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Human retinal pigment epithelium-induced CD4$^+$CD25$^+$ regulatory T cells suppress activation of intraocular effector T cells☆

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Abstract Murine retinal pigment epithelial (RPE) cells suppress T-cell activation by releasing soluble inhibitory factors and promote the generation of regulatory T cells in vitro. These T cells exposed to RPE supernatants (RPE-induced Treg cells) can suppress the activation of bystander effector T cells via the production of transforming growth factor-beta (TGFβ). In the present study, we showed that human RPE-induced Treg cells are also able to acquire regulatory function when human RPE cell lines were pretreated with recombinant TGFβ2. These RPE-induced Treg cells produced TGFβ1 and IL-10 but not IFNγ, and they significantly suppressed the activation of target cell lines and intraocular T-cell clones established from patients with active uveitis. Moreover, CD4$^+$CD25$^+$ RPE-induced Treg cells expressed CTLA-4 and Foxp3 molecules, and the CD25$^+$ Treg cells profoundly suppressed the T-cell activation. Thus, *in vitro* manipulated Treg cells acquire functions that participate in the establishment of immune tolerance in the eye.

Introduction

Suppression of intraocular infiltrating cells is one of the critical functions of the peripheral immune system, which includes the eyes. A subset of T cells expressing high levels of the interleukin IL-2 receptor α-chain (CD25) has immunosuppressive activity [1–4]. CD4$^+$CD25$^+$ T regulatory cells (Treg cells) are naturally arising cells derived from the thymus. In addition to CD25, these cells express the transcription factor Foxp3. Foxp3 is a forkhead transcription factor that appears to be a master gene that controls Treg cell development and function and is thus far the best Treg

Abbreviations PE, pigment epithelium; RPE, retina pigment epithelium.

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marker [5,6]. In the eye, CD25$^+$ Treg cells suppress effector T cells in vitro [7,8] and in vivo [9], and these T cells greatly express Foxp3 [7,8]. Ocular resident cells (e.g., pigment epithelial cells) can convert T cells into CD25$^+$Foxp3$^+$ Treg cells [7,8,10,11], and intraocular cells/tissues and the induced Treg cells can create immune tolerance in the ocular microenvironment. If CD25$^+$ T cells are depleted from mice or are defective, the donors spontaneously have autoimmune disorders [12], suggesting that CD25$^+$ Treg cells control the immune system.

Given that defective Treg cells or low numbers of Treg cells are found in patients with intraocular inflammatory disorders, the replacement of Treg cells is an appealing therapeutic strategy. However, in most individuals, the Treg cell population comprises only 1%-2% of T cells in peripheral blood mononuclear cells (PBMCs), and T cells do not normally exist in the eye. Therefore, there must be reliable methods for cellular expansion to consider Treg cell replacement as a therapy. Although Treg cells do not normally proliferate in vitro, we have developed a technique of expansion while maintaining the classical Treg phenotype and the ability to suppress effector T-cell function (cell proliferation and cytokine production) in vitro.

In the present study, we examined whether human retinal pigment epithelium (RPE)-induced CD4$^+$ regulatory T cells suppress the activation of effector T cells. Since conventional human RPE cell lines do not induce Treg cells in vitro, we used the supernatants of recombinant TGFβ-pretreated RPE cells for induction of Treg cells. The CD4$^+$ T cells exposed to the RPE supernatants express CD25 and Foxp3, and the T cells greatly suppress the activation of inflammatory cells (monocytes, B cells, and T cells) and intraocular T cells obtained from patients with active uveitis. The differentiated human CD4$^+$CD25$^+$ RPE-induced Treg cells suppress bystander effector T cells by secreting active TGFβ immunosuppressive cytokines.

Results

Capacity of human RPE-induced Treg cells

We first examined whether human RPE cells can induce Treg cells in vitro. Murine RPE cells and their supernatants can induce CD4$^+$ Treg cells in vitro [11]. After incubation with anti-CD3 antibody, purified CD4$^+$ T cells exposed to supernatants of a RPE cell line, ARPE-19, were harvested, X-irradiated, and added to secondary cultures containing T cells and anti-CD3 (target responder T cells). CD4$^+$ T cells exposed to RPE supernatants (supernatants of ARPE-19 cells) did not suppress activation of target responder T cells (Fig. 1A). In contrast, murine CD4$^+$ T cells exposed to RPE supernatants from primary cultured murine RPE significantly suppressed the activation of responder T cells (Fig. 1B). Thus, human RPE cell lines do not convert CD4$^+$ T cells into Treg cells in vitro.

Promotion of immunoregulatory factors by recombinant TGFβ-pretreated RPE

When RPE cells are treated with recombinant TGFβ in vitro, their production of immunoregulatory factors such as TGFβ and thrombospondin-1 (TSP-1) is greatly upregulated [11]. Therefore, we studied ARPE-19 cells pretreated with recombinant TGFβ. We used human recombinant proteins of TGFβ2, the dominate isoform of TGFβ in the eye. As expected, we found that the supernatants of ARPE-19 cells in the presence of rTGFβ2 contained significant amounts of the active forms of TGFβ1 and TGFβ2 (Fig. 2A). The rTGFβ2-pretreated ARPE-19 cells also expressed mRNA for TGFβ1 and TGFβ2 at much greater levels than in nontreated cells (Fig. 2B). In contrast, the control cell lines (murine RPE cells and human melanoma cells) in the presence of rTGFβ2 did not produce any TGFβ proteins (data not shown). When RPE cells were treated with 10 ng/ml recombinant TGFβ2 in vitro, their production of TSP-1 (Fig. 2C) and PGE_2 (Fig. 2D) was significantly upregulated. These results suggest that TGFβ produced by retinal pigment epithelium might promote the autocrine production of immunoregulatory factors. Since these immunoregulatory factors are essential for the induction of eye-dependent regulatory T cells [10,11,13], we used the supernatants of human RPE cells in further experiments.

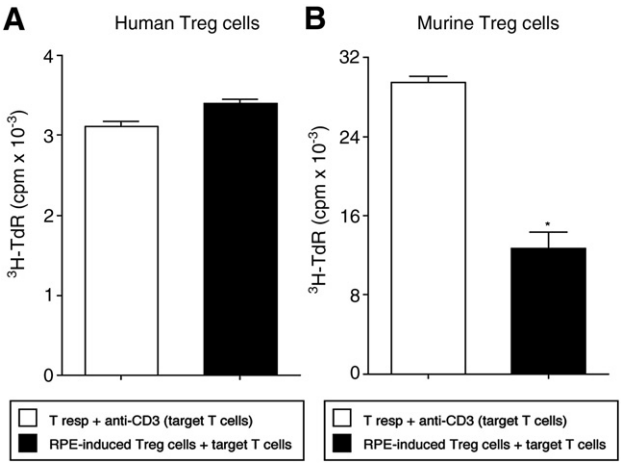

Figure 1 Capacity of RPE cells to convert T cells into regulators. (A) Purified human CD4$^+$ T cells (1×10^6/well) were cultured with supernatants from human RPE cells (ARPE-19) for 24 hours in the presence of anti-CD3 antibody (0.1 μg/ml), harvested, X-irradiated, and used as Treg cells (RPE-induced Treg cells; black bar). The human RPE-induced Treg cells were added to cultures containing naive responder T cells (T resp, open bar) plus anti-CD3. (B) Purified murine CD4$^+$ T cells from a spleen were cultured with supernatants of primary cultured RPE cells for 24 hours in the presence of anti-mouse CD3 antibody, harvested, X-irradiated, and used as Treg cells. The murine RPE-induced Treg cells were added to cultures containing target responder T cells. Error bars represent the SEM. $^*P<0.05$ between positive control cultures and RPE-induced Treg cells.

Detection of TGFβ and its receptors in expanded human RPE-induced Treg cells

We next examined whether CD4$^+$ T cells exposed to supernatants from rTGFβ2-pretreated RPE cells can produce TGFβ, IL-10, or IFNγ. CD4$^+$ T cells exposed to the RPE supernatants secreted significant amounts of the active