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Research Article

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Prostaglandin E_1 Inhibits Effector T Cell Induction and Tissue Damage in Experimental Murine Interstitial Nephritis

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Abstract

Immunosuppressive effects of E-series prostaglandins have been demonstrated in many in vitro assays of immune responsiveness as well as in autoimmune diseases. To explore the mechanisms underlying prostaglandin E_1 (PGE_1)-associated immunosuppression in autoimmunity, we treated SJL mice immunized to produce immune-mediated interstitial nephritis with PGE_1, $PGF_{2\alpha}$, or vehicle alone. Mice receiving PGE_1 treatment do not develop interstitial nephritis, nor do they display delayed-type hypersensitivity (DTH) to the immunizing renal tubular antigen preparation. The observed immunosuppression is critically dependent on PGE_1 administration during the period of effector T cell induction. We therefore investigated the effect of PGE_1 on the in vitro induction of DTH effector T cells reactive to renal tubular antigens (SRTA). PGE_1 inhibits effector T cell induction in a dose-dependent, reversible manner, but has no inhibitory effect on fully differentiated DTH effector cells or SRTA-reactive cell lines. The PGE_1 effect is indirect and mediated via nonspecific suppressor lymphokines. This suppression can be overcome by recombinant interleukin 1 (IL-1), which suggests a mechanism related to either diminished IL-1 secretion or target cell sensitivity to IL-1.

Introduction

Inhibitory effects of E-series prostaglandins (PGE)[1] on lymphoid cells in culture have been described by many investigators (1). Subsequent studies have extended these in vitro observations by examining the protective effect of exogenously administered PGE_1 or PGE_2 on experimental autoimmune diseases, including several murine models of immune complex glomerulonephritis (2–5). Glomerular injury in these models has been attributed to the damage resulting from deposited or locally formed immune complexes, with PGE administration acting either to diminish titers of relevant circulating antibody (4, 5) or to inhibit local antibody or immune complex deposition (2, 3). Although in some studies quantitative changes in T cell subpopulations have been observed concomitant with PGE therapy (4–6), it is uncertain whether these latter findings mediate or have a role in suppression of disease activity. This uncertainty is due in part to lack of a well-defined effector role for auto-sensitized T cells in these models of glomerulonephritis.

We have been interested in characterizing the regulation of antibody and effector T cell function in experimental interstitial nephritis (7–10). This renal lesion can be induced in susceptible rodents by immunization with renal tubular basement membranes (RTA) in complete Freund's adjuvant (CFA). In SJL mice, a prototypic susceptible strain, antibodies to tubular basement membrane (αTBM-Ab) are demonstrable in the serum and kidneys 7–10 d after immunization. Interstitial mononuclear cell infiltrates and progressive tubular atrophy with fibrosis develop 8–10 wk later (11). This lesion can be transferred to naive mice either with antibodies or T cells from immunized syngeneic donors (12), or with long-term cultured tubular antigen-reactive helper T cells (13). These cultured helper cells appear not to directly injure the kidney but rather induce, in the naive recipient, Lyt-2^+, antigen-specific effector T cells, which both mediate delayed-type hypersensitivity (DTH) to soluble tubular antigens as well as tubulo-interstitial injury. The induction of nephritogenic effector T cells can also be carried out in vitro (13), thus facilitating an analysis of the requirements for and the modulation of effector T cell differentiation.

In the present studies we examined the effect of exogenous PGE_1 both on experimental interstitial nephritis, and the in vitro induction of effector cells. Our results demonstrate that PGE_1 can markedly impair histologic interstitial nephritis, as well as DTH reactivity to tubular antigen, but only when administered from the time of immunization. The in vitro studies further indicate that PGE_1-mediated suppression of disease correlates with a dose-dependent and reversible inhibition of effector T cell differentiation. This inhibition, in turn, is mediated by PGE_1-induced soluble spleen cell products that can be overcome with exogenous interleukin 1 (IL-1).

Methods

Animals. SJL mice were purchased from Jackson Laboratory, Bar Harbor, ME and maintained by the Department of Laboratory and Animal Medicine, University of Pennsylvania School of Medicine.

Antigens. Rabbit RTA were isolated by a differential sieving technique. Highly enriched basement membrane fragments were sonicated, lyophilized, and stored at −20°C (14). Soluble renal tubular antigens (SRTA) were made from these lyophilized membranes by collagenase digestion (15). Purified protein derivative (PPD) was purchased from Connaught Laboratories, Ltd., Toronto, Ontario, Canada.

Induction of disease. Mice were immunized to produce disease by

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1. *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; PGE_1, PGE_2, and $PGF_{2\alpha}$, prostaglandins E_1, E_2, and $F_{2\alpha}$, respectively; PITS, PG-induced T suppressor factors; PPD, purified protein derivative; PVC, polyvinyl chloride; RTA, renal tubular basement membrane antigens; SRTA, soluble renal tubular antigens; αTBM-Ab, antibodies to renal tubular basement membrane antigens; ThF^s, soluble helper factors derived from Th^s; Th^s, SRTA-reactive helper T cell line.

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injecting 2 mg of RTA in CFA (2 mg of PPD per animal) in multiple sites (11). Control animals received CFA alone.

In vivo administration of prostaglandins. Groups of mice immunized to produce interstitial nephritis were injected intraperitoneally with (15S)-15-methyl PGE_1, or $PGF_{2\alpha}$ (12 μg in 10% alcohol) 5 of every 7 d until killing 10 wk later. Prostaglandin injections were started either on the day of immunization or 1 wk after immunization. Control mice received 10% alcohol alone. 15-methyl PGE_1 was the gift of G. D. Searle Co., Chicago, IL, and $PGF_{2\alpha}$ the gift of Dr. John Pike, Upjohn Co., Kalamazoo, MI.

Assessment of renal disease in immunized mice. Kidneys were harvested from groups of mice 10 wk after immunization. Kidney tissue was prepared for immunofluorescent and light microscopy by standard methods for this laboratory (12). Direct immunofluorescence of tubular staining by αTBM-Ab was qualitatively graded from 0 to 4+ and expressed as a mean±SEM for each group (12). Kidneys fixed in 10% formalin were sectioned and stained with hematoxylin and eosin, coded, and graded according to the extent of cortical involvement using established criteria (12): 0, normal; 0.5, small focal areas of cellular infiltration; 1, cellular infiltration and tubular damage involving < 10% of the cortex; 2, involvement up to 25% of the cortex; 3, involvement of up to 50–75% of the cortex; and 4, extensive damage involving > 75% of the cortex. Scores for each experimental group were averaged and expressed as a mean±SEM. Sera collected from mice at the time of killing were also assayed for presence and titer of αTBM-Ab using a solid-phase radioimmunoassay (10). 25 μl of SRTA (15 μg/ml) was added to wells of polyvinyl chloride (PVC) microtiter plates and kept at 4°C overnight. Wells were blocked with 4% bovine serum albumin, overlaid with serial dilutions of test antisera, and subsequently incubated with ^{125}I-rabbit anti-mouse IgG. This assay is specific for αTBM-Ab (10).

Adoptive cell transfer and measurement of DTH to SRTA. Draining lymph node cells were harvested from groups of immunized mice 10 wk after treatment with prostaglandins. Single cell suspensions were washed, counted, and 15×10^6 donor cells per mouse were intravenously injected into naive, syngeneic recipients. In some groups these cells were T lymphocyte–depleted with monoclonal αThy-1.2 antibody (from Jlj hybridoma [16]) and a mixture of rabbit and guinea pig complement (C). This treatment kills ~ 60% of SJL lymph node cells. In other studies lymph node cells from different experimental groups of donor mice were admixed in equal numbers and co-transferred intravenously into naive recipients (30×10^6 cells/recipient). Shortly (1–2 h) after intravenous cell transfer the recipients were challenged in the footpad with 25 μg of SRTA or PPD (Connaught Laboratories, Ltd.) in 25 μl phosphate-buffered saline (PBS), or with PBS alone. Footpad swelling as an index of DTH was measured 24 h later with a spring-loaded engineer's micrometer (Schlesinger's for Tools, Brooklyn, NY). The magnitude of swelling was expressed as the increment between the antigen-challenged footpad and that injected with PBS, reported as the mean±SEM (8). A modification of this protocol was used for assessing the in vitro induction of DTH-reactive cells (13). In these latter studies cultured lymphocytes, under various experimental conditions, were harvested after 5 d (except where indicated). Cells were resuspended at 4×10^7 cells/ml in SRTA (1 mg/ml) or PBS, and 25 μl of each cell preparation was then injected into one footpad of syngeneic mice (four recipients per group). Magnitude of swelling was expressed as described above. All DTH measurements were cage blind.

Helper T cell lines. SRTA-reactive helper T cell line (Th^s, SJL origin) was carried by weekly passage at $5–10 \times 10^4$ cells/well in 2 ml of standard media consisting of RPMI 1640 supplemented with IL-2 (MLA-144 [17]), 10% fetal calf serum, SRTA (20 μg/ml), 5% NCTC, 5×10^{-5} 2-mercaptoethanol, and antibiotics with 5×10^6 irradiated feeder cells in 24-well plates. Their characteristics and specificity have been described (13). Supernatants from these cultured cells were used as a source of a soluble helper factor (ThF^s). In selected studies PGE_1 or $PGF_{2\alpha}$ were also added to the culture media. Supernatants at the end of these cultures were extensively dialyzed to remove free prostaglandin before their use as a source of ThF^s (18). The effectiveness of removing prostaglandins from the cultures by dialysis was monitored by radioimmunoassay (19).

In vitro effector T cell induction assay. Th^s cell lines (1×10^6 cells/flask) or ThF^s (10% final volume) were co-cultured in standard media with 30×10^6 nonirradiated SJL splenic feeder cells, 20 μg/ml SRTA, and IL-2 (20% MLA-144 supernatant) in flasks at 37°C, 5% Co_2 for 5–10 d (13). In some experiments, PGE_1 or $PGF_{2\alpha}$ in varying concentrations were added to these flasks for the entire incubation period or for defined time intervals. In other studies the feeder cells were pulsed with PGE_1 for only the first 24 h of culture. After this pulse, the feeder cells were then washed and cultured alone with SRTA, IL-2, and ThF^s for varying periods of time, or co-cultured with fresh spleen cell subpopulations. Fresh spleen cells in some experiments were separated by two sequential incubations on polystyrene plates for 1 h at 37°C in 5% Co_2. 3×10^6 adherent antigen-presenting cells and 27×10^6 nonadherent cells were added in the reconstitution studies. Nonadherent cells in some groups were further treated with optimal dilutions of αThy1.2 (Jlj) or αB (Jlld) (16) cell antibodies and rabbit/guinea pig complement before reconstitution. This treatment kills ~ 35–40% of SJL spleen cells. Cells from such cultures, after incubation, were harvested and assayed for DTH reactivity using the local adoptive transfer assay described above.

Production of prostaglandin-induced T suppressor substances (PITS). Supernatants were harvested from PGE_1 containing effector cell induction flasks after 5 d in culture. These supernatants were extensively dialyzed and then added in varying amounts (% final volume) to fresh induction cultures. Nondialyzed PGE_1 remaining in the day-5 supernatants was quantified (~ 2 ng/ml) and added to control cultures.

Effect of murine recombinant IL-1 on PGE_1-associated inhibition of effector cell induction. Recombinant murine IL-1 (20) was generously supplied by Dr. Peter Lomedico of the Roche Research Center, Nutley, NJ. 7 M guanidine HCl extracts of *Escherichia coli* containing the recombinant plasmid were used as a source of biologically active IL-1. Varying dilutions of this extract (units per millimeter) were added to the effector cell induction flasks described above at day 0. The specific activity of this preparation is 6×10^6 U/mg (20). The final concentration of guanidine HCl in all flasks was 2.5×10^{-4} M. In some experiments heat-inactivated recombinant IL-1 (100°C for 1 h) was used as a control.

Statistical analysis. Differences between experimental groups were determined by Student's *t* test.

Results

Suppression of histologic interstitial nephritis by treatment with PGE_1. In a series of introductory experiments we examined the effect of PGE_1 or $PGF_{2\alpha}$ administration on the expression of disease in SJL mice immunized with RTA/CFA. As seen in Table I and Fig. 1, *A* and *B*, mice receiving PGE_1 from the time of immunization (day 0) displayed a markedly less severe interstitial lesion than the cohorts receiving no treatment, PBS alone, or $PGF_{2\alpha}$ injections. Since previous studies from our laboratory have shown that nephritogenic effector cells are induced in mice during the first week after immunization, we examined whether PGE_1 treatment during this induction period was critical. We were intrigued to find that when PGE_1 treatments were begun on day 7, no protective effect was demonstrable (Table I, Fig. 1 *C*). Regardless of the severity of the interstitial nephritis in the various groups, all groups displayed similar amounts of bound αTBM-Ab by direct immunofluorescence (Table I), and similar serum titers of circulating αTBM-Ab by radioimmunoassay (data not shown).

Previous work from our laboratory has demonstrated the validity and feasibility of using DTH responses to tubular antigen (SRTA) as a measure of the nephritogenic effector T cell repertoire (8, 13). To examine a possible effect of PGE_1 on cellular effector mechanisms, we tested whether the DTH response to SRTA correlated with disease activity in the various treatment

Table I. Prostaglandin Effect on Experimental Interstitial Nephritis

Immunization*	Treatment‡ (start day)	αTBM-Ab (direct IF)	Degree of histologic injury§
RTA/CFA	—	3.5±0.5	3.2±0.3 (4)
RTA/CFA	PBS (day 0)	3.8±0.2	2.6±0.6 (9)
RTA/CFA	PGE_1 (day 0)	3.7±0.2	0.2±0.1 (13)‖
RTA/CFA	PGE_2 (day 7)	3.6±0.3	2.9±0.5 (6)
RTA/CFA	$PGF_{2\alpha}$ (day 0)	3.7±0.1	3.1±0.4 (8)
CFA	—	0.0	0.0 (7)

* Naive SJL mice were immunized in the footpads and subcutaneously with RTA/CFA or CFA as indicated on day 0.

‡ Various groups were injected with 12 μg of PGE_1 or $PGF_{2\alpha}$ in 10% alcohol, or 10% alcohol alone, starting the day of immunization or 7 d after immunization. Injections were given intraperitoneally 5 of every 7 d for 10 wk.

§ Kidneys were harvested 10 wk after immunization. Numbers in parentheses indicate number of mice in each group. One kidney from each animal was harvested for histology and one for immunofluorescence.

‖ $P < 0.0005$ compared with RTA/CFA mice receiving no treatment.

groups. As indicated in Table II, immune lymphocytes from mice immunized 10 wk previously with RTA/CFA transfer a DTH response to SRTA and PPD into naive syngeneic recipients. This reactivity is eliminated by treatment of the lymph node cells with αThy-1.2 and C. These DTH-reactive cells were not functionally present in immunized mice treated with PGE_1 from day 0, but were present in all other experimental groups. To examine if the absence of a DTH response in the PGE_1-treated cohort was attributable to active suppression, we admixed immune lymphocytes from RTA/CFA-immunized mice with cells from mice treated with PGE_1. Previous studies from our laboratory have shown that functional suppression of the DTH response can be demonstrated when suppressor cells are acutely admixed and co-transferred with DTH-reactive cells (21). As indicated in Table II, the absence of a DTH response in the PGE_1-treated group could not be attributed to active suppression.

In vitro induction of tubular antigen-reactive effector cells inhibited by PGE_1. In previous studies we have described an in vitro system that induces nephritogenic effector T cells in 5-d cultures (13). Long-term cultured $L3T4^+$ T helper cells (Th^s) that are specifically reactive to tubular antigen, or soluble factors from these cells (ThF^s) (Hines, W. H., R. Mann, C. J. Kelly, and E. G. Neilson, manuscript in preparation), will induce $Lyt\text{-}2^+$ effector cells from a population of naive SJL splenic feeder cells in the presence of antigen, antigen-presenting cells, and MLA-144 supernatants as a source of IL-2. These culture-induced $Lyt\text{-}2^+$ effector cells, unlike the helper Th^s cells, can mediate DTH to SRTA as well as produce acute tubulo-interstitial injury (13). Since PGE_1 must be administered in vivo on days

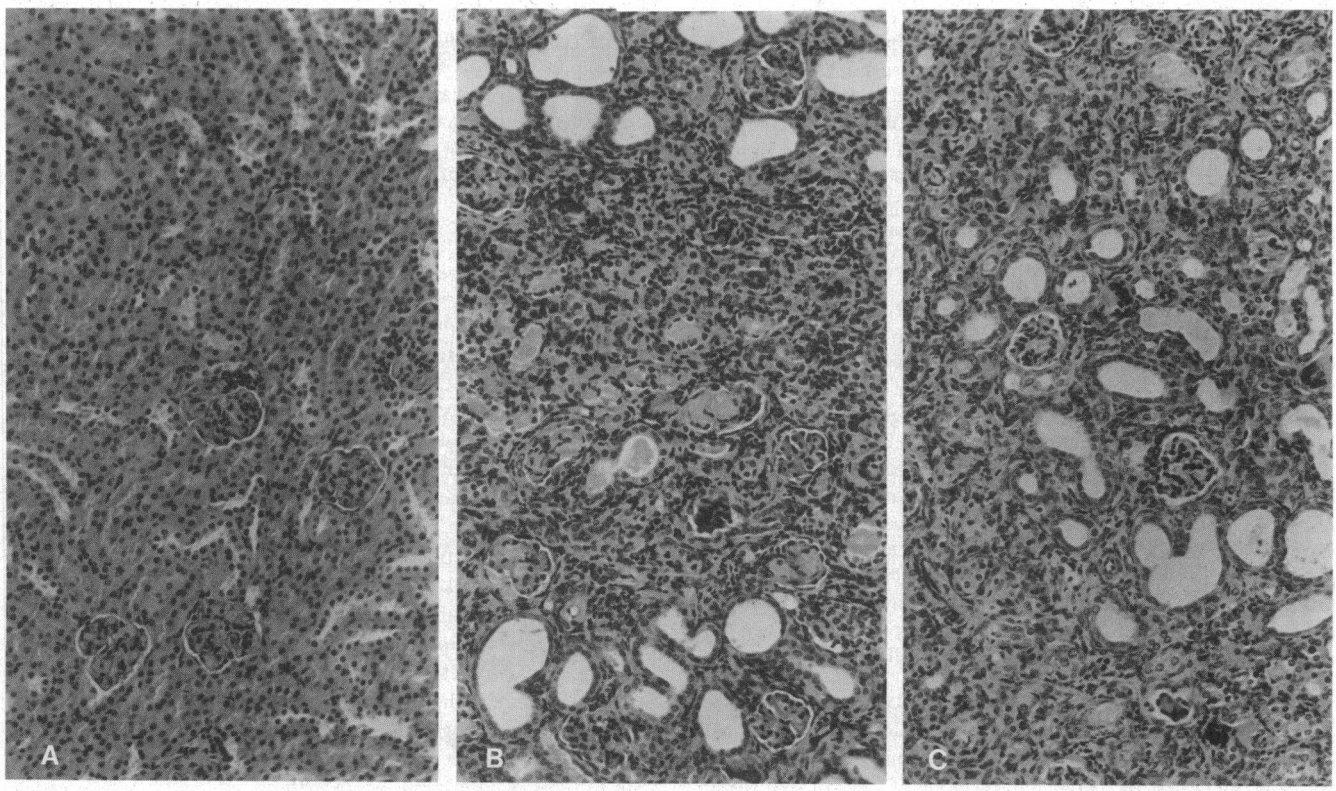

Figure 1. Representative renal lesions from SJL mice immunized with RTA/CFA and treated with prostaglandins. Histologic sections were prepared from animals killed 10 wk after immunization: (*A*) PGE_1 treatment from day 0; the kidneys were normal and indistinguishable from those of animals immunized with CFA alone. (*B*) $PGF_{2\alpha}$ treatment from day 0. There is a diffuse mononuclear cell infiltrate in the interstitium with total obliteration of the interstitial architecture. Severity of the lesions in this group were similar to those in RTA/CFA immunized mice receiving no prostaglandin. (*C*) PGE_1 treatment from day 7. The mononuclear cell infiltration, tubular atrophy, and derangement of the interstitial architecture are comparable to that seen in the $PGF_{2\alpha}$-treated cohort and markedly different from the histology displayed in *A*. × 200.

Table II. PGE_1 Inhibits DTH Reactivity in RTA/CFA Immunized Mice

Donor immunization* (cell treatment)	Treatment of animals‡	DTH response§ PPD	DTH response§ SRTA
RTA/CFA	—	16.2±0.5	15.5±0.8
RTA/CFA (C)	—	16.3±0.5	16.0±0.4
RTA/CFA (αThy-1.2 + C)	—	3.0±1.0‖	4.0±1.2‖
RTA/CFA	PBS	15.3±0.3	14.2±0.6
RTA/CFA	PGE_1 (day 0)	4.5±0.8‖	4.8±0.7¶
RTA/CFA	PGE_1 (day 7)	19.2±0.9	19.4±0.6
RTA/CFA	$PGF_{2\alpha}$ (day 0)	16.5±0.5	15.8±0.4
RTA/CFA	—		
RTA/CFA	PGE_1 (day 0)	—	16.3±0.8
RTA/CFA	—		
RTA/CFA	$PGF_{2\alpha}$ (day 0)	—	16.3±0.8
CFA	—	16.0±0.9	3.8±0.2‖

* Draining lymph node cells were harvested from mice immunized 10 wk earlier with RTA/CFA or CFA alone. Cells were treated, where indicated, with monoclonal antibodies and/or complement before adoptive intravenous transfer into naive syngeneic mice (15×10^6 cells of each group per recipient).

‡ Prostaglandin treatments were performed as described in Table I. Where indicated, lymph node cells from prostaglandin-treated mice were admixed with those from untreated, immunized mice before adoptive transfer.

§ Recipients were footpad challenged with antigens after cell transfer and mean footpad increment in inches $\times 10^{-3}$±SEM ($n = 4$) was determined 24 h later. Preinjection footpad measurements were typically 80–85 in. $\times 10^{-3}$.

‖ $P < 0.0005$, compared with positive control.

¶ $P < 0.001$, compared with positive control.

Table III. PGE_1 Inhibits Effector Cell Induction by Th^s or ThF^s

Group	Th^s/ThF^s*	SRTA (20 μg/ml)	Prostaglandin (12 μg/ml)	DTH response‡
A	Th^s	+	—	16.3±0.5
	Th^s	+	PGE_1	4.8±0.3§
	Th^s	+	$PGF_{2\alpha}$	15.8±0.9
	Th^s	—	—	5.8±0.8§
B	ThF^s	+	—	16.5±0.3
	ThF^s	+	PGE_1	6.0±0.6§
	ThF^s	+	$PGF_{2\alpha}$	17.3±0.8
	ThF^s	—	—	4.5±0.6§

* Th^s cells (1×10^6 per 30×10^6 naive spleen cells) or ThF^s (added at 10% of culture media volume) were added to flasks containing naive syngeneic spleen cells, IL-2, and SRTA or prostaglandins where indicated. Flasks were cultured at 37°C for 5 d. After 5 d, the cells were harvested, washed, and counted. 1×10^6 in 25 μl of SRTA (1 mg/ml) or PBS were injected into footpads of naive syngeneic mice.

‡ 24 h later mean footpad increment in inches $\times 10^{-3}$±SEM between cells injected with SRTA versus PBS was recorded ($n = 4$ for each group).

§ $P < 0.0005$, compared with SRTA-containing flasks without added prostaglandin.

0–7 to be effective (the time during which induction of the effector T cell repertoire occurs) we tested whether PGE_1 had a demonstrable effect in this in vitro induction system. In Table III (A), in the presence of 12 μg/ml (3×10^{-5} M) PGE_1, the SRTA-reactive cell line, Th^s, was unable to induce an effector population mediating DTH. $PGF_{2\alpha}$ had no such inhibitory effect. Part B of Table III demonstrates that the inhibitory effect of PGE_1 was also evident when soluble factors from Th^s cells (ThF^s) were used to induce the effector cells. In either setting, the presence of PGE_1 did not effect the total cell count or cell viability after 5 d in culture (data not shown). The findings in parts A and B of Table III confirm our previous observation that tubular antigen is obligatory for effector cell induction to occur (13).

Further support for the biologic significance of the PGE_1 effect was also obtained by examining the results of serial 10-fold dilutions of PGE_1 on effector cell induction. As shown in Table IV there was a clear dose-response effect of PGE_1, with ~ 50% inhibition of the DTH response seen with 0.12 μg/ml of PGE_1 (3×10^{-7} M). $PGF_{2\alpha}$, as before, had no inhibitory effect on effector cell induction.

No PGE_1 inhibition of the production of ThF^s. Although the results in Table III indicate that the PGE_1 effect cannot be overcome solely by the addition of exogenous helper factors, this did not exclude the possibility that PGE_1 also inhibits ThF^s production in addition to other steps in effector cell induction. To test the effect of PGE_1 on helper factor production, we harvested supernatants from Th^s cells after 5 d in culture with antigen (SRTA), IL-2, and irradiated SJL spleen cells in the presence of PGE_1 or $PGF_{2\alpha}$. After extensive dialysis to remove the free prostaglandin, these supernatants were used as a source of ThF^s in the effector cell induction assay. PGE_1 remaining in the supernatant after dialysis, ~ 2 ng/ml (6×10^{-9} M) by radioimmunoassay, was added to the control flasks (18). As shown in Table V, ThF^s harvested in this manner was capable of inducing SRTA-reactive effector cells mediating DTH regardless of the presence

Table IV. Dose-Response Effect of PGE_1 Inhibition of Effector Cell Induction

Induction cultures* Prostaglandin	Induction cultures* SRTA	DTH response to SRTA‡	Percent inhibition¶
—	+	16.7±0.3	—
—	—	3.0±0.6§	—
$PGF_{2\alpha}$ (12 μg/ml)	+	16.0±0.7	5
PGE_1 (12 μg/ml)	+	3.7±1.2§	95
PGE_1 (1.2 μg/ml)	+	6.0±1.2‖	78
PGE_1 (0.12 μg/ml)	+	9.3±1.0‖	54
PGE_1 (0.012 μg/ml)	+	16.3±0.9	3

* Culture flasks were prepared as described in Table III. Final concentration of alcohol in all flasks was 0.2%.

‡ See Table III.

§ $P < 0.0005$, compared with SRTA-containing flasks without added PGE_1.

‖ $P < 0.005$, compared with SRTA-containing flasks without added PGE_1.

¶ Percent inhibition was calculated as 1 − (experimental DTH response − negative control)/(positive control − negative control) × 100.

Table V. PGE_1 Does Not Affect ThF Production by the Th^s Cells

Induction cultures*		
ThF	SRTA	DTH response to SRTA‡
ThF^s	—	5.3±0.5
ThF^s	+	16.2±0.4
$ThF^s + PGE_1$	+	17.0±0.6
$ThF^s + PGF_{2\alpha}$	+	16.5±0.4

* Induction cultures were prepared with 30×10^6 naive spleen cells, 20% final volume IL-2, and 20 μg/ml SRTA. ThF^s source was supernatants from Th^s cells grown in the presence or absence of prostaglandins as indicated. Supernatants were dialyzed extensively to remove free prostaglandins. Remaining concentrations of PGE_1 or $PGF_{2\alpha}$ were added to control flasks (2 ng/ml). ThF^s was used as 10% final volume.
‡ See Table III.

of PGE_1 during production of the ThF. Since 10% ThF^s is the minimum concentration required to induce the maximum DTH response (data not shown), PGE_1 also did not appear to significantly diminish the magnitude of ThF^s production.

No PGE_1 inhibition of an induced effector population. Previous work from our laboratory has also characterized the precursor population of the DTH effector T cells (Mann, R., C. J. Kelly, W. H. Hines, M. D. Clayman, N. Blanchard, M. J. Sun, and E. G. Neilson, manuscript in preparation). These effector cells are demonstrable in the in vitro induction system after 72 h in culture. To distinguish inhibition of effector cell differentiation from suppression or inactivation of fully formed DTH-reactive cells, we compared the effects of PGE_1 added either at day 0 or day 5 of culture. In Table VI, in sharp contrast to the inhibition seen when PGE_1 is present throughout the 5-d culture period, PGE_1 added at the end of culture did not inhibit the ability of differentiated effector cells to display DTH to tubular antigen.

PGE_1 reversible inactivation of effector T cell precursors. The studies described in the preceding paragraph suggested to us that PGE_1 inhibited one or multiple steps of effector cell differentiation at the precursor T cell level. Conceivably such inhibition could occur by several mechanisms; an effect of prostaglandins on major histocompatibility complex (MHC) antigen expression by antigen-presenting cells (22), lymphokine production (23–25), or a direct inhibitory effect on the precursor T cells. To test these hypotheses, we used strategies of adding back either fresh

Table VI. PGE_1 Does Not Inhibit DTH Reactivity of Induced Effector Cells

Induction cultures*		
Prostaglandins (day added)	SRTA	DTH response to SRTA‡
—	—	4.5±0.8
—	+	15.6±0.5
PGE_1 (12 μg/ml) (day 0)	+	5.8±0.6
PGE_1 (12 μg/ml) (day 5)	+	17.3±0.6

* PGE_1 was added on either day 0 or day 5 of culture.
‡ See Table III.

Table VII. PGE_1 Reversibly Inactivates Effector T Cell Precursors

Induction cultures* (PGE_1 24-h pulse [12 μg/ml])	Reconstitution		Day harvested	DTH response to SRTA‡
	Cells (treatment)	SRTA		
—	—	+	5	15.3±0.2
—	—	—	5	3.3±0.4
+ (12 μg/ml)	—	+	5	3.5±0.6
+	Adherent	+	5	4.0±0.4
+	Nonadherent	+	5	15.0±2.9§
+	Nonadherent (C)	+	5	15.3±0.4‖
+	Nonadherent (αThy1.2)	+	5	3.3±0.8
+	Nonadherent (αB)	+	5	15.5±0.6‖
+	—	+	7	10.5±1.0¶
+	—	+	10	14.8±0.4‖

* Naive spleen cells in the presence of ThF^s, IL-2, and SRTA were cultured with PGE_1 for 24 h where indicated. Cells were then washed and resuspended in fresh media, Il-2, SRTA, and ThF^s. In some experiments fresh spleen cells or spleen cell subpopulations were added to the cells pulsed with PGE_1.
‡ DTH response was measured as described in Table III on various days after reconstitution.
§ $P < 0.02$, compared with PGE_1-pulsed cultures (line 3) not reconstituted with fresh spleen cells.
‖ $P < 0.0005$, compared with line 3.
¶ $P < 0.005$, compared with line 3.

adherent cells or nonadherent cells depleted of T or B cells. These strategies required eliminating exogenous PGE_1 from the culture media to which the splenic cell subpopulations were added. We initially established, therefore, that pulsing the naive spleen cell population with 12 μg/ml of PGE_1 for only the first 24 h of culture also resulted in a suppressed effector cell response when the cells were harvested 5 d later (Table VII). When the cells were harvested 7 or 10 d after the 24-h pulse with PGE_1, however, responsiveness was restored, which indicated that the PGE_1 effect was temporary. Addition of fresh nonadherent, but not adherent cells, after the 24-h PGE_1 pulse reconstituted the DTH response by day 5. Negative selection techniques with monoclonal antibodies and C indicated that the nonadherent cell responsible for restoration of the DTH response was a T cell.

PGE_1 inhibitory effects overcome by recombinant murine IL-1. Recent reports have described the down-regulation of IL-1 production by endogenously produced PGE_2 and PGI_2 (26, 27). Although the PGE_1 concentration used in our in vitro studies exceeds by several logfold that generated endogenously, we examined the effects of recombinant IL-1 on PGE_1 inhibition of DTH effector cell induction. As seen in Table VIII, purified recombinant IL-1, in a dose-dependent manner, eventually overcame the inhibitory effects of PGE_1. Heat inactivation of the IL-1 preparation abrogated its activity.

PGE_1-associated inhibition mediated by PGE_1-induced soluble factors. Although the preceding experiment was consistent with an effect of exogenous PGE_1 on IL-1 production, the studies in Table VII also implicated an effect on T cells rather than antigen-presenting cells. Both Tables VII and VIII, however, were equally consistent, with a primary effect of PGE_1 on T cells that secondarily resulted in suppressed IL-1 production and/or

Table VIII. PGE_1 Inhibitory Effects Are Overcome by Recombinant IL-1

Induction cultures*		DTH response to SRTA‡
PGE_1 (12 μg/ml)	IL-1	
	U/ml	
+	—	2.3±0.3
+	1	3.7±0.6
+	10	10.7±1.0§
+	100	17.3±0.6‖
+	100 (heat inactivated)	4.0±0.5
—	—	18.3±0.7‖

* Culture flasks were prepared as described in Table III. Recombinant murine IL-1 was added as indicated. Final concentration of guanidine HCl was 2.5×10^{-4} M in all flasks.
‡ See Table III.
§ $P < 0.005$, compared with PGE_1 cultures without IL-1 (line 1).
‖ $P < 0.0005$, compared with line 1.

diminished T cell sensitivity to secreted IL-1. Previous work by other investigators has described nonspecific T suppressor factors induced by PGE_1 (28–31). We tested, therefore, the ability of dialyzed supernatants from day 5 effector cell induction cultures treated with PGE_1 to inhibit effector cell induction. As demonstrated in Table IX, inhibition is mediated by a nondialyzable factor in these supernatants that is not PGE_1. Inhibition by these supernatants can also be overcome by recombinant murine IL-1 in a dose-dependent manner, similar to that seen in Table VIII.

Table IX. Inhibition Is Mediated by a Soluble Factor Induced by PGE_1

Induction cultures*			
PGE_1 (12 μg/ml)	Supernatant from PGE_1-treated cultures (% of final volume)	IL-1	DTH response to SRTA‡
		U/ml	
—	—	—	17.7±0.3
+	—	—	3.0±1.0§
—	20	—	3.5±0.4§
—	10	—	10.7±0.3§
—	20	1	3.3±0.7
—	20	10	10.3±1.0‖
—	20	100	18.0±0.5¶

* Culture flasks were set up as described in Table III using 10% ThF^s. Supernatants from PGE_1 containing 5-d cultures were extensively dialyzed to remove unbound PGE_1. Quantitation of nondialyzed PGE_1 was determined by radioimmunoassay and added to control cultures. 20% supernatants (vol/vol) from 5-d induction cultures without PGE_1 had no inhibitory effect (DTH to SRTA, 18.3±0.8).
‡ See Table III.
§ $P < 0.0005$, compared with line 1.
‖ $P < 0.005$, compared with flasks containing 20% supernatant and no IL-1 (line 3).
¶ $P < 0.0005$, compared with line 3.

Discussion

The present experiments indicate that exogenous PGE_1, when administered from the time of immunization, markedly inhibits the development of immune-mediated interstitial nephritis without demonstrable effects on circulating or deposited αTBM-Ab. Immune lymphocytes from PGE_1-treated mice do not exhibit a DTH response to tubular antigen, which is a response that correlates well with the presence of nephritogenic effector T cells (8–10, 21). Inhibition of DTH reactivity, not attributable to active suppression, was a nonspecific effect of PGE_1 treatment, as the PPD response was also inhibited. We were impressed by the marked disparity between these results and those seen in mice commencing PGE_1 therapy on day 7, in whom neither histologic disease nor DTH reactivity was suppressed. Since we had previously observed that nephritogenic effector T cells are induced in vivo during this critical time period, and this induction process can be reliably reproduced in vitro (13), we were in a unique position to study PGE_1 effects on effector T cell induction in an organ-specific autoimmune response. As demonstrated in Tables III–VI, in the presence of PGE_1, effector T cell induction by helper cells or their soluble mediators is inhibited, whereas the functional activity of helper cells (as defined by secretion of soluble mediators) or fully differentiated effector cells (DTH reactivity) is not. The observed inhibition cannot be attributed to a cytotoxic effect of high PGE_1 concentrations as the effect is reversible (Table VII) after removal of PGE_1 from the media. This recovery process can be accelerated by addition to induction cultures of fresh T cells, but not B cells or macrophages. PGE_1 appears to act in part through other soluble factors (Table IX) whose inhibitory action can be overcome by exogenous IL-1 (Tables VIII and IX).

A variety of studies have demonstrated that pharmacologic doses of PGE_1 or PGE_2 have marked suppressive effects on spontaneous or induced immune complex glomerulonephritis (2–5). In some cases this correlates with a decline in relevant antibody titers or a reduction in circulating immune complexes (4, 5). We found no significant depression of either circulating or bound αTBM-Ab in PGE_1-treated mice in the present studies. Such a clear dissociation between the humoral and effector T cell responses in this experimental disease has been demonstrated previously in studies both of antigen-specific and non-specific immunosuppression (32–34). Presumably the B cell response to tubular antigen is T dependent; the mechanism underlying the apparent sparing of T cells providing B cell help, however, remains unclear. Although in our system PGE_1-associated inhibition is nonspecific with regards to evolving immune responses (since DTH to PPD and SRTA were both inhibited), this suppression does not extend to all tested T cell functions. Fully differentiated T cells, such as Th^s, or the effector T cells mediating DTH, are not functionally compromised by PGE_1 (Tables V and VI).

The majority of our studies were done using pharmacologic PGE_1 concentrations (3×10^{-5} M) unlikely to be achieved in vivo by endogenous release of PGE_1 (partial inhibition of effector cell induction was observed, however, at 3×10^{-7} M). The pharmacologic concentrations of PGE_1 that we used were similar to those previously shown, for example, to stimulate T cells to release the suppressor lymphokines, PITS (28–31). This heterogeneous group of suppressor factors has been shown to inhibit mitogen-induced blastogenesis (28–30), mixed lymphocyte re-

actions (29), as well as T-dependent and -independent B cell responses (29, 30). One of the low molecular weight PITS factors may even be a leukotriene (31). The data in this report are consistent with PGE_1 indirectly mediating inhibition through induction of suppressor lymphokines (Table IX). Our studies also suggest that synthesis and/or release of these PITS factors requires the continued presence of PGE_1 in the media, since delayed recovery occurs after removal of PGE_1 (Table VII). Addition of fresh T cells, which have not been induced to synthesize PITS factors, also accelerates recovery.

Several earlier studies of PGE_1- or PGE_2-mediated suppression suggested this effect was attributable in part to inhibition of IL-2 synthesis (23–25). In theory such results could also be explained by a primary inhibitory effect on IL-1 synthesis and/or responsiveness. Coupled with more recent studies suggesting an inhibitory effect of endogenous PGE_1 on IL-1 production, (26, 27) these previous experiments suggested to us that exogenous PGE_1 (or PITS) may also act through inhibition of IL-1 synthesis and/or target cell responsiveness to this lymphokine. Our results in Tables VIII and IX are consistent with this hypothesis, although they do not distinguish between the two aforementioned mechanisms. It is also possible that high concentrations of recombinant IL-1 can overcome other PGE_1-related, IL-1-independent, defects in effector cell differentiation.

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