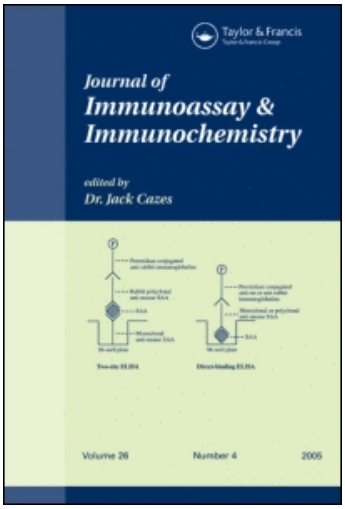

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Bovine IL-18 ELISA: Detection of IL-18 in Sera of Pregnant Cow and Newborn Calf, and in Colostrum

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Abstract: In this study, we examined the concentration of bovine IL-18 in the sera of pregnant cows, their fetuses and newborn calves, and in colostrum in order to examine the role of IL-18 in bovine pregnancy and the neonatal period. A sandwich-ELISA to quantify bovine IL-18 was established using anti-porcine IL-18 monoclonal antibodies, which cross-reacted with bovine IL-18, and used it to measure the concentration of bovine IL-18 in the sera of pregnant cows, their fetuses and newborn calves, and in colostrum. Significant levels of IL-18 were detected in the sera of pregnant cows, but not in the sera obtained from the corresponding fetuses, umbilical arteries and veins. After birth, IL-18 levels in the sera of 1-day and 1-week old calves were low, and significantly increased in the sera of 1-month and 4-month old calves. IL-18 was also detected in colostrum, with the concentration of IL-18 in the first colostrum produced after delivery being the highest, and then decreasing depending on the number of milkings. Furthermore, the serum IL-18 concentration of

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newborn calves was increased after the oral administration of colostrum. These results suggest that IL-18 during bovine pregnancy and in the newborn period may play important roles in the maintenance of pregnancy and in the maturation of neonatal immunity.

Keywords: Bovine, Colostrum, Interleukin-18, Neonatal immunity, Pregnancy

INTRODUCTION

Interleukin-18 (IL-18) first became known as an interferon-γ inducing factor (IGIF) because of its ability to induce IFN-γ production by Th1 cells.[1,2] Recent evidence has shown that IL-18 is a pleiotropic cytokine that regulates both Th1 and Th2 immune responses.[3] IL-18 plays an important role, not only in Th1-dominated immunological reactions,[1,2] but also in antigen presentation,[4] allergic inflammation,[5,6] and mucosal immunity.[7] On the other hand, several studies have indicated that IL-18 is also involved in reproductive immunology, such as the maternal-fetal interface,[8] pregnancy,[9] labor,[9] and neonatal immunity.[10] IL-18 acts on uterine NK cells, and contributes to the maternal-fetal interface.[8] IL-18 mRNA level was seen to be reduced during the first and second trimesters in human pregnancy. IL-18 levels in the serum from pregnant women were gradually elevated from the first trimester until the onset of labor.[9] In addition, human colostrum has been reported to contain significantly higher levels of IL-18 compared with mature milk.[10] These results suggest that IL-18 plays an important role in the Th1/Th2 immunotrophism of the host during pregnancy and the neonatal period. However, no study of IL-18 related with reproductive and neonatal immunity has been performed in domestic animal species so far in spite of the importance of their reproduction and neonatal immunity.

Previously, we identified cDNAs for porcine IL-18.[11] We also generated the recombinant protein of porcine IL-18 and established anti-porcine IL-18 monoclonal antibodies (mAbs) in order to perform a sandwich enzyme-linked immunosorbent assay (sandwich-ELISA) for the detection of porcine IL-18.[12,13] In the present study, we applied the porcine IL-18 ELISA to the detection of bovine IL-18. Since bovine and porcine IL-18 shows 95% identity on the amino acid level,[11,14] our anti-porcine IL-18 mAbs were successfully cross-reacted with bovine IL-18. Using this ELISA, we examined IL-18 levels in the serum of pregnant cows and their fetuses, and in neonatal calves. We also investigated IL-18 levels in bovine colostrum and the changes of IL-18 level in the serum of the newborn calf after drinking colostrum. The results obtained by this study suggested the importance of IL-18 during bovine pregnancy, and its importance to neonatal immunity.

EXPERIMENTAL

Bovine IL-18 ELISA

The bovine IL-18 ELISA used for this study was previously developed by us for the detection of porcine IL-18, except for the use of clone 2-2-B (IgG1, κ) as a capture antibody and biotinylated clone 12-C-12 (IgG2a, κ) as a detection antibody.[12,13] Recombinant bovine IL-18, kindly provided by Dr. Takahiro Yamaguchi (Touhoku University, Sendai, Japan), was used as the standard protein. The ELISA procedures employed were basically the same as those described previously.[13] In order to confirm the cross-reactivity of these mAbs to bovine IL-18, we performed Western blot analysis using 2-2-B or 12-C-12 as described previously.[11] Recombinant bovine precursor and mature IL-18 expressed by a baculovirus system,[15] provided by Dr. Kazuaki Takehara (Kitasato University, Aomori, Japan), was used as the antigen.

IL-18 Level in Pregnant Cow and Fetus

The sera obtained from the peripheral blood and the uterine arteries of nine pregnant cows at 150-285 days of gestation, their umbilical arteries and veins, and the corresponding dams were obtained from a slaughterhouse. The IL-18 concentration in each serum was estimated by means of the bovine IL-18 ELISA described above.

IL-18 Level in Neonatal Calf

To examine the alteration of IL-18 levels in the serum of each individual calf, we collected sera periodically at 1-day, 1-week, 1-month, and 4-months after parturition from 13 Holstein calves bred in the Hokkaido Animal Research Center (Hokkaido, Japan). The IL-18 concentration in each sample was measured by above-described ELISA.

IL-18 Concentration in Colostrum

Colostrum samples were collected from 8 healthy Holstein dairy cows reared on the Senbonmatsu farm (Tochigi, Japan). Colostrum samples were collected twice a day from the day of parturition to day 5 after parturition. These samples were centrifuged at 10,000 rpm for 10 min at 4°C. The whey was separated and stored at −20°C until analysis. The IL-18 concentration in each sample was measured by ELISA.

To evaluate the transfer of colostral IL-18 to the newborn calf serum, we examined the changes of IL-18 level in the sera of newborn calves after drinking colostrum. Eight hundred mL of colostrum (the mean IL-18 concentration in the colostrums was 160 pg/mL as determined by ELISA) was administrated orally to the three newborn calves, and sera was obtained before and after 3 h and 6 h of drinking. The IL-18 concentration in each serum was then estimated by ELISA.

Statistical Analysis

Differences between each group were analyzed by the Student's t-test using Statview software (Abacus Concept, Berkley, CA, U.S.A.). $P < 0.05$ was considered significantly different.

RESULTS

Bovine IL-18 ELISA

Figure 1-A shows that both 2-2-B and 12-C-12, which were used in the sandwich ELISA for bovine IL-18, were cross-reactive with the recombinant bovine IL-18 by Western blotting. Both the precursor and the mature form of IL-18 was detected by 2-2-B and 12-C-12. As shown in Figure 1-B, recombinant bovine IL-18 was measured quantitatively, with a minimum detectable limit of about 20 pg/mL.

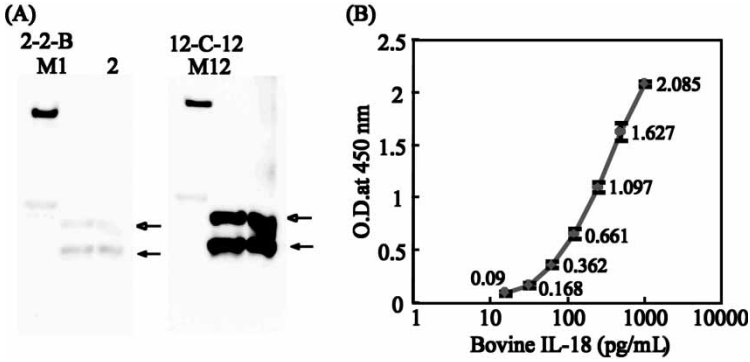

Figure 1. Establishment of bovine IL-18 ELISA. (A) Cross-reactivity of anti-porcine IL-18 mAbs (2-2-B and 12-C-12) to bovine IL-18 by western blotting. White arrows show bovine precursor IL-18. Black arrows show mature bovine IL-18. Lane 1: Reducing condition. Lane 2: Non-reducing condition. M: molecular size marker. (B) Representative standard curve of bovine IL-18 ELISA. Recombinant bovine IL-18 was serially diluted and applied to the ELISA as described in Experimental. Data show the mean absorbance ± standard deviation of four experiments.

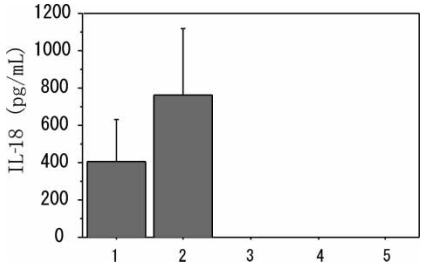

Figure 2. IL-18 concentration in the sera of pregnant cows and their fetuses. Lane 1: Sera from peripheral blood of pregnant cows. Lane 2: Sera from uterine arteries of pregnant cows. Lane 3: Sera from peripheral blood of their fetuses. Lane 4: Sera from umbilical arteries. Lane 5: Sera from umbilical veins. Data represent the mean ± standard error of nine pregnant cows and the corresponding fetuses.

IL-18 Level in Pregnant Cow and Fetus

Using this ELISA, we evaluated bovine IL-18 concentrations in the serum obtained from pregnant cows and in the corresponding fetuses. As shown in Figure 2, significant amounts of IL-18 were detected in the serum obtained from peripheral blood (mean 403.3 pg/mL) and the uterine arteries (mean 760.2 pg/mL) of pregnant cows. However, no IL-18 was detected in the sera obtained from the peripheral blood of their fetuses, and the umbilical arteries and veins (Fig. 2, Lanes 3–5).

IL-18 Level in Neonatal Calf

Figure 3 shows the IL-18 concentration in the serum obtained from the peripheral blood of 13 Holstein calves periodically at 1-day, 1-week, 1-month, and 4-months after parturition. IL-18 levels in 1-day and 1-week-old neonates

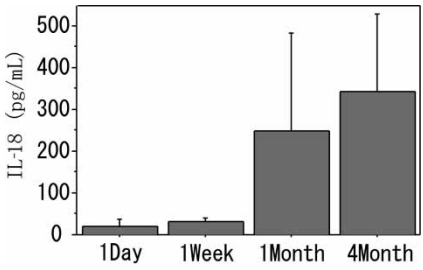

Figure 3. IL-18 concentration in the sera of neonatal calves. Sera were obtained periodically from 13 Holstein calves, and IL-18 concentration of each serum sample was measured. Data represent the mean ± standard error of 13 calves.

were relatively low (1-day: mean 19.7 pg/mL, 1-week: mean 30.8 pg/mL). However, serum IL-18 concentrations at 1-month and 4-months after parturition were increased (1-month: mean 250.2 pg/mL, 4-month: mean 344.0 pg/mL).

IL-18 Concentration in Colostrum

As shown in Figure 4-A, high concentrations of IL-18 were detected in the early colostrum after parturition, and the levels are gradually decreased. The IL-18 level in the first milking colostrum (mean 628.3 pg/mL) was significantly higher than that of the fourth milking and thereafter ($p < 0.05$). The IL-18 level in the colostrum of the second milking (mean 502.1 pg/mL) was also significantly higher than that of the sixth milking

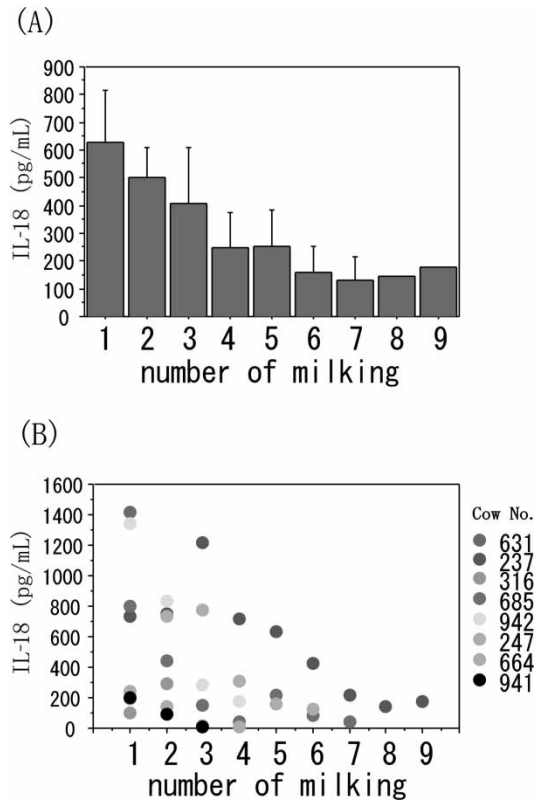

Figure 4. IL-18 levels in bovine colostrum. (A) Mean IL-18 concentration in colostrum. Data represent the mean ± standard error of eight colostrum samples. (B) IL-18 concentration in the colostrum of each cow.

and thereafter ($p < 0.05$). Figure 4-B illustrates the movement of the IL-18 level in the colostrum of each cow, also indicating a similar tendency.

We then examined the changes of IL-18 level in the serum of newborn calves after they had ingested colostrum. As shown in Figure 5, little IL-18 was detected in the serum of newborn calves before they had ingested colostrum (below detection limit). After ingesting colostrum, IL-18 levels were also below detection limit at 3 h, but increased at 6 h (mean 69.3 pg/mL).

DISCUSSION

In this study, we showed that anti-porcine IL-18 mAbs, which we had established previously,[12,13] were successfully applied to the detection of bovine IL-18. Two anti-porcine IL-18 mAbs (2-2-B and 12-C-12) were able to detect bovine IL-18 by Western blot (Fig. 1-A). As well, bovine IL-18 could be detected quantitatively using 2-2-B as a capture antibody and 12-C-12 as a detection antibody (Fig. 1-B). The detection limit of bovine IL-18 using the ELISA was comparable to that using porcine IL-18 ELISA as described previously.[13] These results indicated that the porcine IL-18 mAbs previously developed by us were cross-reactive for bovine IL-18, and useful for the detection of bovine IL-18 in various samples.

Using this ELISA, IL-18 concentrations in the sera of pregnant cows and their fetuses, and of neonatal calves were determined. Interestingly, a high-level of IL-18 was detected in the sera obtained from pregnant cows, but not in the sera obtained from their fetuses. Furthermore, there was a tendency that the sera obtained from pregnant cows near delivery (278–285 days of gestation) contained higher levels of IL-18 (data not shown). It is

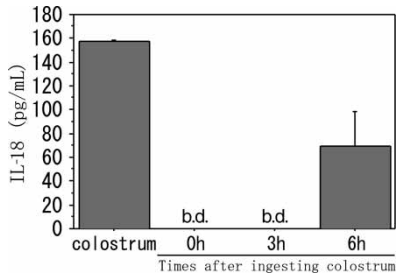

Figure 5. Changes IL-18 level in the sera of neonatal calves after ingesting colostrum. Eight hundred mL of colostrum (mean IL-18 concentration: 160 pg/mL) was administrated orally to each newborn calf, and serum was obtained before and after 3 h and 6 h of drinking colostrum. IL-18 concentration in each serum was estimated by ELISA. Data represent the mean ± standard error using three different calves. b.d. means below detection limit.

well-known that during a normal pregnancy the maintenance of pregnancy is associated with an altered Th1/Th2 balance,[16] and cytokines produced by Th2 cells predominate over those produced by Th1 cells.[17] IL-18 is an IFN-γ-inducing factor that is involved in the Th1 response and inflammatory response.[1–3] Therefore, the expression of IL-18 may also be suppressed on the fetal side during bovine pregnancy.

IL-18 levels after parturition were low in the sera obtained from 1-day and 1-week-old neonates, but the concentration of IL-18 in the same calves was increased at 1-month and 4-months (1-month: 250.2 pg/mL, 4-month: 344.0 pg/mL). Goto et al. reported that IL-1β was not detected in the sera of newborn calves, and that the concentration increased on day 3 after birth.[18] The average IL-18 levels in healthy human serum are about 100 pg/mL.[19] These results suggested that the bovine IL-18 level was also low during the neonatal period, and that the concentration would gradually reach a constant level within several months.

We also investigated the IL-18 levels in bovine colostrum and the changes of IL-18 level in the sera of newborn calves after ingesting colostrum. Bovine colostrum contains high a concentration of IL-18 at the first milking, and the concentration of IL-18 gradually decreases to about 100 pg/mL. Human colostrum also contains a high level of IL-18, with epithelial cells in lactating mammary gland secreting IL-18.[10] The cytokines contained in early colostrum may be absorbed through the gut of neonates, and affect neonatal immunity. In the present study, IL-18 levels were also increased after ingesting colostrum. Indeed, Hagiwara et al. reported that orally administrated IL-1βtransferred passively, and showed immunostimulatory effects in newborn calves.[20] Generally speaking, neonatal animals show decreased interferon-γ response,[21,22] T-cell and antigen presenting cell functions,[23] and cytokine productions that are implicated in the susceptibility to many kinds of pathogens.[24] Nomura et al. reported that cord blood NK cells respond well to IL-18 and produce IFN-γ, and this phenomenon may contribute to the host defense during the neonatal period.[25] We also reported that IL-18 was able to induce IFN-γ from the peripheral blood of 1-day-old newborn piglets.[26] These results suggested that the IL-18 produced in colostrum may also transfer to the neonates orally, and stimulate bovine neonatal immunity through the induction of IFN-γ.

In conclusion, we developed a bovine IL-18 ELISA in order to measure IL-18 concentrations in various bovine samples using anti-porcine IL-18 mAbs, and detected IL-18 in the sera of pregnant cows, and in colostrum samples. These are the first results showing IL-18 levels during pregnancy and neonatal period, and in the colostrum of the cow. Recent evidence has demonstrated that IL-18 is involved in the maintenance of pregnancy,[9,27] in the maternal-fetal interface,[8] in the intra-amniotic infection,[28] and in neonatal immunity[10] in humans. More studies are needed to elucidate the role of IL-18 in pregnancy and in the neonatal immunity of domestic animals.

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