Inhibition of B-Cell Activation by Retinal Pigment Epithelium

Sunao Sugita, Shintaro Horie, Yukiko Yamada, and Manabu Mochizuki

Purpose. To determine whether retinal pigment epithelial (RPE) cells can inhibit B-cell activation in vitro.

Methods. Primary cultured RPE cells were established from normal C57BL/6 mice. Activated target B cells were established from splenic B cells stimulated with anti–mouse CD40 antibody and lipopolysaccharide (LPS) in the presence of recombinant interleukin 4 (rIL-4). B-cell activation was assessed by examining proliferation through [^{3}H]-thymidine incorporation or carboxyfluorescein succinimidyl ester dilution, and antibody production was determined by ELISA. Expression of costimulatory molecules and the receptors on B cells was evaluated by flow cytometry. Neutralizing anti–TGFβ antibodies were used in the assay.

Results. Addition of primary cultured RPE cells suppressed B-cell proliferation in response to anti–CD40, LPS, and rIL-4 stimulation. Similarly, antibody production by these activated B cells was suppressed. Suppression of B-cell activation was mediated by a soluble factor because supernatants from cultured RPE cells were sufficient to inhibit B-cell responses. Moreover, TGFβ was identified as the soluble mediator given that RPE-supernatants failed to suppress B-cell activation if pretreated with neutralizing anti–TGFβ antibodies.

Conclusions. Cultured RPE cells suppress the activation of B cells in vitro. These data support the hypothesis that retinal pigment epithelium has immunosuppressive properties that are capable of suppressing B-cell activation. (*Invest Ophthalmol Vis Sci.* 2010;51:5783–5788) DOI:10.1167/iovs.09-5098

Cognate interactions between B cells and T cells have critical roles during immunologic responses. Activated T cells are able to induce B-cell proliferation and immunoglobulin secretion in a major histocompatibility complex (MHC)–restricted and cell contact–dependent manner. Moreover, B cells play an important role in antigen presentation.[1–3] Activation of B cells results in higher expression of MHC class II and antigen presentation that can elicit proliferation and cognate help from antigen-specific T cells. These cognate interactions between B and T cells elicit reciprocal activation of both cells, resulting in a potentially inflammatory microenvironment. Generation of an inflammatory response in the eye can be detrimental because it may lead to the destruction of vulnerable intraocular tissues.

More than a decade ago, there were many reports of the detection of infiltrating B cells within inflamed eyes.[4–8] For example, granulomatous lesions containing T-helper and B lymphocytes were observed in patients with sympathetic ophthalmia.[4] Histopathologically, most intraocular lymphomas are classified as diffuse large B-cell lymphomas (a B-cell-type, high-grade malignant non-Hodgkin lymphoma) that produce IL-10.[5,6] These abnormal infiltrating B cells can affect the retina, choroid, or vitreous or the optic nerve. In animal models of herpes simplex keratouveitis, immunohistochemical analysis revealed large numbers of infiltrating B cells.[7,8] These results suggest that intraocular B cells, as well as T cells, have an important role in the generation of inflammatory responses during infectious uveitis, noninfectious uveitis, and intraocular lymphoma.

Recently, several investigators reported that resident ocular tissues/cells can suppress effector T cells that have been activated by cross-linking of CD3.[9–15] Primary cultured cells and cell lines established from various intraocular tissues greatly suppress the activation of bystander effector T cells in vitro. The immunosuppressive properties of intraocular cells may create immune tolerance to avoid adverse consequences of intraocular inflammation, such as blindness. However, the mechanisms by which resident ocular cells can suppress infiltrating B cells are not yet elucidated.

Therefore, we designed experiments to investigate whether cultured ocular cells are able to suppress the activation of B cells in vitro. To evaluate this, we used primary cultured retinal pigment epithelial (RPE) cells, which have powerful immunosuppressive properties and create immune tolerance in the posterior segment of the eye. Activated target B cells were generated using stimulators such as anti–mouse CD40 antibody and lipopolysaccharide (LPS) in the presence of rIL-4.

Materials and Methods

Cultured Retinal Pigment Epithelial Cells

Adult C57BL/6 mice, purchased from CLEA Japan Inc. (Tokyo, Japan), were used as donors for ocular RPE cells. RPE cells were cultivated as described previously.[9–12] Eyes were enucleated and cut into halves along a circumferential line posterior to the ciliary process, creating a ciliary body-free posterior eyecup. The eyecup was incubated in 0.2% trypsin (BioWhittaker, Walkersville, MD) for 1 hour. The RPE tissues were triturated to make a single cell suspension and then resuspended in Dulbecco's modified Eagle medium (DMEM) complete medium, placed in 24-well plates, and incubated for 2 weeks. DMEM complete medium containing 20% fetal bovine serum (FBS) was used for the primary RPE cultures.[9,10] We also prepared other ocular PE, iris pigment epithelial (IPE), and ciliary body pigment epithelial (CBPE) cells, as described previously.[9] As determined by flow cytometry, the primary RPE cultures were found to be greater than 98% cytokeratin positive.[9]

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All animal protocols were approved by Tokyo Medical and Dental University (Tokyo, Japan). Animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Preparation and Activation of Purified B Cells

Purified B cells were prepared separately from donor spleens using isolation kits (MACS Cell Isolation Kits; Miltenyi Biotec, Auburn, CA). These B cells, which were purified by a single immunomagnetic depletion step using MACS magnetic beads, proved to be >95% CD40 positive. Purified B cells (1×10^6 cells/well in a 24-well plate) were stimulated with anti–mouse CD40 antibody (clone 1C10; eBioscience, San Diego, CA), LPS (Sigma Chemical Co., St. Louis, MO), and recombinant mouse IL-4 (100 U/mL; eBioscience). Depending on the individual experiment, the concentration of the soluble anti-CD40 in these cultures ranged from 0.01 to 10 μg/mL; for LPS concentrations, it ranged from 0.1 to 100 ng/mL. Proliferation assays lasted from 1 to 5 days. Cultured RPE cells (5×10^4 cells/well) were added to B cells at the initiation of αCD40, LPS, and IL-4 stimulation.

Evaluation of B-Cell Activation

After 72- or 96-hour incubation, the cultures were assayed for uptake of [^{3}H]-thymidine (1 μCi/mL for the terminal 8 hours of culture), as a measure of B-cell proliferation. Incorporated radioactivity was measured by liquid scintillation counting and expressed as counts per minute (cpm).

Labeling of B cells with carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) was performed as described in previous reports.[16] Briefly, 5×10^6 B cells were diluted in 1 mL serum-free HBSS; 1 μM CFSE was added, and the cell suspension was incubated for 8 minutes at room temperature. Purified B cells (5×10^6) were labeled or were not labeled with CFSE at a final concentration of 1 μM (controls) and then were added (1.5×10^6/well in 24-well plates) to wells containing anti–CD40 mAb, LPS, and rIL-4 in the presence or absence of RPE cells. After 96 hours, the B cells exposed to RPE were washed and analyzed by flow cytometry.

The amounts of IgG in the supernatant of B cells exposed to RPE were measured by ELISA (mouse IgG quantitation kit; Bethyl Laboratories, Inc., Montgomery, TX).

Flow Cytometry

Flow cytometry was used to analyze the expression of surface molecules/receptors such as CD40, CD80, CD86, CD152, and TGFβ receptor on B cells. The following antibodies were used to stain the RPE-exposed B cells and control B cells: FITC-conjugated anti–mouse CD40 (clone HM40-3; eBioscience), PE-conjugated anti–mouse CD80 (B7-1, clone 16-10A1; eBioscience), FITC-conjugated anti–mouse CD86 (B7-2, clone GL1; eBioscience), PE-conjugated anti–mouse CD152 (CTLA-4, clone UC10-4B9; BioLegend, San Diego, CA), PE-conjugated anti–mouse TGFβ RII (R&D Systems, Minneapolis, MN), and FITC-conjugated anti–mouse MHC class II antibody (clone M5/114.15.2; eBioscience). The following isotype control antibodies were used: FITC-conjugated hamster IgG, FITC- or PE-conjugated rat IgG, and PE-conjugated goat IgG. After 72 hours of activation with anti–CD40, LPS, and rIL-4, B-cell cultures were harvested, washed twice, and stained with the antibodies. Before staining, the cocultured cells were incubated with mouse Fc block (Fcγ III/II receptor, clone 2.4G2; BD Biosciences, San Diego, CA) for 15 minutes Cells (1×10^6) were stained for 30 minutes at 4°C in the dark. Stained samples were analyzed using a cytometer (FACSCalibur; BD Biosciences).

Expression of TGFβ on RPE cells was detected using the following three-step staining protocol: RPE cells, cultured in the presence or absence of B cells, were stained with a mouse anti–TGFβ1, anti–TGF-β2, or anti–TGF-β3 monoclonal antibody (clone 1D11; R&D Systems) for 1 hour at 4°C in the dark. After washing, a secondary biotin-conjugated anti–mouse IgG (BD Biosciences) was added and incubated as described previously. After a second wash, the final detection step was the addition of FITC-conjugated streptavidin (BD Biosciences), which was incubated as in the previous steps. Stained samples were analyzed with a cytometer (FACSCalibur; BD Bioscience).

Statistical Analysis

Each experiment was repeated at least twice, and comparable results were obtained. All statistical analyses were conducted with Student's *t*-test. Values were considered statistically significant if $P < 0.05$.

Results

Capacity of Primary Cultured RPE Cells to Suppress Activation of B Cells In Vitro

We first tested whether RPE cells could suppress B-cell activation. For the assay, primary RPE cells were cocultured in 24-well plates and added to purified splenic B cells stimulated with anti-CD40 and LPS in the presence of rIL-4. B-cell proliferation was assessed by [^{3}H]-thymidine incorporation after 72 hours. B-cell proliferation stimulated by anti-CD40, LPS, or rIL-4 alone was not suppressed by RPE cells; however, proliferation from combined stimulation (anti-CD40 + LPS + rIL-4) was significantly suppressed (Fig. 1A).

As another measure of proliferation, we examined the suppressive effect of RPE cells on the dilution of CFSE in activated B cells. B cells were labeled with CFSE and stimulated with anti-CD40, LPS, and rIL-4 in the presence of RPE. Cultured B cells were harvested after 96 hours, and flow cytometry was used to evaluate the extent of progressive cell division. As shown in Figure 1B, up to three or four rounds of cell division were evident in positive control cultures without the RPE cells (upper panel). When RPE cells were present, stimulated B cells underwent only one round of division (lower panel).

To confirm the capacity of RPE cells, we used different concentrations of anti–CD40 antibody or LPS. First, B cells were stimulated with anti-CD40, 0.01 to 1.0 μg/mL (LPS 10 ng/mL and rIL-4 100 U/mL) in the presence or absence of RPE. As revealed in Figure 1C, RPE cells significantly suppressed the activation of B cells with 1 or 10 μg/mL anti-CD40, whereas B cells stimulated with 0.01 or 0.1 μg/mL anti-CD40 were not suppressed. Next, we titrated the amount of LPS, ranging from 0.1 to 100 ng/mL LPS (anti-CD40 1 μg/mL and rIL-4 100 U/mL). RPE cells significantly suppressed activated B cells over the entire range of LPS concentrations tested, especially 10 to 100 ng/mL (Fig. 1D). In further experiments, we used target B cells stimulated with 1 μg/mL anti-CD40, 10 ng/mL LPS, and 100 U/mL rIL-4 because these were optimal conditions for RPE suppression.

To determine the duration of RPE cell suppression, B-cell proliferation was followed over time. As can be seen, B-cell proliferation peaked on day 4 (Fig. IE), whereas RPE cells had little effect on the initial burst of B-cell proliferation on day 1, and RPE cells were able to significantly suppress proliferation over the full duration of the time course examined.

We next investigated whether other PE cells located on the anterior segment of the eye, IPE, and CBPE could suppress the activation of B cells. As expected, both types of PE cells significantly suppressed B-cell proliferation, as did RPE cells (Fig. 1F).

Next we examined whether RPE cells could suppress antibody production by activated B cells. As revealed in Figure 1G, IgG production by activated B cells was significantly suppressed. Together, these results indicate that cultured RPE cells have the capacity to suppress the activation of B cells in vitro.

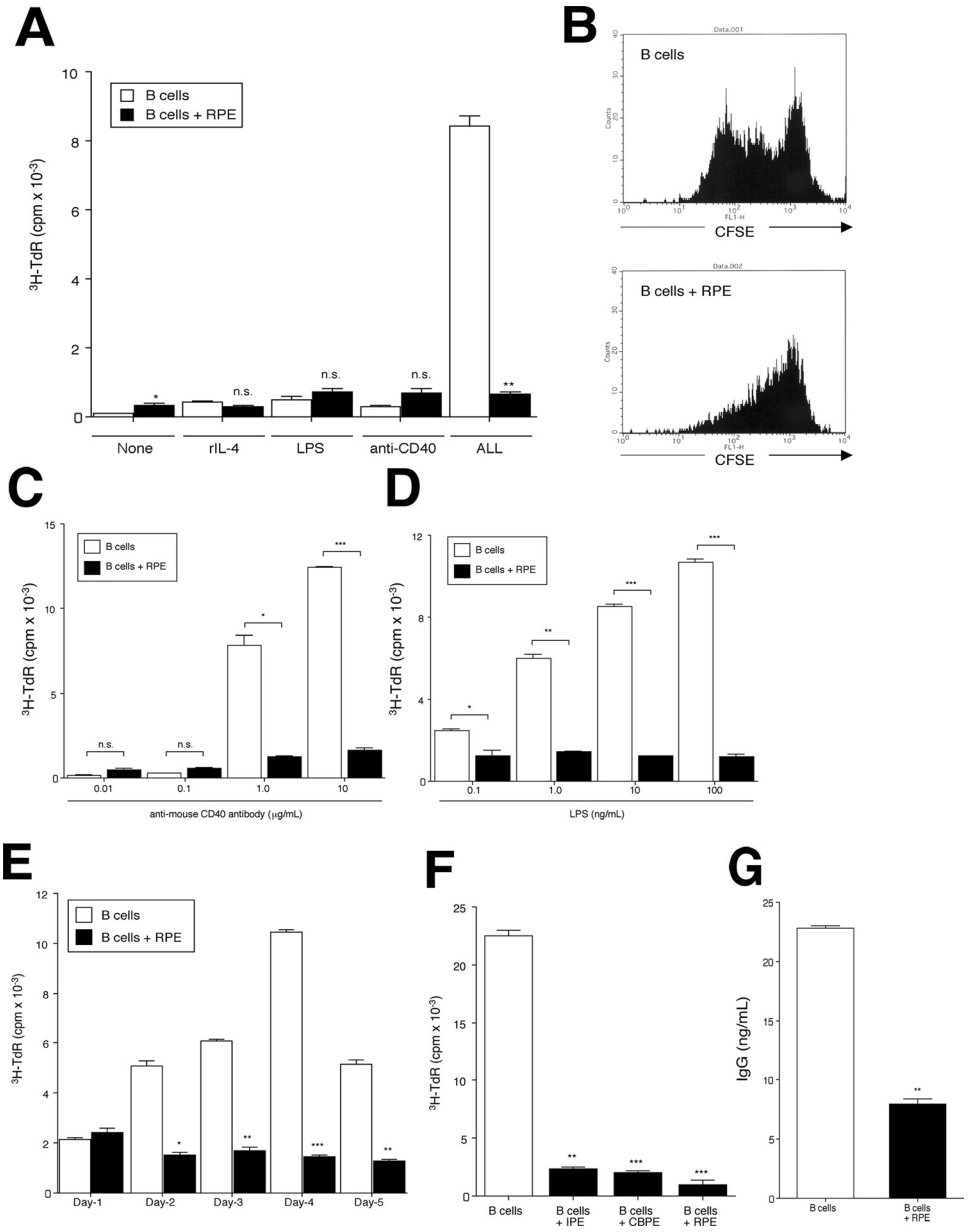

FIGURE 1. Capacity of cultured RPE to suppress the activation of bystander B cells in vitro. (**A**) In the presence of anti-CD40 (1 μg/mL), LPS (10 ng/mL), and rIL-4 (100 U/mL), purified naive B cells were cocultured with primary RPE cells for 72 hours. Mean cpm for triplicate cultures are presented (±SEM). (*open bars*) B cells only as a positive control. (*filled bars*) B cells plus RPE cells. $^*P < 0.05$ and $^{**}P < 0.005$ compared with positive control. n.s., not significant. (**B**) B cells were stimulated as in (**A**) in absence (*upper*) or presence (*lower*) of RPE cells. After 72 hours, the B cells were harvested for flow cytometric analysis. (**C**) B cells in the presence of rIL-4 (100 U/mL) and LPS (10 ng/mL) were cocultured with RPE cells in the presence of increasing concentrations of anti-CD40. (**D**) B cells in the presence of rIL-4 (100 U/mL) and anti-CD40 (1 μg/mL) were cocultured with RPE cells in the presence of increasing concentrations of LPS. (**E**) Activated B cells were cultured as in **A** and then harvested daily over a 5-day time course to measure proliferation. (**F**) Activated B cells were cocultured with primary PE, IPE, CBPE, and RPE cells. $^*P < 0.05$, $^{**}P < 0.005$, and $^{***}P < 0.0005$ compared with positive control (B cells alone). n.s., not significant. (**G**) Activated B cells were cocultured with RPE cells. Then supernatants were harvested after 72 hours and assayed for IgG content by ELISA. Data are the mean ± SEM of three ELISA determinations. $^{**}P < 0.005$ compared with positive control.

Inhibition of B-Cell Proliferation by RPE Cells via Soluble Inhibitory Factors

To elucidate the role of cell contact in the suppression of B-cell activation by RPE cells, we physically separated the cells (Transwell; Corning, Inc., Corning, NY). As expected, in the absence of an appropriate insert (Transwell; Corning, Inc.), activated B-cell proliferation was greatly suppressed, as shown in Figure 2. Interestingly, RPE cells significantly suppressed B-cell proliferation across the membrane (Transwell; Corning, Inc.). To confirm that cell contact was not required, RPE cell supernatants were added, and B-cell proliferation was suppressed by the RPE supernatants in a dose-dependent manner (Fig. 2). These results suggest that RPE cells are able to suppress B-cell activation by soluble factors.

Expression of Costimulatory Molecules by RPE-Exposed B Cells

B cells constitutively express CD40,[17] and B cells stimulated by anti–CD40 antibody and IL-4 upregulate MHC class II and costimulatory molecules, such as CD80 and CD86.[18] We evaluated the expression of costimulatory molecules (ligand/receptors) on B cells activated by anti–CD40 antibody, LPS, and IL-4 in the presence of retinal pigment epithelium. Activated B cells in the presence or absence of RPE cells were stained with anti–mouse CD40, CD80, CD86, CD152 (CTLA-4), and TGFβ receptor monoclonal antibodies (mAbs) and were analyzed by flow cytometry. B cells stimulated with anti-CD40, LPS, and IL-4 showed high CD40, CD80, and CD86 expression (Fig. 3). In contrast, B cells exposed to RPE showed low expression compared with control cultures, indicating that the expression was downregulated when RPE cells were cocultured with activated B cells. The expression of CD152 costimulatory receptor, which binds to CD80 or CD86, was poor in both B cells (Fig. 3). MHC class II expression was upregulated, but similar levels were observed on B cells from both culture conditions (data not shown). In contrast, RPE-exposed B cells expressed TGFβ receptor II (TGFβ RII) at much higher levels than did control B cells (Fig. 3). These studies demonstrate that though exposure to RPE cells results in the downregulation of costimu-

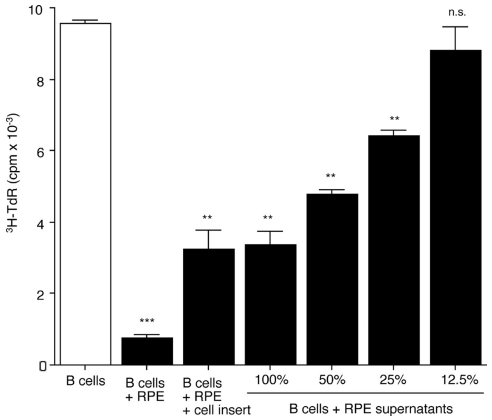

FIGURE 2. Ability of RPE cells to suppress B-cell activation by soluble factors. RPE cells were grown in 24-well culture plates. Cell culture inserts were placed in the wells, and B cells plus activation stimuli (anti-CD40, LPS, and rIL-4) were added. Activated B cells also were cocultured with supernatants from RPE cultures (100%, 50%, 25%, and 12.5% supernatants). After 96 hours, cultures were assayed for the uptake of [^{3}H]-thymidine. Mean cpm for triplicate cultures are presented (±SEM). $^{**}P < 0.005$ and $^{***}P < 0.0005$ compared with positive control. n.s., not significant.

latory molecules (CD40, CD86, CD80), there is a strong upregulation of TGFβ receptor II, suggesting TGFβ may have a role in RPE-mediated suppression of B-cell activation.

Functional Analysis of RPE-Dependent Suppression of Activated B Cells by TGFβ

Primary cultured RPE cells produce and secrete TGFβ, and the soluble factor is a critical mediator of immunosuppression by RPE cells.[10,12,19] Therefore, we confirmed that when exposed to B cells, RPE cells express TGFβ. As expected, RPE cells exposed to activated B cells expressed TGFβ at a higher level than did RPE cells alone (Fig. 4A), suggesting that encountering activated B cells leads to increased TGFβ expression by RPE cells.

We next examined whether recombinant TGFβ can suppress B-cell activation in vitro. Briefly, activated B cells were cocultured with rTGFβ2 for 96 hours. As seen in Figure 4B, the B cells failed to proliferate in the presence of rTGFβ in a dose-dependent manner. Thus, exogenous TGFβ could directly suppress the activation of B cells in vitro.

Finally, we investigated whether TGFβ was the soluble factor that mediates RPE-dependent suppression. Activated B cells were cocultured with supernatants of RPE cells in the presence of blocking anti–TGFβ antibodies or control antibodies. B cells failed to proliferate in the presence of control antibody-treated RPE supernatants. In contrast, B cells proliferated significantly if the supernatants were pretreated with anti-TGFβ (Fig. 4C). These results suggest that soluble TGFβ produced by RPE cells functions as an inhibitory factor against B cells.

DISCUSSION

In this study, we demonstrated that cultured RPE cells greatly suppressed the activation of B cells in vitro. These cultured RPE cells suppressed B-cell activation stimulated by anti-CD40 and LPS in the presence of rIL-4. Although RPE-exposed B cells were inhibited by cell-to-cell contact between RPE and B cells, soluble inhibitory factors, such as soluble TGFβ secreted by RPE cells, might be a critical mechanism by which activated B cells in inflamed eyes are suppressed.

B cells positively regulate immune responses through antibody production and optimal helper T-cell activation. Murine B cells express Toll-like receptor-4 (TLR-4), which binds LPS[20,21] and, therefore, can be activated in response to LPS stimulation. B cells constitutively express CD40, which is another important stimulatory receptor for B-cell function.[17,22] In humans and mice, activated T cells highly express CD40 ligand (CD40L; CD154), and cognate B-cell activation requires CD40-CD40L signals.[23] Activation of B cells by stimulation of both LPS and CD40 leads to upregulated expression of MHC class II and costimulatory molecules, such as CD80 and CD86. In addition, $CD4^+$ T cell–produced IL-4 is necessary for B-cell activation and differentiation.[24] Under CD40 and IL-4 signals, B cells differentiate into antibody-producing cells.[25] Therefore, optimal B-cell activation can be achieved by TLR activation with CD40-CD40L signals in the presence of IL-4. We found that cultured RPE cells fully and significantly suppressed anti-CD40, LPS, IL-4–treated B-cell responses, and we also showed the suppression of immunoglobulin secretion of B cells in the presence of RPE cells. B cells exposed to ocular resident cells or soluble factors such as TGFβ produced by the ocular cells were suppressed.

Ocular pigment epithelia of the retina have been identified as important participants in helping to create and maintain immune tolerance.[26,27] Previously, several investigators demonstrated that cultured RPE cells suppress T-cell activation through the production of immunosuppressive factors.[13,15] Moreover, cultured RPE cells established from healthy mice can suppress the activation of T cells by secreting TGFβ.[10,12] Thus, RPE cells produce and secrete immunoregulatory soluble

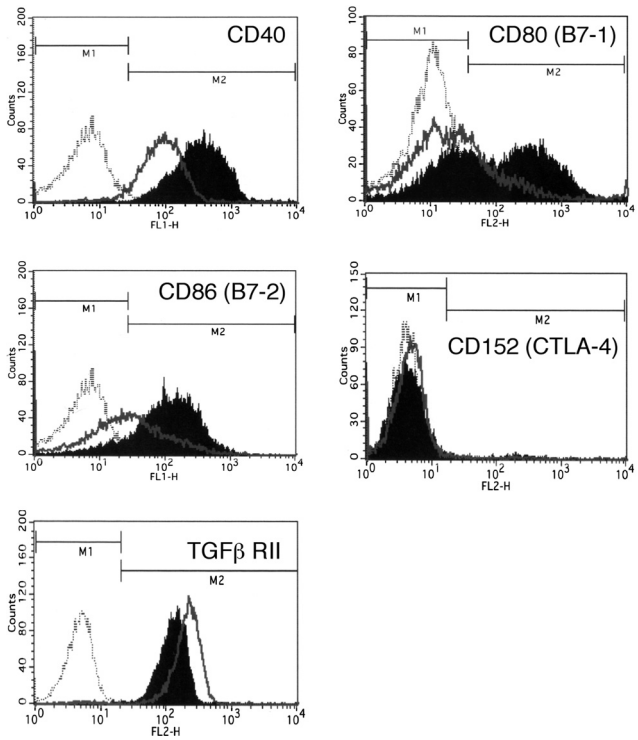

FIGURE 3. Expression of costimulatory molecules by RPE-exposed B cells. (*histograms*) Expression of costimulatory molecules (ligand/receptors) on B cells activated by anti–CD40 antibody, LPS, and rIL-4 in the presence or absence of RPE cells. Cells were stained with anti–mouse CD40 FITC, anti–CD80 PE (B7–1), anti–CD86 FITC (B7–2), anti–CD152PE (CTLA-4), and anti–TGFβ receptor II PE mAbs (TGFβ RII) and were analyzed by flow cytometry. (*dotted histograms*) Isotype control staining. (*solid histograms*) Cultures without RPE cells. (*open histograms*) RPE-cocultured B cells.

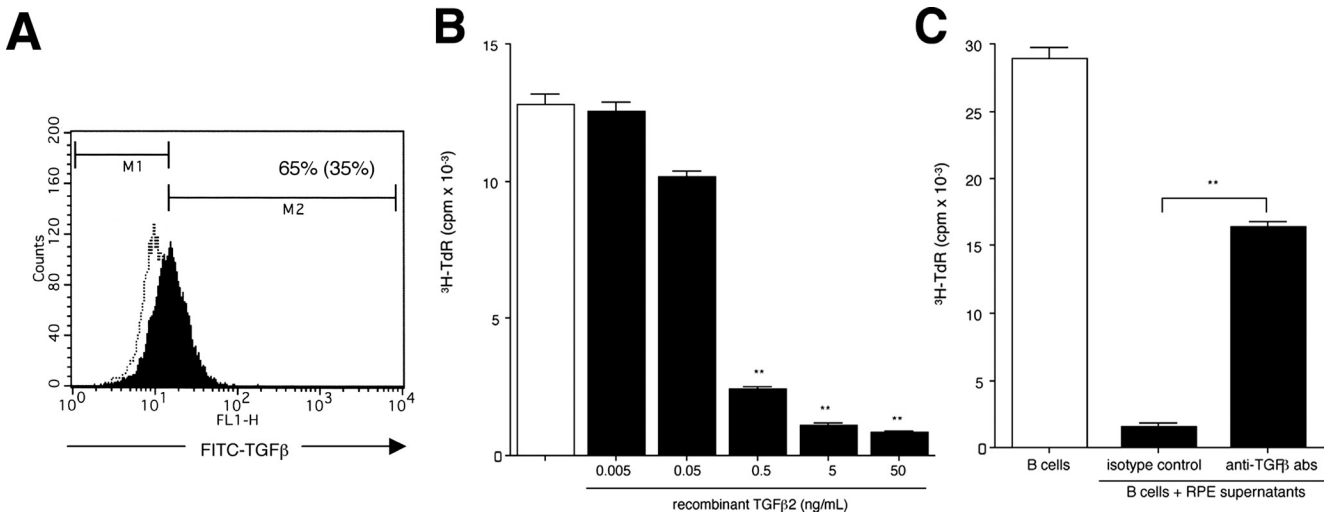

FIGURE 4. Effect of TGFβ by RPE cells to suppress B-cell activation. (**A**) Cultured RPE cells were prepared for detection of TGFβ. In flow cytometric analysis, B cell–exposed RPE cells (*filled histogram*) and control RPE cells (not exposed to B cells; *open histogram*) were stained with anti–TGFβ antibodies or control mouse IgG. Then cells were stained with secondary anti–mouse IgG-biotin followed by staining with FITC-conjugated streptavidin. Number indicates percentage positive cells for TGFβ in B cell–exposed RPE cells, and number in parenthesis indicates percentage of positive cells in control RPE. (**B**) B cells in the presence of anti–CD40 antibody, LPS, and rIL-4 were cocultured with titrated amounts of rTGFβ2 (0, 0.005, 0.05, 0.5, 5, and 50 ng/mL). $^{**}P < 0.005$ compared with positive control (B cells in the absence of rTGFβ). (**C**) Activated B cells were cocultured with supernatants from RPE cultures (100% supernatants) in the presence of anti–TGFβ1, anti–TGF-β2, or anti–TGF-β3 monoclonal antibody or isotype control (10 μg/mL, respectively). After 96 hours, cells were assayed for the uptake of [^{3}H]-thymidine. Mean cpm for triplicate cultures are presented ($\pm$SEM). $^{**}P < 0.005$, compared between the two groups.

factors. For this reason, we focused in this present study on TGFβ production by RPE cells. We demonstrated that B cells expressed TGFβ receptor in the presence of RPE (Fig. 3) and that B-cell suppression by RPE was dependent on soluble TGFβ. For example, B cells proliferated significantly in the presence of RPE supernatants pretreated with anti–TGFβ blocking antibody (Fig. 4C). However, B-cell proliferation was only partially restored if the supernatants were pretreated with anti-TGFβ alone, suggesting that other soluble inhibitory factors or other cell surface molecules expressed on RPE are involved in the suppression of B-cell activation. Optimal activation of both B and T cells requires the receipt of reciprocal stimulatory signals during the cognate interaction between B and T cells. Given that RPE cells have been shown to regulate T cells[10,12,13,15] and now B cells, ocular pigment epithelium makes important contributions to maintaining immune tolerance in the eye.

TGFβ has potent immunoregulatory effects that act on both T cells and B cells. It strongly inhibits immunoglobulin secretion in human and murine activated B cells[28–30] and can induce apoptosis in human B cells.[31] Recombinant TGFβ significantly suppresses B-cell function and antigen-specific T-cell responses in vitro, suggesting that this factor may play an important role in the regulation of the antigen-dependent immune responses.[28] In antigen-dependent immune responses, B cells and dendritic cells (DCs) function as antigen-presenting cells. DCs, which are well-known for their T cell–stimulatory properties, strongly affect B-cell growth and immunoglobulin secretion.[32] TGFβ can suppress DC functions and interfere with DC maturation, indicating its ability to suppress the expression of costimulatory molecules such as CD40, CD80, and CD86 in DCs.[32] In addition, TGFβ plays critical roles in T-cell suppression and regulatory T-cell induction in the inflamed eye.[27] Thus, TGFβ is a common denominator in various suppressive mechanisms elicited by cultured RPE cells.

In recent studies, [12,33] we investigated how T cells that are cultured in the presence of RPE are able to acquire the capacity to regulate other T cells (T-regulatory [Treg] cells). $CD4^+$ T cells acquire regulatory activity when exposed to RPE, and this conversion occurs in the presence of a novel inhibitory factor, cytotoxic T lymphocyte antigen-2α (CTLA-2α). Cultured RPE cells constitutively express CTLA-2α, whereas $CD4^+$ T cells exposed to RPE are able to upregulate the expression of FoxP3 (the marker for Treg cells). CTLA-2α molecules are able to convert latent TGFβ into active forms.[12] In addition, we previously showed that RPE-secreting thrombospondin-1 can bind to TGFβ and promote TGFβ activation in the eye.[34] Taken together, these results suggest that the TGFβ pathway plays a critical role in regulatory T-cell generation in the eye. However, for a long time, there was no evidence for that B cells have regulatory activities. Recently, several investigators found "regulatory B cells" as inhibitors of immune responses and inflammation.[35–38] These B cells produce high levels of IL-10, an inhibitory cytokine critical for immunologic tolerance. Optimal IL-10 production by regulatory B cells requires both LPS and anti-CD40 signals.[35] We are now conducting experiments to determine whether B cells exposed to RPE cells or to recombinant TGFβ produce IL-10 and acquire regulatory function.

In conclusion, cultured RPE cells, through soluble inhibitory factors, inhibit B cells that have been simultaneously activated through CD40, TLR-4, and the IL-4 receptor. The TGFβ/TGF RII pathway, known to be a powerful inhibitory factor within the eye,[39,40] is one of the important immunomodulatory mechanisms by which infiltrating B cells are suppressed in the eye during inflammation.

Acknowledgments

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