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Human iris pigment epithelial cells suppress T-cell activation via direct cell contact☆

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ABSTRACT

The purposes of the present study were to investigate whether cultured human iris pigment epithelial (hIPE) cells acquire the ability to modify T-cell activation, and if so, to identify the mechanism. Human IPE cells were prepared from patients who underwent glaucoma surgery, and were cultured in RPMI 1640 medium containing 10% fetal calf serum for 4–7 days. Expression of MHC molecules and co-stimulatory molecules on cultured hIPE cells either unstimulated or stimulated with IFN-γ was examined by FACS. In addition, peripheral blood T cells were incubated with cultured hIPE cells prepared from the same patients and anti-CD3 antibody in a transwell culture system, or in the presence of anti-PD-L1 and PD-L2 antibodies, and T cell proliferation was assessed by [^{3}H]-thymidine incorporation. The hIPE cells inhibited anti-CD3-driven T-cell activation but the inhibition was diminished when tested in the transwell culture system, indicating that a contact-dependent mechanism is important in the immunoregulatory roles of hIPE. Although cultured hIPE cells expressed Class I and PD-L1 but not Class II or PD-L2, all these molecules were observed on hIPE cells cultured in the presence of IFN-γ. Blocking antibodies against both PD-L1 and PD-L2 reduced the immunoregulatory activity of hIPE cells. Our data indicates that cultured hIPE cells inhibit T-cell activation by T-cell receptor ligation, which is mediated by cell-to-cell contact in part via the PD-L1 and PD-L2 pathways.

1. Introduction

Ocular tissue is known as a site of immune privilege, which provides the eye with immune protection against pathogens by minimizing the risk of inflammation-induced vision loss (Streilein, 2003). Every T cell that enters the intraocular compartments (anterior chamber, vitreous cavity, subretinal space) carries with it the threat of immunogenic inflammation. If the infiltrating T cells encounter an antigen that binds their receptor, the cells may become activated, leading to release of proinflammatory cytokines with the potential to deteriorate visual acuity. To protect vision, a special anatomic, cellular and systemic environment exists in the eye (Streilein, 2003). The blood–ocular barrier and absence of lymphatic drainage pathways in the eye are anatomical specializations for shielding against antigens. However these are not enough for protecting inflammation in ocular tissues. Aqueous humor contains an abundance of immunosuppressive factors including transforming growth factor (Wilbanks et al., 1992), alpha-melanocyte-stimulating hormone (Namba et al., 2002; Taylor et al., 1992), vasoactive intestinal peptide (Taylor et al., 1994), calcitonin gene-related peptide (Taylor et al., 1998) and thrombospondin (Niederkorn and Streilein, 1983). These various immunosuppressive and anti-inflammatory factors suppress the effector functions of adaptive and innate molecules and cells that threaten vision by triggering intense inflammation, angiogenesis, and bystander cell injury and death. In addition to such immunosuppressive molecules, anterior chamber-associated immune deviation (ACAID) which is a type of immune deviation also cooperates in the suppression of ocular inflammation (Streilein, 2003; Streilein et al., 1980) and contributes to protecting ocular inflammation. ACAID induces a systemic immune response that includes the generation of suppressor T cells (Kaplan and Streilein, 1977, 1978; Streilein, 2003; Streilein et al., 1980).

Recent studies revealed that murine pigment epithelial cells from the iris, ciliary body and retina display immunomodulatory features, suggesting that this layer of cells contributes to ocular immune privilege (Carron et al., 2000; Griffith et al., 1995; Knisely et al., 1991; Liversidge et al., 1993; Miyajima-Uchida et al., 2000; Streilein and Bradley, 1991; Sugita and Streilein, 2003; Tanihara et al., 1993). They express cell surface molecules such as CD95

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ligand (Griffith et al., 1995) and CD86 (Sugita and Streilein, 2003) and secrete soluble factors such as TGF (Knisely et al., 1991; Streilein and Bradley, 1991; Tanihara et al., 1993), thrombospondin (Carron et al., 2000; Miyajima-Uchida et al., 2000) and prostaglandin E2 (Liversidge et al., 1993). Using these molecules, mouse PE cells inhibit T-cell activation. Among the mouse pigment epithelial cells, iris pigment epithelial (IPE) cells inhibit T-cell activation in a contact-dependent manner (Sugita and Streilein, 2003; Yoshida et al., 2000a,b). Mouse IPE cells express CD86, which interacts with cultured T cells that express cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). When murine T cells are cultured with a stimulator of T cell receptor ligands in the presence of IPE cells, not only T cell proliferation is profoundly suppressed but regulatory T cells are also generated by the interaction of CD86 with CTLA-4 (Sugita et al., 2006, 2004; Sugita and Streilein, 2003). Thus mouse IPE cells have the ability to protect visual function by reducing intraocular inflammation. However, the immunoregulatory roles of human IPE cells (hIPE cells) have not yet been reported.

Programmed death-1 (PD-1) is a co-stimulatory molecule that interacts with its ligands, PD-L1 and PD-L2. PD-L1 is expressed not only on lymphoid tissues but also on non-lymphoid tissues such as endothelial cells in the heart (Ishida et al., 2002), glial cells in the inflamed brain (Salama et al., 2003) and b cells in the pancreas (Ansari et al., 2003). Studies using blocking antibodies or knockout mice proved that PD-L1 is a negative regulator of T-cell activation (Ansari et al., 2003; Nishimura et al., 1996, 1998). Recent studies have demonstrated that interaction of PD-1 and PD-L1 is related to immune privilege in the placenta (Guleria et al., 2005; Petroff et al., 2003) and cornea (Hori et al., 2006; Shen et al., 2007; Watson et al., 2006).

In this study, we investigated whether hIPE cells inhibit T-cell activation via T-cell receptor (Tcr), and if so, whether immunosuppressive signals via PD-L1 or PD-L2 are involved in the immunoregulatory role.

2. Materials and methods

2.1. Culture media

RPMI 1640 complete medium was used for primary cultures of hIPE cells and mixed lymphocyte reactions (MLRs). It was composed of RPMI 1640, 10% FCS, 10 mM HEPES, 2 mM glutamine, penicillin, and streptomycin.

2.2. Preparation of hIPE cells

The study protocol was approved by the Tokyo Medical University Ethics Committee and informed consent was obtained from all patients. The tenets of the Declaration of Helsinki concerning human experimentation were followed.

We prepared iris samples from 47 patients. All patients had either normal tension glaucoma or primary open angle glaucoma. Patients with secondary glaucoma were excluded from this study. All patients used at least one topical antiglaucoma drug, but none used topical or systemic steroid.

IPE cells were prepared when blood sample could be obtained from the patient. Iris samples were collected during trabeculectomy, after informed consent was obtained. The samples were incubated in PBS containing 1 mg/ml dispase and 0.05 mg/ml DNase1 at 37 °C for 1 h. The tissues were triturated several times with 18-guage, 21-guage and 23-guage needles to obtain single-cell suspensions. IPE cells were washed twice with RPMI 1640 medium and cultured in 5% CO_2/95% air at 37 °C. Single-cell suspension of IPE cells was seeded into 3.5 mm dish, and culture medium was changed twice a week. Adherent cells were considered as viable cells and nonadherent cells as dead cells. After culture for 1 week, IPE cells were 10–20% confluent. IPE cell cultures with cell numbers less than 2.5×10^3 were excluded from the study. Using our methods, 95% of cultured cells were cytokeratin-positive when analyzed by flow cytometry and these cultured cells were regarded as IPE cells. Our culture method yielded good adherent IPE cell cultures in 39 patients but failed to obtain adherent cells in around 8 patients. The former were used in subsequent experiments while the latter were not used.

For the flow cytometry study, two eye balls obtained from American eye bank were used in one experiment and whole irises were used. Single cell suspension of IPE cells was seeded into 10-mm dishes and culture medium was changed twice a week. Dishes are almost confluent after 2 weeks culture. We used these cells in flow cytometry analysis. In our culture methods, we performed one or two passages. When cells were cultured for 4 weeks (4 passages were performed), pigments disappeared and cell shape was altered to resemble fibroblast. Two-week cultures (2 passages) were considered suitable for our experiments.

2.3. Preparation of T cells

Twenty ml of peripheral blood was collected from glaucoma patients, and mononuclear cells (PBMCs) were isolated from these samples using HISTOPAQUE-1077 and 1119 (Sigma, St. Louis, USA). T cells were prepared from these PBMCs using a Pan T cells Isolation Kit and autoMACS (both from Miltenyi Biotech, CA, USA).

2.4. Proliferation analysis (MLR)

The hIPE cells were seeded in flat-bottomed 96-well plates and cultured for 4–7 days. T cells (2.5×10^5/well) were cultured with or without hIPE cells in the presence of anti-CD3 antibody (0.5 μg/ml). The T cell to hIPE cell ratio was 50:1 or 100:1. After culturing for 3 days, the cells were pulsed with 0.5 μCi [^{3}H]-thymidine for the last 8 h. Radioactivity was assessed by liquid scintillation spectrometry, and the data expressed as counts per minute (cpm). MLRs were also studied with a transwell culture system (Corning, MA, USA), using anti-PD-L1 (0.5 μg/ml) and anti-PD-L2 (0.5 μg/ml) antibodies (eBioscience, San Diego, CA, USA). Rat IgG2a (eBioscience) at 0.5 μg/ml was used as isotype control.

2.5. Flow cytometry

The hIPE cells were stained with fluorescein-isothiocyanate-labeled anti-pan keratin antibody (Clone PCK-26, SIGMA). Cells were fixed and permeabilized and washed twice. Thereafter, the cells were blocked with PBS containing 2% FBS for 20 min on ice, incubated with anti-pan keratin antibody for 30 min on ice, and washed twice, after which they were analyzed by flow cytometry. Anti-HLA-A, B, C-FITC; anti-DR, DP, DQ-FITC; anti-CD80-FITC, anti-CD86-PE; anti-PD-L1 (B7H1)-FITC; and anti-PD-L2 (B7H2)-FITC (eBioscience) antibodies were used for immunofluorescent staining. FITC and PE-conjugated rat IgG2a (eBioscience) was used as an isotype control. A single-cell suspension of hIPE cells was prepared from irises of human eyes obtained from America eye bank. hIPE cells were cultured with RPMI 1640 medium containing 10% FCS. Two weeks later, these cells were incubated with medium alone or in the presence of IFN-γ (1000 U/ml). Cells were first incubated with Fc Block (BD Pharmingen, San Jose, CA) to block non-specific binding before the staining with antibody. Stained cells were analyzed using FACScalibur (Nippon Becton Dickinson Company, Tokyo, Japan) and CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA).