostral origin.

KEY WORDS: calf-serum, colostrum, cytokine.

Resistance to infection is determined by the host immune state. The immune state of neonatal calves is largely dependent on the colostrum they are fed. Immunoglobulin, one of resistant factors present in colostrum, is known to play a protective role. The presence of several cytokines in bovine colostrum has been shown by several investigators [4–6, 15]. In addition, oral administration of recombinant bovine (rb) IL-1β, one of the colostral cytokines, to neonatal calves resulted in the appearance of the cytokine in circulation and the activation of circulating lymphocytes and neutrophils [7]. Furthermore, IL-6 [12] and TNF-α and IFN-γ [17], which are also present in bovine colostrum [6], have been reported to potentiate immunological functions. Therefore, cytokines transferred to neonates via colostrum are considered to contribute to maturation of neonatal immune functions.

To obtain basic information on the state of cytokines in neonatal calves, we measured the levels of five cytokines (IL-1β, IL-6, TNF-α, IFN-γ and IL-1 receptor antagonist, IL-1ra) in serum samples and expression of these cytokine mRNAs in peripheral blood mononuclear cells (PBMC) obtained from neonatal calves after they had been fed colostrum.

Specimens were collected from Holstein Friesian cattle that were kept at the dairy farm of Rakuno Gakuen University. Blood was obtained by cervical vessel puncture using a sterile vacuum syringe from healthy calves at birth before being fed colostrum (n=21) and at 12 hr (n=19), 1 day (n=21), 2 days (n=18), 3 days (n=18), 5 days (n=17), 7 days (n=16), 14 days (n=13), 21 days (n=11) and 28 days (n=11) after birth. Newborn calves were fed about 2 l of colostrum from respective dams (n=10) or pools (n=11) within 2 hr after birth and then fed colostrum from respective dams two times a day by 5 days after birth. The other 4 calves, as controls, were fed formula as substitute for colostrum for 3 days and their blood were collected during this period. Sera were stored at –30°C until use. PBMC from newborn calves (n=10) were prepared from heparinized peripheral blood of healthy calves at birth to 28 days after birth as described previously [21].

The concentrations of five cytokines (IL-1β, IL-6, TNF-α, IFN-γ and IL-1ra) in sera were determined by sandwich ELISA using affinity-purified IgGs specific for each cytokine as described previously [5, 6, 20]. These affinity-purified IgGs from rabbit immunized with each recombinant bovine cytokine were used for capture ELISA. These antibodies were labeled with Biotin by a commercially available kit (Amersham, UK) and used for the detection of antibodies. The detection limits of the ELISA were 2 ng/ml for IL-1ra, 0.2 ng/ml for IL-1β and 0.1 ng/ml for IL-6, TNF-α and IFN-γ.

Expression of cytokine mRNAs in PBMC were determined by reverse transcription (RT)-PCR. Briefly, total cellular RNAs were extracted from PBMC using a TRizol reagent (GIBCO BRL, U.S.A), and the RNA (1 µg) was reverse-transcribed using ReverTra Dash™ (TOYOBO, Osaka, Japan) and random primers for 10 min at 30°C, 20 min at 42°C and 5 min at 99°C. Cytokine-specific sequences corresponding to each cytokine were amplified by PCR as described previously [8, 14]. The PCR products were analyzed by 1.5% agarose gel electrophoresis and stained with ethidium bromide. The PCR products were semi-quantitatively detected with Scion Image (Scion Corp., Frederick, MD, U.S.A.). The results are expressed as the ratio of cytokine mRNA/β-actin mRNA expression.

The statistical difference between two groups were determined by the Mann-Whitney U-test, and significant difference was evaluated at a probability level of <0.05.

At birth, none of the five cytokines, except for IL-1β in one serum sample, were detected in any of the serum samples. However, they became detectable by 12 hr after being fed colostrum and the mean concentrations of the cytokines...
reached peak levels at 12 hr (IL-1ra, 32.54 ± 13.23; IFN-γ, 0.192 ± 0.04) to 1 day (IL-1β, 46.9 ± 31.4; IL-6, 1.23 ± 0.34; TNF-α, 0.69 ± 0.22) after birth and then gradually decreased and became almost undetectable by 28 days after birth (Fig. 1). Furthermore, none of the cytokines in sera from colostrum-deprived 4 calves was detected until 3 days after birth (data not shown).

In addition, to determine whether cytokines are produced in the peripheral blood of newborn calves, we examined cytokine mRNA expressions in PBMC at birth and at 1, 7 and 28 days after birth by RT-PCR (Fig. 2). The results shown in Fig. 2 are those in PBMC from 3 calves, out of 10 calves examined, since the expressions in PBMC from the other 7 calves were similar to those in the PBMC from 3 calves. The results of semi-quantitation of those in PBMC from these 10 calves were also shown in Fig. 3. Although all of the cytokine mRNAs were detected in PBMC of newborn calves at birth and 28 days after birth, serum cytokines were undetectable or very low concentrations at these time points. Inversely, mRNA expressions were significantly reduced in IL-6 and tended to reduce in IFN-γ and TNF-α at 1 day after birth at which time serum cytokine levels were high. In human infants, low levels of cytokine production in cord blood cells [11, 16] were found to be associated with a decreased post-transcriptional mRNA stability [19]. Therefore, serum cytokines were not considered to be produced by newborn PBMC. Furthermore, our previous study showed that orally administered cytokines to newborn calves were easily transferred to circulation [7]. Therefore our present results suggested that serum cytokines are considered to be colostral origin.

Colostrum also contains immunosuppressive factors and cytokine inhibitors such as receptor antagonists and soluble receptors [1, 2, 4, 6, 15]. However, mitogenic activities of PBMC from colostrum-fed calves were significantly higher than those of PBMC from colostrum-deprived calves after stimulation with ConA [7]. Indeed, we confirmed that coexistence of IL-1ra with IL-1β in colostrum had no effect on the mitogenic response of neonatal PBMC [21]. The cytokine inhibitors in colostrum may play a role in amelioration of the adverse effects of cytokines as suggested by Buescher and Malinowska [1].

In general, it is considered that the susceptibility of newborns to infection is higher than adults. Possible reasons are functional immaturity of lymphocytes, monocytes and natural killer cells in neonates [10, 13, 18]. However, it was well known that cytokines such as IL-1β, IL-6, TNF-α and IFN-γ activate immune cells, and oral administration of rbIL-1β to newborn calves resulted in activation of circulating lymphocytes and neutrophils [7]. Therefore, transfer of colostral cytokines to neonates may contribute to the potentiation of neonatal immune functions.
Finally, some investigators indicated that measurement of plasma cytokine levels in newborn infants might be useful in predicting neonatal morbidity and mortality and that cytokine levels in sera of newborn infants deserve evaluation as prognostic parameters in certain neonatal diseases such as perinatal asphyxia, neonatal respiratory distress syndrome and neonatal sepsis [3, 9]. Since serum cytokine levels were variable among individual newborns in the present study, it would be very interesting if monitoring health state of these calves were done. This issue will be a future research theme.

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