学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
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Research on disease progression and immunoinhibitory pathway using sheep model of persistent bovine leukemia virus infection
（牛伝染性リンパ腫ウイルス持続感染羊モデルにおける病態進行および免疫抑制因子に関する研究）

Bovine leukemia virus (BLV) is a retrovirus that infects B cells in ruminants and can lead to lymphoma in some infected cattle after a long latent period. Previous studies have characterized the T-cell exhaustion status of BLV-naturally infected cattle, which is mediated by immunoinhibitory molecules such as programmed death-1 (PD-1), PD-ligand 1 (PD-L1), and T cell immunoglobulin and mucin domain 3 (TIM-3). However, the understanding of disease and immune status across all stages of BLV infection remains challenging due to the long latency of the virus in cattle. Sheep is one of the optimal experimental models for BLV infection since they develop the clinical disease in a shorter period with a higher frequency compared to natural hosts. Using sheep as an experimental model, this research aimed to elucidate the utility of molecular diagnosis and immunopathological status during BLV infection.

In Chapter I, blood samples were collected periodically from BLV-experimentally infected sheep to compare clonality value (Cv) kinetics, measured by the 'RAISING-CLOVA' method, with proviral load (PVL) levels, in relation to the lymphoma diagnosis. Cv provided a substantial prognostic lead time for lymphoma development compared to PVL. Blood and tissue samples analyses also confirmed identical BLV integration sites on chromosome 15 in all samples. In Chapter II, the nucleotide and amino acid sequences of the immunoinhibitory factors PD-1 and PD-L1 were identified in sheep. A flow cytometric assay showed that anti-bovine PD-L1 monoclonal antibodies (mAbs) cross-react with ovine PD-L1, confirming their utility for detecting ovine PD-L1. Immunohistochemistry further demonstrated PD-L1 expression on barin macrophages in ovine listeriosis. In Chapter III, peripheral blood mononuclear cells (PBMCs) were isolated from the blood of BLV-experimentally infected sheep and analyzed by flow cytometry to confirm PD-L1 expression on ovine B cells. The proportion of PD-L1^{+} B cells in PBMCs increased over time after BLV infection. Subsequently, genomic DNA was extracted from

PBMCs to quantify BLV PVL, which rose at later time points as disease progressed. The expression levels of PD-L1 and TIM-3 on PBMCs were upregulated during BLV experimental infection in sheep. PBMCs were then cultured with anti-bovine PD-L1 mAbs and anti-bovine TIM-3 mAbs under the stimulation with concanavalin A. The combined blockade with anti-TIM-3 and anti-PD-L1 mAbs increased T-cell activation markers, CD25 and CD69, and enhanced production of cytokines, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), in T cells. IFN-γ production was also increased by TIM-3 inhibition and was higher with combined TIM-3 and PD-L1 blockade.

In conclusion, the results suggest that Cv derived from the RAISING-CLOVA method is a useful indicator for lymphoma prognosis compared with PVL. The immunoinhibitory molecules, PD-L1 and TIM-3, contribute to T-cell exhaustion during BLV infection in sheep, and blocking the TIM-3 and PD-L1 pathways enhances antiviral immune responses. Further elucidation of immune exhaustion and antiviral effects will require analysis of additional cytokines and T-cell functions in BLV-infected sheep. Using sheep as a model, this research improves the understanding of immune exhaustion in cattle and contributes to the development of therapeutic interventions for chronic infectious diseases.