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Human Follicular Dendritic Cells Express Prostacyclin Synthase: A Novel Mechanism to Control T Cell Numbers in the Germinal Center

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Stromal cells in the lymphoid organs provide a microenvironment where lymphocytes undergo various biological processes such as development, homing, clonal expansion, and differentiation. Follicular dendritic cells (FDCs) in the primary and secondary follicles of the peripheral lymphoid tissues interact with lymphocytes by contacting directly or producing diffusible molecules. To understand the biological role of human FDC at the molecular level, we developed a mAb, 3C8, that recognizes FDC but not bone marrow-derived cells. Through expression cloning and proteome analysis, we identified the protein that is recognized by 3C8 mAb, which revealed that FDC expresses prostacyclin synthase. The 3C8 protein purified from FDC-like cells indeed displayed the enzymatic activity of prostacyclin synthase and converted PGH2 into prostacyclin. In addition, prostacyclin significantly inhibited proliferation of T cells but delayed their spontaneous apoptosis. These findings may help explain why T cells constitute only a minor population compared with B cells in the germinal center.

Materials and Methods

Abs and reagents

The mAb 3C8 was developed as previously described (21), purified, and conjugated with biotin using a biotinylation kit (Pierce) according to the manufacturer’s instructions. Abs and reagents used in this work were anti-β-actin, biotin-conjugated mouse IgG1, streptavidin-FITC (DAKO), mouse IgG1, PGI2 (Sigma-Aldrich), alkaline phosphatase (AP)-conjugated goat anti-mouse IgG1, beraprost, PGH2, rabbit anti-human PGIS (Cayman Chemical), unconjugated anti-human CD3 (64.1, Dr. Y. S. Choi, Ochsner Medical Foundation, New Orleans, LA), and HRP-conjugated anti-rabbit Ig (Pierce).

Culture of HK cells and T cells

HK cells were prepared and maintained as described by Kim et al. (11). Synovial fibroblasts were a kind gift from Dr. C.-S. Cho (Division of Rheumatology, Center for Rheumatic Diseases in St. Mary’s Hospital, Seoul, Korea). HK cells and fibroblasts were grown in RPMI 1640 (Invitrogen).
Expression cloning of 3C8 Ag

A Uni-ZAP XR expression cDNA library of human rheumatoid arthritis-positive synoviocytes was purchased from Stratagene. The cDNA library was immunoscreened according to the manufacturer’s protocols. Briefly, the library at the concentration of 1 × 10^7 plaques in 1 μl of SM buffer (100 mM NaCl, 10 mM MgSO_4, 50 mM Tris-HCl, pH 7.5) was preincubated with 600 μl of XL1-Blue Escherichia coli (OD_600 = 0.5) resuspended in 10 mM MgSO_4 for 15 min at room temperature. Then 7 ml of top agar was added, and the mixture was plated immediately into a 150-mm agar plate. Plates were incubated at 37°C until small size plaques appeared. Nitrocellulose membranes (Schleicher & Schuell) impregnated in 10 mM phosphate buffered saline (PBS) were placed on the agar surface for at least 5 hr at 37°C. The membranes were removed, washed in TTNT (10 mM Tris- HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20), and blocked with 3% skim milk in TTNT for 1 hr at room temperature. The membranes were then incubated overnight at 4°C with 3C8 ascites diluted 1/1000 in blocking buffer. After thorough washing three times for 10 min in TTNT, AP-conjugated goat anti-mouse IgG (0.3 mg/ml diluted 1/5000 in blocking buffer) was applied for 2 hr at room temperature. The membranes were washed five times and precipitated in AP buffer (0.1M Tris-HCl, pH 9.5, 0.1M NaCl, 5 mM MgCl_2, 0.05% Tween 20), and blocked with 3% skim milk in TTNT for 1 hr at room temperature. The membranes were then incubated overnight at 4°C with 3C8 Ags diluted 1/10000 in blocking buffer. To rule out the possibility that 3C8 Ab bound the isolated cDNA clone, we purified 3C8 protein from HK cells infected with PGIS-specific siRNA. The construction of siRNA was conducted according to the manufacturer’s instructions (Ambion). For the construction of PGIS siRNA, sense-(5'-AAGGTTCTAGACAACACACCTCCTGTCTC-3') and antisense-(5'-AAATGCAGTCTGCTGAGACACCCTGTGTC-3') oligonucleotides were synthesized. The sequences of control siRNA with random PGIS nucleotides are as follows; sense-(5'-AAATGACTGTCCTTCTCCACAGACCTGTC-3') and antisense-(5'-AAATGACTGTCCTTCTCCACAGACCTGTC-3'). These siRNAs were transfected into HK cells by using FuGENE6 transfection reagent (Boehringer Mannheim) according to the manufacturer’s directions. The degree of gene silencing was assayed by Western blotting.

**Results**

*3C8 Ag is prostacyclin synthase*

Because the 3C8 Ag was expressed in synovial fibroblasts as well as in FDC (24, 25), we attempted to isolate 3C8 cDNA from the synoviocyte cDNA library by expression cloning. A 3.2-kb cDNA insert was obtained from a positive plaque (Fig. 1). The sequence of the 3C8 cDNA insert matched exactly that of PGIS (data not shown).

To rule out the possibility that 3C8 Ab bound the isolated cDNA clone by cross-reactivity, we purified 3C8 protein from HK cells by immunoprecipitation for biochemical analysis of the 3C8 Ag.

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**FIGURE 1.** Expression cloning with cDNA library of synovial fibroblasts reveals the molecular identity of the 3C8 Ag. With a cDNA library of human rheumatoid arthritis positive synoviocytes, expression cloning was conducted by immunoscreening with 3C8 mAb. A positive plaque (A) was subjected to the second (B) or third (C) rounds of immunoblotting. A replica of B was immunoblotted with isotype-matched control Ab (D).
SDS-PAGE followed by immunoblotting with 3C8 Ab revealed two specific bands (Fig. 2A), a major and a minor band with molecular masses of around 55 kDa. The nominal molecular mass of PGIS is 57 kDa. These two specific bands were identified both as PGIS by MALDI-TOF-MS analysis (Fig. 2B and C), confirming the expression cloning result. The appearance of 3C8 as a doublet may have resulted from protein fragmentation during immunoprecipitation procedure since a single band was obtained with Western blot analysis that was performed with 3C8 Ab and commercially available polyclonal Ab against PGIS (Fig. 3A). Immunoprecipitation with 3C8 Ab followed by immunoblotting with PGIS polyclonal Ab further confirmed that 3C8 mAb and anti-PGIS polyclonal Ab recognize the same molecule (Fig. 3B). Finally, because 3C8 Ag was successfully isolated in pure forms by immunoprecipitation, we investigated whether the isolated Ag would exhibit the enzymatic activity of PGIS, that is, conversion of PGH₂ into PGI₂. After incubation of 3C8 Ab with HK cell lysates followed by precipitation with protein A-Sepharose beads, we measured the PGIS activity of precipitates and supernatants. Compared with the control precipitate that had been incubated with isotype-matched control Ab, the 3C8 precipitate converted 15 times more PGH₂ into PGI₂ (Fig. 4A). At the same time, the PGIS activity of the control supernatant that had been incubated with control Ab was comparable to that of 3C8 precipitate while 3C8 supernatant lost most of the PGIS activity after incubation with 3C8 Ab (Fig. 4A). The production of PGI₂ by 3C8 precipitate was dependent on the concentration of PGH₂. However, increased addition of PGH₂ to control precipitate did not result in a significant increase of PGI₂ production (Fig. 4B). These results suggest that the protein that precipitated with 3C8 Ab is indeed the enzyme PGIS.

**Prostacyclin inhibits the proliferation of T cells but promotes their survival**

Widespread distribution of PGIS expression in the GC suggests its critical role in the GC reaction. The report that indomethicin reversed FDC-induced inhibition of T cell proliferation (12) prompted us to investigate the effect of prostacyclin on T cell proliferation. Because prostacyclin is a very unstable molecule (29),

![FIGURE 2. MALDI-TOF-MS analysis of immunoprecipitated 3C8 protein confirms the cloning result. A, 3C8 molecules were purified from HK cells by immunoprecipitation, and then the presence of 3C8 molecules in the supernatants (lanes 1, 2, 5, 6) or precipitates (lanes 3, 4, 7, 8) was examined by Coomassie blue staining (lanes 1–4) and Western blotting (lanes 5–8) after SDS-PAGE under the reducing condition. The supernatants or precipitates were obtained after incubation with control Ab (lanes 1, 3, 5, 7) or 3C8 mAb (lanes 2, 4, 6, 8). Arrows indicate specific bands obtained with 3C8 mAb. B, MALDI-TOF mass spectrum of a digest of the upper band of the gel (band 1) with trypsin was obtained as described in Materials and Methods. C, The amino acid sequences of eight selected peptides of the two 3C8-specific bands were aligned in boxes with the sequence of human PGIS protein.]

![FIGURE 3. mAb 3C8 and anti-PGIS polyclonal Ab recognize the same molecule. A, Immunoblotting of HK cell lysate was conducted with 3C8 mAb (lane 1) or anti-PGIS polyclonal Ab (lane 2) after SDS-PAGE under the reducing condition. B, Immunoblotting with anti-PGIS polyclonal Ab after immunoprecipitation of HK cell lysate with control (lane 1) or 3C8 mAb (lane 2). SDS-PAGE was conducted under the nonreducing condition.]

![FIGURE 4. Immunoprecipitated 3C8 Ag exhibits PGIS activity. A, After incubation of HK cell lysates with control or 3C8 mAb, the ability of supernatants and precipitates to convert PGH₂ to 6-keto PGF₁α was measured by ELISA. B, Dose-response result of control or 3C8 precipitates. The results are expressed as the ability of each fraction relative to control precipitate and in terms of the mean ± SEM of three independent experiments. Asterisks indicate significant difference compared with controls (**, p < 0.01; ***, p < 0.001).]
we also examined the effect of beraprost, a more stable prostacyclin analog. T cells were stimulated to proliferate on the plate coated with anti-CD3 Ab and continuously proliferated up to 80 h poststimulation (Fig. 5A). Addition of prostacyclin resulted in a slight decrease of T cell proliferation, whereas beraprost significantly reduced the proliferation from 48 h (Fig. 5C). The inhibition of T cell proliferation was dose-dependent, which was more evident with beraprost than prostacyclin at the equivalent concentrations (Fig. 5, B and D). This inhibitory effect of prostacyclin and beraprost was confirmed because addition of beraprost gave rise to ~50% inhibition of [3H]thymidine uptake by proliferating T cells (Fig. 5E).

We next examined the effect of beraprost on T cell survival. T cells underwent spontaneous apoptosis, and only small numbers of viable cells were observed after 48 h of the culture. In contrast to its inhibitory effect on T cell proliferation, beraprost enhanced T cell survival during the 3-day culture period. The presence of beraprost resulted in significantly higher number of viable cells at 24 and 48 h (Fig. 6A). Beraprost enhanced the T cell survival in a dose-dependent manner, and significant difference was obtained at the concentration of 1 μM (Fig. 6B). Because HK cells express PGIS and beraprost enhances T cell survival, we investigated whether knockdown of PGIS from HK cells would modulate the effect of HK cells on T cell survival. The PGIS gene was silenced by transfection with siRNA against PGIS. The HK cells that were transfected with 100 nM PGIS siRNA expressed significantly reduced PGIS proteins, whereas endogenous expression of PGIS protein was slightly modulated by transfection with control siRNA (Fig. 6C). Freshly isolated T cells were cultured with equal numbers of normal or transfected HK cells. T cells cultured in the absence of HK cells exhibited 48% of viable cell recovery at the end of 48 h culture. Both normal HK cells and HK cells that were transfected with control siRNA increased the recovery to 57%. However, PGIS siRNA-transfected HK cells did not support the T cell survival.

**FIGURE 5.** Prostacyclin and beraprost suppress T cell proliferation. Kinetics and dose response of the effect of PGI2 (A and B) or beraprost (C and D) on tonsillar T cell (1 × 10^5 cells/well) proliferation stimulated by immobilized anti-CD3 Ab. The degree of cellular proliferation was measured colorimetrically by cell counting kit-8 at the indicated time points or at the end of 72 h culture (B and D). E, The effect of prostacyclin and beraprost on the [3H]thymidine uptake by proliferating T cells. Tonsillar T cells (1 × 10^5 cells/well) were cultured in the presence or absence of prostacyclin or beraprost for 72 h, and [3H]thymidine incorporation during the last 16 h was measured. Results are presented as the mean ± SEM of three independent experiments. Asterisks indicate significantly different effect compared with controls (*, p < 0.05; **, p < 0.01; ***, p < 0.001).
cell survival and yielded 49% of viable cell recovery (Fig. 6D). This result suggests that HK cells enhance T cell survival by producing prostacyclin. The effect of transfected HK cells on the T cell proliferation was not examined because the coating of culture plate with anti-CD3 Ab was technically problematic due to the presence of adherent HK cells.

**Discussion**

Based on the following results presented previously (25, 26) and in this paper, we conclude that human FDCs express prostacyclin synthase: 1) FDCs express 3C8 Ag. 2) Molecular cloning and proteome analysis reveal that 3C8 Ag is prostacyclin synthase. 3) 3C8 Ag is recognized by anti-PGIS polyclonal Ab. 4) Purified 3C8 protein displays PGIS activity.

Although PGs, particularly PGE2, have been suggested as potent modulators of immunity (30), their cellular source and biological functions at the culminating site of the immune response, the GC, were not studied. Strong expression of PGIS throughout the GC suggests that prostacyclin is produced by FDC and is the major PG species in this microenvironment. This is, to our best knowledge, the first report that FDC expresses PGIS. Therefore, 3C8 is the first mAb raised against human PGIS. Furthermore, the result that prostacyclin and beraprost inhibited T cell proliferation is extending the previous report by Butch et al. (12) that PGs are involved in FDC-mediated inhibition of T cell proliferation and specifies the molecular identity of the responsible PG. Our results may explain how T cell numbers are controlled in the GC. GC T cells are mostly CD4+ and specific to the inducing Ag (31, 32), suggesting cognate interactions between T and B cells. Considering that B cells are efficient APCs to T cells, it has been puzzling that GC T cells do not normally proliferate and are defective for IL-2 production in response to Ag stimulation (12, 33). Our current data and recent results by Tenner-Racz and colleagues (31) that showed 70–90% GC T cells express CTLA-4 suggest that the number of T cells in the GC appears to be tightly regulated by FDC and B cells. B cells may induce negative signals in T cells during the cognate interactions through CTLA-4. At the same time, FDCs in close contact with T cells inhibit T cell proliferation by producing prostacyclin.

FDC expression of PGIS is more evidence supporting the view that FDC originates from local fibroblast as reviewed by Heinen and Bosseloir (34). Ultrastructural (35, 36), enzymological (35, 37), and functional studies (38, 39) support this view. We demonstrated recently that fibroblasts as well as FDCs express 3C8 Ag (25). Because 3C8 Ag has turned out to be PGIS in the current study, fibroblast and FDC exhibit another common feature of expressing PGIS. Fibroblasts are the major producer of prostacyclin (40). When the requirement for FDC increases in the GC during immune response, fibroblasts in connective tissues may migrate to lymphoid follicles in response to certain chemokines and differentiate to FDCs as a result of interaction with GC B cells. FDC may produce prostacyclin, which controls the number of T cells by inhibiting their proliferation but promoting their survival. Lindhout et al. (41) have demonstrated that synovial fibroblasts could be
induced with IL-1β and TNF-α to express the FDC phenotype. Because fibroblasts are ubiquitous cells, it remains to be elucidated whether all kinds of fibroblasts are capable of differentiating to FDCs or whether this property is limited to certain types of fibroblasts. The distinct staining pattern of 3C8 Ab from those of other fibroblast-specific Abs (25) implies that only a subset of fibroblasts may differentiate to FDCs.

Disclosures

The authors have no financial conflict of interest.

References


