論文の内容の要旨

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論文題目 Studies on association of bovine major histocompatibility complex (BoLA)-*DRB3* polymorphism with bovine leukemia virus infection outcome
(ウシ主要組織適合遺伝子複合体(BoLA)-*DRB3* 遺伝子の多型性と牛伝染性リンパ腫ウイルス感染症の相関性に関する研究)

*Chapter 1: Introduction and research aim*

Bovine leukemia virus (BLV) infects cattle worldwide and causes enzootic bovine leucosis, the B-cell lymphoma in cattle. During infection, the DNA copies of BLV genome integrates into host genome called as provirus leading to lifelong infection. The provirus load (PVL) is positively related with viral transmission as well as disease progression. So far, no therapy and prevention methods have yet been established. Therefore, cattle breeding selection based on disease resistance gene might be a promising strategy to reduce BLV-induced damages. *BoLA-DRB3* is a highly polymorphic gene which is responsible for antigen presentation and therefore associated with several cattle diseases. This research aimed to investigate the relationship between *BoLA-DRB3* polymorphisms with BLV infection outcome (PVL level and lymphoma development) in cattle.

*Chapter 2: Differential association between BoLA-DRB3 Polymorphism with BLV-Induced Lymphoma and Proviral Load*

Polymorphism of *BoLA-DRB3* is reported to associate with PVL; however, little is known about the

relationship of *BoLA-DRB3* polymorphism with BLV-induced lymphoma. Furthermore, whether or not PVL-associated *BoLA-DRB3* allele is linked to lymphoma-associated *BoLA-DRB3* allele has not been clarified. In this chapter, using Holstein cows as a model, I compared the association between *BoLA-DRB3* polymorphism with BLV-induced lymphoma and with PVL. I found that two *BoLA-DRB3* alleles were specifically associated with lymphoma resistance (**010:01* and **011:01*); Two other alleles, **002:01* and **012:01*, were associated with PVL resistance and susceptibility, respectively. In contrast, lymphoma and PVL shared two resistance-associated (*DRB3*014:01:01* and **009:02*) *BoLA-DRB3* alleles. Interestingly, we found that PVL associated alleles, but not lymphoma associated alleles, are related with the anti-BLV gp51 antibody production level in cows. Overall, this study is the first to demonstrate that the *BoLA-DRB3* polymorphism confers differential susceptibility to BLV-induced lymphoma and PVL.

*Chapter 3: Association of BLV -induced lymphoma with BoLA-DRB3 polymorphisms at DNA, amino acid, and binding pocket property levels*

The study In Chapter 2 shows that the DNA sequence polymorphisms of *BoLA-DRB3* allele have exhibited a correlation with BLV-induced lymphoma in Holstein cows. However, the association may vary between different cattle breeds and the information in Japanese black cattle is not yet available. In this chapter, I comprehensively analyzed the correlation between BLV-induced lymphoma and DRB3 allele types at DNA, amino acid, and binding pocket property levels in Japanese black cattle. I found

that *DRB3*011:01* was identified as a resistance allele, whereas *DRB3*005:02* and *DRB3*016:01* were susceptibility alleles. Amino acid association studies showed that positions 9, 11, 13, 26, 30, 47, 57, 70, 71, 74, 78, and 86 were associated with lymphoma susceptibility. Structure and electrostatic charge modeling further indicated that binding pocket 9 of resistance DRB3 was positively charged. In contrast, alleles susceptible to lymphoma were neutrally charged. Altogether, this is the first association study of BoLA-DRB3 polymorphisms with BLV-induced lymphoma in Japanese black cattle. In addition, these results further contribute to understanding the mechanisms regarding how *BoLA-DRB3* polymorphisms mediate susceptibility to BLV-induced lymphoma.

*Chapter 4: BoLA-DRB3 heterozygote advantage against the outcome of BLV infection*

Host genetic heterozygosity at major histocompatibility complex is thought to have enhanced ability against infectious diseases due to recognizing a more divert antigen pool. However, whether heterozygote of *BoLA-DRB3* has advantage against BLV induced-lymphoma and PVL still unclear. In this chapter, I found heterozygote at *BoLA-DRB3* has advantage against BLV PVL and BLV-induced lymphoma in both Holstein cows and Japanese black cattle.

*Chapter 5: General conclusions and perspectives*

First, I found that BLV-induced lymphoma compared with PVL is associated with differential *BoLA-DRB3* polymorphisms in cattle. This result could be an important reference for accurate cattle

breeding selection to combat BLV-induced lymphoma. Second, I identified the electrostatic charge difference between lymphoma resistant and susceptible *BoLA-DRB3* at the encoding DRBβ binding pocket 9 (a structure for antigen binding). This finding potentially contributes in future vaccine development for BLV-induced lymphoma as the charge of binding pocket is a key factor for antigen interaction and triggering effective immune response and therefore, the charge of peptide vaccine should be taken in account to fit with the charge of BoLA-DRB3 binding pockets. Third, I found that *BoLA-DRB3* heterozygous status was significantly associated with both PVL and lymphoma resistance irrespective of cattle breeds. This result suggests that cattle breeding in BoLA-DRB3 heterozygous setting could potentially reduce the occurrence rate/ level of BLV-induced lymphoma and PVL.