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Human Follicular Dendritic Cells Interact with T Cells via Expression and Regulation of Cyclooxygenases and Prostaglandin E and I Synthases

In Yong Lee,2*† Whajung Cho,*† Jini Kim,*† Chan-Sik Park,§ and Jongseon Choe3*†‡

PGE2 inhibits mature T cell proliferation and protects T cells from activation-induced cell death (AICD). We have previously demonstrated that human follicular dendritic cells (FDC) strongly express PGI synthase. In this study, we hypothesized that FDC have regulatory roles on germinal center T cells by controlling production of PGE2 and PGI2. Confocal microscopic analyses of human tonsil tissues revealed that FDC indeed expressed PGE synthase in addition to PGIS. To confirm these results, we studied the regulation mechanism of PG production in FDC, using an established human FDC-like cell line, HK. Specifically in response to TNF-α, TGF-β, and LPS, protein expression of cyclooxygenase (COX)-2 and downstream PGE synthase was up-regulated with coordinate kinetics, whereas COX-1 and PGIS were constitutively expressed. The increase of these enzymes was reflected in actual production of PGE2 and PGI2. Interestingly, IL-4 almost completely abrogated the stimulatory activity of TNF-α, TGF-β, and LPS in PG production. Furthermore, the up-regulation of PGE2 and PGI2 production was markedly down-regulated by indomethacin and a selective COX-2 inhibitor. PGI2 analog and PGE2 inhibited proliferation and AICD of T cells in dose- and time-dependent manners. Finally, coculture experiments revealed that HK cells indeed inhibit proliferation and AICD of T cells. Put together, these results show an unrecognized pathway of FDC and T cell interactions and differential mechanisms for PGE2 and PGI2 production, suggesting an important implication for development and use of anti-inflammatory drugs. The Journal of Immunology, 2008, 180: 1390–1397.

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ntigen-presenting cells initiate, support, and control the diverse immunological activities of lymphocytes. Follicular dendritic cells (FDC) are stromal cells specialized in presenting unprocessed Ags to B cells. FDC are known to derive from the nonhemopoietic cells (1), provide a microenvironmental niche for B and T cells in the primary and secondary follicles of lymphoid organs (2), and are indispensable for a fully robust humoral immune response. Several investigative groups, by using transgenic and knockout animals, have clearly demonstrated that FDC are required for normal development and maintenance of germinal center (GC) reactions (3–5). The GC, microanatomic site for the generation of memory B cells and plasma cells with high-affinity Abs, consists of the dark and light zones, surrounded by the follicular mantle zone (6). The dark zone contains actively dividing centroblasts that differentiate into centrocytes in the light zone (7). The light zone is rich in FDC in addition to the major component centrocytes (8–11). Although accumulated evidence maintains that FDC play central roles in GC B cell differentiation into memory and plasma cells (2, 12–14), the regulatory role of FDC in T cells is poorly understood. T cells are a minor cellular component in the light zone, constituting ~10% of total cells (15). Castan et al. (16) demonstrated that T cells do not undergo active proliferation in the GC. Another perplexing puzzle is how T cells are insensitive to activation-induced cell death (AICD), considering the physiological milieu wherein they are exposed to continued antigenic insults.

PGs are lipid mediators derived from endogenous arachidonic acids that are released from the cell membrane phospholipids in response to various stimuli, including inflammatory ones. Arachidonic acids are converted to PGs through the sequential enzyme actions of cyclooxygenases (COXs) and terminal PG synthases whose expression is cell specific (17). As well as the well-known activities of the major PGs, PGE2 and PGI2 as inflammatory and vascular mediators, respectively (18, 19), the importance of these molecules, particularly PGE2, in immunity is becoming recognized (20, 21). PGE2 inhibits T cell proliferation (22), protects from TCR-mediated AICD (23), and deviates T cell responses toward Th2 by inhibiting the production of Th1 cytokines (21). Furthermore, PGE2 exhibits numerous roles in the development and activities of B cells, dendritic cells, and macrophages (20). We have recently reported that human FDC and an FDC cell line, HK, strongly express functionally active PGI synthase (PGIS) (24).

Based upon the data presented by us and other research groups, we hypothesized that FDC suppress the proliferation and AICD of T cells in the GC by paracrine secretion of PGE2 and PGI2. To test this possibility, we examined the expression and regulation of the

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4Abbreviations used in this paper: FDC, follicular dendritic cell; AICD, activation-induced cell death; COX, cyclooxygenase; GC, germinal center; mPGES-1, microsomal PGE synthase-1; PGES, PGE synthase; PGIS, PGI synthase; PI, propidium iodide; TXB2, thromboxane B2.

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enzymes responsible for the production of these PGs by using human tonsil sections and HK cells. Our results show that FDC and HK cells express COXs and PGE synthase (PGES) in addition to PGIS; these enzymes are differentially regulated by inflammatory stimuli, and PGI2 analog, PGE2; and HK cells inhibit the proliferation and AICD of T cells.

Materials and Methods

Abs and other reagents

The mAb against PGIS, clone 3C8, was developed, as previously described (25), and labeled with biotin, according to the manufacturer’s instructions (Pierce). Other mAbs were against human microsomal PGES (mPGES)-1, COX-1, COX-2 (Cayman Chemical), β-actin (Sigma-Aldrich), and CD3 (clone 64.1; Y. Choi, Ochsner Clinic Foundation, New Orleans, LA). unconjugated mouse IgG1, propidium iodide (PI), Con A, PGE2, and LPS were obtained from Sigma-Aldrich. Biotin-conjugated mouse IgG1 and streptavidin-FITC were purchased from DakoCytomation. Goat anti-mouse IgG FITC, goat anti-mouse IgG PE, alkaline phosphatase-conjugated goat anti-mouse IgG, and HRP-conjugated goat anti-mouse IgG were obtained from Jackson ImmunoResearch Laboratories. Indomethacin, CAY10404, beraprost, and enzyme immunoassay kits for PGE2, 6-keto-PGF1α, and thromboxane B2 (TXB2) were purchased from Cayman Chemical. Recombinant IL-1β, IL-8, IL-13, TNF-α, TGF-β, and IL-10 were purchased from R&D Systems. IL-2 was obtained from Hoffmann-La Roche. Recombinant IL-4 and IL-6 were prepared in our laboratory (26).

Confocal scanning fluorescence microscopy

Cryostat sections of human tonsils were fixed in cold acetone for 10 min. The sections were rehydrated in PBS and blocked for 10 min with protein block (DakoCytomation). The slides were double stained first with anti-PGES Ab, followed by PE-labeled goat anti-mouse IgG Ab. Unoccupied Fab sites of goat Abs were blocked with mouse IgG1 Abs. The stained slides were incubated with biotin-conjugated 3C8 or biotin-conjugated mouse IgG1 Abs and then with FITC-conjugated streptavidin. The coverslips were mounted onto slides using fluorescent mounting medium (DakoCytomation). The relative positional distribution of the two fluorochromes was visualized and scanned using a confocal laser microscope (Fluoview FV300; Olympus). HK cells cultured in chamber slides were fixed with cold acetone for 10 min and incubated with 3C8 or anti-PGES Abs for 1 h at room temperature. After wash, the slides were incubated with FITC-conjugated goat anti-mouse Ig and PI for nuclear staining.

Culture of HK cells

HK cells were prepared and maintained, as described by Kim et al. (27). HK cells were maintained in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FBS, 2 mM l-glutamine (Invitrogen Life Technologies), and 80 μg/ml gentamicin (Sigma-Aldrich). To examine the regulation of COX, PGES, and PGIS, HK cells were cultured in the presence of IL-1β (10 ng/ml), IL-2 (100 U/ml), IL-4 (100 U/ml), IL-6 (30 ng/ml), IL-8 (10 ng/ml), IL-10 (50 ng/ml), IL-13 (5 ng/ml), TNF-α (50 ng/ml), TGF-β (1 ng/ml), or LPS (1 μg/ml).

RT-PCR

Total RNA was extracted from HK cells using the Easy-blue RNA extraction kit (Intron Biotechnology). Reverse transcription was conducted using the GeneAmp RNA PCR kit (Applied Biosystems) in 20 μl of mixture containing 2 U of RNase inhibitor, 1 mM dNTP, 25 μM oligo(dT), and 2.5 U of reverse transcriptase. After denaturation for 5 min at 65°C, the reaction was conducted at 42°C for 45 min, followed by the inactivation at

FIGURE 1. Human FDC express COX, PGES, and PGIS. A, Frozen tonsil sections were double stained with anti-PGIS and anti-PGES Abs for analysis with a confocal microscope. Lower panels, Magnified pictures of a part of GC area. The scale bar, 100 μm. B, HK cells were cultured in chamber slides and stained with either anti-PGIS or anti-PGES Abs. The slides were observed with a confocal microscope after nuclear staining with PI. Scale bars, 50 μm. C, Expression of COX-1, COX-2, PGES, and PGIS in HK cells was examined by RT-PCR. GAPDH represents a positive control of the assay. D, Expression of COXs, PGES, and PGIS in unstimulated HK cells was confirmed by a chromogenic immunoblotting. β-Actin served as a loading control. The arrows indicate the specific location of each protein on the immunoblot. Representative of at least two similar experiments is shown.
99°C for 5 min. DNA amplification was performed using the DFS-Taq DNA polymerase (Bioron) in a 25 μl mixture of 100 ng of cDNA, 0.4 μM each primer, 0.4 mM dNTPs (Promega), and PCR buffer (Bioron). The primer sets used are as follows: COX-1, TTACACTCGTATTCTGCCCT (sense), ATTGTCTCCATAAATGTGGC (antisense); COX-2, TTCAAATGAGATTGTGGAAAAATTGCT (sense), AGATCATCTCTGCCTGAGTATCTT (antisense); mPGES-1, ATGCCTGCCCACAGCCTG (sense), TCACAGGTGGCGGGCCGC (antisense); PGIS, GGAGCAAATGGCTGGAGAGTTAC (sense), ATCCGTCAGGGTTCAGGAATCG (antisense); GAPDH, CCCTCCAAAATCAAGTGGGG (sense), CGCCACAGTTTCCCGGAGGG (antisense).

Thirty cycles of amplifications were performed with the annealing temperature of 50°C for COX-1, 55°C for GAPDH, 56°C for COX-2, and 63°C for mPGES-1 and PGIS. The GAPDH control was amplified with 25 cycles. The reaction products were subjected to 1% agarose gel electrophoresis and visualized with Gel documentation system (Ultraviolet Products Bioimaging Systems).

Immunoblotting

Immunoblotting was conducted, as previously described (28). When HRP-conjugated anti-mouse IgG was used as a secondary Ab, the membranes were incubated for 2 min with ECL solution (Amersham) and exposed to x-ray film.

Enzyme immunoassay to measure prostanoids

The produced amounts of PGE2, TXB2, and 6-keto-PGF1α, chemically stable metabolite of prostacyclin, were measured using enzyme immunoassay kits, as described previously (24).

T cell proliferation assay

T cells were isolated from tonsillar mononuclear cells by rosetting with SRBC; the resulting cells contained >98% CD3+ cells as analyzed by a FACScalibur (BD Biosciences). T cells were cultured under the various conditions, as described in the figure legends. For the proliferation assay, 96-well flat-bottom microtiter plates were coated with 10 μg/ml 64.1 mAb, followed by incubation with T cells for the indicated periods of time. The cellular proliferation was measured by pulsing with 0.5 μCi of [3H]thymidine (PerkinElmer) during the last 12-h culture period. The cultures were harvested onto glass-fiber filters, and [3H]thymidine incorporation was measured by a liquid scintillation counter (Packard Instrument). In coculture experiments, isolated T cells were labeled with a CFSE kit (Invitrogen Life Technologies), according to the manufacturer’s instructions, and added with or without Con A or COX inhibitors to HK cells prepared 1 day earlier. Con A was used for T cell proliferation because it was not feasible to stimulate T cells with plate-bound anti-CD3 Abs due to the adherent HK
cells. CFSE dilution in T cells was measured by counting 20,000 viable cells with a FACSCalibur.

**T cell AICD**

AICD was induced, as described by Porter and Malek (23). T cells were cultured in a 100-mm dish in the presence of Con A (5 g/ml) and IL-2 (30 U/ml) for 3 days. Viable cells were purified by Ficoll density-gradient centrifugation and incubated with IL-2 alone for additional 48 h, followed by 12- to 24-h incubation with Con A in the presence or absence of prostanoids with or without HK cells. Viability of T cells was measured by direct cell counting after trypan blue staining or by a flow cytometer after staining with annexin V and PI (Trevigen) (13). Analysis was conducted on a FACSCalibur with the CellQuest software.

**Statistical analysis**

Statistical analysis and graphic presentation were conducted with GraphPad Prism 4.0 (GraphPad). Results are presented as means of triplicate assays plus SEM. The statistical significance of differences was determined by Student's t test; p < 0.05 was considered significant.

**Results**

**Human FDC express PGES in addition to PGIS**

Because mPGES-1 is responsible for the induction of PGE₂ in inflammation (29), we investigated FDC expression of mPGES-1 by immunohistochemical staining of frozen tonsil tissues with anti-mPGES-1 and anti-PGIS. Anti-PGIS mAb 3C8 (30) stained FDC in the GC, but not extrafollicular areas (Fig. 1A). mAb 3C8 was demonstrated to be a better marker for FDC because, unlike other mAbs, it was not cross-reactive with other cells derived from hematopoietic stem cells (28, 30). In contrast to PGIS, PGES staining was not restricted to GCs, but extrafollicular area was also stained. The cells that were stained with anti-PGES Ab in the GC were FDC based upon the colocalization of anti-PGES and anti-PGIS mAbs that was expressed by a yellow coalescence of green (PGIS) and red (PGES) fluorescence. Under the higher magnification of a GC, the coalescence was clearly determined on a single cell level (Fig. 1A, lower panels). To extend the results obtained in situ with human tonsil and to perform intensive experiments, we conducted further investigations using HK cells. HK cells expressed PGES mainly in the cytoplasm and slightly in the nucleus. The expression levels were as intense as those of PGIS (Fig. 1B). The cytoplasmic localization of PGES was reproduced in flow cytometric analyses by a dramatic shift of staining fluorescence after membrane permeabilization of HK cells (data not shown). Isotype-matched control Abs did not stain either tonsil sections or HK cells. This observation was further confirmed by RT-PCR and immunoblotting (Fig. 1, C and D). These assays revealed further the expression of COX-1 and -2, upstream enzymes of PGES and PGIS, in unstimulated HK cells. Taken together, our results clearly argue that FDC are equipped with the enzyme system that produces the major PGs, PGE₂ and PGI₂, from substrate arachidonic acid.

**HK cells regulate the expression of COX-2 and PGES with different kinetics**

Because FDC reside in both primary and secondary follicles of the peripheral lymphoid tissues, they may participate in the inflammatory immune response by regulating production of various inflammatory molecules, including PGE₂ and PGI₂. We asked...
whether HK cells regulate protein expression of COXs, PGIS, and PGES by incubating with a variety of cytokines in chamber slides. The expression levels of COX-1 and PGIS did not change and were constitutive irrespective of cytokine stimuli (Fig. 2A), which is consistent with the previous results (26). However, COX-2 and PGES responded to TNF-α, TGF-β, and LPS, but not to other cytokines tested by significantly increasing their protein expression levels. Time-course experiments showed that COX-2 induction reached the plateau between 4 and 12 h. In contrast, PGES induction required 18–48 h to reach the maximum (Fig. 2, B–D). The increase of COX-2 and PGES occurred in dose-responsive manners (Fig. 2, E and F). These results suggest that COX-2 and PGES expression is regulated to allow coordinate enzyme reactions. 

The regulation of COX-2 and PGES correlates with production of PGE₂ and PGI₂

To determine whether the up-regulation of COX-2 and PGES indices leads to increase of PGE₂ and PGI₂ production, we measured their concentrations in the culture supernatants of HK cells after stimulation with various cytokines. PGI₂ secretion was assessed by measuring 6-keto-PGF₁α, chemically stable metabolite of PGI₂. Among the stimuli tested, TNF-α, TGF-β, and LPS again significantly increased production of PGE₂ and PGI₂ by 2- to 3-fold (Fig. 3A). The increase was specific because TXB₂ production did not change in response to these cytokines. There are two isoforms of COX, COX-1 and COX-2, which are responsible for PGE₂ and PGI₂ production upstream of PGES and PGIS. We examined the impact of indomethacin and CAY10404, COX, and COX-2 inhibitor, respectively, on PGE₂ and PGI₂ production to determine the isoform that contributes to production of these PGs. Indomethacin and CAY10404 suppressed PGE₂ and PGI₂ production almost completely, both the constitutive and inducible secretion (Fig. 3, B and C). Indomethacin and CAY10404 did not affect the normal growth of HK cells (data not shown). These results indicate that COX-2 is responsible for PGE₂ and PGI₂ production in HK cells.

IL-4 and IL-13 inhibit the induced production of PGE₂ and PGI₂

Because synovial fibroblasts are related to FDC and anti-inflammatory cytokines inhibit TNF-α-induced PGE₂ production (31, 32), we examined the effect of IL-4, IL-10, and IL-13 on the production of PGE₂ and PGI₂ that is stimulated by TNF-α, TGF-β, and LPS. The addition of the optimal concentration of IL-4 resulted in an almost complete abrogation of the stimulatory effect on PGE₂ production (Fig. 4A). IL-4 also inhibited PGI₂ secretion significantly, although the inhibition was partial (Fig. 4B). The optimal concentration of IL-4 inhibited TNF-α-induced PGI₂ production by ~50%. Another member of IL-4 family cytokines, IL-13 exhibited a similar inhibitory activity on PGE₂ production, although the efficacy of inhibition by IL-13 was lower. Furthermore, IL-13 did not inhibit PGI₂ production consistently. In contrast to IL-4 and IL-13, IL-10 did not display any inhibitory effect on PGE₂ and PGI₂ production. These results argue that IL-4 specifically counteracts the stimulatory activity of TNF-α, TGF-β, and LPS in PGE₂ and PGI₂ production.

PGE₂ and PGI₂ analog repress proliferation and AICD of T cells

Because PGI₂ inhibits T cell proliferation (24), we examined the effect of PGE₂ on T cell proliferation to compare their effects in the
same experimental system. Purified T cells were activated by immobilized anti-CD3 Abs, and their proliferation was assessed by [3H]thymidine incorporation. Due to the very unstable nature of PGI2 (33), we used beraprost, a more stable PGI2 analog. Consistent with our previous results, beraprost inhibited T cell proliferation significantly. The effect of PGE2 was not different from beraprost and inhibited T cell proliferation (Fig. 5A). The inhibition by PGE2 was greater than that by beraprost when compared at the same molar concentrations (Fig. 5B). For example, 30% inhibition was obtained with 10^{-6} M PGE2 compared with 19% with beraprost. Control PG 6-ke to-PGF1α did not display any inhibitory effect on T cell proliferation, indicating the specificity of PGE2 and beraprost.

Because T cells are thought to receive continued stimulation from cognate Ags in the light zone in which FDC dendrites are most well developed, we next asked whether PGE2 and PGI2 have any modulating effect on AICD of T cells. AICD was induced by culturing T cells with Con A and IL-2, followed by resting and then restimulating with Con A. The level of AICD was assessed by directly counting the number of viable cells. Both PGE2 and beraprost partially repressed the AICD in a dose-dependent manner (Fig. 6A). The partial inhibition was reproducible when the AICD was assessed by flow cytometric analysis after staining with annexin V and PI (Fig. 6B). The increase of annexin V^−PI^- viable cells occurred with concomitant decrease of annexin V^+PI^+ apoptotic cells. However, control PG 6-keto-PGF1α did not show any modulating effect.

HK cells recapitulate the inhibitory activities

To examine whether HK cells indeed inhibit the proliferation and AICD of T cells by producing PGs, we conducted coculture experiments. CFSE-labeled T cells remained unproliferative for 3 days when cultured in the presence or absence of HK cells (Fig. 7A and data not shown). The addition of Con A gave rise to a potent cellular proliferation by generating T cells that underwent cell divisions up to three times. The stimulatory effect of Con A was nullified by the addition of PGE2, confirming the results obtained with [3H]thymidine incorporation assays. Stimulation of T cells with Con A in the presence of HK cells resulted in increased numbers of T cells that underwent cell division. However, the addition

![FIGURE 5. PGE2 and beraprost inhibit T cell proliferation. Tonsillar T cells (1 × 10^5 cells/well) were stimulated with immobilized anti-CD3 Abs in the presence or absence of 10 μM concentration of PGE2, beraprost, or 6-keto-PGF1α up to 84 h (A). Dose-response experiments (B) were obtained by culturing T cells for 72 h. An asterisk(s) indicates significantly different effect compared with 6-keto-PGF1α control (*, p < 0.05; **, p < 0.01; ***, p < 0.001). Representative of three reproducible experiments is shown.]

![FIGURE 6. PGE2 and beraprost partially suppress the AICD of T cells. Purified tonsillar T cells (1 × 10^7 cells) were cultured in a 100-mm dish in the presence of Con A (5 μg/ml) and IL-2 (30 U/ml) for 3 days. Viable cells were purified by Ficoll density-gradient centrifugation and incubated with IL-2 alone for additional 48 h, followed by a 12 (A)- or 24 (B)-h incubation with Con A after washing in the presence or absence of indicated PGs (10^{-7} – 10^{-5} M). Viability of T cells was measured by direct cell counting after trypan blue staining (A) or by a flow cytometer after staining with annexin V and PI (B). □, indicate annexin V^−PI^- live cells; ■, indicate annexin V^+PI^+ apoptotic cells. An asterisk(s) indicates significantly different effect compared with AICD induction control (*, p < 0.05; **, p < 0.01; ***, p < 0.001). Representative of three reproducible experiments is shown.]}
FIGURE 7. HK cells inhibit the proliferation and AICD of T cells. A, CFSE-labeled tonsillar T cells (1 × 10^6 cells/well) were cultured in a 96-well plate with or without Con A (5 μg/ml), PGE2 (10 μM), indomethacin (10 μM), or CAY10404 (10 μM) in the presence or absence of HK cells (1 × 10^4 cells/well) that were prepared 1 day earlier. T cell proliferation was assessed after 72 h by analyzing the dilution of CFSE in the same number of viable cells (20,000 cells each condition) with a FACSCalibur. B, The percentages of T cells that underwent cell division as indicated by markers in A are presented. C, AICD of T cells was induced and measured, as described in Fig. 6. To induce accumulation of PGs in the supernatants, HK cells were prepared 48 h before the addition of T cells for the last 24-h coculture. An asterisk(s) indicates significantly different effect for each comparison (*, p < 0.05; **, p < 0.01; ***, p < 0.001). Representative of at least two reproducible experiments is shown.

of indomethacin or CAY10404 further enhanced T cell proliferation (40.8 vs 52.3 or 51.6%, respectively; Fig. 7B). In the AICD of T cells, the presence of HK cells significantly increased the numbers of viable T cells (60.5 vs 81.5%), which were reversed by the addition of indomethacin or CAY10404 (81.5 vs 64.5 or 72.3%, respectively; Fig. 7C). Collectively, these results suggest that PGs are responsible for the suppressive effect of HK cells on T cell proliferation and AICD.

Discussion

The GC is the anatomic site for generating humoral immune responses to T cell-dependent Ags. Recent investigations using in vivo imaging techniques revealed its dynamic features of cellular interactions (11, 34), confirming the conventional view of the GC functions, but also challenging classical models for GC reactions that are mostly based on experimental results obtained with frozen snapshots of immunohistochemistry. For example, the physical boundaries of the secondary follicles were considered as obstacles that restrict free migration of lymphocytes between different anatomic compartments. However, the cellular tracks of centroblasts, centrocytes, and follicular mantle B cells are overlapping (11). These data underscore the important role of resident stromal cells in regulating the physiology of differentiating lymphocytes in the vicinity.

Our data show that human FDC express PGES and PGIS in addition to COXs. FDC are fully equipped to produce the major PGs, suggesting that FDC in response to stimuli such as bacterial infections (LPS or TNF-α) may enhance expression of COX-2 that provides a sufficient supply of the substrates for PGES and PGIS. PG12 may be the major PG produced if PGES is not induced in a timely manner. Constitutive PGIS is available at high levels. Our data argue that FDC produce significant amounts of both PGE2 and PG12 by a coordinate up-regulation of COX-2, followed by PGES. In addition to FDC and HK, a similar sequential induction of COX-2 and PGES was demonstrated in other cell types such as chondrocytes, synoviocytes, and vascular smooth muscle cells (35–37). These results suggest that PGs are involved in FDC-mediated inhibition of T cell proliferation, as reported previously (38), and specify the molecular identity of the responsible PGs. Furthermore, we show that PGE2 and PG12 production are regulated differentially in FDC. PGE2 production is controlled by proximal PGES, whereas PG12 generation is determined by distal COX-2. In other words, the checkpoint of PG12 production is substrate availability, whereas PGE2 production depends on enzyme availability as well.

FDC appear to participate in GC reactions by producing PGE2 and PG12. These PGs displayed overlapping activities on the proliferation and AICD of T cells. However, the fact that expression of PGES is not limited to the GC, but expressed also in the extrafollicular areas, whereas that of PGIS is specific to the GC, argues that T cells may require both PGs for the optimal effect. In support of this speculation, the requirement of both PGE2 and PG12 for the development of collagen-induced arthritis was demonstrated in knockout animals (39).

IL-4 is the cytokine that is consistently expressed by GC T cells (40, 41). We previously suggested that IL-4 plays an important role in the generation of memory B cells (12). In addition, IL-4 dramatically repressed production of PGE2 and PG12 by HK cells. IL-4 appears to mediate cross-talk between FDC and T cells by regulating production of PGs. Because Butch et al. (38) demonstrated that CD54 and CD106 are involved in FDC inhibition of T cell proliferation, FDC-T cell interactions appear to involve both direct cell-to-cell contacts and diffusible cytokines. IL-4 abrogated TNF-α-mediated PGE2 induction in synovial fibroblasts, and COX-2 down-regulation was presented as a mechanism (32). In adenocarcinoma cells, IL-4 inhibited COX-2, but not PGES expression (42). We are currently investigating the molecular mechanism of the inhibitory effect of IL-4 on PG production in FDC.

Stimulation of T cells with Con A in the presence of HK cells resulted in increased numbers of T cells that underwent cell division. The unexpected increase of T cell proliferation on HK cells
suggests that HK cells provide both negative and positive factors that affect T cell survival or proliferation. Positive factors such as the soluble mediator secreted by fibroblasts (43, 44) appear to dominate over the negative factors, including PGE₂ and PGI₂. The production of these negative factors by HK cells and their inhibitory activities are clearly demonstrated by the enhanced T cell proliferation when COX inhibitors were present in the cocultures. Although our data highlight the role of extracellular factors in the regulation of GC T cell activities, it is worthwhile to note that GC T cells are intrinsically different from conventional T cells. CD57⁺ GC T cells express CTLA-4 (16, 45), suggesting another means of containing T cells by FDC and B cells. Cognate interactions with B cells may lead to negative signals in T cells through CTLA-4. In addition, regulatory T cell function of GC T cells has been recently demonstrated (45).

In conclusion, this study reveals FDC and T cell interactions in molecular terms unrecognized previously. FDC, by regulating production of PGs, may prevent T cell expansion in the nonproliferating area of the GC, and at the same time protect them from the cell death caused by frequent encounters with cognate Ags. Furthermore, this observation implies that there is a potential risk of perturbing physiological functions of FDC by the administration of specific inhibitors of COX or mPGES-1 during infection or vaccination.

**Disclosures**

The authors have no financial conflict of interest.

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