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

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学位論文題名
The title of the doctoral dissertation

Studies on the mechanism of the immunosuppression caused by bovine mycoplasmosis and the development of its novel control method

（牛マイコプラズマ感染症における免疫抑制機序の解明と新規治療法の開発）

Bovine mycoplasmosis, caused by *Mycoplasma bovis*, is a disease of cattle, such as chronic pneumonia, therapy-resistant mastitis. There is no effective vaccine to control *M. bovis*. Thus, the elucidation of the mechanisms underlying the disease progression and the development of the novel strategy to control *M. bovis* infection are required. In this study, to elucidate the mechanisms of immunosuppression during *M. bovis* infection, the immunoinhibitory molecules, programmed death 1 (PD-1) and PD-ligand 1 (PD-L1) which induce T-cell exhaustion and prostaglandin E_2 (PGE_2) were investigated. In addition, clinical tests targeting both the PD-1/PD-L1 pathway and PGE_2 were performed in *M. bovis*-infected cattle.

CHAPTER I: The role of the PD-1/PD-L1 pathway in *M. bovis* infection were investigated. The proportions of PD-1^+ T cells as well as PD-$L1^+$ monocytes were increased in peripheral blood of *M. bovis*-infected cattle. The increases in the proportions of PD-1^+ T cells were negatively correlated with IFN-γ production from peripheral blood mononuclear cells (PBMCs) during *M. bovis* infection. On the other hand, the blockade of the PD-1/PD-L1 pathway *in vitro* by anti-bovine PD-1/PD-L1 antibodies (Abs) significantly upregulated the IFN-γ production from *M. bovis*-specific cells. These results suggest that the PD-1/PD-L1 pathway could be involved in immunosuppression of *M. bovis*-specific T cells.

CHAPTER II: The role of PGE_2 in the immunosuppression and the relationship between PGE_2 and the PD-1/PD-L1 pathway during *M. bovis* infection were investigated. The stimulation with *M. bovis in vitro* upregulated the expressions of PGE_2 and PD-L1 presumably via Toll-like receptor 2 in bovine PBMCs of healthy cattle. PGE_2 levels of peripheral blood from *M. bovis*-infected cattle were significantly increased in line with increased proportions of PD-$L1^+$ monocytes. On the other hand, the

dual blockade, using anti-bovine PD-L1 Ab and a cyclooxygenase (COX)-2 inhibitor *in vitro*, significantly enhanced the *M. bovis*-specific IFN-γ response. These observations show that PGE_2 could be one of the inducers of PD-L1 expression.

CHAPTER III: The effect of dual blockade of the PD-1/PD-L1 pathway and PGE_2 in *M. bovis* infection *in vivo* was investigated using anti-bovine PD-L1 rat-bovine chimeric antibody (chAb) and a COX-2 inhibitor in seven calves naturally infected with *M. bovis*. *M. bovis*-specific IFN-γ response in cattle treated with anti-PD-L1 chAb and a COX-2 inhibitor was significantly increased. In addition, bacterial loads in nasal discharge and BALF were decreased in cattle treated with alone and combination of anti-PD-L1 chAb with a COX-2 inhibitor. These results suggest that the combination of anti-PD-L1 chAb with a COX-2 inhibitor is a candidate for therapeutic applications to prevent *M. bovis* infection.

In conclusion, this study showed that the immunosuppression during *M. bovis* infection is caused by T-cell exhaustion induced by the upregulation of PD-1/PD-L1. In addition, PGE_2 was one of the inducers of PD-L1 expression. Furthermore, the combination of anti-PD-L1 Ab with a COX-2 inhibitor reactivates *M. bovis*-specific immune reaction effectively both *in vitro* and *in vivo*. Taken together, the combined treatment of therapeutic Ab specific for PD-1/PD-L1 with a COX-2 inhibitor is a promising strategy for the control of *M. bovis* infection.