7. Function of Clara Cell Secretory Protein (CSSP) and Mice with Ablation of Clara Cells

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Clara cells are non-ciliated secretory epithelial cells in the pulmonary airway and their function includes secretion of CSSP. *In vitro* studies suggest that CSSP is able to regulate the inflammatory response, however, the function of CSSP *in vivo* has not been identified. We generated CSSP knockout mice to investigate the role of this protein in lung injury of mice. Inflammatory cytokine gene expression was studied in CSSP −/− mice and wild type (CSSP +/+) controls exposed to silica.

Wild type and knockout mice were exposed to 50 μg crystalline silica or saline intratracheally and then were sacrificed at 12, 24 and 48 hrs and RNA isolated from lung tissue. RNA expression of TNFα, IL1, IL6, TGFβ1 and IFNγ was analyzed using an RNAse protection assay.

Compared to the control (no silica exposure) and saline goups, lungs from animals exposed to silica had increased TNFα mRNA levels in both CSSP −/− and CSSP +/+ mice. The maximum response in the CSSP −/− was seen at 24 hrs and at this time point TNFα mRNA was increased significantly in CSSP −/− mice compared to CSSP +/+ controls. Gene expression of the other cytokines assayed was not significantly different between CSSP −/− and CSSP +/+ mice.

To induce ablation of the Clara cells a transgene was constructed containing the herpes simplex thymidine kinase (TK) gene under the control of the CSSP promoter, thus resulting in Clara cell-specific expression of TK. Transgenic animals expressing the TK transgene were treated with gancyclovir leading to negative selection of TK-expressing cells in the animal, that is the Clara cells, leading to generation of inducible destruction of Clara cells in the mouse. Two founder females were identified that had germ line expression of the transgene.

These animals were then crossed with wild type mice and F_2 animals were assessed for expression of the transgene by RT-PCR. Transgene expression was observed exclusively in the lung and not in the liver. Further studies are being carried out to characterize these animals and assess CSSP function *in vivo*.

Discussion

Dr Ichinose: Is CSSP the same as urine protein 1 or CC-10?

Dr Morimoto: Yes.

Dr Ichinose: Several years ago we looked at urine protein 1/CC-10 in the BALF. In patients with sarcoidosis or in smokers the levels of the protein were increased. At that time we thought that it may be a regulatory protein in the peripheral lung fluid.

Dr: The studies that Dr Morimoto did suggested that urine protein 1 had some kind of endogenous anti-cytokine activity or anti-inflammatory activity and therefore was reflective of a 'curing stage' in sarcoidosis.

Dr Ichinose: At the time we thought that there was some inhibition of the inflammation protein.

Dr Rennard: When the transgenic mice that you have generated are exposed to gancyclovir the Clara cells will presumably die and so things other than CC-10 will be lost. How will you interpret the specific role of CC-10 in that experimental system?

Dr Morimoto: That is a difficult question.