Pregnancy specific regulation of lysosomal cathepsins in bovine blood leukocytes

(ウシ末梢白血球におけるリソソームカテプシンの妊娠特異的応答機構に関する研究)

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Introduction

In most mammalian species, an embryo secretes signals to specify its presence to the mother for successful establishment of pregnancy. The interferon tau (IFNT), a key pregnancy recognition signal in ruminants, is secreted from the trophectoderm of the conceptus at the blastocyst stage and its level increases with the elongation of the conceptus. The IFNT during the maternal recognition period (MRP) is important for successful establishment of pregnancy in ruminants.

During the pregnancy recognition period, IFNT acts to uterine tissues to silence the expression of estrogen receptor (ESR) alpha and oxytocin receptor (OXTR), which prevents the oxytocin-dependent release of luteolytic pulses of prostaglandin F2α (PGF2α)secretion by uterine tissue. Therefore, the corpus luteum (CL) continues to produce progesterone required for maintenance of pregnancy. IFNT may act other organs and cells than that the uterine tissue. Recently, it has reported that pregnancy induced up-regulation of interferon stimulated gene 15 (*ISG15*) and myxovirus resistance 1 (*MX1*) genes in bovine liver on d18 and peripheral blood leukocytes (PBLs). Therefore, it is necessary to discovery other novel markers which have the ability to detect pregnancy at earlier period with easy and clear manner.

Recently, it has been reported that lysosomal cysteine proteases are activated by type 1 IFN (IFN-β) in in vitro culture of mouse macrophages. Type 1 IFN stimulated pathway may be involved in several types of cell like blood leukocytes because type 1 IFN pathway exists in many types of somatic cells. The ISGs may be activated in lysosomal CTSs through type 1 IFN cell signaling pathway in PBLs and possibly to play important roles during early

pregnancy in ruminants. Interestingly, up-regulation of ISGs expression has been identified in circulating immune cells during implantation, making these factors a potential source of non-invasive biomarkers for early pregnancy.

Therefore, this study was conducted to explore the dynamics of lysosomes and lysosomal CTSs in PBLs collected from pregnant and non-pregnant dairy cows, and conducted in vitro IFNT-stimulation of blood leukocytes from cyclic cows.

Experimental design and results

1. Pregnancy specific response of lysosomal CTSs in bovine leukocytes

This study aimed to explore the IFNT-mediated lysosomal activation in PBLs during early pregnancy of cows. Multiparous Holstein Friesian cows were subjected to artificial insemination (AI). Leukocytes collected from peripheral bloods on d18 of pregnant and non-pregnant cows were separated and used for the measurement of lysosomal acidification, activities of CTSs B and K, expressions of *LAMP-1*, *-2* and CTSs *B* and *K* genes. Lysosomal acidification and CTS B and K activities were significantly increased in the pregnant leukocytes than those collected from non-pregnant cows. Gene expression levels of *LAMP-1*, *-2*, and CTSs *B* and *K* were also significantly increased in the pregnant leukocytes than non-pregnant cells. Besides, immunodetection showed significant increase of LAMP-1 (Fig 1a, b) and CTSK (Fig 1c, d) in the leukocytes of pregnant cows. These results showed the first observation of pregnancy-specific lysosomal activation in bovine PBLs.

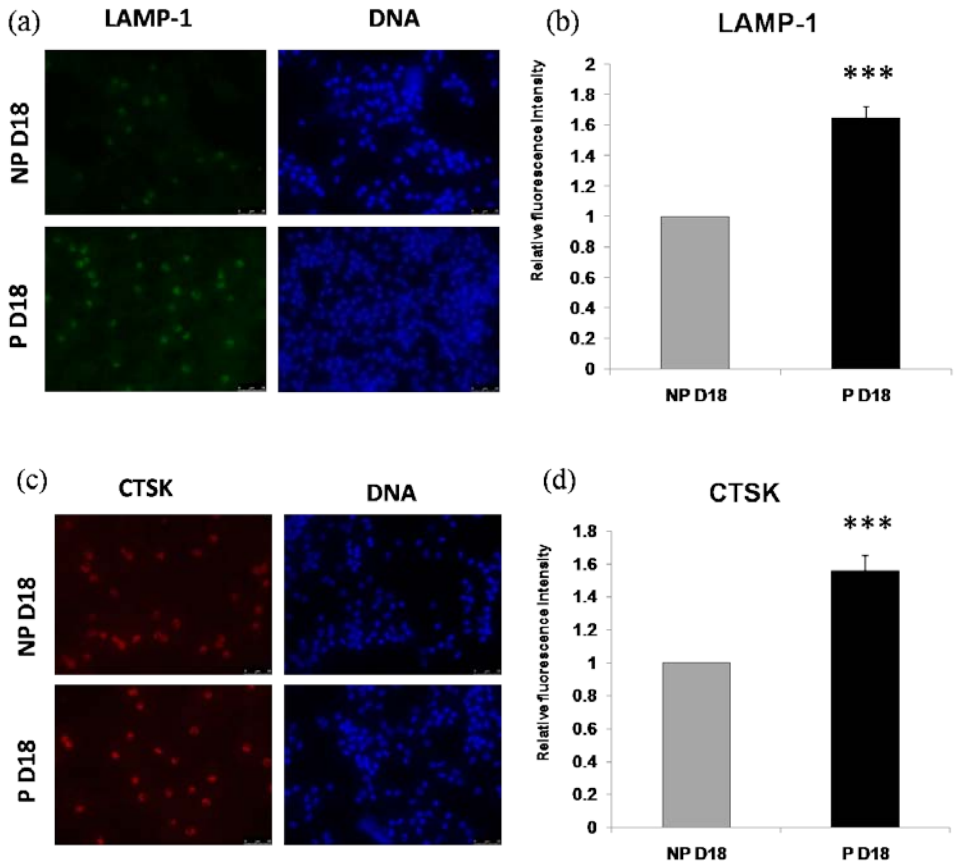

Figure 1. Immunostaining and detection of LAMP-1 and CTSK protein in leukocytes after AI.

2. Status of lysosomes and lysosomal CTSs in bovine PBMCs and PMNs during early pregnancy

This study aimed to investigate the cell specific difference of lysosomal activation in the separated peripheral blood mononuclear cells (PBMCs) and polymorphonuclear granulocytes (PMNs). Blood samples were collected on d0, 7, 14 and 18 after AI. After 40 days of AI, pregnancy was confirmed by ultrasonography. The activities of CTS B, K and L were increased significantly both in PBMCs and PMNs in the progress of pregnancy.

Expressions of CTS *B* and *K* genes were significantly increased both in PBMCs or PMNs in pregnant cows compared to non-pregnant cows. Assessment of lysosomal activity was also showed higher and increased significantly in PBMCs (Fig 2a, b) and PMNs (Fig 2c,d) in pregnant cows. Expression of *LAMP-1* (Fig 3a, b) and *LAMP-2* (Fig 3c,d) increased significantly in pregnant cows compared to non-pregnant cows on d18. Immunodetection analysis revealed that, CTSB protein was detected and significantly increased in PBMCs and PMNs in pregnant cows compared to non-pregnant cows on d18. The findings of this study suggest that, lysosomal CTSs could be reactive to IFNT in PBMCs and PMNs. It also suggested that, PMNs have higher sensitive than PBMCs and are suitable for potential marker cells for detection of early pregnancy.

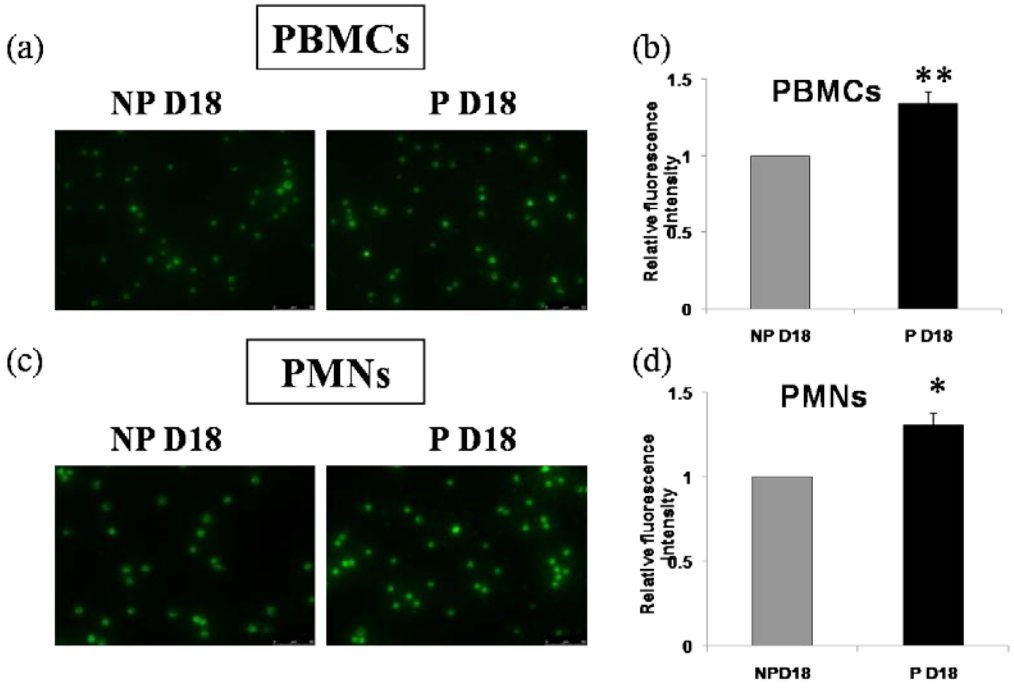

Figure 2. Lysosomal activity in PBMCs and PMNs after AI.

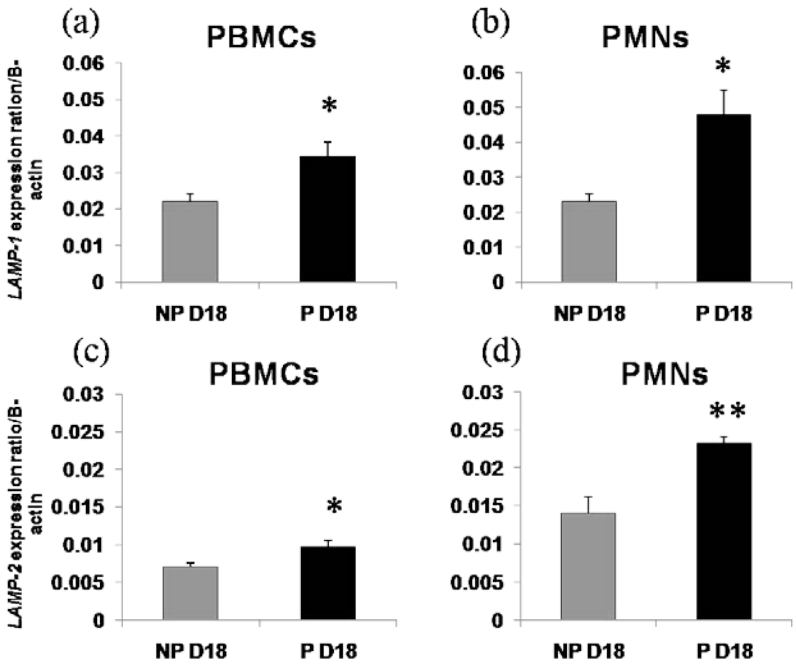

Figure 3. Expression of *LAMP-1* and *LAMP-2* mRNA in PBMCs and PMNs after AI.

3. Effect of IFNT on stimulation of the activity of lysosomes and lysosomal CTSs in bovine PBMCs and PMNs

To elucidate the direct effect of IFNT secreted from conceptus for the activation of lysosomal functions, *in vitro* experiment with recombinant IFNT was performed. Addition of IFNT significantly increased the activities of CTSs B, K and L both in PBMCs and PMNs. Gene expression levels of CTS *B* and *K* were also increased significantly both in PBMCs and PMNs by IFNT stimulation. Lysosomal activity was increased significantly in PBMCs (Fig 4a, b) and PMNs (Fig 4c, d) after IFNT stimulation. Immunodetection analysis revealed that CTSB protein was detected and significantly increased in PBMCs (Fig 5a, b) and PMNs (Figure 5a, c). These results suggest that CTSs activity is increased by the direct IFNT stimulation supported the lysosomal function.

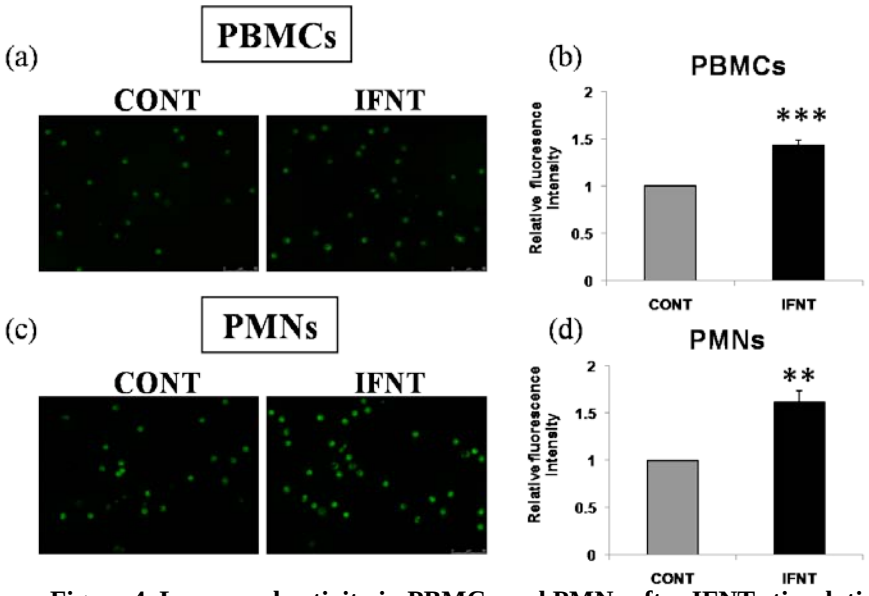

Figure 4. Lysosomal activity in PBMCs and PMNs after IFNT stimulation.

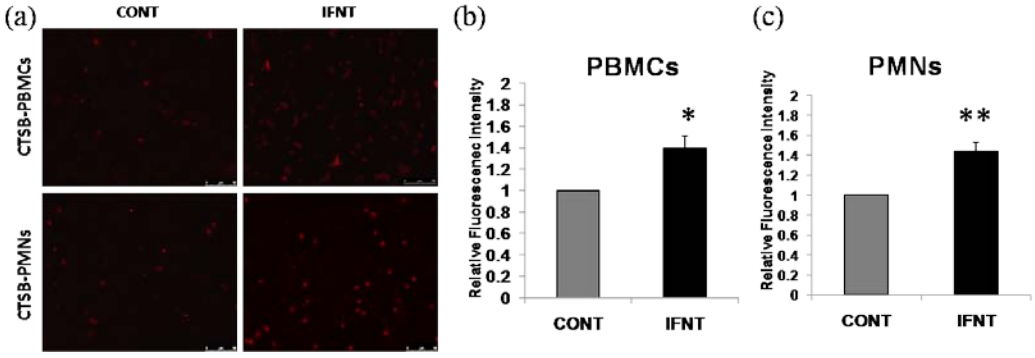

Figure 5. Immunostaining and detection of CTSB protein in PBMCs and PMNs after IFNT stimulation.

General discussion

Accordingly, this research aimed to address the lysosomal CTSs activation by conceptus secretory protein IFNT and to specify the role of implantation of the conceptus. It is clear that there is no precise pathway of IFNT mediated lysosomal CTSs activation.

It is summarized that, the lysosomes and lysosomal CTSs activity, expressions and protein level could be responsive to IFNT during maternal-fetal recognition period of pregnancy in PBLs via type 1 IFN signal transduction pathway and also lysosome and lysosomal CTSs potentially be useful biomarkers for early pregnancy detection.

Conclusion

It could be suggested that, in early pregnancy PBLs gene expression may be altered through the influence of pregnancy signal of IFNT. An increased knowledge of the interaction between conceptus and maternal immune system during early pregnancy in ruminants is necessary to understand and elucidate the true cause for successful establishment of pregnancy. This study also provided the basis for new strategies for improving pregnancy outcomes and reproductive efficiency. In this study, we defined for the first time that IFNT induces lysosomal activation as well as the expressions of lysosomal proteins (CTSB, CTSK and LAMP-1). It is still remaining to address whether lysosomal function is regulated by the IFNT via type 1 IFN pathway in bovine leukocytes during maternal-fetal recognition period. Future study will explore the route of IFNT transport to lysosomes in leukocytes during the implantation period and expression of lysosomal CTSs in leukocytes and discover new functions of lysosomal CTSs by IFNT.