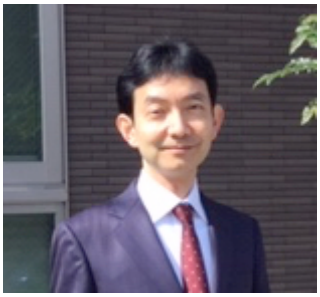
Principal Investigator: Ken-ichi Nishijima
Grant Title: Modification of sialic acids in chickens by gene manipulation

Abstract

1. Introduction.



Chickens are important livestock as the source of eggs or meats. The effects of sialic acids on the immune regulation are known in mammals: for example, eating some types of sialic acids affects inflammation. This indicates that understanding the regulatory mechanisms by sialic acids and/or the modification of chicken glycans may be useful to modulate inflammation. Chickens also used in the production of several pharmaceuticals including influenza vaccine, which can be improved by glycan optimization. This study aimed to provide efficient chicken genetic alteration method for chicken glycan modification and to analyze Siglecs, sialic acid-binding lectins that are expressed on immune cells.

2. Methods

Chicken primordial germ cells (PGCs) were cultured in Knockout DMEM supplemented with activin, BMP, FGF and B27. Long term cultured PGC line was edited by CRISPR/Cas9 and transplanted to bloodstream of 2.5-d recipient embryos. Hatched chickens were mated after sexual maturation and transgenic progeny was screened by genome PCR.

Primary mouse macrophages were differentiated from bone marrow cells in the presence of M-CSF for 7-9 days and used for the assay to evaluate the expression of Siglecs.

3. Results.

(1) Genetic manipulation of chickens using PGC line

Cultured PGCs were transfected by electroporation and sorted by reporter expression (GFP) under the control of endogenous promoter. When these cells were transplanted into recipient embryos, cells transmigrated to gonads and finally differentiated into sperms. Mating experiments revealed the high percentages of offspring were derived from transplanted PGC, indicating the usefulness of this cell line to generate various glycan-manipulated chickens.

(2) Analysis of cell surface lectin recognizing sialic acid

Siglecs, which had been shown to have anti-inflammatory activity, can be induced in mouse macrophages by stimulation. GM-CSF, IL-3 but not IL-5, all share common βc receptor for signal transduction, induced Siglec-F expression on mouse macrophages. To examine the function of Siglec-F in macrophages, siRNAs against Siglec-F were transfected and several cytokine responses were measured. qRT-PCR analysis revealed that the knockdown affected the IL-4-induced gene expression. Further study is needed to clarify the function of Siglec-F in relation to inflammation.