Detection of Interleukin-1β in Sera and Colostrum of Dairy Cattle and in Sera of Neonates

Masamitsu GOTO, Mitsuhiro MARUYAMA, Kentaro KITADATE, Rikio KIRISAWA, Yuji OBATA, Masateru KOIWA1, and Hiroshi IWAI*

Departments of Veterinary Microbiology and 1Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyo-dai Midorimachi, Ebetsu 069, Japan

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ABSTRACT. In order to obtain basic information about bovine interleukin-1 (IL-1β), levels of IL-1β in sera and milk of clinically normal mature Holstein cattle before and after parturition and in sera of newborn calves were examined by ELISA. The level of IL-1β was undetectable in sera of mature cattle around the time of artificial insemination, but the concentration gradually increased and reached a peak at parturition and then decreased again to an undetectable level. IL-1β in milk was detected on the day of parturition but not thereafter. IL-1β mRNA was detected by reverse transcription-polymerase chain reaction in the cells from milk collected during 20 days before and 2 to 3 days after parturition, but was not detected thereafter. Although IL-1β was not detected in all the sera of newborn calves, the concentration transiently increased with peak titers on day 3 and became undetectable by day 14 after birth. Newborns that showed serum IL-1β on day 3 had been fed on colostrum in which the IL-1β concentration was significantly higher than that in colostrum that had been fed to newborns having no detectable IL-1β on day 3. These results indicate that IL-1β is induced in association with pregnancy in healthy dairy cattle and that the cytokine might be transferred to neonates via colostrum. — KEY WORDS: cattle, colostrum, interleukin-1, pregnancy.


Interleukin-1 (IL-1) is one of proinflammatory cytokines and is thought to be induced by various type of cells in response to injuries or infections and not to be detected in normal healthy animals [14]. However, we encountered many newborn calves that had a variety of serum IL-1 activities without any clinical abnormality (unpublished observation) using A375 cell growth inhibition assay [9]. Since presence of IL-1β in human colostrum [16] and production of IL-1β by bovine milk cells stimulated with lipopolysaccharide [18] have been reported, we postulated that IL-1 activities in the sera of newborns might be transmitted from dams via colostrum. Although detection of bovine IL-1 in association with pneumonia has been reported [9, 23], there is no report describing the cytokine in clinically normal cattle. The present studies were carried out to obtain basic information about bovine IL-1β in sera and milk of clinically normal cattle and to determine the origin of IL-1 activities in newborn sera mentioned above.

In the preliminary experiment, we failed to evaluate IL-1 activity in colostrum using the A375 assay, since milk whey itself was cytotoxic to A375 cells (unpublished observation). Therefore, we established a sandwich enzyme-linked immunosorbent assay (ELISA) for bovine IL-1β using polyclonal anti-recombinant bovine IL-1β rabbit IgG. Using the ELISA, we estimated IL-1 levels in sera and milk obtained from dams before and after parturition, and in sera from newborns and heifers. The results indicate that IL-1β is induced in association with pregnancy in healthy dairy cattle and that the cytokine might be transferred to neonates via colostrum.

MATERIALS AND METHODS

Cattle and specimens: Specimens were collected from Holstein Friesian cattle that were kept at Rakuno Gakuen University dairy farm. Blood was obtained by cervical vessel puncture using sterile vacuum syringe from healthy pregnant and/or lactating cows of various ages, calves aged 0 to about 180 days and heifers of around 1 year old. Sera were stored at -20°C until use. Milk was collected from healthy cows during 20 days before and 14 days after parturition by manual milking into sterile plastic tubes. Milk was centrifuged at 2,000 rpm for 10 min at 4°C and aqueous phase (nonfat milk) was recovered. Precipitated cells were suspended in RPMI 1640 medium and stored at -20°C. The nonfat milk was centrifuged at 10,000 rpm for 15 min at 4°C to obtain whey. Whey was stored at -20°C. Sampling was performed in October 1994 through April 1995. Sampling numbers and intervals are shown in Results.

Recombinant bovine interleukin-1β (rbIL-1β): The bIL-1β sequences (462 bp) encoding the mature IL-1β were amplified by reverse transcription-polymerase chain reaction (RT-PCR), as described below, using mRNA of concanavalin A (ConA, Sigma Chemical, U.S.A.)-stimulated bovine peripheral blood mononuclear cells (PBMC) and the following primers: 5′ GCACCCGTGACGATCAATAGT 3′ and 5′ TTAG GAGAGAGGGTTTCCATTCTGAAGT 3′ which were designed by using published sequences for bIL-1β [15]. The recombinant plasmid was constructed by insertion of bIL-1β cDNA into plasmid pGEX-4T-2 (Pharmacia, Sweden).
The rbIL-1β was produced in Escherichia coli (E. Coli) strain NM522 (Stratagene, U.S.A.) by recombinant DNA technology and purified to homogeneity by a protein purification kit (Pharmacia, Sweden). The purified rbIL-1β was analyzed by sodium dodecyl sulfate polyacrylamid gel electrophoresis (SDS-PAGE) and Western blot using anti-human IL-1α and β antibody (R & D systems, U.S.A), because anti-bIL-1β antibody could not be available at that time and bovine and human IL-1β share common antigenic determinant [9]. The biological activity of rbIL-1β was confirmed by A375 cell growth inhibition assay.

*Polyclonal antibody to rbIL-1β and its purification*: Japanese white rabbits were immunized by intramuscular injection of rbIL-1β (50 µg/ml) emulsified in an equal volume of complete Freund’s adjuvant. Two subsequent immunizations of 50 µg of rbIL-1β emulsified in incomplete Freund’s adjuvant were given at 3 to 4-week intervals. After confirming antibody titer against IL-1β higher than 1:10,000 dilution of sera by ELISA, the rabbits were bled and sera were collected. Specific anti-rbIL-1β rabbit IgG was purified from ammonium sulfate precipitation fraction of the immune sera by affinity chromatography using cyanogen bromide-activated Sepharose 4B (Pharmaca, Sweden) coupled with rbIL-1β. The purified IgG was used as a capture antibody for IL-1β without any modification and as a detecting antibody after biotinization using a commercially available kit (Amersham, U.K.).

*Sandwich ELISA for bovine IL-1β*: Immunoreactive IL-1β in sera and colostrum was tested by using a capture ELISA. A flexible microtest plate III (Becton Dickinson, U.S.A.) was coated with 50 µl of purified rabbit anti-rbIL-1β IgG (18 µg/ml) in PBS and incubated for 30 min at room temperature. The wells were washed 5 times with PBS containing 0.05% Tween-20 (T-PBS) and the plate wells were filled with PBS containing 2% bovine serum albumin and kept at 37°C for 1 hr to block non-specific absorbance. After one washing with T-PBS, samples, including standards of known rbIL-1β content, control specimens, and unknowns, were added into the wells, and the plate was incubated for 30 min at room temperature. Then, after 5 washings with T-PBS, appropriately diluted biotinylated anti-rbIL-1β rabbit IgG (which was determined by a preliminary test) was added to wells and incubated for 30 min at room temperature. After a further washings, appropriately diluted streptavidin horse radish peroxidase conjugate (Pharmacia, Sweden) was added to the wells and incubated for an additional 30 min at room temperature. After 5 washes with T-PBS, 100 µl of 2 mM 2,2’-3-ethylbenzthiazoline-sulfonic acid and substrate (H2O2) mixture was added to each well and left for 30 min at room temperature to detect bound enzyme that produced color, and the intensity of the colored product was measured by a Immuno Mini NJ-2300 (Nihon Intermed Co., Tokyo) at 405 nm. The concentration of IL-1β in unknown samples was read from the plotted standard curve. The detection limit of the ELISA was 0.5 ng/ml for rbIL-1β diluted in serum, medium or whey.

**Detection of bIL-1β mRNA in cells obtained from milk by RT-PCR**: Total RNA was extracted from milk cells without any stimulation and ConA-stimulated PBMC as positive control by the method of Chomczynski and Sacchi [6]. cDNAs were synthesized using the Moloney murine leukemia virus RNAse H’ reverse transcriptase and random primer (Superscript; Gibco BRL, U.S.A). PCR reaction was performed in a final volume of 50 µl, containing 100 ng each of the upstream and downstream PCR primers for the bIL-1β, cDNA (2 µl), 1 x PCR reaction buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2), dNTPs, and AmpliTaq DNA polymerase (Perkin Elmer, U.S.A.). The cycling parameter was a pre-soaked at 94°C for 6 min followed by 35 cycles at 94°C for 1 min, at 55°C for 1 min, and at 72°C for 3 min. Aliquots of each PCR product were analyzed by 2% agarose gel electrophoresis. As a positive control for RNA quality, PCR was also performed on all samples using bovine β-actin specific primers: 5’ ACGTCGCCCTTGACCTTCGAGCAGG 3’ and 5’ GCTGGAAGGTGGACAGGGAGGCCAGGA 3’, which were reported by Ito and Kodama [12].

**RESULTS**

**IL-1β levels in sera of cattle**: As shown in Fig. 1, IL-1β was detected in 90% of the animals during a period from 2 days before to 14 days after parturition. However, the mean concentration of IL-1β was gradually decreased and became undetectable 90% of the animals by 7 to 9 weeks postpartum. The cows came into estrus about 10 weeks postpartum and were inseminated artificially. After conception, the number of IL-1β positive cases and the mean IL-1β concentration gradually increased.

In 10 to 14 newborn calves at ages indicated in Fig. 2, serum IL-1β was transiently detected with peak titers on day 3 during the first 2 weeks of life and became undetectable thereafter. Of seven heifers aged around 1 year, only one showed IL-1β, of which concentration was the detection limit, 0.5 ng/ml (data not shown).

**IL-1β in milk**: IL-1β concentrations in milk collected on days 0, 3, 7 and 14 post partum are shown in Fig. 3. IL-1β was detected in milk collected on day 0 but not on or after day 3. To examine the possible transfer of colostrous IL-1β to neonates, a comparison was made of the IL-1β concentration between in colostrum given to neonates with a detectable level of serum IL-1β on day 3 (Fig. 4A) and in colostrum fed to newborns without detectable IL-1β (Fig. 4B). IL-1β concentrations in colostrum given to neonates with detectable IL-1β were significantly higher than those in colostrum given to neonates without detectable IL-1β (P<0.01).

**IL-1β mRNA in milk cells**: To determine a possibility of IL-1β to be produced in udder, IL-1β mRNA in cells isolated from milk that was collected during 20 days before and 30 days after parturition was examined by RT-PCR. The results are shown in Fig. 5. IL-1β mRNAs were detected only in cells from milk collected during 20 days before and 2–3
IL-1β was transiently detectable in sera of some neonates with a peak titer on day 3 after birth and became undetectable by about 2 weeks of age. In addition, the IL-1β concentration in colostrum fed to newborns that had days after parturition.

DISCUSSION

The present studies showed that IL-1β was detected in sera and colostrum of mature dairy cattle and sera of newborns within 2 weeks after birth, but not in almost all sera of heifers around one-year-old and calves aged 3 to 25-week-old. The ovulatory process could be considered as an inflammatory reaction [7], and leukocytes, especially neutrophils and monocytes/macrophages, have been shown to infiltrate the ovaries just before ovulation [2, 5, 8]. It has been suggested that IL-1β derived from infiltrating leukocytes enhanced ovulation [3, 10, 20, 21]. Therefore, IL-1β is indicated to begin to appear in serum of dairy cattle at around artificial insemination, probably at ovulation, and to persist at higher level during pregnancy and to decline to undetectable level after parturition. Moreover, IL-1β has been reported to involve with regulation of progesterone [1, 13, 17, 22] which is essential for maintaining pregnancy [19]. Therefore, presence of IL-1β in sera of healthy mature dairy cattle is considered as physiologically normal condition associated with reproduction.

Pattern of IL-1β detection in colostrum but not in postcolostrous milk is similar to that of transient transfer of IgG from serum to colostrum [4]. However, presence of IL-1β mRNA in cells isolated from colostrum without any stimulation indicates the possibility that IL-1β is produced locally, in udder, although further studies are required for this issue.

IL-1β was transiently detectable in sera of some neonates with a peak titer on day 3 after birth and became undetectable by about 2 weeks of age. In addition, the IL-1β concentration in colostrum fed to newborns that had
serum IL-1β on day 3 was significantly higher than that in colostrum fed to newborns with no detectable serum IL-1β. Although the presence of IL-1β in the sera of some neonates before consumption of colostrum and the presence of IL-1α in commercially available fetal bovine sera [11] indicate the

Fig. 3. Changes in IL-1β levels in the milk of cows after parturition. Dots indicate individual IL-1β concentrations and cross bar indicates mean values of positive cows with standard deviations.

Fig. 4. Comparison of IL-1β concentrations in colostrum fed to newborns with (A) or without (B) serum IL-1β on day 3 after birth. Dots indicate individual IL-1β concentrations and cross bars indicate mean values of positive cows with standard deviations in each group.

Fig. 5. Expression of IL-1β mRNA in cells isolated from milk collected during the period from 20 days before to 30 days after parturition by RT-PCR. Lane 1: ConA-stimulated bovine PBMC for positive control. Lanes 2 to 9: unstimulated milk cells for -18, -16, 1, 2, 3, 14, 20, and 30 days after parturition, respectively, from different cows. Lanes 10 to 13 and Lanes 14 to 17: unstimulated milk cells collected -20, 0, 3, and 7 days after parturition, respectively, from two cows.
transfer of IL-1 across the placenta or the production of cytokines in fetus, the present results indicate that IL-1β might be transferred from dams to newborns via colostrum. In any case, the fact that IL-1β is found only in colostrum, not in post-colostrous milk is considered to mean that the cytokine is produced to be ingested by neonates. To obtain direct evidence, precise experiments to determine transfer of orally administered rbIL-1β to sera of neonates are required.

In conclusion, the present studies showed presence of IL-1β in sera and colostrum of healthy mature dairy cattle associated with reproduction and in sera of neonates. These results suggest one possibility that colostrous IL-1β might be transferred to neonates.

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REFERENCES


