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Roles of Toll-like Receptors on Islets in Pig to Mouse Islet Transplantation

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Purpose : Innate immunity contributes to xenograft rejection. However, there have been few studies for the role of Toll-like receptor (TLR), an important receptor of innate immunity, in xenotransplantation. Moreover, most studies in allotranplantation focused on the recipient' s TLRs. Therefore, we investigated whether TLRs on porcine islets can contribute to islet xenograft rejection.

Materials and methods : Isolation and purification of porcine islets were performed by the modified-Ricordi method. Polyinosinic:polycytidylic acid (Poly I:C) or lipopolysacchride (LPS) was used to stimulate TLR3 or TLR4 on porcine islets, respectively. Induction of cytokines and chemokines in islets in response to TLR stimulation was assessed using reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). Transmigration assays were performed in order to measure the chemotactic activity of islet culture supernatant. Procoagulant induction was also assessed using RT-PCR, tissue factor assay and thrombin assay. Lentiviral siRNA were constructed in order to knockdown expression of porcine MyD88 in islets. Porcine islets were transplanted to renal subcapsular space of MyD88 knockout, TLR4 knockout and wild type C57BL6/J mice. LPS was injected together with anti-CD154 antibodies (MR-1) after pig to mouse islet transplantation.

Results : Porcine islets expressed the TLRs at resting status. In vitro TLR stimulation induced the expression of both chemokines (MCP-1, RANTES, IP-10, IL-8) and cytokines (IL-6, type I interferon). Islet culture supernatant after stimulation by TLR agonists induced transmigration of human leukocyte across the species barrier. In vitro TLR stimulation also induced expression of VCAM-1 and ICAM-1 on porcine islets. However, TLR stimulation did not influence insulin secretion. Myd88 knockdown decreased TLR-mediated induction of proinflammatory cytokines/chemokines and procoagulants. LPS stimulation interfered with tolerance induction by MR-1 in pig to TLR4 knockout or MyD88 knockout mouse islet transplantation.

Conclusion : TLR activation on porcine islets induced both proinflammatory response and procoagulant response, and thereby contributed to xenograft rejection. Considering important roles of TLRs on porcine islets, production of genetically modified pigs for TLR pathways could be a promising option for the reduction of islet xenograft rejection.

Key Words : 토르양 수용체, 췌도, 이종이식
Toll-like receptor, Islet, Xenotransplantation