Macrophages as an Effector Mechanism to Reject Encapsulated Hepatoma Cells

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THE IMMUNOSUPPRESSIVE effect of cytotoxic T-lymphocyte antigen 4-Ig (CTLA4-Ig) is mainly to block the CD28 (CTLA4)/B7 costimulatory pathway between T cells and antigen presenting cells (APC).¹ When T-cell activation is deficient in CD28 (CTLA4)/B7 costimulation, it will produce a circumstance of T-cell anergy.² Several researchers reveal that CTLA4-Ig will result in indefinite survival of islet,³ cardiac,⁴ liver,⁵ and skin xenografts.⁶ However, it is too capricious to establish the conclusion that only blocking the CD28 (CTLA4)/B7 costimulation will enhance transplantation-specific tolerance and eliminate the rejection.^{5,7}

Furthermore, interleukin-1 (IL-1) receptor antagonist (IL1-Ra), an antiinflammatory protein or acute-phase protein, can prevent IL-1 α and IL-1 β from binding to type I and type II IL-1 receptors by competitive inhibition, and it can ease the inflammatory symptom caused by infection or tissue damage.⁸ The structure of IL1-Ra remained unknown until 1997.⁹

In recent years, researchers have used alginate-poly(L-lysine)-alginate (A-P-A) microcapsules to protect the grafted islets from being attacked by the immune system.¹⁰ Surface modification of A-P-A microcapsules will further result in less cell attachment.¹¹ It is the purpose of this preliminary study to evaluate the effects of viral infection and microencapsulation on the biocompatibility and function of hepatoma cells.

MATERIALS AND METHODS

Materials

HepG2/C3A (CCRC 60177) hepatoma cell was purchased from Culture Collection & Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan. Adenoviruses carrying genes encoding human CTLA4-Ig (AdHCMVhCTLA4Ig), mouse IL1-Ra (AdHCMVmIL1ra), or E₂Ftk (AdHCMVE₂Ftk) were provided by Chang Gung Memorial Hospital and used as vectors to transfer the genes into HepG2/C3A. Male 4- to 6-week-old BALB/c mice were purchased from animal culture center, National Taiwan University Hospital, Taipei, Taiwan.

Methods

HepG2/C3A cells were cultured in MEM (Eagle) medium containing 0.1 mmol/L nonessential amino acids, 1 mmol/L sodium pyruvate, and 10% fetal bovine serum under 37°C, 5% CO₂ environment. HepG2/C3A was infected with virus for 24 to 48

hours and encapsulated in A-P-A microcapsules with a field effect microparticle generator. The multiplicity of infection (MOI) ratio of virus to hepatoma cell was set to 100. The cells were transplanted into the intraperitoneal cavity of BALB/c mice for 2 to 4 weeks, after which the cells were retrieved for in vitro evaluation. Cells with no infection but with microencapsulation were used as controls. Retrieved cells were examined under an anatomy microscope to calculate the degree of fibrosis. A score of 0 to 4 was assigned to each individual microcapsule according to the proportional area of neogrowth, 0 for no cell overgrowth; 1 for less than 25% area; 2 for 25% to 50% area; 3 for 50% to 75% area; 4 for greater than 75% area with overgrowth. The score was averaged over all retrieved cells. To assess the liver-specific function of transplanted hepatoma cells, cells retrieved at 2 and 4 weeks were cultured in vitro up to 2 weeks, and the level of albumin and alpha-fetoprotein (α FP) in the culture media were determined. Analysis of albumin and α FP were carried out in the clinical laboratories of Chang Gung Memorial Hospital. Data were expressed as mean $\pm$ standard error. The differences of means were analyzed using Student's *t* test. *P* < .05 was considered significant. *P* > .05 was considered not significant (NS).

RESULTS AND DISCUSSION

Pericapsular overgrowth of cells was observed in all cases, and histology examinations indicate macrophages being the main cellular component. In contrary to what had been expected, the degree of fibrosis on A-P-A surface cannot be reduced by genetically engineering the cells to carry hCTLA4-Ig or mIL1-Ra gene. The mean overgrowth score of grafts retrieved at 2 and 4 weeks was 2.32, 2.82, 3.96, 3.90 and 3.11, 3.61, 3.96, 3.98, respectively, for four different preparations (control, E₂Ftk, hCTLA4Ig, mIL1-Ra). Overgrowth of cells on the capsule surfaces with these engineered cells became even worse than with the control. It is

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possible that the high MOI led to lysis of HepG2/C3A or CD28 (CTLA4)/B7, and CD40/CD40L must be blocked at the same time before the immune response could be completely shut off. These hypotheses will be tested in future studies.

The mean concentration of α FP (ng/mL) secreted by HepG2/C3A in vitro after being transplanted into mice for 2 and 4 weeks was 1223, 1052, 18, 15 and 463, 285, 6, 15, respectively, for four different preparations (control, E₂Ftk, hCTLA4Ig, mIL1-Ra). There were no detectable α FP in the culture media of all groups using bare cells. The four different preparations of encapsulated cells secret different levels of α FP, a lower α FP secretion capability being correlated with a higher score of cell overgrowth. A longer transplantation period in vivo also led to a higher score of cell overgrowth and a lower α FP concentration in the culture medium. Microencapsulation therefore is important for hepatoma cells to show liver-specific function in vivo.

CONCLUSION

The immunomodulating effect of A-P-A membrane protects encapsulated liver cells. The graft survival of A-P-A

encapsulated cells is the outcome of the degree of capsular neogrowth. The cellular component of pericapsular overgrowth involves mainly macrophages.

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