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Oral Presentations

O-6

Production of recombinant human leukemia inhibitory factor protein and its immunologic and anti-fertility impacts as a contraceptive candidate vaccine in mice model

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Background: Contraceptive vaccines are one of the methods studied to prevent fertility in mammals. Various factors are involved in the establishment and maintenance of the pregnancy and can be targeted for antifertility vaccine design. The human leukemia inhibitory factor (hLIF) is considered as a cytokine of the interleukin-6 family. LIF is also involved in the embryo implantation process.

Objective: The production and functional competence of the LIF, as the immunocontraceptive vaccine in Balb/c mice, was investigated in this experimental study.

Materials and Methods: Recombinant hLIF (rhLIF) was generated in a variety of host-vectors system. The protein expression rate and functional activity of rhLIF were assessed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and tetrazolium reduction assay, respectively. The production and characterization of Rabbit polyclonal antibody (pAb) to rhLIF was performed applying enzyme-linked immunosorbent assay and western blot techniques. The Balb/c mice were classified into two study groups. In group 1, each mouse was intraperitoneally inoculated by purified rabbit anti-rhLIF in 3th day and day 4 following vaginal plaque observation; after sacrificing on day 7, the number of implantation sites was quantified. Mice in second group were subdivided

into two vaccinated and controls groups. The rhLIF protein as well as phosphate buffer saline was emulsified with Freund's adjuvant and injected into both vaccinated and control groups, respectively. The inhibitory rate of implantation was investigated in the uterus of mice. The secreted levels of interferon- γ and interleukin-4 were determined in cultured splenocyte of mice induced by rhLIF. Also, the mRNA levels of *immune responsive gene 1 (IRG-1)*, *cochlin (COCH)*, *amphiregulin (Ar)*, and heparin-binding EGF-like growth factor (*HB-EGF*) genes were evaluated. The inhibition of fertility after delivery, reversibility of immune response against rhLIF, and survival rate of mice were assessed.

Results: Our data showed that pET32b/hLIF and pColdI/hLIF vectors could successfully express rhLIF in all hosts. The produced rhLIF was functionally active and the produced anti-rhLIF pAb could specifically bind to commercial rhLIF and native LIF extracted from mouse uterus. Passive immunization outcomes indicated that anti-rhLIF antibody entirely inhibited the fertility potential in all vaccinated mice compared to controls. Active immunization of Balb/c mice with rhLIF led to the implantation and fertility reduction rate up to 80.49% and 75%, respectively. All mice produced a high amount of anti-rhLIF antibodies in both serums and vaginal fluids wash after 16 weeks; while, these antibodies were disappeared from vaginal fluid washes six months later. The findings of splenocyte stimulation with hLIF demonstrated a significant increased level of both cytokines in vaccinated mice compared to the controls. A significant decreased gene expression of *IRG-1*, *Ar*, and *HB-EGF* was observed in vaccinated group compared to control group; however, the mRNA level of *COCH* gene showed no significant change.

Conclusion: rhLIF could inhibit pregnancy in a high rate of female mice. The immunization of female Balb/c mice with rhLIF prevented fertility and the gene expression associated with rhLIF. To investigate the side effects of this vaccine in a wide range, further studies are needed.

Key words: Leukemia inhibitory factor, Contraception, Vaccine, Active immunization, Mice.

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