Transforming growth factor-β1 derived from biliary epithelial cells may attenuate alloantigen-specific immune responses

Abstract Immune response to liver allografts may be different from that to other organ transplants since immunological manipulation easily attenuates immune-response to liver allografts. Numerous studies on the alloantigen-specific immune response have been carried out, however, the precise mechanisms involved in this attenuation are not clear yet. Here we suggest the attenuation of alloantigen-specific immune response by TGF-β1 derived from biliary epithelial cells. The transforming growth factor-β1 (TGF-β1) expression in rat liver was examined immunohistologically. Rat biliary epithelial cells (BEC) were purified from the perfused liver and added to allogeneic mixed lymphocyte reaction (allo-MLR) to assess their attenuating potential on allo-MLR and alloantigen-specific cytotoxic T lymphocyte (allo-CTL) induction. Immunohistological investigation revealed the expression of TGF-β1 in biliary epithelial cells. Both purified biliary epithelial cells and TGF-β1 attenuated allo-MLR and allo-CTL induction in a dose-dependent manner, and anti-TGF-β1 antibody partially relieved this attenuation. This study reveals that biliary epithelial cells, the major target cells of allo-antigen specific immune response, contain TGF-β1 and that they have a capacity to attenuate allo-MLR and allo-CTL induction.

Key words Biliary epithelial cells · TGF-β1 rat · Mixed lymphocyte reaction · CTL

Introduction

Transplantation in the adult is often complicated by rejection episodes, whereas newborn children injected with donor cells frequently become immunologically tolerant of donors’ organs. The immune system of adult recipients reacts to transplantation antigens on the allograft, and this process includes cellular and humoral components: T-cells recognize and react to alloantigens, and B-cells produce antibodies [6, 25]. Indeed, the regulation of alloantigen-specific cytotoxic T-lymphocyte (allo-CTL) generation is under the control of a net of regulatory signals and cytokines that modulate the clonal expansion and differentiation [4, 6, 20, 26]. Therefore, the adult immune system needs, in contrast to that of the newborn child, intensive immunosuppressive measures, such as administering FK-506, antilymphocyte globulins, monoclonal antibodies for T cells, and total body irradiation, to accept allografts and maintain tolerance. However, the immune response of the host to liver allografts may be less intensive than that to other organ transplants. Therefore, immunological manipulations such as donor-specific blood transfu-
sion (DST) modulate the immunological response to liver allografts with the result of higher donor-specific tolerance and better acceptance of other organ transplants in a donor-specific manner [7, 21]. This phenomenon attracted much attention by immunologists, and immunomodulation of alloantigen-specific immune response have been investigated extensively. However, the precise mechanisms involved in the attenuated alloantigen-specific immune response are not clear yet.

Here, we suggest that TGF-\(\beta\) derived from biliary epithelial cells (BEC), the major target cells of allo-antigen specific immune response, have a capacity to attenuate the alloantigen-specific immune response.

Materials and methods

Chemicals, proteases, cytokines, antibody, and medium

Human TGF-\(\beta\), recombinant human IL-2, pronase E, collagenaase, and trypsin-ethylenediaminetetraacetate (EDTA) solution were purchased from Calbiochem (CA), Takeda Pharmaceutical (Japan), and Merck (Germany). Wako Pure Chemical (Japan), and Gibco (NY), respectively. Rabbit anti-TGF-\(\beta\) antibody, streptavidin-biotin peroxidase kit (Histofine SAB-PO kit), and L15 medium were purchased from Promega (WI), Nichirei (Japan), and Dainippon Pharmaceutical (Japan). Chemicals were purchased from Biochem, Sigma-Aldrich Japan, Wako Pure Chemical (Japan), and Nakarai Tesque (Japan).

Animals

Male inbred Lewis rats (RT1\(^{l}\))(LEW) and male inbred Brown-Norway (RT1\(^{n}\)) (BN) were purchased from Charles River Japan. Male inbred ACI rats (RT1\(^{a}\)) were purchased from Japan SLC. All rats were provided with standard laboratory chow and water ad libitum and housed in compliance with the Guide for Animal Experimentation of Ehime University School of Medicine.

Immunohistological detection of TGF-\(\beta\)1 on BEC

Normal livers and liver allografts of ACI rats 2 months after orthotopic liver transplantation (OLT) in DST-treated LEW rats were excised and fixed with periodate-lysine-paraformaldehyde. Rabbit anti-TGF-\(\beta\)1 antibody and streptavidin-biotin peroxidase kit were used for immunohistological examination.

Donor specific blood transfusion (DST) and liver transplantation

LEW rats were transfused with 1 ml heparinized ACI donor blood through the penile vein. Seven days later, donor surgery of ACI rats was performed under ether anesthesia according to the technique of Kamada and Calne [11]. Livers were skeletonized and flushed with 4 ml of cold histidine-tryptophane-ketoglutaratate (HTK) solution through the portal vein. Livers were excised and fixed with periodate-lysine-paraformaldehyde. Rabbit anti-TGF-\(\beta\)1 antibody and streptavidin-biotin peroxidase kit were used for immunohistochemical examination.

Preparation of stimulator and responder cells for allogeneic mixed lymphocyte reaction (allo-MLR)

Stimulator spleen cells were \(\gamma\)-irradiated (2,000 rads from \(^{137}\)Cs source) and resuspended at a density of 4 \(\times\) 10^6 cells/ml in MLR medium. For the preparation of responder cells, spleen cells were suspended at a density of 10^6 cells/ml in RPMI 1640 medium supplemented with 20% rat serum and applied to a nylon wool column. The column was incubated at 37°C for 1 h, and non-adherent spleen cells were collected to repeat this process in another nylon wool column. Twice treated non-adherent spleen cells were resuspended at a density of 4 \(\times\) 10^6 cells/ml in MLR medium as responder cells.

Optimization of allo-MLR

Stimulator cells (2 \(\times\) 10^5) and responder cells (2 \(\times\) 10^5) were incubated in 0.2 ml MLR medium on a 96-well tissue culture plate (Iwaki Glass, Japan) at 37°C in 5% CO2 and 95% air for 24–120 h. Eighteen hours before harvest, 0.5 µCi of 3H-thymidine (\(^{3}\)H-TdR) (Amersham) was added to each well. The results were calculated for uptake of \(^{3}\)H-TdR and expressed as mean ± SD in pentaplicate.