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Article

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Receptors for prostaglandin E_2 that regulate cellular immune responses in the mouse

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Introduction

Prostaglandin E_2 (PGE_2) is generated by the sequential metabolism of arachidonic acid by cyclo-oxygenase and prostaglandin E synthase (1, 2). This lipid mediator has pleiotropic actions in a range of tissues, including the immune system (3). Within the immune system, PGE_2 modulates the functions of cell populations, such as T cells and macrophages, which are critical to the immune response. For example, PGE_2 suppresses proliferation of human T cells (4, 5). In macrophages, PGE_2 inhibits production of cytokines such as TNF-α, and IL-12 (6, 7) and alters antigen presentation by inhibiting expression of MHC class II proteins (8). Thus, the overall actions of PGE_2 on in vitro models of cellular immune responses tend to be inhibitory and suppressive (9). Along with its actions to inhibit cellular functions, PGE_2 may also affect the overall character of an immune response. PGE_2 may polarize cellular response toward a Th2 phenotype enhancing IL-4 and IL-5 production (10, 11) and facilitating immunoglobulin class switching to IgE (12).

These actions of PGE_2 can dramatically alter the outcome of immune responses in the intact organism. For instance, PGE_2 has inhibitory and protective effects in autoimmune disease. In murine lupus models, administration of PGE_2 and its analogues improves survival (13, 14). This improvement in survival is accompanied by reduced auto-Ab production and a substantial reduction in immune-mediated kidney injury. Similarly, PGE_2 may delay or prevent allograft rejection. In a rat model of kidney transplantation, administration of PGE_1 markedly prolonged graft survival and reduced systemic cellular alloimmune responses (15). Analogous effects of PGE_2 to ameliorate rejection have been observed in animal models of heart, intestinal, and skin transplantation (16–19). In human renal transplant recipients, a reduced number of kidney allograft rejection episodes has also been reported with PGE_2 analogues (20).

The biological actions of PGE_2, including its effects on immunity, are mediated by G protein–coupled receptors, by convention designated EP (for E prostanoid) receptors (21, 22). The EP receptors can be divided into four distinct pharmacological classes, EP1–4, and EP receptors from each of these subtypes have been cloned and sequenced (21). The EP receptor isoforms have unique expression patterns, and they couple to distinct signaling pathways. The EP1 receptor is coupled to intracellular calcium, while the EP2 and EP4 receptors are coupled to G's and signal by stimulating adenylyl cyclase. Signaling by the EP3 receptor is more complex. Multiple EP3 receptor isoforms are generated by alternative splicing from a single EP3 receptor gene, and these EP3 receptor isoforms

couple to different signaling pathways including G_i, G_s, and calcium. The existence of this family of EP receptors coupled to distinct intracellular signals provides a molecular basis for the diverse physiological actions of PGE_2.

EP receptor isoforms are expressed by the major cellular constituents of the immune system (3). However, the precise EP receptor isoforms that mediate the immunoregulatory actions of PGE_2 are not known. Thus, the objective of our studies was to define the expression of EP receptors by immune cell populations in the mouse and to determine their contribution to the regulation of cellular immune responses. Using a combination of pharmacological and genetic approaches, we find that the actions of PGE_2 to suppress antigen-specific proliferation are complex. To a significant extent, these actions are mediated by EP2 receptors on T cells and by EP2 and EP4 receptors on macrophages.

Methods

Animals. The production of mouse lines with targeted disruptions of the four EP receptor genes are described elsewhere (23–26). The EP1-deficient line was produced on an inbred DBA/1 background using an embryonic stem (ES) cell line derived directly from DBA/1 mice (25). Thus, controls for the EP1 experiments were age-matched DBA/1 mice that were purchased from The Jackson Laboratories (Bar Harbor, Maine, USA). Mice with targeted mutations of the *EP2* and *EP3* genes were produced on a 129/SvEv background. Controls for these studies were wild type 129/SvEv littermates. On inbred backgrounds, most EP4-deficient mice die within 24 hours from complications of patent ductus arteriosus (24). However, by selective breeding on a mixed background, EP4-deficient lines have been produced in which the ductus closes and the animals survive normally (24). $EP4^{-/-}$ mice from these selected mixed breedings were used in our experiments. Controls for these studies are wild type littermates. Animals were bred and maintained in the animal facility of the Durham VA Medical Centers under the NIH guidelines.

Identification of EP receptor mRNA expression by RT-PCR. Expression of EP receptor mRNA was assessed by RT-PCR as described (27). Splenocyte suspensions were prepared from wild-type and EP-deficient mice by gently grinding the spleen between glass slides. The cells were washed once in PBS and then resuspended in ice-cold PBS containing 10 mM Tris HCl (pH 7.4), 1% BSA. Splenic T cells were isolated using a commercial separation column (R&D Systems Inc., Minneapolis, Minnesota, USA), splenic B cells were isolated by panning using a polyvalent anti-mouse IgG Ab, and splenic macrophages were isolated by plastic adherence. Purity of the cell populations was confirmed by cytofluorometry. Total RNA was isolated from these cell preparations using Tri-Reagent (Sigma Chemical Co., St. Louis, Missouri, USA), and 0.5 μg was reverse-transcribed using oligo-dT primers. EP receptor cDNA was amplified in PCR reactions using the following primers: EP1 sense 5′-TTAACCTGAGCCTAGCGGATG-3′ and anti-sense 5′-CGCTGAGCGTATTGCACACTA-3′; EP2 sense 5′-GTGGCCCTGGCTCCCGAAAGTC-3′ and anti-sense 5′-GGCAAGGAGCATATGGCGAAGGTG-3′; EP3 sense 5′-TGACCTTTGCCTGCAACCTG-3′, EP3α anti-sense 5′-AGCTGGAAGCATAGTTGGTG-3′, EP3β anti-sense 5′-GACCCAGGGAAACAGGTACT-3′, EP3γ anti-sense 5′-AGACAATGA-GATGGCCTGCC-3′; EP4 sense 5′-AGTAGCTAAAGGGGGAA-TCTT-3′, anti-sense 5′-AACACTTTGGCCTGAACTTGT-3′. The PCR products were size fractionated on 1.8% agarose in Tris-borate/EDTA gels, stained with ethidium bromide, and photographed.

Mixed lymphocyte responses. Primary one-way mixed lymphocyte responses (MLRs) were performed as described previously (27). Splenocyte suspensions and isolated T cells were prepared as described above. Suspensions of responder splenocytes or T cells were reconstituted at various concentrations and were mixed with irradiated stimulator splenocytes from H-2 disparate mice at the indicated ratios. Fifty microliters of each cell suspension was added to individual wells of a 96-well plate along with various concentrations of EP agonists or vehicle. Plates were incubated at 37°C in a humidified incubator containing 5% CO_2. After varying periods in culture, cells were pulsed with 0.5 μCi of ^{3}H-thymidine per well for the final 18 hours of culture. The amount of ^{3}H-thymidine incorporated in cells was assessed by harvesting cells onto a glass fiber filtermat using an automated cell harvester (Tomtek, Hamden, Connecticut, USA). Filter-bound radioactivity was measured using a scintillation counter. Values are expressed as specific counts per minute, which are calculated from counts in wells with responders alone subtracted from counts in wells with responders and stimulators. Within each experiment, individual conditions were examined in triplicate or quadruplicate samples.

In some experiments using isolated T cell responders, purified populations of macrophages were added to the cultures. To prepare purified cultures of macrophages, bone marrow was harvested from EP2-deficient and control mice. B cells and natural killer (NK) cells were removed from the suspension by sequential panning, with anti-Ig for B cells and anti-asialo GM1 for NK cells, followed by complement lysis. The resulting cell population was highly enriched for macrophages as determined by cytofluorometry with MAC-1 and F480 Ab's. These pure populations of macrophages were used to reconstitute responder populations in MLR by adding 6×10^4 macrophages to cultures containing 4×10^5 T cells. Splenic T cells were isolated using a commercial separation column (R&D Systems Inc.). Allospecific proliferative responses were then assessed as described above.

Prostanoid compounds used in MLR experiments. Prostanoid compounds including PGE_2, misoprostol, and sulprostone were obtained from Cayman Chemical (Ann Arbor, Michigan, USA) as crystalline solids of greater than or equal to 99% purity. Based on the manufacturer's recommendations, stock solutions were prepared in organic solvent (ethanol) and were stored

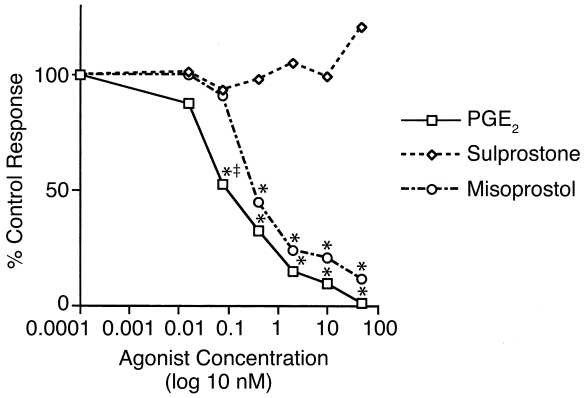

Figure 1

Effects of PGE_2 analogues on proliferation in the MLR. A range of concentrations of PGE_2, misoprostol, and sulprostone were added to the MLR, and their effects on proliferation were determined. The data are expressed as a percentage of the control response in MLR with vehicle alone. The agonist concentrations are shown on the *x*-axis as log 10 nM. PGE_2 and misoprostol caused significant inhibition of the response. *$P < 0.001$ vs. media alone; ‡$P < 0.001$ vs. misprostol.

at –20°C between experiments. The compounds are stable in these solutions at this temperature for up to 6 months. For the studies, fresh dilutions were prepared in media on the day of study to produce the desired experimental concentrations. A similar concentration of ethanol vehicle alone was added to the control cultures.

LPS-stimulated production of TNF-α and IL-12. Macrophages were prepared by culturing bone marrow from $EP2^{-/-}$, $EP4^{-/-}$, and wild-type mice in Petri dishes with media supplemented with GM-CSF (30% L929 supernatants). Nonadherent cells were discarded from these plates after 3 days, and cultures were maintained in GM-CSF–enriched media for an additional 4–5 days. Macrophages predominate in these cultures as adherent cells. Purity was confirmed by cytofluorometry using MAC-1 and F480 Ab's. Macrophages (4×10^4) were cultured in 96-well tissue culture plates in the presence 10 nM LPS along with vehicle or PGE_2 in the indicated concentration. Supernatants were removed from these wells after 8 and 24 hours. TNF-α production was assessed using a bioassay and IL-12 production was measured by ELISA.

Statistical analysis. The values for each parameter within a group are expressed as the mean plus or minus the SEM. For comparisons between EP-deficient and wild-type groups, statistical significance was assessed using an unpaired *t* test for normally distributed data. A paired *t* test was used for comparisons within groups. For nonparametric analyses, a Mann-Whitney *U* test was used.

Results

EP receptor isoform mRNA expression in murine lymphocyte subsets. Because there is limited information regarding expression of EP receptor isoforms by various immune cell populations in the mouse, we used RT-PCR to examine EP receptor mRNA in cell populations isolated from mouse spleen. Using this RT-PCR assay, expression of EP1 receptor mRNA was easily detected in T and B lymphocytes, as well as macrophages (data not shown). Similarly, expression of mRNA for EP2 and EP4 receptors was present in all three splenocyte subsets (not shown). While all three isoforms of the EP3 receptors were detected in B cells and macrophages, the α and β isoforms could not be reproducibly amplified from splenic T cells (not shown). Thus, with the exception of α and β isoforms of the EP3 receptor in T cells, mRNA from all of the known EP receptors was detected in the major immune cell populations in mouse spleen.

PGE_2 analogues suppress proliferative responses of lymphocytes to alloantigens. To begin to define the actions of specific EP receptors to regulate immune responses, we first examined the effects of PGE_2 and several of its analogues upon the MLR, a model of the cellular alloimmune response. The MLR is designed to mimic the conditions that might occur in a transplanted organ when recipient immune cells are activated by recognition of foreign MHC antigens expressed on the donor tissue. One-way MLRs were performed using responders from wild-type (129SvEv) $H\text{-}2^b$ mice and irradiated stimulators from wild-type (C3H-HeJ) $H\text{-}2^k$ animals. Proliferative responses were compared between cultures containing PGE_2 analogues and those that received vehicle alone. The various PGE_2-agonists differ in their affinities for EP receptor isoforms (21). PGE_2 binds with high affinity to all four EP receptor subtypes. Misoprostol, at lower concentrations, exhibits relative specificity for EP3 and EP4 receptors (K_i = 67 nM), but it may also activate EP1 and EP2 receptors at higher concentrations (K_i = 120–250 nM) (21). Sulprostone, on the other hand, is a relatively specific agonist for the EP1 (K_i = 21 nM) and EP3 receptors (K_i = 0.6 nM) (21).

As depicted in Figure 1, PGE_2, among the compounds tested, caused the most potent inhibition of the cellular immune response; concentrations of 5–10 nM caused 50% inhibition of proliferation. Although misoprostol also inhibited the MLR, it was significantly less potent than PGE_2, requiring almost log dose-higher concentrations to produce 50% inhibition. In contrast, concentrations of sulprostone up to 50 μM did not substantially affect the vigor of the proliferative response. These data confirm the actions of PGE_2 to suppress cellular immune responses and suggest that EP2 and/or EP4 receptors may mediate these effects.

Identification of EP receptors that inhibit MLR using genetically altered mice. To more precisely identify the EP receptor isoforms that regulate proliferation in MLR, we compared the actions of PGE_2 upon responder cell populations derived from mice with targeted disruption of each of the individual EP receptor genes. Figure 2 summarizes the results of experiments with EP1-deficient (Figure 2a), EP3-deficient (Figure 2b), and EP4-deficient (Figure 2c) responders. PGE_2 caused potent inhibition of allospecific proliferation by the EP1- and EP3-deficient splenocytes, and this effect was virtually identical to that observed in their respective wild-type

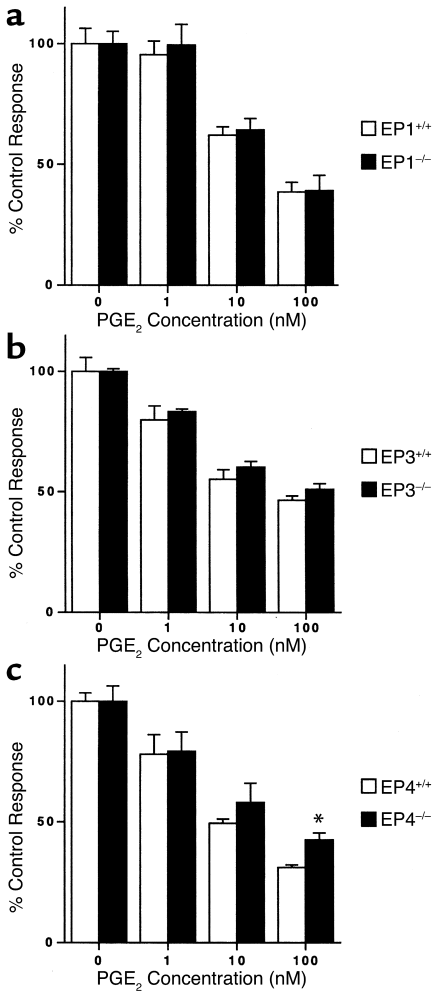

Figure 2

PGE_2 inhibits proliferation in MLR with EP1-, EP3-, and EP4-deficient lymphocytes. MLR experiments were performed comparing responders that were lacking (**a**) EP1, (**b**) EP3, and (**c**) EP4 receptors with their respective wild-type controls. On the *x*-axis the effects of various concentrations of PGE_2 upon proliferation were determined. The data are expressed as a percentage of the control response in MLR with vehicle alone. $*P = 0.008$ vs. $EP4^{+/+}$.

controls. In the lower concentration range, the inhibitory actions of PGE_2 on EP4-deficient cells were also similar to controls. However, at concentrations of 100 nM and above, the absence of EP4 receptors was associated with a modest, but significant reduction in sensitivity to the antiproliferative effects of PGE_2. By contrast, as depicted in Figure 3, EP2-deficient responders were markedly resistant to the inhibitory actions of PGE_2. At the lowest concentrations, PGE_2 had no effect on proliferation of the EP2-deficient cultures. At higher concentrations of PGE_2 that inhibited more than 60% of the response in wild-type cultures, only modest inhibition of the EP2-deficient responders was observed. These data indicate that EP2 receptors expressed by the responder population mediate most of the actions of PGE_2 to inhibit cellular immune responses in the MLR.

MLR with purified T cells. In our initial experiments, we studied responder cells consisting of a mixed population of splenocytes including T cells, B cells, and macrophages. Since all of these cell populations express various EP receptor isoforms, including EP2, EP-receptor–mediated effects in any or all of these cells might contribute to the actions of PGE_2 to modulate the cellular response to alloantigens. To determine whether PGE_2 influences allospecific proliferation by direct effects on T cells and to examine the role of the EP2 receptor in these actions, we performed additional MLR experiments using isolated T cells as responders. As shown by Figure 4 and similar to its actions on mixed splenocyte responders, PGE_2 caused significant inhibition of the MLR when the responders were a purified population of T cells isolated from wild-type mice. In marked contrast, concentrations of PGE_2 up to 30 μM had no effect on proliferation of isolated EP2-deficient T cells. Compared with the mixed population of EP2-deficient splenocytes, EP2-deficient T cells had a more complete resistance to PGE_2. The difference in PGE_2 responsiveness between $EP2^{-/-}$ mixed splenocytes and isolated T cells suggests a contribution of other (non-T cell) populations to the regulatory actions of PGE_2 in the MLR.

Distinct actions of PGE_2 on T cells and macrophages. To determine whether EP receptors on macrophages might contribute to the regulation of cellular immune responses by PGE_2, we performed reconstitution experiments with macrophages and isolated T cells. MLR cultures were set up with T cells from EP2-deficient splenocytes in the presence of exogenous PGE_2 (Figure 5). Some of the cultures were reconstituted with macrophages derived from wild-type or EP2-deficient mice. As shown previously, EP2-deficient T cells alone were completely resistant to the actions of PGE_2. Addition of syngeneic $EP2^{+/+}$ macrophages restored some sensitivity to the antiproliferative actions of

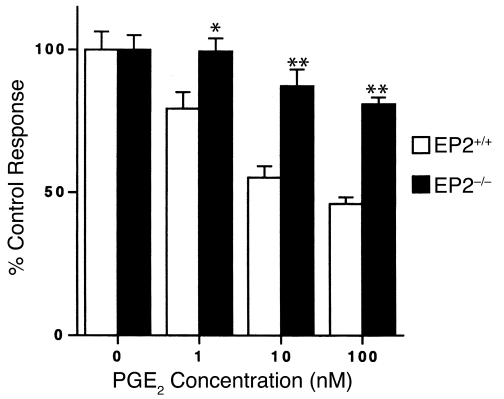

Figure 3

EP2-deficient lymphocytes are resistant to the antiproliferative actions of PGE_2 in the MLR. MLR experiments were performed comparing responders that were lacking EP2 receptors with their respective wild-type controls. On the *x*-axis, the effects of various concentrations of PGE_2 upon proliferation were determined. The data are expressed as a percentage of the control response in MLR with vehicle alone. $*P = 0.004$ vs. $EP2^{+/+}$; $**P < 0.0001$ vs. $EP2^{+/+}$.

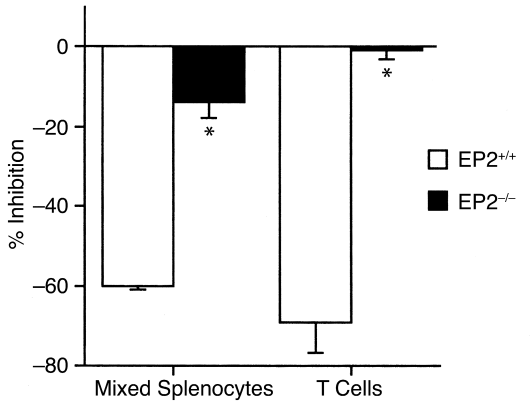

Figure 4
Comparison of PGE_2 effects on allospecific proliferation of EP2-deficient mixed splenocytes and T cells. MLR experiments were performed comparing responders that were lacking EP2 receptors (filled bars) with their respective wild-type controls (open bars). Responder cells were either unseparated populations of mixed splenocytes (left) or T cells that had been purified from the spleen (right). Allospecific proliferation was measured in the presence of 30 nM PGE_2 or vehicle. The data are expressed as percentage of inhibition compared with the MLR with vehicle alone. *$P < 0.001$ vs. $EP2^{+/+}$.

PGE_2. Inhibitory actions of PGE_2 were also conferred by $EP2^{-/-}$ macrophages, but the magnitude of inhibition was less prominent than with $EP2^{+/+}$ macrophages (13% vs. 27%; $P = 0.03$). These data suggest that EP receptors on autologous macrophages contribute to the regulation of the cellular alloimmune response.

EP receptors that regulate cytokine release by macrophages. Macrophages shape cellular immune responses in their capacity as antigen-presenting cells and by producing a variety of cytokines that can influence the proliferation and function of antigen-specific T cells. Because of this central role, along with our data implicating EP receptors on macrophages in modulating the alloimmune response, we examined the relative contributions of EP2 and EP4 receptors to the regulation of macrophage function. We focused on two cytokines, TNF-α and IL-12, which are known to regulate T cell function. As shown in Figure 6, PGE_2 caused dose-dependent inhibitions of TNF-α and IL-12 release in LPS-stimulated wild-type cells, with substantial reductions in cytokine release at concentrations greater than 30 nM PGE_2. In the EP2-deficient macrophages, the inhibitory actions of PGE_2 were virtually identical to those seen in the wild-type cells with 50% inhibition of cytokine release at concentrations of 1–3 nM PGE_2. In contrast, EP4-deficient macrophages were more resistant to the inhibitory actions of PGE_2 upon IL-12 release (Figure 6a). Furthermore, across the concentration range tested, PGE_2 had no detectable effect on TNF-α production by EP4-deficient, LPS-stimulated macrophages (Figure 6b).

Discussion

The actions of PGE_2 to regulate immune responses have been long recognized. PGE_2 modulates a wide range of T cell functions, and these actions are largely suppressive or inhibitory (3). For example, PGE_2 inhibits antigen-induced proliferation, cytokine production, and cell surface expression of cytokine receptors (9). A variety of effector functions are also inhibited by PGE_2, including the development of antigen-specific cytotoxic T cells (28). The capacity of PGE_2 to suppress many of the responses that are triggered by T cell receptor activation suggests that it acts at a central site in the biochemical cascade associated with T cell receptor stimulation. In this regard, Paliogianni and associates found that PGE_2 antagonizes the actions of calcineurin phosphatase to stimulate the transcription of cytokine genes (29), such as the IL-2 and IFN-γ genes, that are an important part of the genomic activation program in T cells. The inhibition of IL-2 and IFN-γ by PGE_2 may also contribute to its ability to skew cellular responses toward a Th2 phenotype.

These inhibitory actions of PGE_2 upon T cell functions can impact immune responses in the whole animal. In murine models of autoimmune disease, chronic administration of PGE_2 has dramatic effects to ameliorate the manifestations of autoimmunity and to prevent immune-mediated end organ injury (13, 14). Likewise, PGE_2 and its analogues dramatically suppress rejection of organ and tissue transplants (15–19). In some models, long-term allograft survival can be induced by treatment with PGE_2 and no other immunosuppressive therapy. Beneficial effects of E-series prostanoids in human transplant recipients have also been reported (20, 30, 31). Nonetheless, the precise role of individual EP receptor isoforms in mediating these actions is not clear. Because of the potent actions of PGE_2 to inhibit transplant rejection, we chose to first examine the role of EP receptor isoforms in the MLR, an in vitro model of the cellular alloimmune response.

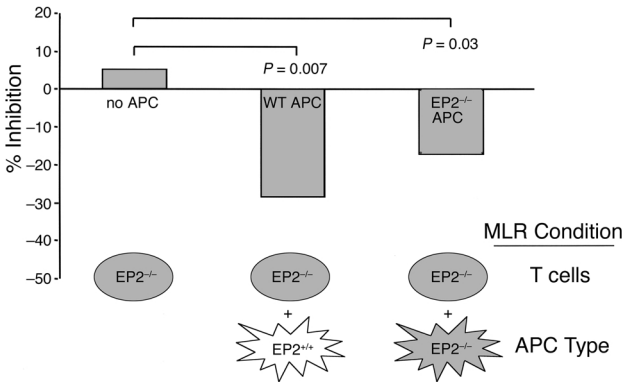

Figure 5
Relative contributions of T cells and macrophages to the inhibitory actions of PGE_2 in the MLR. MLR experiments were performed using T cells isolated from spleens of EP2-deficient mice. In some studies, bone marrow macrophages from $EP2^{+/+}$ or $EP2^{-/-}$ mice were added to the cultures as indicated. Allospecific proliferation was measured in the MLR in the presence or absence of 10 nM PGE_2. The data are expressed as percentage of inhibition compared with the corresponding control MLR with vehicle alone. WT, wild-type; APC, antigen presenting cells.

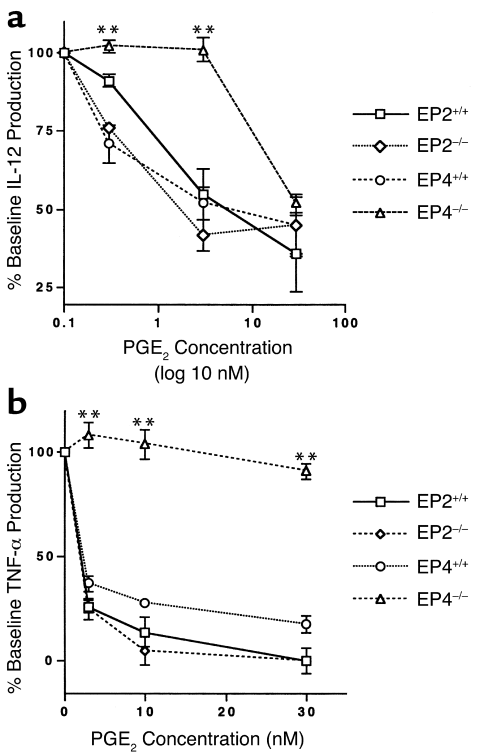

Figure 6
The EP4 receptor mediates inhibitory actions of PGE_2 on cytokine release by macrophages. Bone marrow macrophages were isolated from $EP2^{+/+}$, $EP2^{-/-}$, $EP4^{+/+}$, and $EP4^{-/-}$ mice. The macrophages were stimulated with 10 nM LPS, and various concentrations of PGE_2 were added (*x*-axis). Supernatants were collected, and release of IL-12 (**a**) and TNF-α (**b**) were measured. For clarity, the *x*-axis in **a** is expressed as log 10 nM. $EP4^{-/-}$, but not $EP2^{-/-}$, macrophages were resistant to the actions of PGE_2 to inhibit cytokine release. $^{**}P < 0.001$ vs. $EP4^{+/+}$.

Our studies using genetically manipulated mice indicate that the regulatory actions of PGE_2 in cellular immunity are complex, involving more than one receptor and different immune cell populations. Our data show that the EP2 receptor is a dominant mediator of the inhibitory actions of PGE_2 in the MLR. In T cells, absence of the EP2 receptor confers a resistance to PGE_2 that is virtually absolute. It is possible that the deletion of the EP2 receptor may have uncovered stimulatory actions of other EP receptors, such as EP3, that might contribute to the apparent resistance of EP2-deficient cells to PGE_2. However, no exaggerated effect of PGE_2 was observed in the EP3-deficient cells, which would be expected if the EP3 receptor has a major effect to antagonize EP2 signaling in this circumstance. Thus, despite the presence of multiple EP isoforms in T cells, the G_s-linked EP2 receptor alone seems to mediate the inhibition of antigen-specific proliferation by PGE_2. This finding is consistent with previous work showing that many of the inhibitory actions of PGE_2 upon T cells can be reproduced by cAMP or maneuvers that increase intracellular cAMP concentration (15, 29, 32). Although EP4 receptors are also expressed by murine T cells and these receptors also couple to adenylyl cyclase, our studies indicate that EP4 receptors do not directly modulate antigen-stimulated proliferation of T cells. At this point, our findings are most clearly relevant to murine systems. It is possible that there may be differences in expression and functions of EP receptors in human leukocytes.

While recognition of antigens by T cells initiates and drives cellular immune responses, macrophages also play several critical roles. They can efficiently present peptide antigens to the T cell in the context of MHC class II proteins and provide additional costimulatory signals that are required for T cell activation. In addition, macrophages produce a variety of cytokines and other mediators that shape the T cell response. PGE_2 has the capacity to inhibit or suppress many of these macrophage functions. For example, PGE_2 inhibits expression of MHC class II proteins (8) and thus may interfere with antigen-presenting functions of the macrophage. Cytokine production is likewise inhibited by PGE_2 (6, 7). Our studies have demonstrated that the actions of PGE_2 to suppress cytokine production by macrophages are mediated almost exclusively by the EP4 receptor; PGE_2 has no effect on LPS-stimulated cytokine release in EP4-deficient macrophages. In MLR, enhanced production of cytokines such as TNF-α and IL-12 contributes to the vigor of the proliferative response (33, 34), and therefore suppression of monokine production by PGE_2 might contribute to the overall actions of PGE_2 to reduce proliferation. The modest resistance of EP4-deficient mixed splenocytes to PGE_2 may be explained by a failure to inhibit TNF-α and/or IL-12 production by macrophages. If so, the apparent contribution of this pathway to the overall actions of PGE_2 in the MLR is relatively small. However, in other circumstances where monokine production has a more critical role, the inhibitory actions of EP4 receptors may have more profound consequences. Furthermore, IL-12 production by antigen-presenting cells drives $CD4^+$ T cell differentiation toward the Th1 phenotype (35). Accordingly, inhibition of IL-12 production by PGE_2 may contribute to its propensity to promote Th2 responses.

Along with regulation of cytokine release, the reconstitution MLR experiments suggest other actions of PGE_2 in the macrophage that modulate cellular immunity. In these studies (Figure 5), the addition of macrophages to the cultures of EP2-deficient T cells partially restored the inhibitory actions of PGE_2 on alloantigen-induced proliferation. These findings are most consistent with active suppression and suggest that PGE_2 may induce a factor in macrophages that inhibits the MLR. As the magnitude of suppression was less with EP2-deficient compared with wild-type macrophages, the EP2 receptor seems to contribute to this activity. Because of the mixed genetic background of the EP4-deficient mice, an analogous reconstitution experiment to determine the contribution of the EP4 receptor could not be carried out. Nonetheless, our findings suggest that the macrophage plays a complex role in mediating inhibitory actions of PGE_2 in cellular immunity. Furthermore, in the macrophage as in the T cell, EP2 and

EP4 receptors have divergent functions despite their similar intracellular signaling pathways. A mechanism to explain this apparent compartmentalization of signal-effector coupling remains to be defined.

Our studies have identified distinct actions of different EP receptor isoforms to regulate cellular immune responses. These receptors, EP2 and EP4, therefore represent potential targets for immunomodulatory therapies. Although the immunosuppressive actions of PGE_2 are well recognized, problematic side effects related to its broad biological activities along with difficult pharmacokinetics have made it impractical to use as a therapeutic agent. Identification of small molecules that interact specifically with immunoregulatory EP receptor isoforms may allow a wider, more practical exploitation of these pathways in the therapy of autoimmune diseases and transplant rejection. The distinct separation of functions of these receptors within individual cells provides a potential mechanism for modulating immune responses with relatively fine specificity affecting a range of immunological functions.

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1. Needleman, P., Turk, J., Jakschik, B., Morrison, A., and Lefkowith, J. 1986. Arachidonic acid metabolism. *Annu. Rev. Biochem.* **55**:69–102.
2. Smith, W. 1992. Prostanoid biosynthesis and mechanisms of action. *Am. J. Physiol.* **263**:F181–F191.
3. Tilley, S.L., Coffman, T.M., and Koller, B.H. 2001. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J. Clin. Invest.* **108**:15–23.
4. Goodwin, J., Bankhurst, A., and Messner, R. 1977. Suppression of human T-cell mitogenesis by prostaglandin. Existence of a prostaglandin-producing suppressor cell. *J. Exp. Med.* **146**:1719–1734.
5. Minakuchi, R., Wacholtz, M., Davis, L., and Lipsky, P. 1990. Delineation of the mechanism of inhibition of human T cell activation by PGE2. *J. Immunol.* **145**:2616–2625.
6. Scales, W., Chensue, S., Otterness, I., and Kunkel, S. 1989. Regulation of monokine gene expression: prostaglandin E2 suppresses tumor necrosis factor but not interleukin-1 alpha or beta m-RNA and cell associated bioactivity. *J. Leukoc. Biol.* **45**:416–421.
7. van der Pouw Kraan, T., Boeije, L., Smeenk, R., Wijdenes, J., and Aarden, L. 1995. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J. Exp. Med.* **181**:775–779.
8. Snyder, D., Beller, D., and Unanue, E. 1982. Prostaglandins modulate macrophage Ia expression. *Nature.* **299**:163–166.
9. Goetzl, E., An, S., and Smith, W. 1995. Diversity and specificity of the eicosanoid mediators of normal physiology and disease. *FASEB J.* **9**:1051–1058.
10. Betz, M., and Fox, B. 1991. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J. Immunol.* **146**:108–113.
11. Katamura, K., et al. 1995. Prostaglandin E2 at priming of naive CD4+ T cells inhibits acquisition of ability to produce IFN-gamma and IL-2, but not IL-4 and IL-5. *J. Immunol.* **155**:4604–4612.
12. Roper, R., Brown, D., and Phipps, R. 1995. Prostaglandin E2 promotes B lymphocyte Ig isotype switching to IgE. *J. Immunol.* **154**:162–170.
13. Kelley, V., Winkelstein, A., and Izui, S. 1979. Effect of prostaglandin E on immune complex nephritis in NZB/W mice. *Lab. Invest.* **41**:531–537.
14. Zurier, R., Damjanov, I., Sayadoff, D., and Rothfield, N. 1977. Prostaglandin E1 treatment of NZB/NZW F1 mice. *Arthritis Rheum.* **20**:1449–1456.
15. Strom, T., and Carpenter, C. 1983. Prostaglandin as an effective anti-rejection therapy in rat renal allograft recipients. *Transplantation.* **35**:279–280.
16. Anderson, C., Jaffe, B., and Graff, R. 1977. Prolongation of murine skin allogratfs by prostaglandin E1. *Transplantation.* **23**:444–447.
17. Fabrega, A., Blanchard, J., Rivas, P., Moran, A., and Pollak, R. 1992. Prolongation of rat heart allograft survival using cyclosporine and enisoprost, a PGE1 analog. *Transplantation.* **53**:1363–1364.
18. Kamei, T., Callery, M.P., and Flye, M. 1991. Intragraft delivery of 16,16-dimethyl PGE2 induces donor-specific tolerance in rat cardiac allograft recipients. *Transplantation.* **51**:242–246.
19. Koh, I., et al. 1992. The effects of 16,16-dimethyl prostaglandin E2 therapy alone and in combination with low-dose cyclosporine on rat small intestinal transplantation. *Transplantation.* **54**:592–598.
20. Moran, M., et al. 1990. Prevention of acute graft rejection by the PGE1 analog misoprostol in renal-transplant recipients treated with cyclosporine and prednisone. *N. Engl. J. Med.* **322**:1183–1188.
21. Narumiya, S., Sugimoto, Y., and Ushikubi, F. 1999. Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* **79**:1193–1225.
22. Sugimoto, Y., Narumiya, S., and Ichikawa, A. 2000. Distribution and function of prostanoid receptors: studies from knockout mice. *Prog. Lipid Res.* **39**:289–314.
23. Fleming, E., et al. 1998. Urinary concentrating function in mice lacking EP3 receptors for prostaglandin E_2. *Am. J. Physiol.* **275**:F955–F961.
24. Nguyen, M., et al. 1997. The prostaglandin receptor EP_4 triggers remodeling of the cardiovascular system at birth. *Nature.* **390**:78–81.
25. Stock, J., et al. 2001. The EP1 receptor for PGE_2 mediates inflammatory pain responses and regulates blood pressure. *J. Clin. Invest.* **107**:325–331.
26. Tilley, S., et al. 1999. Reproductive failure and reduced blood pressure in mice lacking the EP2 prostaglandin E_2 receptor. *J. Clin. Invest.* **103**:1539–1545.
27. Nataraj, C., et al. 1999. Angiotensin II regulates cellular immune responses through a calcineurin-dependent pathway. *J. Clin. Invest.* **104**:1693–1701.
28. Leung, K., and Mihich, E. 1980. Prostaglandin modulation of development of cell-mediated immunity in culture. *Nature.* **288**:597–600.
29. Paliogianni, F., Kineaid, R., and Boumpas, D. 1993. Prostaglandin E2 and other cyclic AMP elevating agents inhibit interleukin 2 gene transcription by counteracting calcineurin-dependent pathways. *J. Exp. Med.* **178**:1813–1817.
30. Henley, K., et al. 1995. A double-blind, randomized, placebo-controlled trial of prostaglandin E1 in liver transplantation. *Hepatology.* **21**:366–372.
31. Tancharoen, S., Jones, R., Angus, P., and Hardy, K. 1993. Beneficial effect of PGE1 on hepatic allograft rejection following orthotopic liver transplantation. *Transplant. Proc.* **25**:2890–2891.
32. Goetzl, E., An, S., and Zeng, L. 1995. Specific suppression by prostaglandin E2 of activation-induced apoptosis of human CD4+CD8+ T lymphoblasts. *J. Immunol.* **154**:1041–1047.
33. Christensen, P., et al. 1993. Characterization of the production of monocyte chemoattractant protein-1 and IL-8 in an allogeneic immune response. *J. Immumol.* **151**:1205–1213.
34. Ohshima, Y., and Delespesse, G. 1997. T cell derived IL-4 and dendritic cell-derived IL-12 regulate the lymphokine-producing phenotype of alloantigen-primed naïve human CD4 T cells. *J. Immunol.* **158**:629–636.
35. Abbas, A., Murphy, K., and Sher, A. 1996. Functional diversity of helper T lymphocytes. *Nature.* **383**:787–793.