THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Peyer's Patch Plasmacytoid Dendritic Cells (pDCs) Produce Low Levels of Type I Interferons: Possible Role for IL-10, TGFβ, and Prostaglandin E_2 in Conditioning a Unique Mucosal pDC Phenotype[1]

Nikhat Contractor,[*‡] *Jennifer Louten,*[†§] *Leesun Kim,*[*] *Christine A. Biron,*[†] *and Brian L. Kelsall*[2*]

***The organized lymphoid tissues of the intestine likely play an important role in the balance between tolerance to harmless mucosal Ags and commensal bacteria and immunity to mucosal pathogens. We examined the phenotype and function of plasmacytoid dendritic cells (pDCs) from murine Peyer's patches (PPs). When stimulated with CpG-enriched oligodeoxynucleotides in vitro, PPs and spleen pDCs made equivalent levels of IL-12, yet PP pDCs were incapable of producing significant levels of type I IFNs. Three regulatory factors associated with mucosal tissues, PGE_2, IL-10, and TGFβ, inhibited the ability of spleen pDCs to produce type I IFN in a dose-dependent fashion. These studies suggest that mucosal factors may regulate the production of type I IFN as well as IL-12 by pDCs. In the intestine, this may be beneficial in preventing harmful innate and adaptive immune responses to commensal microorganisms. The Journal of Immunology,* 2007, 179: 2690–2694.**

Immune responses within the intestine are tightly regulated to prevent untoward inflammation against harmless Ags and commensal bacteria and yet allow for the induction of immunity to pathogens. A dysregulation of this balance results in abnormal immune responses against commensal bacteria and intestinal inflammation. Peyer's patches (PPs)[3] are representative sites for the induction of immune responses in the intestine. Subsets of conventional dendritic cells (cDCs), some with PP-specific properties, have previously been identified (for review see Ref. 1). However, little is known about plasmacytoid dendritic cells (pDCs) within normal PPs. We chose to examine the phenotype and function of pDCs within PPs, because the control of the production of type I IFNs in response to both viral and bacterial nucleic acids by these cells may be important in the induction of immunity to mucosal pathogens as well as in the regulation of abnormal immune responses to commensal bacteria.

Both human and murine pDCs express TLRs that recognize viral RNA (via TLR7), and CpG-rich unmethylated DNA from bacteria and DNA viruses (via TLR9). Signaling by TLRs expressed on pDCs drives the production of type I IFN that can directly activate antiviral responses and augment both innate and adaptive immunity to viral as well as nonviral infections (2).

We characterized the phenotype of pDCs in PPs and found that following stimulation with CpG-enriched oligodeoxynucleotides (CpG ODN) or influenza virus, PP pDCs were incapable of producing significant amounts of type I IFN while they were fully capable of producing IL-12. Furthermore, three factors present in the mucosal microenvironment, IL-10, PGE_2, and TGFβ, inhibited type I IFN production by spleen pDCs in response to CpG ODN, suggesting that the mucosal microenvironment may condition pDCs for poor type I IFN production. Finally, in studies using spleen pDCs from IFNαR1$^{-/-}$ mice, we determined that TGFβ acts to inhibit initial TLR9-induced type I IFN mRNA expression while IL-10 acts at a later stage, most likely by blocking autocrine signaling via the type I IFN receptor. Together, these studies suggest a role for the mucosal microenvironment in preventing type I IFN production from pDCs.

Materials and Methods

Mice

Female BALB/c and C57BL/6 mice obtained from the National Cancer Institute (Frederick, MD) and in-house bred IFNαR1$^{-/-}$ mice on a C57BL/6 background were housed at the National Institutes of Health (Bethesda, MD), maintained in accordance with institutional guidelines for animal welfare, and used at 6- to 12-wk of age. In some experiments, 4×10^5 Fms-like tyrosine kinase 3 ligand (FLT3L)-expressing B16 cells (3) were injected s.c. 11 days before dendritic cell isolation.

*Mucosal Immunobiology Section, Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; †Department of Molecular Microbiology and Immunology, Brown University, Providence, RI 02912; ‡Wyeth Nutrition, Collegeville, PA 19426; and §Schering-Plough Biopharma, Palo Alto, CA 94304

Received for publication November 6, 2006. Accepted for publication July 5, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This research was supported by the Intramaral Research Program of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (NIH). In addition, C.B. and J.L. were supported by NIH Grant number RO1 AI 55677.

[2] Address correspondence and reprint requests to Dr. Brian Kelsall, 10/11N111, 10 Center Drive, Bethesda, MD 20892. E-mail address: kelsall@nih.gov

[3] Abbreviations used in this paper: PP, Peyer's patch; cDC, conventional dendritic cell; FLT3L, FMS-like tyrosine kinase 3 ligand; int, intermediate; LP, lamina propria; mPDCA, murine pDC Ag; ODN, oligodeoxynucleotide; pDC, plasmacytoid dendritic cell.

Antibodies

Abs used include: 120G8 (4), anti-murine pDC Ag (mPDCA) (clone JF05-1C2.4.1; Miltenyi Biotec), anti-CD11c (clone HL3 or N418), anti-B220 (clone RA3-6B2), anti-Gr-1 (clone RB6-8C5), anti-CD19 (clone 1D3), anti-CD40 (clone 3/23), anti-CD80 (clone 1G10), anti-CD86 (clone GL1), anti-MHC class II (clone AMS-32.1), anti-CD4 (clone GK1.5), and anti-CD8 (clone 53-6.7) (BD Biosciences).

Confocal microscopy

Primary Abs were detected in frozen sections using Alexa 488 and Alexa 594 tyramide signal amplification (TSA) kits (Molecular Probes). Nuclei were counterstained with Hoechst (Sigma-Aldrich). Images were collected on a Leica TCS-SP2 confocal microscope (Leica Microsystems) at ×40 magnification.

Cell isolation

Spleen and PP pDCs were obtained from single cell suspensions as previously described (5) except that collagenase was replaced with 420 μg/ml Liberase CI (Roche Applied Science). pDCs were enriched by negative selection (first step of pDC isolation kit; Miltenyi Biotec) and analyzed using a FACSCalibur cytometer and FlowJo software (Tree Star) or sorted using a FACS Vantage SE with DIVA option or FACS Aria cytometer (BD Biosciences).

Cell culture

Enriched pDCs or FACS-purified pDCs were cultured at 10^6 cells/ml in complete RPMI 1640 containing 10% FCS (BioSource International) and antibiotics at 37°C and 5% CO_2. Cells were stimulated with UV-irradiated influenza virus (strain PR8; 40 hemagglutinating units/ml; gift of J. Yewdell, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) and 1 μM CpG ODN 1826 (TCCATGACGTTCCT GACGTT) or 2216 (GGGGGACGATCGTCGGGGGG), both synthesized on a phosphorothioate backbone, and both of which have been demonstrated to induce type I IFN production in murine pDC (6, 7). Where indicated, cells were preincubated for 1 h with TGFβ 1, IL-10 (Peprotech), PGE_2 (Sigma-Aldrich), anti-IL10R or control Ab (clones 1B1.3a and GL113, kindly provided by DNAX Research). Culture supernatants were collected 20 h after the addition of CpG ODN and assayed by ELISA for IFNα or β (PBL Biomedical Laboratories), IL-12 p40 or p70 (R&D Systems), or bioactive type I IFNs (8).

Quantitative RT-PCR

Spleen mPDCA$^+$ (Miltenyi Biotec) pDCs isolated from B16-FLT3L-treated mice were pretreated as described above and stimulated with CpG ODN for a subsequent 4 h. RNA was isolated (Qiagen), and cDNA was synthesized (SuperScript III; Invitrogen). Real-time RT-PCR was performed on an Applied Biosystems 7900 HT instrument. FAM-labeled IL-12 p40 probe/primers were obtained from Applied Biosystems (Assays-on-Demand). SYBR Green (Applied Biosystems) was used to detect IFNα (all genes) and IFNβ (9). GAPDH was the endogenous control. Fold difference in gene expression was determined with the formula $2^{\Delta\Delta Ct}$, where Ct is threshold cycle.

Statistical analysis

Graphing and statistical analysis was conducted using Prism 4 software (GraphPad Software). Data were analyzed with a two-tailed, unpaired Student's *t* test.

Results and Discussion

pDCs are found in the subepithelial dome and interfollicular regions of the PP

Luminal Ags and commensal bacteria can enter the organized mucosal lymphoid tissues of the Peyer's patches and isolated colonic follicles via transport by M cells in the follicle-associated epithelium or via direct sampling by cDCs with intraepithelial extensions (1). Therefore, immune responses must be controlled at these inductive sites (10). We explored the role of pDCs in the immune regulation in PPs. We first identified these cells in tissue sections by immunofluorescence staining using the pDC-reactive Ab mPDCA (Fig. 1*A*) and the 120G8 Ab (Ref. 4 and data not shown). pDCs were found in the subepithelial dome and interfollicular regions of PPs, as well as in the lamina propria (LP) of PP-adjoining villi, but were not found in B cell follicles, consistent with prior studies (4, 11). Therefore,

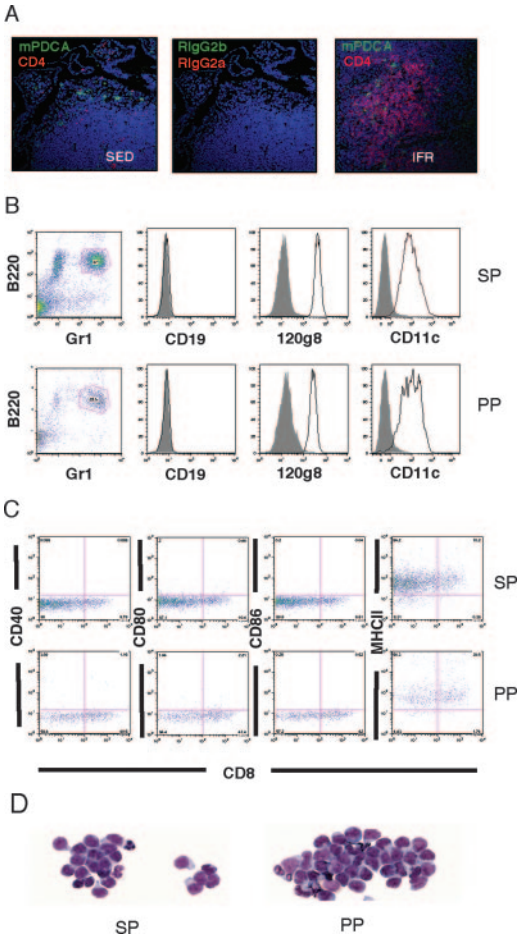

FIGURE 1. Location and phenotype of PP pDCs. *A*, PP sections were stained for mPDCA (green) and CD4 (red) or isotype controls (*middle panel*) and the nuclear dye Hoechst 33258 (blue). mPDCA$^+$ cells were present in the PP subepithelial dome (SED) (*left panel*) and interfollicular regions (IFR) (*right panel*). *B*, Flow cytometry of spleen (SP) and PP cells enriched for pDCs. *C*, Flow cytometry of B220$^+$GR1$^+$ cells. *D*, Cytospins of FACS-purified CD11c^{int}B220$^+$Gr1$^+$CD19$^-$ spleen (SP) and PP pDCs after Wright-Giemsa staining. Data are representative of at least three independent experiments.

PP pDCs are present at sites of initial Ag entry and within regions where they could influence T cell responses.

PP pDCs contain both CD8$^+$ and CD8$^-$ populations and express low levels of costimulatory molecules

pDCs from PPs were further characterized by the flow cytometry of pDC-enriched cell populations (see *Materials and Methods*; Fig. 1, *B* and *C*). As with pDCs from other tissues, PP pDCs were identified as CD19$^-$, CD11c^{int}, B220$^+$, and Gr1$^+$ cells that were uniformly positive for the 120G8 Ab (Fig. 1*B*) and mPDCA (data not shown). Prior studies identified CD8$^+$CD11b$^-$, CD8$^-$ CD11b$^+$, and CD8$^-$CD11b$^-$ populations of cDCs in PPs, all of which express high levels of CD11c and do not express B220 (1). Therefore, CD11c^{int} and B220$^+$ PP pDCs are not included in these previously identified populations. Costimulatory molecule expression was low, whereas the expression of MHC class II was intermediate on pDCs from both spleen and PP (Fig. 1*C*). PP pDCs contained a greater percentage of CD8$^+$ cells than their splenic counterparts (40 vs 10%). However, both CD8$^-$ and CD8$^+$ pDC populations from the spleen and PPs expressed similar levels of

CD40, CD80, CD86, and MHC class II Ags, suggesting that $CD8^+$ cells are not a more mature population of $CD8^-$ cells (7). Finally, purified pDCs from the spleen and PP had similar morphology on Wright-Giemsa staining (Fig. 1*D*).

PP pDCs are deficient in their ability to produce type I IFNs

PP pDCs purified to ≥98% by flow cytometry produced IL-12 p40 and IL-12 p70 at similar levels to those of spleen pDCs when stimulated with influenza virus strain PR8 or CpG ODN (Fig. 2, *A* and *B*). In contrast, the production of IFNα and IFNβ by PP pDCs was only a small fraction of that produced by spleen pDCs (Fig. 2, *C* and *D*). These results were confirmed with a bioassay for type I IFN (Fig. 2*E*). Consistent with these findings, IFNα production was undetectable from whole PP cells depleted of B cells, T cells, NK cells, and macrophages stimulated with CpG ODN whereas it was readily detectable from the depleted spleen preparations (Fig. 2*F*), indicating it was unlikely that a population of IFN-producing cells that did not express classic pDC markers was present in the PP.

Two prior studies indicated that PP pDCs were capable of producing type I IFN in response to in vitro stimulation with CpG ODN (11, 12); however pDCs in these experiments were isolated following expansion in vivo by treatment with FLT3L. To explore this issue, spleen and PP pDC were purified from mice treated with FLT3L and stimulated with CpG ODN (Fig. 2*G*). Both spleen and PP pDCs from FLT3L-treated animals produced significantly more IFNα than cells from untreated mice. However, the levels from PP pDCs were still at least 5- to 10-fold less than those from spleen pDCs. Therefore, despite the higher levels of type I IFN produced, the differential production between PPs and spleen pDCs was consistent with results from unmanipulated mice.

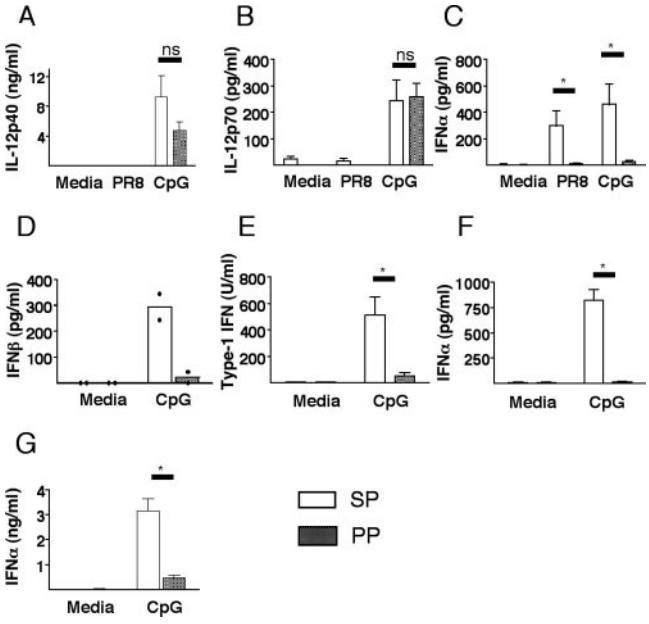

FIGURE 2. Lack of type I IFN production by PP pDC. Supernatants of FACS purified (*A–E*) or enriched (*F* and *G*) pDCs were assayed by ELISA for IL-12 p40 (*A*), IL-12 p70 (*B*), IFNα (*C* and *G*), or IFNβ (*D*) or by viral inhibition assay (*E*). Data indicate the means ± SD for five or six (*A–C*), two (*D*), or three (*E-G*) independent experiments. *F*, The percentage of pDC was similar in both spleen (SP) and PP preparations. *G*, pDCs were enriched from the spleens and PPs of mice treated with FLT3L. *, $p \leq 0.05$; ns, $p \geq 0.05$.

TGFβ, PGE_2, and IL-10 can inhibit IFNα production by spleen pDC

To address the mechanism by which PP pDCs cannot produce type-I IFN in response to CpG ODN, we initially determined whether differences in the expression of TLR9 or IRF7, a major transcriptional regulator of type I IFN, could account for differences in type I IFN production by PP and spleen pDCs. We found that mRNA for TLR9 or IRF7 were expressed and <2-fold different between purified spleen and PP pDCs (data not shown). In addition, surface level expression of TLR9, as indicated by flow cytometry, was similar between the two cell types (data not shown).

We next evaluated the ability of the regulatory factors most commonly associated with mucosal tissues to affect IFNα production from purified spleen pDCs (Fig. 3). Pretreatment of spleen pDCs with TGFβ, PGE_2, or IL-10 resulted in a dose-dependent reduction in IFNα production (Fig. 3*A*). In addition, IL-10, PGE_2, and, to a lesser extent, TGFβ inhibited the production of IL-12 p40 (Fig. 3*B*). Finally, an additive effect on type I IFN production was seen with PGE_2 and IL-10 or TGFβ. Baseline survival of pDCs was low in overnight cultures with only 25% of cells surviving after 24 h. IL-10 (10 ng/ml) and TGFβ (10 ng/ml) resulted in an enhancement of cell death by 14–27% at 8 h after culture, a time when the majority of type I IFN is present in the culture supernatants; however, at this time point type I IFN production is blocked by >80% in these cultures (data not shown). Therefore, the increase in cell death could not account for the observed suppression of type I IFN.

Inhibition of IFNα production by IL-10 is consistent with studies of human pDCs (13). Furthermore, suppression of

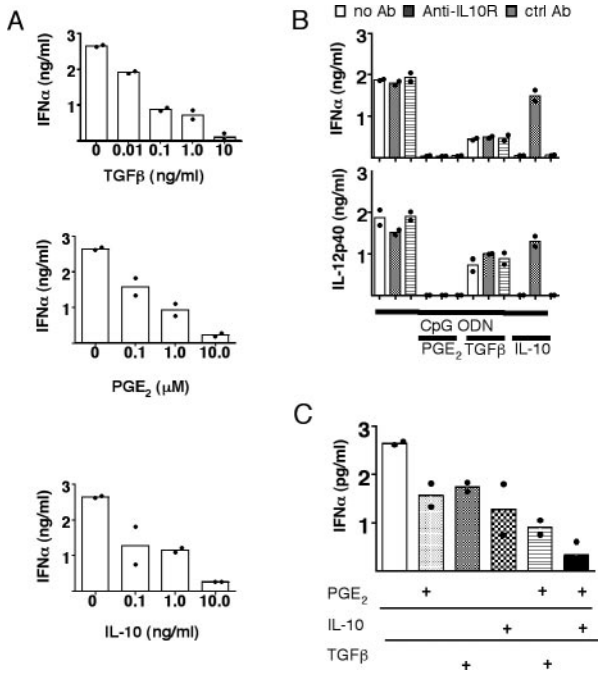

FIGURE 3. TGFβ, PGE_2, and IL-10 inhibit type I IFN production by murine pDCs. *A*, Supernatants of FACS-purified spleen pDCs were assayed for IFNα after preincubation for 1 h and stimulation for 20 h with CpG ODN. *B*, Spleen pDCs were preincubated with PGE_2 (10 μM), TGFβ (10 ng/ml), or IL-10 (10 ng/ml) plus anti-IL-10R, control (ctrl) Ab (2.5 μg/ml), or no Ab. *C*, spleen pDCs were preincubated with PGE_2 (0.1 μM) with or without TGFβ (0.01 ng/ml) or IL-10 (0.1 ng/ml). Data are representative of two independent experiments.

IL-12 production by IL-10 and PGE_2 is consistent with effects on cDCs (14).

These data suggest that factors present in the mucosal microenvironment have direct effects on the ability of pDC to produce type I IFN as well as IL-12. Of the factors tested, treatment with TGFβ most closely recapitulated the phenotype observed in PP pDCs in that type I IFN was suppressed while IL-12 production was maintained, at least to some degree. Furthermore, TGFβ is constitutively expressed in the intestine (15) and plays a key role in the PP for inducing B cell switching to IgA (16), supporting the possibility that TGFβ is involved in vivo in conditioning PP pDCs for low type I IFN production.

Additional roles for IL-10 and PGE_2 in conditioning mucosal pDCs are suggested because cDCs (1) and T cells (17) from mucosal tissues produce IL-10, and IL-$10^{-/-}$ mice develop colitis (18). Furthermore, PGE_2 is expressed by stromal cells in the intestinal LP and at low levels in PPs (19); and PGE_2-deficient Cox-$2^{-/-}$ mice are less susceptible to the induction of oral tolerance (20). Whether pDCs in the LP of the intestine are similar to PP pDCs in their inability to produce significant amounts of type I IFN is not yet known, but TGFβ expressed by intestinal epithelial cells, PGE_2 by stromal cells, and IL-10 by activated LP dendritic cells and macrophages suggests that this may be the case. It has been reported that mesenteric lymph node pDCs can produce type I IFN after exposure in vivo to R-848 given orally and drive cDC activation (21). In addition, pDCs do not appear to readily migrate from the intestine to the mesenteric lymph node (22), indicating that direct exposure to the intestinal microenvironment may be important for the phenotype of PP pDCs.

We are currently exploring the direct role of IL-10, TGFβ, and PGE_2 in conditioning pDCs in vivo. We found that pDCs from IL-$10^{-/-}$ mice still had a defect in type I IFN production so that IL-10 does not appear to be required for the suppression of PP pDC type I IFN production (data not shown). TGFβ-$1^{-/-}$ as well as wild-type and IL-$10^{-/-}$ mice treated with the cyclooxygenase inhibitor piroxicam develop intestinal inflammation. Therefore, to date, we have been unable to directly determine the in vivo role of TGFβ and/or PGE_2, with or without IL-10, on PP pDC type I IFN production. However, given their additive effects on pDC function in vitro (Fig. 3*C*), it is likely that a combined effect of these factors may be important to control pDC responses in PPs.

TGFβ but not IL-10 inhibits the induction of mRNA for type I IFN production by pDCs from IFNαR1$^{-/-}$ mice

We next addressed whether two factors likely affecting type I IFN in the PP, TGFβ and IL-10, act to inhibit type I IFN production by spleen pDCs by preventing primary signaling via TLR9 or by blocking autocrine stimulation of the type I IFN receptor. To do this, we tested the ability of TGFβ and IL-10 to suppress the early induction of type I IFN from the pDCs of wild-type mice without functional type I IFN receptors (IFNαR1$^{-/-}$ mice; Fig. 4). Although both TGFβ and IL-10 inhibited the transcription of mRNA for IFNβ by CpG ODN stimulation in pDCs from wild-type mice, only TGFβ was able to suppress the expression of IFNβ mRNA in pDCs from IFNαR1$^{-/-}$ mice. In contrast, TGFβ was not able to suppress the expression of mRNA for IL-12 p40 in pDCs from either mouse strain, whereas IL-10 suppressed IL-12 p40 from both. These studies imply that in the regulation of type I IFN production, TGFβ acts primarily on proximal signaling via TLR9 while IL-10 acts on type I IFN production downstream of initial TLR signaling and type I IFN production, most likely by blocking autocrine type I IFN signaling.

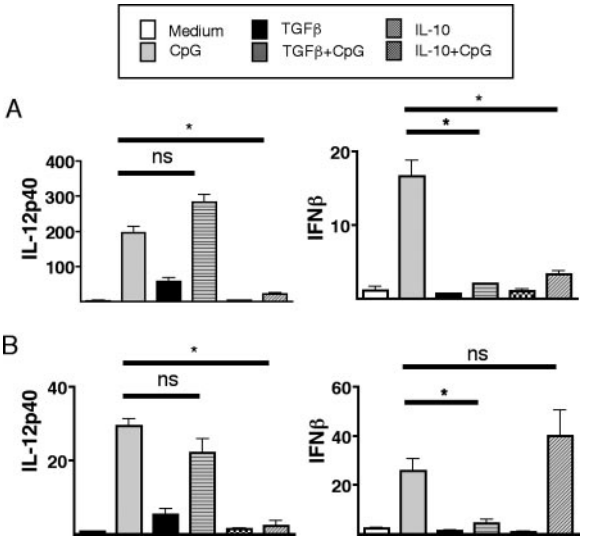

FIGURE 4. TGFβ, but not IL-10, inhibits the induction of mRNA for IFNβ in pDCs from IFNαR1$^{-/-}$ mice. mRNA expression by mPDCA$^+$ spleen pDCs isolated from FLT3L-treated wild-type (*A*) or IFNαR1$^{-/-}$ (*B*) mice pretreated with TGFβ (10 ng/ml) or IL-10 (10 ng/ml) and stimulated with CpG ODN for 4 h is shown. Data are representative of three independent experiments. *, $p \leq 0.05$; ns, $p \geq 0.05$.

In addition to its well known role as an antiviral cytokine, type I IFN can activate both innate and adaptive immune mechanisms (see Ref. 2). Type I IFN can act together with IL-12 to augment NK cell cytotoxic activity and induce Th1-mediated immunity via the innate production of IFN-γ by NK cells and $CD8^+$ T cells. Alternatively, type I IFN can act independently of IL-12 to activate cDC maturation and cross-presentation and to enhance $CD8^+$ T cell responses. An enhanced Th1 response or possibly an aberrant $CD8^+$ T cell response (23, 24) to commensal bacteria driven by type I IFN could contribute to intestinal inflammation. Therefore, one consequence of the lack of type I IFN production by pDC may be to shift the immunological balance away from potentially damaging immune responses against commensal organisms.

A second possible consequence of our findings is that pathogens dependent on the production of type I IFN by pDCs for their clearance may gain a selective advantage by replicating in cells of the mucosa. The most intriguing possibility in this regard is HIV, which readily infects mucosal tissues (25), activates pDCs to produce type I IFN (26), and progresses coincidentally with a decline in pDC numbers (27). However, the actual role of type 1 IFN in HIV infection or progression is not yet clear. In contrast, during certain viral infections such as those caused by type I reovirus (28) or *Herpes simplex virus*-2 (29) or during intestinal inflammation (30), pDCs may be newly recruited from the blood, and thus may be fully capable of producing type I IFN. Therefore, local microenvironmental conditions may dictate whether type I IFN-competent pDCs are present within mucosal tissues.

Together, our studies support the hypothesis that dendritic cells are conditioned by their tissue microenvironment to drive tissue-specific immune responses. Further studies are needed to

elucidate the in vivo mechanism by which PP pDC type I IFN production is inhibited and to determine the specific signaling pathways involved in the TGFβ-, PGE_2-, and IL-10-mediated suppression of type I IFN production by pDCs.

Acknowledgments

We thank Warren Strober for critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

1. Kelsall, B. L., and F. Leon. 2005. Involvement of intestinal dendritic cells in oral tolerance, immunity to pathogens, and inflammatory bowel disease. *Immunol. Rev.* 206: 132–148.
2. Colonna, M., G. Trinchieri, and Y. J. Liu. 2004. Plasmacytoid dendritic cells in immunity. *Nat. Immunol.* 5: 1219–1226.
3. Mach, N., S. Gillessen, S. B. Wilson, C. Sheehan, M. Mihm, and G. Dranoff. 2000. Differences in dendritic cells stimulated in vivo by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or Flt3-ligand. *Cancer Res.* 60: 3239–3246.
4. Asselin-Paturel, C., G. Brizard, J. J. Pin, F. Briere, and G. Trinchieri. 2003. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. *J. Immunol.* 171: 6466–6477.
5. Iwasaki, A., and B. L. Kelsall. 1999. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J. Exp. Med.* 190: 229–239.
6. Omatsu, Y., T. Iyoda, Y. Kimura, A. Maki, M. Ishimori, N. Toyama-Sorimachi, and K. Inaba. 2005. Development of murine plasmacytoid dendritic cells defined by increased expression of an inhibitory NK receptor, Ly49Q. *J. Immunol.* 174: 6657–6662.
7. Blasius, A., W. Vermi, A. Krug, F. Facchetti, M. Cella, and M. Colonna. 2004. A cell surface molecule selectively expressed on murine natural interferon-producing cells that blocks secretion of interferon-α. *Blood* 103: 4201–4206.
8. Orange, J. S., and C. A. Biron. 1996. Characterization of early IL-12, IFN-$\alpha\beta$, and TNF effects on antiviral state and NK cell responses during murine cytomegalovirus infection. *J. Immunol.* 156: 4746–4756.
9. Gautier, G., M. Humbert, F. Deauvieau, M. Scuiller, J. Hiscott, E. E. Bates, G. Trinchieri, C. Caux, and P. Garrone. 2005. A type I interferon autocrine-paracrine loop is involved in Toll-like receptor-induced interleukin-12p70 secretion by dendritic cells. *J. Exp. Med.* 201: 1435–1446.
10. Mowat, A. M. 2003. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat. Rev. Immunol.* 3: 331–341.
11. Castellaneta, A., M. Abe, A. E. Morelli, and A. W. Thomson. 2004. Identification and characterization of intestinal Peyer's patch interferon-α producing (plasmacytoid) dendritic cells. *Hum. Immunol.* 65: 104–113.
12. Bilsborough, J., T. C. George, A. Norment, and J. L. Viney. 2003. Mucosal $CD8\alpha^+$ DC, with a plasmacytoid phenotype, induce differentiation and support function of T cells with regulatory properties. *Immunology* 108: 481–492.
13. Duramad, O., K. L. Fearon, J. H. Chan, H. Kanzler, J. D. Marshall, R. L. Coffman, and F. J. Barrat. 2003. IL-10 regulates plasmacytoid dendritic cell response to CpG-containing immunostimulatory sequences. *Blood* 102: 4487–4492.
14. Trinchieri, G. 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* 3: 133–146.
15. Babyatsky, M. W., G. Rossiter, and D. K. Podolsky. 1996. Expression of transforming growth factors α and β in colonic mucosa in inflammatory bowel disease. *Gastroenterology* 110: 975–984.
16. Borsutzky, S., B. B. Cazac, J. Roes, and C. A. Guzman. 2004. TGF-β receptor signaling is critical for mucosal IgA responses. *J. Immunol.* 173: 3305–3309.
17. Kamanaka, M., S. T. Kim, Y. Y. Wan, F. S. Sutterwala, M. Lara-Tejero, J. E. Galan, E. Harhaj, and R. A. Flavell. 2006. Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. *Immunity* 25: 941–952.
18. Kuhn, R., J. Lohler, D. Rennick, K. Rajewsky, and W. Muller. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75: 263–274.
19. Newberry, R. D., J. S. McDonough, W. F. Stenson, and R. G. Lorenz. 2001. Spontaneous and continuous cyclooxygenase-2-dependent prostaglandin E2 production by stromal cells in the murine small intestine lamina propria: directing the tone of the intestinal immune response. *J. Immunol.* 166: 4465–4472.
20. Newberry, R. D., W. F. Stenson, and R. G. Lorenz. 1999. Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. *Nat. Med.* 5: 900–906.
21. Yrlid, U., S. W. Milling, J. L. Miller, S. Cartland, C. D. Jenkins, and G. G. MacPherson. 2006. Regulation of intestinal dendritic cell migration and activation by plasmacytoid dendritic cells, TNF-α and type 1 IFNs after feeding a TLR7/8 ligand. *J. Immunol.* 176: 5205–5212.
22. Yrlid, U., V. Cerovic, S. Milling, C. D. Jenkins, J. Zhang, P. R. Crocker, L. S. Klavinskis, and G. G. MacPherson. 2006. Plasmacytoid dendritic cells do not migrate in intestinal or hepatic lymph. *J. Immunol.* 177: 6115–6121.
23. Liu, Z., and L. Lefrancois. 2004. Intestinal epithelial antigen induces mucosal CD8 T cell tolerance, activation, and inflammatory response. *J. Immunol.* 173: 4324–4330.
24. Oki, M., H. Ohtani, Y. Kinouchi, E. Sato, S. Nakamura, T. Matsumoto, H. Nagura, O. Yoshie, and T. Shimosegawa. 2005. Accumulation of $CCR5^+$ T cells around $RANTES^+$ granulomas in Crohn's disease: a pivotal site of Th1-shifted immune response? *Lab. Invest.* 85: 137–145.
25. Brenchley, J. M., D. A. Price, and D. C. Douek. 2006. HIV disease: fallout from a mucosal catastrophe? *Nat. Immunol.* 7: 235–239.
26. Beignon, A. S., K. McKenna, M. Skoberne, O. Manches, I. DaSilva, D. G. Kavanagh, M. Larsson, R. J. Gorelick, J. D. Lifson, and N. Bhardwaj. 2005. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. *J. Clin. Invest.* 115: 3265–3275.
27. Muller-Trutwin, M., and A. Hosmalin. 2005. Role for plasmacytoid dendritic cells in anti-HIV innate immunity. *Immunol. Cell Biol.* 83: 578–583.
28. Johansson, C., J. D. Wetzel, J. He, C. Mikacenic, T. S. Dermody, and B. L. Kelsall. 2007. Type I interferons produced by hematopoietic cells protect mice against lethal infection by mammalian reovirus. *J. Exp. Med.* 204: 1349–1358.
29. Lund, J. M., M. M. Linehan, N. Iijima, and A. Iwasaki. 2006. Cutting edge: plasmacytoid dendritic cells provide innate immune protection against mucosal viral infection in situ. *J. Immunol.* 117: 7510–7514.
30. Katakura, K., J. Lee, D. Rachmilewitz, G. Li, L. Eckmann, and E Raz. 2005. Toll-like receptor 9-induced type I IFN protects mice from experimental colitis. *J. Clin. Invest.* 115: 695–702.