論文の内容の要旨

論文題目　Depletion of $CD4^+CD25^-LAG\text{-}3^+Egr2^+$ Regulatory T cells Using DNA Vaccination Results in Lupus-like Severe Systemic Autoimmunity
（DNA ワクチンによる LAG-3 陽性 Egr2 陽性制御性 T 細胞の除去と、ループス様自己免疫病態）

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Regulatory T cells (Treg) are crucial to maintain immune homeostasis and by maintaining homeostasis it helps to avoid immune-mediated pathology. $CD4^+CD25^+$ Treg cells which characteristically express transcriptional factor FoxP3 are important for immunological tolerance. Recently, $CD4^+CD25^-LAG3^+$ Tregs are reported as a new subset that plays a significant role in suppressing peripheral inflammatory reaction and their function is IL-10 dependent and FoxP3 independent. Lymphocyte activation gene-3 (LAG-3) is a type 1 membrane protein with four extracellular Ig-like domains and mainly expressed on activated T cells. $CD4^+CD25^-LAG\text{-}3^+$ Tregs have a strong correlation with transcriptional factor Egr2, which was reported as a negative regulator of T cell activation and necessary for clonal anergy induction. Transduction of Egr2 give $CD4^+$ naïve T cells LAG-3 expression and regulatory activity, therefore it is speculated that suppressive activity of $CD4^+CD25^-LAG\text{-}3^+$ Treg is regulated by Egr2.

In order to elucidate the immunological function of $CD4^+CD25^-LAG\text{-}3^+$ Treg in vivo, analyzing mice without $CD4^+CD25^-LAG\text{-}3^+$ Treg is important. LAG-3 knockout mice displayed apparently normal pathological condition and showed no defect on T cell function. Furthermore, administration of monoclonal antibody against mice LAG-3 did not induce depletion of $CD4^+LAG\text{-}3^+$ T cells.

In order to deplete $CD4^+CD25^-LAG\text{-}3^+$ Tregs in mouse efficiently, I adopted DNA vaccination procedure. DNA vaccination needs the plasmid in which eukaryotic and synthetic sequences administered, and it stimulates both humoral and cellular immune responses. After immunization with DNA vector, gene sequence enters into the cell and antigen is expressed by APCs which causes the activation of all arms of immune response; as a consequence antibody is produced.

I constructed pCAGGS-LAG-3 vector in which murine LAG-3 D1-D3 portion was inserted into the cloning region of pCAGGS vector. Female C57BL/6 mice were immunized with 100 μg pCAGGS-LAG-3 vector intravenously. Within eight months of vaccination, a part of mice immunized with pCAAGS-LAG-3 vector had developed lupus-like histological and functional abnormalities including alopecia, dermatitis and proteinuria while control mice did not. Moreover, pCAGGS-LAG-3 immunized mice with lupus-like lesion had high titer of anti-dsDNA antibody in their serum. On sacrifice, these mice had splenomegaly and histopathological analysis revealed dermatitis with hydrophic degeneration of basal

cells and glomerulonephritis with IgG/C3 deposition. Pathological analysis of lung presented lymphocyte infiltration around bronchiole and lung injury score shows remarkable tissue damage in these mice

FACS analysis of splenocytes revealed that $CD4^{+}LAG\text{-}3^{+}Egr2^{+}$ T cells were depleted in pCAGGS-LAG-3 immunized mice with severe lupus-like lesions. Control mice or pCAGGS-LAG-3 immunized mice without lupus-like lesions did not show the depletion of $CD4^{+}LAG\text{-}3^{+}Egr2^{+}$ T cells. Moreover, ELISA of anti-LAG-3 antibody revealed that $CD4^{+}LAG\text{-}3^{+}Egr2^{+}$ T cells depleted mice developed high titer of anti-LAG-3 antibody. Most probable working hypothesis is that DNA vaccinations with pCAGGS-LAG-3 vector induced the production of anti-LAG-3 antibody, and this antibody depleted $CD4^{+}LAG\text{-}3^{+}Egr2^{+}$ T cells and caused lupus-like systemic autoimmunity.

The intricate part of this experiment is that the anti-LAG-3 antibody production depleted only $CD4^{+}LAG\text{-}3^{+}Egr2^{+}$ T cells, but not $CD4^{+}LAG\text{-}3^{+}Egr2^{-}$ T cells. The reason why only $CD4^{+}LAG\text{-}3^{+}Egr2^{+}$ T cells were depleted needs further investigation. In my speculation one reason of this discrepancy is, the epitope expression of $CD4^{+}LAG\text{-}3^{+}Egr2^{+}$ T cells might be suitable for depletion with anti-LAG-3 antibody.

This procedure is the first report about the efficient depletion of $CD4^{+}CD25^{-}LAG3^{+}Egr\text{-}2^{+}$ T cells in mice model using DNA vaccination procedure. This depletion model will help to analyze how LAG3 Treg cells exert regulatory activity *in vivo* and will clarify what cells are important to express lupus-like phenotype in mouse model.