ABSTRACT Prostaglandin E2 (PGE2) is a potent lipid molecule with complex proinflammatory and immunoregulatory properties. PGE2 can shape the immune response by stimulating the production of IgE antibody by B lymphocytes and the synthesis of T-helper type 2 cytokines [e.g., interleukin (IL)-4, IL-10], while inhibiting production of Th1 cytokines (e.g., interferon-γ, IL-12). It is unknown what type of receptor binds PGE2 and modulates these responses. Recent analyses in nonhematopoietic cells have identified six PGE2 receptors (EP1, EP2, EP3a, EP3b, EP3γ, and EP4). This investigation examines quiescent B lymphocytes and reports that these cells express mRNA encoding EP1, EP2, EP3b, and EP4 receptors. The immunoregulatory functions of each receptor were investigated using small molecule agonists that preferentially bind EP receptor subtypes. Unlike agonists for EP1 and EP3, agonists that bound EP2 or EP4 receptors strongly inhibited expression of class II major histocompatibility complex and CD23 and blocked enlargement of mouse B lymphocytes stimulated with IL-4 and/or lipopolysaccharide. PGE2 promotes differentiation and synergistically enhances IL-4 and lipopolysaccharide-driven B-cell immunoglobulin class switching to IgE. Agonists that bound EP2 or EP4 receptors also strongly inhibited class switching to IgE. Experiments employing inhibitors of cAMP metabolism demonstrate that the mechanism by which EP2 and EP4 receptors regulate B lymphocyte activity requires elevation of cAMP. In conclusion, these data suggest that antagonists to EP2 and EP4 receptors will be important for diminishing allergic and IgE-mediated asthmatic responses.

Prostaglandin E2 (PGE2) is a potent lipid molecule that regulates a broad range of physiologic processes in the cardiovascular, endocrine, gastrointestinal, neural, pulmonary, reproductive, visual, and immune systems (1, 2). PGE2 binds specific protein receptors on a diverse array of target cells. Pharmacologic and cDNA cloning studies have identified six different PGE2 receptors (EP3s), each of which is G protein-associated and belongs to the rhodopsin family of serpentine receptors. Sequences encoding the extracellular domains of EP receptors are highly conserved and each binds PGE2 with similar affinity. However, the intracellular domains of these receptors are unique. Based on heterogeneous intracellular domains and their association with different intracellular signaling pathways (3), EP receptors are divided into four different subtypes (EP1-EP4). EP1 receptors activate phospholipase C and phosphatidylinositol turnover and stimulate release of intracellular calcium via a poorly characterized G protein-mediated mechanism (3, 4). EP2 receptors activate adenylate cyclase via a cholera toxin-sensitive, stimulatory G protein (Gαs) and signal in response to butaprost, an agent (agonist) that selectively binds PGE receptors (3, 5). Molecular analysis of the mouse EP2 subtype has revealed three different isoforms (α, β, and γ), each of which is an alternate splice variant of the same gene. Sequence differences occur within the 3' terminus and determine the isoform-specific association with G proteins and characteristic regulatory effects on adenylate cyclase and phospholipase C (3, 7, 8). EP3a, EP3b, and EP3γ stimulate release of calcium and inhibit cAMP metabolism via inhibitory G proteins (7, 8). Under certain conditions, EP3γ can also stimulate cAMP metabolism via Gαs (8). Lastly, EP4 receptors function similar to EP2 receptors, activating adenylate cyclase via Gαs. However, EP4 receptors are insensitive to the receptor agonist butaprost (3, 6).

PGE2 is a complex immunomodulator that shifts the balance of the cellular immune response away from T-helper type 1 (Th1) and toward Th2 and drives humoral responses to IgE (2, 9, 10). While lymphocytes themselves do not synthesize PGE2, nonlymphoid inhabitants of the B-cell microenvironment such as macrophages, follicular dendritic cells, fibroblasts, and vascular endothelial cells produce PGE2. Moreover, production dramatically increases in response to a variety of immunological stimuli including interleukin (IL)-1, tumor necrosis factor-α, antigen-antibody complexes, and lipopolysaccharide (LPS) (11). Newly synthesized PGE2 directly regulates activation and differentiation of mature B lymphocytes. For example, PGE2 inhibits certain activation events such as enlargement and hyperexpression of class II major histocompatibility complex (MHC) and FcεRII (a low-affinity IgE receptor), and it diminishes IgM production. However, PGE2 dramatically increases production of IgE by synergistically enhancing IL-4 and LPS-induced isotype switching to the ε heavy chain locus (2, 9–14). PGE2 indirectly modulates humoral responses by modulating the production of cytokines by non-B-lineage cells. PGE2 profoundly inhibits production of Th1-type cytokines such as, IL-2, interferon-γ, and IL-12 (2, 9, 15, 16). In contrast, PGE2 does not inhibit Th2 cytokine production, and depending on the mode of Th cell activation, can increase production of IL-4, IL-5, and IL-10 (2, 9, 15–17). Thus by enhancing isotype switching to the ε locus and promoting secretion of Th2 cytokines, PGE2 shifts Ig production to IgE. This relationship is of particular interest because an overproduction of PGE2 (as high as 10-4 M) correlates with elevated Th2 and IgE responses in a number of disorders (AIDS, allergy, hyper-IgE response) non-B-lineage cells.

Abbreviations: PGE, E-series prostaglandin; EP, PGE receptor; Th1 and Th2, T-helper types 1 and 2; FcεRII, low-affinity IgE receptor; MHC, major histocompatibility complex; LPS, lipopolysaccharide; IL, interleukin; dbcAMP, N5O4'-dibutyryladenosine 3',5'-cyclic monophosphate.

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syndrome, Hodgkin lymphoma, sepsis, trauma, and transplantation of autologous bone marrow; refs. 2, 9, 10, and 18).

The complex immunoregulatory effects of PGE2 are receptor-mediated. Pharmacologic analyses using [3H]PGEs indicate that lymphocytes express a high-affinity receptor that specifically binds PGE2 (9, 19–21). Little is known regarding which subtype(s) of EP receptor are expressed by normal lymphocytes (22, 23). Moreover, the diverse immunoregulatory effects of PGE2 could be explained by heterogeneous patterns of EP receptor expression in hematopoietic cells and tissues. Herein is the first demonstration that primary quiescent B lymphocytes express EP1, EP2, EP3, and EP4 receptors. Moreover, this report demonstrates that each subtype contributes differently to immunoregulation by PGE2.

MATERIALS AND METHODS

EP Agonists and CAMP Reagents. PGE1, PGE2, and PGE2α were purchased from Sigma. EP-selective agonists were obtained from a number of sources. 17-Phenyl-ω-trinor-PGE2 was purchased from Cayman Chemicals (Ann Arbor, MI). Butaprost was provided by M. P. Kotnick (Miles), misoprostol and SC46275 from P. W. Collins (Searle), cicaprost, iloprost, and sulprostone from R. A. Wohl and Berlex Laboratories (Cedar Knolls, NJ). Each subtype of PGE2 receptor exhibits a unique profile of activation by these agonists (3). The relative potency of each agonist at activating PGE receptor subtypes is: EP1, PGE2 ω-17-phenyl-ω-trinor-PGE2 ω-cicaprost ω-iloprost > sulprostone; EP2, PGE2 ω-misoprostol > butaprost; EP3, SC4675 > PGE2 ω-sulprostone > misoprostol; and EP4, PGE2 ω-misoprostol > sulprostone.

Desacetylated agonists were reconstituted in absolute ethanol and serially diluted in medium. Agonists were confirmed to be active by the source and were stored according to their instructions. Dibutyryl-CAMP (N9,N9-dibutyladenosine-3′,5′-cyclic monophosphate; Sigma), forskolin (Sigma), cholera toxin, SQ22536 (9-(tetrahydro-2-furanyl)-9H-purin-6-amine), and RpCAMP (R9-adenosine 3′,5′-cyclic monophosphothioate triethylammonium; Biomol, Plymouth Meeting, PA) were reconstituted in phosphate-buffered saline.

Polycyclic Activators. Escherichia coli LPS was purchased from Sigma and recombinant mouse IL-4 from Genzyme. Anti-IGM antibody was rabbit F(ab′)2 anti-mouse IGM (heavy chain-specific) and was obtained from Jackson ImmunoResearch.

Culture Conditions. B lymphocytes were cultured in RPMI medium 1640 (GIBCO) supplemented with 5% fetal bovine serum (HyClone), 5 x 10^{-3} M 2-mercaptoethanol (Eastman Kodak), 10 mM Heps (United States Biochemical), 2 mM L-glutamine (GIBCO), 1 mM sodium pyruvate, 0.1 mM non-essential amino acids (Sigma), and 50 μg of gentamicin per ml (GIBCO).

Isolation of Quiescent Mouse B Lymphocytes. Rigorously purified, normal mouse B lymphocytes were isolated from spleens of 7- to 22-week-old C57BL/6 x DBA/2I [B6D2F1] male mice (The Jackson Laboratory) using a negative selection and adherence protocol that has been previously described (24). B lymphocytes isolated in this manner are >98% surface IgM-positive, >99% class II MHC-positive, Thy-1.2-negative, nonphagocytic, do not express esterase, and do not proliferate in response to Con A. Purified B cells were fractionated into quiescent and activated subfractions via a discontinuous Percoll density gradient (Pharmacia) that was modified to isolate the smallest, most dense B lymphocytes (24). Cells from the lowest interface were harvested and quiescence verified by particle size distribution analysis with a Coulter Channelizer 256 (24).

Isolation of RNA, Reverse Transcription, and PCR. Total RNA was isolated from cells by the acid guanidinum thiocyanate/phenol/chloroform method (Tri-Reagent, Molecular Research Center, Cincinnati). cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase and oligo(dT) primer as previously described (23). For each cDNA synthesis reaction, a parallel reaction was performed without reverse transcriptase (RT) and the “product” was employed in PCR as a negative control. PCR protocols were specifically tailored for each subtype of EP receptor. Protocols for EP1, EP2α, EP3, EP3β, EP4, and glyceroldehyde-3-phosphate dehydrogenase were performed as previously described (23). EP2 cDNA was amplified by adding an aliquot of the cDNA synthesis reaction to 10× Taq DNA polymerase PCR buffer (Boehringer Mannheim), 1 mM dNTPs, 1 μl oligonucleotide primers (Genosys, The Woodlands, TX) specific for EP2 (sense nucleotides 962–982): 5′-TTCGGCATATGCTCTTGCT-3′, antisense (nucleotides 1446–1466): 5′-ACGACAGCAATTTGCTGC-3′, 2.5 units of Taq DNA polymerase (Boehringer Mannheim), and water to a final volume of 100 μl. PCR samples were overlaid with mineral oil, initially denatured at 94°C for 2 min and run for 30–40 cycles (94°C for 30 sec, 62°C for 1 min, and 72°C for 2 min) with a final extension at 72°C for 7 min, in a DNA thermal cycler (Perkin-Elmer). Products were fractionated by electrophoresis through a 2% agarose gel containing ethidium bromide. The identity of EP-specific cDNA was confirmed via restriction endonuclease digestion and sequence information using the BLAST sequence comparison program. Sequences were elucidated via dideoxynucleotide terminator cycle sequencing (Applied Biosystems; ref. 23).

Activation Assays. Quiescent B lymphocytes were cultured in flat-bottomed 24-well microtiter plates (Becton Dickinson) at 1 x 10^6 cells per ml and preincubated with EP agonist or ethanol control for 1 hr. Ethanol controls are cultures that did not receive EP agonist but did receive an equivalent dose of the ethanol solvent in which desacetylated agonists were reconstituted. Anti-IGM or LPS and/or IL-4 were added and cultures were incubated at 37°C in a humidified atmosphere with 7% CO2. Twenty-four hours after addition of LPS and IL-4, B lymphocyte cultures were washed with medium. Cell volumes for both cultures were determined by particle size distribution analysis with a Coulter Channelizer 256. Cells were analyzed until 200 cells had been recorded in the peak channel (24). Class II MHC and FcεRII expression was monitored via flow cytometry as previously described (12). Briefly, cultures were pelleted and washed in cold PBS containing 0.1% NaNO3 and 1% BSA and incubated at 4°C with saturating concentrations of primary antibody for 1 hr. Class II MHC was detected with supernatant from the M5/114.15.2 hybridoma (rat IgG2b mAb, American Type Culture Collection). FcεRII was detected with the mAb B34 (rat IgG2a gift of D. Conrad, Medical College of Virginia, Richmond). After incubation with primary antibody, cells were washed three times and then incubated at 4°C for 1 hr with phycoerythrin-labeled affinity purified goat anti-rat antibody (Fisher). B cells were washed three times and cellular fluorescence was detected with a Coulter Epics Profile (Coulter Electronics) using forward scatter to gate out debris and counting 10,000 events per sample. Irrelevant isotype-matched rat antibody controls (Zymed) and secondary-only (phycoerythrin-labeled goat anti-rat Ig) staining were run as negative controls to establish background fluorescence. For immunofluorescence data, the percentage of B cells in each gate was calculated by CYTOTOC software from Coulter.

Proliferation and Differentiation Assays. Quiescent B lymphocytes were cultured at 1 x 10^6 cells per ml in flat-bottomed 96-well microtiter plates (Falcon; Becton Dickinson) and were stimulated as outlined for activation assays. For proliferation assays, [3H]thymidine (1 μCi well per ml; American Radiolabeled Chemicals) was added to each culture 40 hr after addition of mitogen and plates were then incubated for 8 hr at 37°C in a humidified atmosphere with 7% CO2. Cells were harvested from the cultures with a Packard Micromate 196 harvester and [3H]thymidine incorporation determined via a Packard Matrix 96

Immunology: Fedyk and Phipps

Results

Quiquescent B Lymphocytes Express mRNA Encoding the EP1, EP2, EP3, and EP4 Subtypes of PGE Receptors. Reverse transcriptase-PCR assays were developed to unambiguously determine which EP receptors are expressed by B lymphocytes. As illustrated in Fig. 1, both quiescent splenic B lymphocytes, as well as the clonal B cell line 70Z/3, yielded reverse transcriptase-PCR products of the anticipated size for EP1 (501 bp), EP2 (693 bp), EP3 (320 bp), and EP4 (539 bp). These products were not obtained in reverse transcriptase-negative reactions (data not shown). The identities of these products were confirmed by restriction endonuclease digestion and nucleotide sequence analysis (data not shown). Quiescent B lymphocytes and 70Z/3 do not express mRNA encoding EP5 or EP6 (Fig. 1) despite expressing mRNA encoding other EP receptor subtypes (Fig. 1) and glyceraldehyde-3-phosphate dehydrogenase (data not shown).

EP-selective agonist only, had little effect on basal expression of class II MHC, FcεRII, or cell volume (data not shown). Stimulation of quiescent B lymphocytes with LPS and IL-4 induces hyperexpression of class II MHC (Fig. 2A), a subset of quiescent B lymphocytes (12, 29). Pretreatment of quiescent B cells with PGE2 (1 × 10^{-5} M) almost completely inhibits hyperexpression of class II MHC (92% inhibition, Fig. 2B) and FcεRII (data not shown). Pretreatment with butaprost (an EP1-agonist) or with misoprostol (primarily an EP2- and EP4-agonist, some EP3-activity) also inhibits hyperexpression of class II MHC (95%, Fig. 2F; and 97%, Fig. 2G, respectively). Viability assays demonstrated that these agonists were not toxic at concentrations up to 1 × 10^{-4} M (data not shown). Furthermore, agonists that target EP1 (Figs. 2 C–E), EP3 receptors (Fig. 2 H and I) or prostaglandin F receptor (PGF2α) (data not shown) did not inhibit hyperexpression of class II MHC. A similar profile of inhibition was observed when FcεRII expression was measured (data not shown). Lower doses of PGE2 or EP-selective agonists also inhibited hyperexpression of class II MHC and FcεRII and inhibition occurs at concentrations as low as 1 × 10^{-8} M (data not shown). In comparing dose–response profiles for class II MHC and FcεRII expression, EP2- or EP3- and EP4-selective agonists were most effective at inhibiting expression of these activation antigens. The order of agonist potency was: misoprostol ≥ PGE2 ≥ butaprost ≥ 17-phenyl-ω-trinor-PGE2 > iloprost > cicaprost > sulprostone = SC46275 = PGF2α. Similar results were also obtained when B cells were activated with LPS or IL-4 only (data not shown), or with anti-IgM antibody (data not shown).

Flow cytometric analyses also suggested that PGE2, butaprost, and misoprostol prevented enlargement (hallmark of transition from G0 to G1 in the cell cycle) of B lymphocytes.
were treated with...cell independent > 5-

Misoprostol. Incubation with comparing induction, cytokine-directed isotype basis, of (Fig. 4A) decreases PGE2 enhances (data not shown). EP1-selective agonists and EP3-selective agonists were dramatically less effective (IC50 > 5 x 10^-3 M). At this later time point (48 hr after stimulation with agonist), EP agonist concentrations at 1 x 10^-4 M and above decreased the viability of B lymphocytes (data not shown). Overall, the order of agonist potency was: misoprostol = butaprost = PGE2 >> sulprostone = SC46275 > 17-phenyl-ω-trinor-PGE2 = iloprost = PGF2α. A similar order of agonist potency was observed for B lymphocytes stimulated with anti-IgM antibody (data not shown).
PGE2, EP2-, or EP2- and EP4-Selective Agonists Enhance IL-4-Directed Isotype Switching to IgE. Despite inhibitory effects on certain aspects of B-cell activation, PGE2 enhances cytokine-directed isotype class switching. Pretreatment with PGE2 enhances production of IgE ~3.5-fold (Fig. 4A) and decreases production of IgM (data not shown) from cultures of B lymphocytes stimulated with LPS and IL-4. On an equimolar basis, the EP2- and EP4-selective agonist misoprostol was as potent as PGE2, while the EP2-selective agonist butaprost was somewhat less effective than PGE2 (Fig. 4A and B). EP1-selective agonists modestly increase IgE production (Fig. 4A) while EP3-selective agonists have no effect (Fig. 4B). In comparing the overall dose-response profiles for IgE production, the order of agonist potency was: misoprostol ≥ PGE2 > butaprost > 17-phenyl-ω-trinor-PGE2 = iloprost > cica-
prost > sulprostone = SC46275 = PGF2α.

cAMP Mediates the Inhibitory Effect of PGE2, Butaprost, and Misoprostol. Incubation with PGE2 increases the level of cAMP 5- to 6-fold in B lymphocytes (27). PGF2α, which does not bind EP receptors, has no effect (27). Moreover in nonlymphoid cells, activation of EP2 or EP3 receptors increases cAMP (3, 5 and 6). To determine whether a cAMP signal mediates the effects of PGE2, butaprost and misoprostol on quiescent B lymphocytes, cells were treated with agents that manipulate cAMP metabolism. Agents that elevate cAMP, such as cholera toxin (an activator of Gsα), forskolin (an activator of adenylate cyclase) or dibutyryl-cAMP (dbcAMP, a membrane-permeant analog of cAMP), mimic inhibition of class II MHC hyperexpression by PGE2, butaprost, or misoprostol (Fig. 5 B–D, G, and data not shown). To determine if the inhibitory effects of PGE2, butaprost, and misoprostol require cAMP, we employed SQ22536 (an inhibitor of adeny-
late cyclase) and Rp-cAMP (a competitive inhibitor of cAMP-dependent protein kinases) to block cAMP-dependent signaling. Pretreatment with SQ22536 or Rp-cAMP alone has negligible effects on LPS and IL-4-induced hyperexpression of class II MHC (data not shown). In contrast, PGE2-induced inhibition of class II MHC hyperexpression (71% inhibition, Fig. 5D) is abrogated by pretreatment with SQ22536 (18% inhibition, Fig. 5E) or Rp-cAMP (21% inhibition, Fig. 5F). Likewise, inhibition by butaprost (data not shown) or misoprostol (72% inhibition, Fig. 5G) was prevented by SQ22536 (17% inhibition, Fig. 5H) and Rp-cAMP (12% inhibition, Fig. 5I).
were stained for PGE2 cells (5). Regulation in each receptor subtypes of EP1-, EP2-, EP3-, and EP4-selective agonist misoprostol dramatically inhibited activation of quiescent B lymphocytes by LPS and/or IL-4 or anti-IgM antibody (Figs. 2 F and G and 3 data not shown). The agonist potency ranking for inhibition of B cell activation indicates that both EP2 and EP3 receptors mediate this effect. The EP2-, EP3-, and EP4-selective agonist misoprostol is as potent as PGE2 over a wide range of concentrations, whereas EP3-selective agonists are ineffective (Figs. 2 and 3 data not shown), indicating that signaling through both EP2 and EP3 receptors is required for mediating the inhibitory effect of PGE2. While butaprost (EP2) was as effective as PGE2 at high concentrations (Fig. 2), it is less effective at concentrations below 1 × 10−9 M (data not shown). These data indicate that EP2 receptors alone are not sufficient for mediating the overall inhibitory effect of PGE2. None of the agonists affect hyperexpression of class II MHC or FcεRII by intermediate density B lymphocytes (activated) that were separated from high density (quiescent) B lymphocytes. These B cells were activated in vivo (by unknown stimuli) prior to stimulation with PGE2, Butaprost, or Misoprostol in vitro. This indicates that the mechanism by which PGE2, butaprost, and misoprostol inhibit activation is not reversion of activated cells to a quiescent phenotype. Rather, these agonists prevent events leading to activation. Finally, PGE2 also has a mild inhibitory effect on proliferation of B lymphocytes stimulated with LPS and IL-4. Butaprost and misoprostol mimic the effect of PGE2, while the remaining agonists have no effect (data not shown). These data support the conclusion that signaling through both EP2 and EP3 is responsible for inhibiting B lymphocyte activation.

Stimulation with IL-4 and LPS is a well-established system for inducing immunoglobulin class switching to IgE (28). PGE2 enhances this class switch (14). EP2- or EP3- and EP4-selective agonists strongly enhance LPS and IL-4 induced IgE production (Fig. 4 A and B) while decreasing IgM production ~20%. The EP2- and EP4-selective agonist misoprostol was at least as effective as PGE2 whereas EP3-selective agonists are ineffective (Fig. 4B), suggesting that EP2 and EP4 receptors are sufficient for mediating enhancement by PGE2. An EP2-selective agonist (butaprost) was less effective than PGE2 (Fig. 4A), suggesting that EP2 receptors alone are not sufficient for enhancing IgE production. EP1-selective agonists only weakly enhanced IgE production (Fig. 4A), suggesting that EP1 receptors are not primarily responsible for mediating enhancement by PGE2.

Previous investigations examining signaling pathways activated by EP2 and EP3 receptors in nonhematopoietic cells reveal that both EP2 and EP3 receptors elevate cAMP via activating a Gα protein and adenylate cyclase (3, 5, 6). PGE2 also elevates cAMP in B lymphocytes (19, 27), and agents that elevate cAMP mimic the effects of PGE2 on B-cell responses (29). In agreement with these investigations, the cAMP- elevating agents cholera toxin, forskolin, and dbcAMP mimicked the effect of PGE2, butaprost, or misoprostol on B-cell activation (Fig. 5 B–D and G and data not shown). To determine if cAMP is required for mediating the effect of EP2- or EP3- and EP4-selective agonists, quiescent B cells were pretreated with agents that inhibit cAMP metabolism. SQ22536 is an inhibitor of adenylate cyclase and blocks the effects of PGE2, butaprost, or misoprostol on B-cell activation (Fig. 5 D, E, G, and H and data not shown). Rp-cAMP is a nonhydrolyzable competitive inhibitor of cAMP-dependent protein kinases. Pretreatment of quiescent B lymphocytes with Rp-cAMP also prevented inhibition of class II MHC hyperexpression by PGE2, butaprost, or misoprostol (Fig. 5 D, F, G,
and I, and data not shown). Collectively, these data indicate that PGE₂ binds EP₂ and EP₄ receptors on B lymphocytes, which activates a Gαₛ protein, leading to an increase in cAMP that is required for inhibiting class II MHC hyperexpression. It is also likely that this pathway is responsible for inhibiting hyperexpression of FceRII and cell enlargement because RpcAMP blocks inhibition of FceRII expression (data not shown) and cell enlargement (29). It has been reported that inhibition of interferon-γ-induced transcription of the class II MHC and FcyRII loci by PGEs operates by a similar cAMP-dependent mechanism (30). This mechanism also appears responsible for enhancing IgE production because dbcAMP and cholecalciferol also mimic the effect of PGE₂ (12, 13, 27). In contrast, the mechanism by which EP₄-selective agonists modestly enhance IgE production (Fig. 4A) remains unknown. EP₄ receptors activate phosphatidyl inositol turnover resulting in calcium release in nonlymphoid cells (3, 4). However, calcium mobilization was not observed when B lymphocytes were stimulated with PGE₂ or the EP₄-selective agonist 17-phenyl-α-tri-nor-PGE₂ (data not shown). In contrast, stimulation with anti-IgM stimulated a large calcium release (data not shown). While many explanations exist for failing to detect a calcium signal, it is possible that EP₁ receptors stimulate calcium mobilization in B lymphocytes; however, due to low levels of EP₁ expression, this signal may be below the detection limit of the calcium assay.

EP₃β receptors do not regulate activation, proliferation nor differentiation of B lymphocytes (Figs. 2–4). The presence of mRNA encoding EP₃β (Fig. 1) suggests that this subtype regulates an activity other than those assayed herein (i.e., activation antigens, cell volume, proliferation and Ig production). While the identity of this activity is unknown, one possibility is that EP₃β regulates the effects of EP₂ and EP₄ receptors. In contrast to EP₂ and EP₄ receptors, EP₃β receptors reduce elevated levels of cAMP by activating an inhibitory G protein that interferes with adenylate cyclase activity (3, 7). Therefore, EP₃β may serve as a regulatory counterbalance by directly antagonizing the cAMP-elevating effects of EP₂ and EP₄ receptors. Thus, the abundance of EP₃β receptors relative to EP₂ and EP₄ receptors could influence the effect of PGE₂ on B cell activation and differentiation.

The results of this investigation provide new insight as to how PGE₂ regulates the immune response. Binding of PGE₂ to EP₂ and EP₄ receptors elevates intracellular cAMP in B lymphocytes that ultimately promotes differentiation (isotype switching) of these cells, while inhibiting antigen presenting function (class II MHC expression) and clonal expansion (proliferation). Thus, PGE₂ acts as a differentiation agent. It is interesting to note that these results also illuminate a novel mechanism by which LPS may drive B-cell differentiation in vivo. Previous data demonstrate that LPS stimulates the production of PGE₂ by monocytes (11) and increases the number of PGE₂ receptors on B cells (19). Additional analysis reveals that LPS increases the amount of mRNA encoding EP₃, but not EP₁ or EP₃β receptors in B-cell lymphomas (23). Therefore, by stimulating synthesis of PGE₂ and up-regulating expression of EP receptor subtypes that promote differentiation (i.e., EP₃), LPS could drive differentiation and class switching. Although the effect of LPS on expression of EP₂ receptors is unknown, the ability of EP₂ receptors to elevate cAMP (3, 5) and enhance IgE production (Fig. 4A) suggests that EP₂ receptors also participate in this novel mechanism. Moreover, this mechanism agrees with other investigations examining the effect of cAMP-elevating agents on IgE production. In these studies, β₁-adrenergic receptor agonists, cAMP analogs, cholecalciferol, forskolin, histamine, and phosphodiesterase inhibitors all increase IgE production in mouse and human systems (2, 9, 10, 12, 13) and emphasize the importance of cAMP in regulating IgE production. Therefore, antagonists to EP₂ and EP₄ receptors could emerge as important therapeutics for diminishing allergic, asthmatic, and atopic disorders mediated by IgE.

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