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Short communication

Age-related changes in mRNA expression of selected surface receptors in lymphocytes of dairy calves

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Abstract

The aim of this study was to determine age-related changes in the mRNA expression of four clusters of differentiation (CD: e.g. CD5, CD21, CD22 and CD23) in lymphocytes of calves. Blood samples were collected from 10 Holstein heifers on day 2, 22 and 56 of life and used for lymphocyte isolation. Subsequently, the mRNA was isolated from lymphocytes and the relative expression of CD5, CD21, CD22 and CD23 was investigated using quantitative real-time PCR with GAPDH as a reference gene. CD5, CD21 and CD23 mRNA expression increased linearly ($p \leq 0.04$) with calf age, whereas CD22 mRNA expression did not change in the investigated period ($p > 0.05$). Age related changes in CD5, CD21 and CD23 mRNA expression suggest their importance in the process of lymphocyte maturation in calves.

Key words: immune system development, clusters of differentiation, marker, lymphocyte maturation

Introduction

The calf immune system is fully developed at birth; however, it is immature and many components of the immune system are not functional until 2-4 weeks of life, which makes newborn calves particularly susceptible to infections (Chase et al. 2008). Maturation of lymphocytes is associated with the emergence of specific surface molecules and their presence indicates stage of differentiation (Tizard 2004). The activity of lymphocytes may be modulated by several factors, including nutrition or vaccination (Chase et al. 2008, Ballou 2012). However, in order to study factors affecting calf immune system maturation and lymphocyte functions, and given the complexity of the immune system, there is a need to identify markers of lymphocyte development. In this study, we attempted to determine age-related changes in the mRNA expression of four clusters of differentiation (CD5, CD21, CD22 and CD23) in lymphocytes of calves, which could serve as potential markers of lymphocyte maturation and differentiation. Only the surface receptors which, according to the literature, appear on the mature lymphocytes B (CD21, CD22, CD23) and T (CD5) were selected for the study. Besides their role in the regulation of differentiation, proliferation and activity of lymphocytes, it is assumed that these molecules take part in lymphocyte maturation

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Table 1. Genes and primers used in the study.

Gene	Gene description	GenBank Accesion no.	Forward primer	Reverse primer	Amplicon length (bp)
CD 5	cluster of differentiation 5	NM_173899.2	GGTGGTGAAGAAATTCCGCC	CGTGTGGTTGCGATGGAAAG	91
CD 21	complement component 3d receptor 2 (CR2)	GQ497281.2	GCAGGGCAGTCCGATGTAAT	GGAGGGGCTTGACAATCCTT	79
CD 22	cluster of differentiation 22	XM_015467434.1	TCCAAGTGCTGTATGCTCCC	CTCTCACAGGTCAGGACTGC	94
CD 23	Fc fragment of IgE receptor II (FCER2)	XM_010798252.2	TTCGAGCTGACCTGAGTGAC	GCTCGATCCACAACTTTCCC	108
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	NM_001034034	CAACGGCACAGTCAAGGCAGAGAA	TCAGCACCAGCATCACCCCACTTG	111

(Tedder et al. 1997, Chattha et al. 2009, Hayashi et al. 2010, Soldevila et al. 2011).

Materials and Methods

The study protocol was approved by a Local Ethics Committee (Krakow, Poland, protocol no. 12/2016). Ten Holstein heifers were allocated to the trial at birth. Each calf was in the study till the age of 56 days. On the first day of life, the calves received colostrum in two equal doses, with the first dose (2 L) administered within 1 h after birth and the second dose (2 L) administered by 8 h of life. Calves were subsequently fed transition milk and then whole milk (2 × 2 L) from day 4 to 8 of life, and milk replacer (3 × 2 L; Sprayfo, Sloten, Poland) thereafter, up to day 56 of life. From day 14 of life the calves were also fed commercial pelleted starter mixture for *ad libitum* consumption (Kalber Starter; Blattin, Poland).

Blood samples were collected from the jugular vein on day 2 (beginning of the study), 22 and 56 of life (last day of liquid feed administration before weaning). Day 22 of life was chosen since it covers a period of immunity depression in calves resulting from a decreasing amount of circulating colostral immunoglobulins but insufficient production of the calf's own antibodies (Nonnecke et al. 2012). Immediately after collection, blood samples were used for lymphocyte isolation with Gradisol L according to Darmochwal-Kolarz et al. (2014), with minor modifications. The mRNA was isolated from lymphocytes using the method of Chomczyński and Sacchi (1987) and instantly subjected to the reverse transcription reaction (High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, Thermo Fisher Scientific, USA). The relative expression of CD5, CD21, CD22 and CD23 in lymphocytes was investigated using quantitative real-time PCR and GAPDH as a reference gene (Table 1). Target and reference gene mRNA expression was analyzed using a StepOne Plus™ Real-Time PCR System (Life Technologies Corporation, Carlsbad, California, USA) and PowerUp™ SYBR® Green Master Mix (Thermo Fisher Scientific, Waltham, USA) according to manufacturer instructions. Data were calculated using the $2^{-\Delta\Delta Ct}$ method with a pooled sample as a calibrator and analyzed using the PROC MIXED statement of the SAS (version 9.4). The statistical model included the effect of calf age as a classifying variable and calf was used as a random term. Polynomial contrasts (linear – L and quadratic – Q) were used to determine age-related changes in mRNA expression of CD5, CD21, CD22 and CD23.

Results and Discussion

In the present study, mRNA expression of CD21 was very low on day 2 of life and increased thereafter ($p < 0.01$; Fig. 1). A similar increase in CD21 expression, but at the protein level, was noted in bovine B cells (Kampen et al. 2006, Chattha et al. 2009). Simultaneously, CD5 and CD23 mRNA expression also increased linearly ($p \leq 0.04$) with calf age, whereas no age-related change was found in CD22 mRNA expression. To our knowledge, this study is the first to evaluate age-related changes in the mRNA expression of CD5, CD21, CD22 and CD23 in dairy calves during the first two months of life. In mice, an increasing level of CD5 and CD23 protein along with progression in lymphocyte T and B maturation was noted, respectively (Azzam et al. 1998, Hayashi et al.

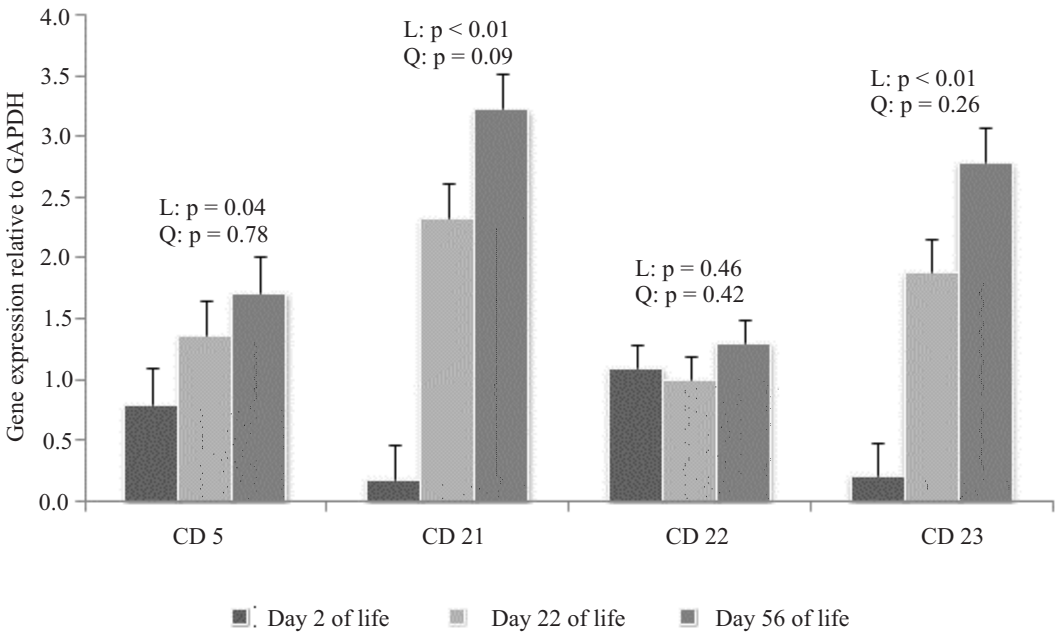

Fig. 1. Effect of age on the mRNA expression of surface molecules in bovine lymphocytes.

2010). CD5, as an activation marker, was also observed to appear on activated B cells in calves due to infection (Stabel et al. 2011). However, in the current study there was no recorded health issue. On the other hand, CD22 – a marker specific for B cells – was shown to appear only on mature B cells in mice and humans (Erickson et al. 1996, Tedder et al. 1997), but its expression may precede CD21 and CD23 appearance and seems to take place before the release of lymphocytes to the blood.

In conclusion, age-related changes in surface molecule CD5, CD21 and CD23 mRNA expression suggest their significance in the process of peripheral blood lymphocyte maturation in calves and are promising targets for further studies.

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