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ABSTRACTS

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The TT2016 meeting is hosted by: the Czech Centre for Phenogenomics (CCP), BIOCEV, Prumyslova 595, 25242, Vestec, Czech Republic

Czech Centre for Phenogenomics

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IMG

was observed from 9 to 10 weeks after birth in CD80CD86$^{-/-}$ mice. In histological examination for 8.5 month old CD80CD86$^{-/-}$, destruction of hair follicle was observed. In flowcytometry analysis, CD80CD86$^{-/-}$ mice showed significant low CD4 + Foxp3 + Treg cell population than wild type. Furthermore, CD80CD86 deficient mice also showed high serum concentrations of T immune related cytokines such as IL2, IL4, IL10, IL12p70, IFNg. This result indicates that CD80CD86 deficiency caused impaired Treg homeostasis, and induce Th1 and Th2 over-activation. To confirm the relation between Treg and hairloss, additional CD25 monoclonal antibody treatment was conducted to C57BL/6. After CD25 depletion, 8-months old C57BL/6 mice showed a similar hairloss with C57BL/6.CD80CD86, and also showed hair follicle destruction. But female mouse with CD25 depleted didn't develop hairloss.

There were few animal models for human autoimmune alopecia. Especially in rodent, C3H mice is usually used as an autoimmune alopecia model. In this study, we have newly found that CD80CD86 deficient mice showed a similar phenotype with human autoimmune alopecia. Furthermore, CD80CD86 deficient mice showed early onset of alopecia (7–9 weeks old), and the incidence was relatively higher than C3H model.

Keywords: alopecia areata, mouse model, regulatory T cell, Autoimmunity

Comparation of three different cell transfection reagents in ram spermatozoa transfection to obtain transgenic sheep embryos

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Cell transfection reagents have been used in transgenic embryo production studies. Lipofectamine is one of used for that purpose in many species as they are known to be small molecules that made of cationic lipids which makes complex structures with negatively charged nucleic acids to carry into cell nuclei. TurboFect^{TM} is a cationic polimer dissolved in water is recently being used in sperm mediated gene transfer (SMGT) in mammalian transgenesis. Lastly poli-β-aminoesters are nanopolymers are being used as alternative to poliethilenimines.

In our study we used Lipofectamin^{TM}2000, TurboFect^{TM} and poli-β-aminoester to transfect ram spermatozoa with plasmid DNA including eGFP. Intracytoplasmic sperm injection (ICSI) is used to fertilize oocytes from slaughterhouse. After trials to determine the optimum amount to use in SMGT, we tried 3, 4, 10 and 20 μg pDNA was mixed with appropriate amounts of Turbofect #R0531 Fermentas, 2, 2, 6 and 10 μl in respect and incubated at room temperature for 20 min. 1×10^6 spermatozoa was added and incubated at room temperature for 1 h. Lipofectamin^{TM} 2000 Reagent groups we similar to those in Turbofect, we used 3, 4, 8 and 10 μl Lipofectamin in order to carry 3, 4, 8 and 10 μg of p DNA, and incubated as above. Poli_β-aminoesters were synthesized at Department of Chemical Engineering, after centrufugation both supernatant and pellet groups were used, the pDNA was attached to them and incubated with spermatozoa at a concentration of 200 ng pDNA/2×10^5. In all gropus samples were mixed with 10 % PVP and used in ICSI. Except activation (only ionomycin activation was used in this study) and fertilization SOF media were used after fertilization, all embryonic procedures were made as in Birler et all 2010.

Results in lipofectamin group was 10 μg pDNA carrying group gave us the highest transgenic embryo rates 37.73 % ($p > 0.05$), but fragmentation rates 9.43 % were seen in this group statistically unimportantly ($p > 0.05$). As similar to those in Turbofect groups 10 μg pDNA carrying group gave us the highest transgenic embryo rates 42.30 % ($p > 0.05$) and had the highest degeneration rate of 48.64 % ($p > 0.05$). In poli_β-aminoesters groups pellet group had the highest transgenic embryo rate 26.31 % and degeneration and fragmentation rates as 48.71 and 21.05 % in sequence.

Keywords: SMGT, ram spermatozoa, transfection

The Czech Centre for Phenogenomics: International research infrastructure for mouse model production, archiving and phenotyping

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The Czech Centre for Phenogenomics (CCP, http://www.phenogenomics.cz/) is a newly built infrastructure consisting of state-of-the-art configurations necessary for transgenic model production, their archiving and phenotyping. As member of INFRAFRONTIER and the IMPC (International Mouse Phenotyping Consortium) the CCP is embedded in an associated global network that aims to analyze effects of knockout gene mutations in mice systematically and in detail.

Our service at the transgenic module at CCP comprises nuclease-induced genetic mutagenesis and transgenesis, ES cell manipulation and injection, cryopreservation of embryos and spermatozoa, rederivation of pathogen-infected mouse lines as well as breeding and genotyping on demand. The phenotyping module houses a comprehensive collection of tools for the physiological and morphological assessment of mice and rats. CCP offers a wide variety of standardized tests and services, including those of IMPReSS (International Mouse Phenotyping Resource of Standardised Screens), mandated by our active partnership in the IMPC. Notable is our capacity for conducting comprehensive phenotyping pipelines, providing a wide breadth of clinical information per experimental animal, and thereby minimizing overall animal usage.

The newly raised and recently obtained €24 million building with 7200 m^2 floor space and maximum capacity of 13000 cages for mice (over 30 000 when combining with capacity at established campus) and 4000 cages for rats is planned to run in full production and phenotyping state during 2016. The CCP is