

LEVEL OF NATURAL AUTOANTIBODY BINDING CARDIOLIPIN IN COLOSTRUM AND NAÏVE CALVES, IN RELATION WITH DIFFERENT MATERNAL DRY PERIOD LENGTH

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Abstract

Natural autoantibodies may prevent infections, clear aging cells and metabolic waste, and also play a role in anti-tumor surveillance, anti-inflammatory activity and maintenance of homeostasis. The objective of this study was to study the presence of natural autoantibody (NAAb) IgM binding cardiolipin in colostrum of cows subjected to different dry period lengths and in plasma of their calves prior colostrum intake. Holstein-Friesian dairy cows were randomly assigned to 2 dry period lengths (0 or 60 days). Colostrum was sampled directly after calving. Blood samples of female calves were collected before colostrum intake. The titers of IgM in plasma or colostrum were determined by an indirect ELISA method. Natural autoantibody titers IgM binding cardiolipin are present in colostrum and plasma of naive calves prior colostrum intake. Cows with a 0-d dry period had lower IgM titers binding cardiolipin in colostrum compared with cows with a 60-d dry period. Naive calves have NAAb and omission of the dry period resulted in lower NAAb levels in colostrum and, but did not affect NAAb levels in plasma of calves before colostrum intake.

Key words: dry period, natural autoantibodies, colostrum and calf

INTRODUCTION

Natural antibodies (NAb) are defined as antibodies present in animals without deliberate or known antigenic stimulation [1], and have been found in cows as well [7, 12, 19]. In mammals, NAb are poly-reactive with low affinity binding with various antigens [2]. Natural antibodies have been divided into two classes: overt and cryptic NAb. Overt NAb bind antigens that the individual has never encountered before, such as keyhole limpet hemocyanin (KLH). Cryptic NAb or so-called natural autoantibodies (NAAb) are antibodies that bind to self-antigens or slightly changed self-antigens (neo-epitopes). In humans [1, 10], rodents [1], chickens [14], and cattle [7, 11, 19], NAAb including their isotypes (IgM, IgG and IgA) were identified.

NAAb provide the first line of defence against infections, physiological homeostasis and bind antigens to promote internalization by antigen-presenting cells [8]. Cardiolipin is a phospholipid almost exclusively localized in the mitochondrial inner membrane and it is involved in mitochondrial energy metabolism [5]. The phenomenon that cows with clinical mastitis have tendency to have less NAAb binding transferrin but more NAAb binding myosin was reported by [19]. In this study the NAAb binding cardiolipin in colostrum and plasma of calves was studied.

In human, NAAb may have a role in the reduction of allergic, inflammatory and autoimmune diseases and lymphomas [21]. The concentration of immunoglobulines in newborn calves prior to first colostrum uptake is low. Maternal antibodies are transferred to calves through colostrum, and may help calves to prevent disease [12]. Little is known of the routes that lead to the production of NAAb in healthy young

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individuals. In earlier study, no IgM and IgG binding keyhole limpet hemocyanin (KLH) and human serum albumin (HuSA) in plasma of calves before colostrum intake (naïve calves) [12]. NAAb titers binding glutamate dehydrogenase (GD), carbonic anhydrase (CA), myosin (MYO) and transferrin (TRANS) were detected in colostrum and in plasma of naïve calves [11]. Earlier study showed the effect of maternal dry period on titers of NAb and NAAb in plasma of cows, colostrum and in plasma of calves [11, 12]. In the present study, we evaluated natural autoantibody binding cardiolipin in colostrum and naïve calves with a different maternal dry period length (0-d vs. 60-d).

MATERIAL AND METHOD

Experimental design, animals and rations

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. The registration number of the experimental protocol is 2010026. Blood and colostrum samples originated from an experiment that was designed to evaluate the effect of dry period length and ration composition on health of cows and calves. The experimental design, treatments of dry period lengths and ration composition in cows were described earlier by Van Knegsel [20]. In short, Holstein-Friesian dairy cows (N=167) were selected from the Dairy Campus research herd (WUR Livestock Research, Lelystad, the Netherlands) blocked according to parity, calving date, milk yield in previous lactation and body condition score, and randomly assigned to treatments. Treatment consisted of three dry period lengths: 0, 30 or 60 days; and two lactation rations (glucogenic or lipogenic ration). For the current study, we evaluated only two dry period lengths (0 vs 60-d). Female calves born to these cows (n=37) were monitored.

Management of calves and colostrum sampling

Management of colostrum and blood sampling of the calves were described earlier [15]. Colostrum was collected and weighed directly after calving, except when the cow calved between 10.00 p.m. and 05.00 a.m. Colostrum samples (10 ml) obtained after parturition were agitated and stored at -20°C,

until analysis. Immediately after birth, calves were removed from the dam. When calves were born between 10.00 p.m. and 05.00 a.m., calves were removed from the dam at 05.00 a.m. Calves were weighed and within 24 h of life, they received four liter of colostrum in two portions from their mother. Disease incidences and treatments of the female calves were recorded during trial.

Blood sampling and immunization

For calves, blood samples were taken from jugular vein for NAAb measurement before colostrum intake (n=37). Blood samples were taken in Vacutainer tubes (10 ml; Greiner Bio-One GmbH, Kremsmunster, Austria) containing heparin as anticoagulant. Blood samples were centrifuged at 3000 x g at 4°C, for 15 min within two hours after collection. Plasma was transferred and stored at -20°C until analysis.

Analysis of NAAb in colostrum and plasma of calves

Natural autoantibody titers to cardiolipin in plasma of calves in colostrum were measured by an indirect enzyme-linked immunosorbent assay (ELISA) technique as outlined by Van Knegsel [12]. In brief, plates were coated with 4 µg/ml of cardiolipin TRANS (T1408, Sigma), in 100 µl/well. Natural autoantibodies of the IgM isotype in plasma of calves and in colostrum were detected using 1:20,000 diluted rabbit polyclonal anti-bovine IgM-whole molecule conjugated to PO (Cat. No. A10-100P, Bethyl). Four step serial dilutions for IgM in colostrum and in plasma samples of calves started at 1:40. After washing, a substrate containing tetra methyl benzidine (Sigma Aldrich Chemie, Steinheim, Germany) and 0.05 percent hydrogen peroxide was added, and incubated for 10 minutes at room temperature. The reaction was stopped by adding 1.25 M sulfuric acid. Extinctions were measured with a Multiskan reader (Lab Systems, Helsinki, Finland) at a wavelength of 450 nm. Titers were expressed as log₂ values of the dilutions that gave an extinction closest to 50 percent of Emax, where Emax represents the highest mean extinction of a standard positive (pooled) serum present on every microtiter plate [15].

Statistical analyses

All data of NAAb titers in colostrum and in plasma of calves approximated normality of residuals by examining whether skewness and kurtosis were in a range of -2 until 2. Moreover, the data was analysed using PROC MIXED in SAS 9.2 (SAS Inst. Inc. Cary, NC). The NAAb titers for IgM binding cardiolipin in colostrum and in plasma of calves before colostrum intake were analysed with dry period length (0 or 60 days), parity of the cow (2, 3 or >3) and their 2-way interaction included as fixed effect. To assess the relationship between NAAb titers in colostrum with NAAb titers in plasma of female calves (before colostrum intake), the regression coefficients (β) from the statistical model were used and the *P*-value corresponding to the β are displayed.

RESULTS

The titers of IgM binding cardiolipin in colostrum of cows with 60-d dry period higher compared with cows with a 0-d dry period ($P < 0.01$) (Figure 1). NAAb titers IgM binding cardiolipin increased with parity (10.81 vs. 11.06 vs. 11.17 ± 0.23 for parity 2 vs. 3 vs. >3, respectively; $P = 0.51$).

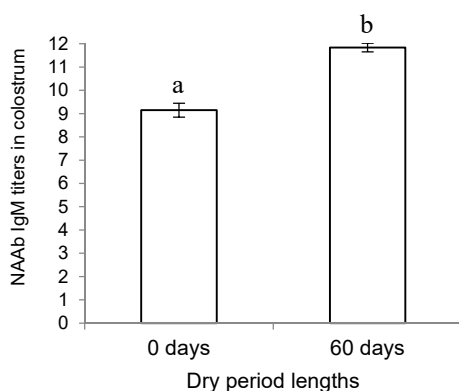


Fig 1 Natural auto antibody titers IgM binding cardiolipin in colostrum from cows with 2 different dry period lengths (0-d or 60-d). Values are means ($\pm$ SEM) per dry period length

NAAb for isotypes IgM binding cardiolipin existed in plasma of newborn calves (Figure 2). Even though there was no significant difference in the titers of NAAb in

plasma of newborn calves, which were given birth by cows with different dry period length, calves from cows with 0-d dry period numerically, have lower IgM titers binding cardiolipin compared with calves from cows with 60-d dry period. There were no relationships between NAAb titers to cardiolipin in colostrum with NAAb titers to cardiolipin in plasma of calves before colostrum intake.

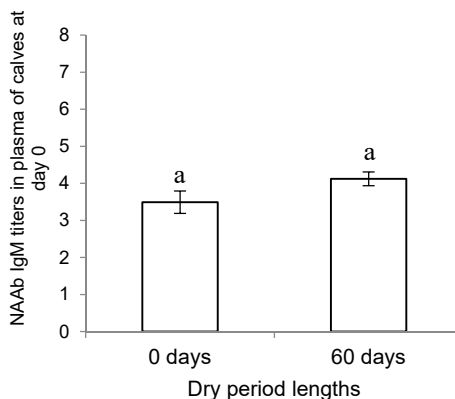


Fig 2 Natural autoantibodies for isotype IgM titers binding cardiolipin in plasma of calves at day 0 before colostrum uptake with 2 different dry period lengths (0-d (n=20) vs a 60-d (n=17)). Values are means per dry period length

DISCUSSIONS

In the current study, NAAb of IgM binding cardiolipin was detected in colostrum and plasma of calves (newborn). Previous studies showed the presence of NAAb of IgG and IgM binding liver cell lysate in plasma of healthy calves after colostrum uptake [7], NAAb of IgG binding myosin and transferrin in plasma of cows [12]. Moreover, NAAb of IgG and IgM binding glutamate dehydrogenase (GD), carbonic anhydrase(CA), myosin (MYO) and transferrin (TRANS) were detected in plasma of cows before calving, in colostrum and in plasma of calves before and after colostrum intake [11]. There was no overt NAb (binding mannan) found in plasma of newborn calves [17]. It was suggested that antibodies in cattle cannot be transferred through the placenta, the antibodies existed in newborn calves are speculated to be produced by bovine fetus [17]. In contrast with the present study, NAAb of IgM binding cardiolipin were present in plasma of calves

before colostrum intake. However, this study is in line with earlier study, NAAb binding the auto-antigens GD, CA, MYO and TRANS were found in plasma from calves before colostrum intake [11]. The study of Mayasari et al [11] suggested that NAAb may be already initiated by self-antigens during the fetal phase and NAAb do reflect auto-responsiveness. The presence of NAAb in calves before colostrum intake may reflect cross reactivity to the (intestinal) microbiota [6].

Cows with 0-d dry period had the lowest NAAb titers of IgM binding cardiolipin in colostrum. In line with previous studies, IgG and IgM binding KLH and human serum albumin (HuSA) [12] and NAAb IgG and IgM binding GD, CA, MYO or TRANS were lower in colostrum from cows with 0-d dry period compared with colostrum from cows a 60-d dry period [11]. Newborn calves from cows with 0-d dry period have the lowest titers and calves from cows with 60-d dry period have the highest titers of IgM binding cardiolipin. A possible reason is the dry period can lead more energy and nutrition transfer from cows to fetal development when there is no requirement to milk production. This was supported by a higher birth weight of calves from cows with 60-d dry period compared with calves from cows with 0-d dry period [12].

In our study, age of the mother affected the level of NAAb IgM binding cardiolipin in plasma of naïve calves. Older cows had higher IgM binding cardiolipin in plasma of their calves compared with younger cows. Previous studies reported older cows had higher NAb titers binding exo-antigens like KLH and HuSA in colostrum than young cows [12, 16]. The present study is in line with Conneely et al. [4] who suggested that older cows have higher antibody levels because they are likely to be exposed more to a greater number of antigens in their life [9]. In mice, the level of IgM binding self-antigens was high in plasma during fetal development and in newborns [13]. In humans, high titers of NAAb binding self-antigens in plasma of the mother might reduce the incidence of diseases of their offspring [18]. Our data indicate that calves did not result in enhanced disease sensitivity during the trial.

CONCLUSIONS

In conclusion, IgM antibodies binding cardiolipin were detected in colostrum and in plasma of calves before colostrum intake. The level of NAAb in plasma of calves before colostrum intake was not affected by dry period of their mother. Future studies should reveal the consequences of low or high levels of NAAb binding cardiolipin from either neonatal or maternal of origin on disease sensitivity of calves later in life.

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