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Transforming growth factor β-producing Foxp3$^+$CD8$^+$CD25$^+$ T cells induced by iris pigment epithelial cells display regulatory phenotype and acquire regulatory functions

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Abstract

The ocular pigment epithelial (PE) cells convert T cells into T regulators (Tregs) in vitro. The PE-induced Tregs fully suppress activation of bystander responder T cells. Iris PE (IPE) cells from anterior segment in the eye produce costimulatory molecules and transforming growth factor β (TGFβ) that is delivered to CD8$^+$ Tregs. We have now examined whether T cells exposed to cultured IPE express CD25 and Foxp3, and to determine if the CD25$^+$ IPE-exposed T cells display regulatory functions in vitro. We have found that cultured B7-2$^+$ IPE converted CTLA-4$^+$ T cells into CD25$^+$ Tregs that suppress the activation of bystander T cells. The CD8$^+$ IPE-induced Tregs constitutively expressed CD25. Through TGFβ–TGFβ receptor interactions, the IPE converted these T cells into CD25$^+$ Tregs that express Foxp3 transcripts. The CD8$^+$ IPE-induced Tregs produced immunoregulatory cytokines, e.g., interleukin-10 and TGFβ. In addition, IPE-exposed T cells that downregulated Foxp3 mRNA failed to acquire the regulatory function. In conclusion, ocular pigment epithelial cells convert CD8$^+$ T cells into CD25$^+$ Tregs by inducing the transcription factor Foxp3. Thus, T cells that encounter ocular parenchymal cells participate in the T-cell suppression.

Keywords: iris pigment epithelium; T regulatory cells; suppression; transforming growth factor β; immune privilege

1. Introduction

Ocular immune privilege protects the delicate internal structures of the eye from the blinding consequences of innate and adaptive immune inflammation (Niederkorn, 2002; Streilein, 2003). One of the factors responsible for creating ocular immune privilege is the pigment-containing epithelium of the iris, the ciliary body, and the neural retina. The ocular pigment epithelial (PE) cells contribute to the integrity of the blood-ocular barrier, and thereby secure immune privilege within the eye. T cells that encounter PE cells *in vitro* are inhibited from undergoing TCR-triggered activation (Sugita and Streilein, 2003; Sugita et al., 2004) and are converted into regulators (Yoshida et al., 2000). We recently showed that iris PE (IPE) cells produce B7 costimulatory molecules and membrane-bound transforming growth factor β (TGFβ) that is delivered to CD8$^+$ T regulators (CD8$^+$ IPE-induced Tregs) (Sugita et al., 2006a). The CD8$^+$ IPE-induced Tregs engage CTLA-4$^+$ bystander T cells by B7 interactions for targeted delivery of membrane-bound TGFβ. Importantly, IPE-induced Tregs express their own B7 and membrane-bound TGFβ in order to suppress bystander T cells. In addition, we showed that thrombospondin-1 (TSP-1) produced by IPE and the IPE-induced Tregs binds and activates TGFβ (Futagami et al., 2007). The TSP-1 is essential to the induction of eye-specific Tregs and the subsequent suppression of bystander T cells in vitro. The CD8$^+$ T cells that encounter ocular PE express immunoregulatory molecules to achieve suppression of T-cell activation.

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$CD4^+CD25^+$ T regulatory cells have emerged as a unique population of suppressor T cells that maintain peripheral immune tolerance (Sakaguchi, 2000; Shevach, 2000). These regulatory T cells develop spontaneously in the thymus and suppress T- and B-cell activation *in vitro* by a cell contact-dependent mechanism (Mason and Powrie, 1998; Nakamura et al., 2001; Suri-Payer et al., 1998). More importantly, mice depleted of $CD4^+CD25^+$ T cells are vulnerable to a variety of autoimmune diseases (Maloy and Powrie, 2001; McHugh and Shevach, 2002; Salomon et al., 2000). Mice that are thymectomized on their 3rd postnatal day (a procedure that depletes their $CD4^+CD25^+$ T-cell population) develop immune-mediated inflammation in a variety of organs, including the uveal tract of the eye (Asano et al., 1996; Takeuchi et al., 1998). This finding raises some critical questions: (I) can the ocular PE cells induce regulatory T cells that express CD25 molecules, (II) can the PE-induced Tregs be induced or converted from peripheral naturally arising $CD25^+$ Tregs, and (III) if this latter situation occurs, which molecules and/or cytokines are responsible for the conversion?

Because the T cells exposed to ocular PE (PE-induced Tregs) suppress bystander effecter T cells, we questioned whether the PE-induced Tregs are related to, or perhaps even derived from, the natural $CD25^+$ T-cell population. We designed a series of experiments to examine this possibility. We found that T cells exposed to IPE proliferate due to TCR-trigged activation via costimulatory interactions. The T cells exposed to IPE are converted into $CD25^+$ T regulatory cells. The Tregs that emerge in the presence of IPE are not necessarily derived from naturally arising $CD25^+$ Tregs. Instead, IPE-induced Tregs can arise independently from T cells that are $CD25^-$ precursors. These eye-derived $CD25^+$ Tregs greatly express *Foxp3* transcripts through TGFβ–TGFβ receptor interactions, and produce immunoregulatory cytokines.

2. Materials and methods

2.1. Mice

Adult C57BL/6 mice, purchased from CLEA Japan Inc. (Tokyo, Japan), were used as donors of lymphoid cells and ocular PE. Mice of the C57BL/6 background with disrupted CD28 genes were purchased from Jackson Laboratories. Drs. Philip J. Lucas and Ronald E. Gress (National Cancer Institute, Bethesda, MD) kindly provided dominant negative TGFβ type II receptor (TGFβ RII) transgenic mice (Lucas et al., 2000). Dr. James P. Allison (Univ. California at Berkeley) kindly provided C57BL/6 background CTLA-$4^{+/-}$ heterozygote mice, as described previously (Sugita and Streilein, 2003).

2.2. Preparation of cultured pigment epithelium from iris

IPE cells were cultivated separately, as previously described (Sugita and Streilein, 2003). Iris tissues were incubated in PBS containing 1 mg/ml Dispase and 0.05 mg/ml DNaseI (both from Boehringer–Mannheim, Mannheim, Germany) for 1 h. These tissues were cultured with RPMI 1640 complete medium composed of RPMI 1640 and 10% fetal bovine serum (FBS). The primary cultures for iris were >99% cytokeratin positive (Clone PCK-26, Sigma) as determined by flow cytometry.

2.3. Preparation of purified T cells and assays of T-cell activation

Suspensions of responder cells were pressed through nylon mesh (Immulan mouse T-cell kit, Biotex Laboratories, Houston, TX) to produce a single cell suspension of cells that were >95% CD3 positive. Regulatory T cells were prepared from purified $CD8^+$ T cells exposed to IPE and referred to as $CD8^+$ IPE-induced Tregs. T cells were enriched for $CD8^+$ cells by using MACS beads (MACS cell isolation kits, Miltenyi Biotec, Auburn, CA, >95% of cells expressed the relevant surface marker), cultured for 24 h in the presence of IPE cells with anti-CD3 (0.1 μg/ml), and then harvested, X-irradiated, and used as Tregs. Flow cytometry with anti-cytokeratin antibodies showed that the amount of IPE cells contaminating the harvested T cells was <0.97%.

For anti-CD3-driven T-cell activation, purified T cells were added (1×10^5 cells/well) to culture wells with or without IPE or X-irradiated IPE-induced Tregs. Anti-CD3 antibody (Clone 2C11, BD PharMingen, San Diego, CA) was added to the wells, and the cultures were maintained for 24 or 72 h. Then, the cultures were assayed for the uptake of [^{3}H]thymidine (1 μCi/ml for the last 8 h of culture), as a measure of cell proliferation. For all T-cell assays, serum-free media were used in cultures and in assays involving T cells stimulated by anti-CD3 antibodies, as previously described (Sugita and Streilein, 2003).

2.4. Depletion of $CD25^+$ T-cell population

T cells were depleted of $CD25^+$ cells using FITC-conjugated anti-CD25 antibodies (IL-2 Receptor α chain, BD PharMingen) and anti-FITC Micro beads (MACS system, <1% of cells expressed CD25 by flow cytometry). The CD25-depleted T cells were added or not added to IPE, harvested after 24 h, X-irradiated, and added to secondary cultures containing T cells and anti-CD3. In a second set of assays, the CD25 population was depleted using the same method after culture with IPE, then X-irradiated and added to secondary cultures.

2.5. Separation of $CD25^+$ and $CD25^-$ IPE-induced Tregs

Purified $CD8^+$ T cells were cultured for 24 h in the presence of IPE, then harvested and stained as IPE-induced Tregs. Before staining, the T cells were incubated with anti-CD16/CD32 at 4 °C for 15 min. FITC-conjugated anti-CD25 antibody was used to stain IPE-induced Tregs at 4 °C for 30 min. Cells were then separated using anti-FITC MACS beads. The separated $CD25^+$ or $CD25^-$ IPE-induced Tregs were added to secondary cultures.