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Human Benign Prostatic Hyperplasia Stromal Cells As Inducers and Targets of Chronic Immuno-Mediated Inflammation

Giuseppe Penna,* Benedetta Fibbi,† Susana Amuchastegui,* Chiara Cossetti,* Francesca Aquilano,* Gilles Laverny,* Mauro Gacci,‡ Clara Crescioli,† Mario Maggi,‡ and Luciano Adorini2*

Benign prostatic hyperplasia (BPH), a highly prevalent prostate condition, could involve an inflammatory component in disease pathogenesis. In this study, we show that human stromal prostate cells obtained from BPH tissue can actively contribute to the inflammatory process by secreting proinflammatory cytokines as well as chemokines able to recruit lymphomononuclear cells and by acting as APCs. BPH cells express all of the TLRs and their ligation leads to the secretion of CXCL8/IL-8, CXCL10, and IL-6. In addition, BPH cells express costimulatory as well as class I and class II MHC molecules, which activate alloreactive CD4+ cells that in turn markedly up-regulate IL-12/IL-23p40 and IL-12p75 secretion by BPH cells. Alloreactive CD4+ cells activated by BPH cells secrete IFN-γ and IL-17. These cytokines up-regulate IL-6, IL-8, and CXCL10 production by BPH cells, creating a positive feedback loop that can amplify inflammation. IL-8 induces autocrine/paracrine proliferation of BPH cells, indicating also a growth-promoting activity of this chemokine in disease pathogenesis. These results show that human BPH cells represent non-professional APCs able to induce and sustain chronic inflammatory processes, supporting the relevance of inflammation in BPH pathogenesis. The Journal of Immunology, 2009, 182: 4056–4064.

Benign prostatic hyperplasia (BPH) is the most common age-related disease of the male, occurring clinically in about half of all men at 70 years of age (1). BPH is defined by hyperproliferation of stromal and epithelial cells of the prostate caused by complex cellular alterations, including changes in proliferation, differentiation, apoptosis, and senescence (2). A variety of growth factors have been implicated in the pathogenesis of BPH and two members of the fibroblast growth factor (FGF) gene family, FGF-2 and FGF-7, are considered to play a key role in prostate cell growth (3). However, the complex regulatory mechanisms of growth control in BPH are still incompletely understood.

Although primarily characterized by prostatic cell proliferation, an inflammatory component has been extensively documented in BPH (4). Histological evidence of prostate inflammation was identified in all patients tested irrespective of bacterial infection (5), and, in a series of 284 BPH patients, only 23% of prostate biopsies were free of infiltrating inflammatory cells (6). It is not clear whether an infectious origin is the primary event for inflammation in the human prostate (7). Microbial organisms demonstrated so far in BPH appear rather heterogeneous and no study has reported a single strain of microorganisms present in all BPH samples, while viruses have been detected less commonly in association with BPH (7). Interestingly, analysis of prostate biopsies in a subgroup of >1000 randomly selected patients from the Medical Therapy Of Prostatic Symptoms study, indicates that the presence of inflammatory infiltrates in the prostate of BPH patients is associated with increased rate of disease progression and higher risk of acute urinary retention (8). In addition, analysis of baseline data from the REduction by DUtasteride of Prostate Cancer Events trial indicates an association between inflammation and BPH symptoms (9).

Inflammatory infiltrates in BPH have been found to consist primarily of T cells, mostly CD4+CD45RO+ cells, B cells, and macrophages (10). Up-regulation of several proinflammatory cytokines has been described in BPH, in particular IL-2 and IFN-γ (11), IL-15 (12) and IL-17 (13), leading to the hypothesis that BPH may represent an “immune inflammatory” disease (7). This is an attractive hypothesis, because the association of BPH with chronic inflammation could offer a sound framework to understand the pathogenesis of the disease. However, immune mechanisms leading to chronic inflammation in BPH have not yet been clearly defined.

In this study, we have examined the capacity of prostate stromal cells obtained from BPH tissue to actively contribute to the organ-specific inflammatory process by acting as APCs or as targets of TLR agonists, leading to the production of proinflammatory cytokines and chemokines able to mediate prostate chronic inflammation and hyperplasia. We show here that BPH cells express all of the TLRs and their triggering by bacterial or viral agonists leads to the secretion of proinflammatory cytokines like IL-6 and chemokines like CXCL10 and IL-8.

Notably, human BPH cells express MHC class II and costimulatory molecules, critical features of APCs, produce high levels of IL-12/IL-23p40 and IL-12p75, and are able to present alloantigen
to CD4+ T cells, inducing secretion of IFN-γ and IL-17. These cytokines strongly enhance production by BPH cells of IL-8, which acts as an autocrine/paracrine growth factor for BPH cells, providing a plausible association among TLR-mediated immune responses, chronic prostate inflammation, and sustained prostatic cell growth in BPH.

Materials and Methods

**BPH cell cultures**

Human BPH cells prepared, maintained, and used as previously described (14) were obtained from prostate tissues derived from five patients who underwent suprapubic adenomectomy for BPH after informed consent and approval by the local ethical committee. In addition, after informed consent...
and approval by the local ethical committee, BPH tissue samples were obtained from prostate tissues derived from 13 patients undergoing transurethral resection of the prostate (TURP) for BPH. Patients did not receive any pharmacological treatment in the 3 mo preceding surgery. BPH cells were prepared as previously described, and characterized as fibromuscular stromal cells (14). Briefly, surgical specimens were cut in small fragments and treated overnight with 2 mg/ml bacterial collagenase type IV (Sigma-Aldrich). Fragments were then extensively washed in PBS and cultured in a DMEM-F12 1:1 mixture supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 \( \mu \)g/ml streptomycin in a fully humidified atmosphere of 95% air and 5% CO\( _2 \). Cells began to emerge within 1 wk and were used within the 10th passage.

BPH cells were stimulated with a TLR agonist kit (InvivoGen) containing Pam3CSK4 (0.5 \( \mu \)g/ml), heat-killed Listeria monocytogenes (10\(^9 \) cells/ml), poly(I:C) (25 \( \mu \)g/ml), LPS Escherichia coli K12 (100 ng/ml), Flagellin Salmonella typhimurium (100 ng/ml), FSL1 (100 ng/ml), LPS Escherichia coli K12 (100 ng/ml), Poly(I:C) (25 \( \mu \)g/ml), LPS Escherichia coli K12 (100 ng/ml), and oligodeoxynucleotide 2006 (5 \( \mu \)g/ml). In addition, BPH cells were stimulated with 10 ng/ml IFN-\( \gamma \), IL-17, and/or TNF-\( \alpha \), (optimal cytokine concentrations to induce in BPH cells IL-8 and IL-6 production as determined in separate experiments), or with the indicated concentrations of IL-8 (BD Pharmingen). After 48 h, cell culture supernatants were analyzed for cytokine and chemokine production, as indicated in the figure legends.

**BPH cell proliferation assay**

The protocol used was essentially as described previously (14). Briefly, 2 \( \times \) 10\(^4 \) cells were seeded onto 12-well plates in growth medium. After 24 h, the growth medium was removed, the cells were washed twice in PBS, and incubated in phenol red- and serum-free medium containing 0.1% BSA. After 24 h, BPH cells were stimulated, as previously reported (15), with 10 nM testosterone (Sigma-Aldrich), 10 ng/ml keratinocyte growth factor (KGF; PeproTech), 10 ng/ml Des(1–3) insulin growth factor 1 (Des(1–3) IGF-1; GroPep), or as indicated in the text. After 48 h, cells were trypsinized, and each experimental point was obtained by hemocytometer counting, and then averaging at least six different fields for each well. In the same experiment, each experimental point was repeated in quadruplicate. Cell growth results are expressed as percentage (± SE) of cell number increase compared with controls.

**CD4\(^+ \) T cell purification and culture**

CD4\(^+ \) T cells were purified from PBMC by negative selection with a CD4\(^+ \) T cell isolation kit (Miltenyi Biotec). BPH cells treated or untreated with 50 ng/ml recombinant human IFN-\( \gamma \) (BD Pharmingen) were cocultured with allogeneic CD4\(^+ \) T cells. After 5 days, cell culture supernatants were analyzed for cytokine production as indicated. CD4\(^+ \) T cells labeled with a Vybrant CFDA SE cell tracer kit (Molecular Probes) were cocultured with BPH cells as indicated, and after a 6-day culture cells were recovered and analyzed with an LSR flow cytometer (BD Biosciences) using CellQuest software.

In blocking experiments, BPH cells were cocultured with allogeneic CD4\(^+ \) T cells in the presence of 10 \( \mu \)g/ml anti-HLA-class I mAb, anti-CD40, anti-CD80, anti-CD86 (BD Pharmingen), anti-IL-12 mAbs 17F7 and 20C2 (16), mouse IgG1, mouse IgG2a, mouse IgG2b-purified mAbs, or with CD134-muIg fusion protein (Ancell).

**FIGURE 3.** Constitutive and IFN-\( \gamma \)-up-regulated expression of MHC class I and class II molecules by BPH cells. A, BPH cells were cultured for 48 h with medium alone (light gray histograms) or containing 50 ng/ml IFN-\( \gamma \) (dark gray histograms). After washing, BPH cells were stained with anti-HLA class I or class II mAbs and analyzed by cytofluorometry. Open histograms represent isotype controls. Data are from one representative experiment of six performed. B, BPH cells were cultured for 48 h with medium alone (left panel) or containing 50 ng/ml IFN-\( \gamma \) (right panel). After washing, BPH cells were stained with anti-HLA class II mAb and analyzed by confocal microscopy. Original magnification, \( \times \) 630. Results are from one representative experiment of three performed. C, Frozen prostate samples obtained by TURP from a representative BPH patient of 13 tested were stained with anti-HLA class II mAb. Original magnifications: left panel, \( \times \) 200 and right panel, \( \times \) 400.
CONCENTRATIONS OF IFN-γ, IL-17, IL-6, CXCL10, IL-12/23p40, and IL-6/12p75 IN CELL CULTURE SUPERNATANTS WERE EVALUATED BY SANDWICH ELISA. CONCENTRATIONS OF IFN-γ (MILES LABORATORIES) AND STORED AT 80°C. FROZEN SECTIONS (5-µM THICK) WERE AIR DRIED AND THEN FIXED IN ACETONE FOR 10 MIN. ENDogenous PEROXIDASE ACTIVITY WAS BLOCKED BY INCUBATION WITH AN AVIDIN SOLUTION MIXED IN 1% BSA IN PBS FOR 15 MIN, FOLLOWED BY A BIOTIN SOLUTION MIXED IN 1% BSA IN PBS FOR 15 MIN (VECTOR LABORATORIES). SECTIONS WERE STAINED WITH H&E OR WITH POLYCLONAL RABBIT ANTI-HUMAN IL-8, CXCR1, OR CXCR2 (SANTA CRUZ BIOTECHNOLOGY), POLYCLONAL RABBIT ANTI-HUMAN IK-67 (ABCAM), AND MONOCLONAL MOUSE ANTI-HUMAN CD15, HLA-DR (BD PHARMINGEN), FOLLOWED BY GOAT ANTI-RABBIT IgG OR GOAT ANTI-MOUSE IgG (SANTA CRUZ BIOTECHNOLOGY) AND BIOTINYLATED STREPTAVIDIN-PEROXIDASE CONJUGATE. 3-Amino-9-ethylcarbazole (DakoCytomation) was used as chromogen and hematoxylin as a counterstain. To stain cultured BPH cells, they were first adhered to poly-L-lysine-coated slides, processed as described above, and then incubated with Abs specific for cytokeratin (pan-cytokeratin; Novocastra Laboratories), smooth muscle α-actin, and desmin or vimentin (Santa Cruz Biotechnology), followed by biotinylated anti-rabbit IgG, anti-mouse IgG or anti-goat IgG (Vector Laboratories), and streptavidin-peroxidase. 3-Amino-9-ethylcarbazole (DakoCytomation) was used as chromogen and hematoxylin as a counterstain. Negative controls were performed by incubation with appropriate isotype-matched primary mAbs.

REAL-TIME QUANTITATIVE RT-PCR
RNA was extracted using TRIZol (Invitrogen) according to the manufacturer’s instruction, followed by a cleanup with the RNeasy Kit (Qiagen). Reverse transcription was performed and real-time quantitative RT-PCR of total CDNA using specific primers was conducted using an Applied Biosystems PRISM 7000 Sequence Detection System and TaqMan chemistry. The primers used are commercially available from Applied Biosystems as assays-on-demand. Relative quantification of target cDNA was determined by arbitrarily setting the control value to 1 and changes in cDNA content of a sample were expressed as a multiple thereof. Differences in cDNA input were corrected by normalizing to β-actin or GAPDH signals. To exclude amplification of genomic DNA, RNA samples were treated with DNase (Sigma-Aldrich).

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TURP-derived BPH surgical specimens were snap frozen in Tissue-Tek (Miles Laboratories) and stored at −80°C. Frozen sections (5-µM thick) were air dried and then fixed in acetone for 10 min. Endogenous peroxidase activity was blocked with 2% hydrogen peroxide and 0.1% sodium azide in cold TBS. Endogenous biotin was blocked by incubation with an avidin solution mixed in 1% BSA in PBS for 15 min, followed by a biotin solution mixed in 1% BSA in PBS for 15 min (Vector Laboratories). Sections were stained with H&E or with polyclonal rabbit anti-human IL-8, CXCR1, or CXCR2 (Santa Cruz Biotechnology), polyclonal rabbit anti-human Ki-67 (Abcam), and monoclonal mouse anti-human CD15, HLA-DR (BD Pharmingen), followed by goat anti-rabbit IgG or goat anti-mouse IgG (Santa Cruz Biotechnology) and biotinylated streptavidin-peroxidase conjugate. 3-Amino-9-ethylcarbazole (DakoCytomation) was used as chromogen and hematoxylin as a counterstain. To stain cultured BPH cells, they were first adhered to poly-L-lysine-coated slides, processed as described above, and then incubated with Abs specific for cytokeratin (pan-cytokeratin; Novocastra Laboratories), smooth muscle α-actin, and desmin or vimentin (Santa Cruz Biotechnology), followed by biotinylated anti-rabbit IgG, anti-mouse IgG or anti-goat IgG (Vector Laboratories), and streptavidin-peroxidase. 3-Amino-9-ethylcarbazole (DakoCytomation) was used as chromogen and hematoxylin as a counterstain. Negative controls were performed by incubation with appropriate isotype-matched primary mAbs.

CONFocal immunoFLUORESCENCE
To stain BPH cells, adherent BPH cell monolayers were incubated with ImageIT FX signal enhancer (Invitrogen), followed by overnight incubation with anti-CD40, anti-CD80, anti-CD86, or anti-HLA-DR-purified anti-human mAb (BD Pharmingen). After three washes with wash buffer (0.45 M NaCl, 0.24 M NaHPO₄, and 0.3% Triton X-100), BPH cells were incubated for 120 min with Cy2-conjugated AfinPure donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories), washed again, and stained with 5 μg/ml 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma-Aldrich) for 15 min at room temperature. Slides were then washed and mounted with ProLong Gold antifade reagent (Invitrogen) and read with an Ultra-view confocal microscope (PerkinElmer). Negative controls were performed by incubation with appropriate isotype-matched primary mAbs.

RESULTS
PRIMARY LINES OF HUMAN BPH Stromal CELLS
We have first established primary cell lines of human BPH cells and characterized them by staining with specific Abs as previously described (14). Cultured BPH cells showed positive staining for α
smooth muscle actin, vimentin, and desmin, suggesting fibromuscular morphological features. Conversely, they were negative for epithelial markers such as cytokeratin (Fig. 1A). Therefore, the BPH cells used in this study appear to be constituted by prostate stromal cells and they were used between the 4th and 10th in vitro passage. Stromal BPH primary cell cultures are devoid of contaminating professional APCs as well as of any leukocyte population tested (Fig. 1B). Staining with specific mAbs and analysis by flow cytometry shows in BPH cell cultures absence of cells expressing CD3 (T cells), CD14 (macrophages/monocytes), CD16 (NK cells, macrophages/monocytes, mast cells, neutrophils), CD19 and CD20 (B cells), CD56 (NK cells), CD11c (myeloid dendritic cells), and CD1a (Langherans cells/monocytes).

Functional TLR expression by human BPH stromal cells

The early events in the pathogenesis of BPH are still poorly understood, but infectious agents could be implicated in disease provocation (7). The host defense against microbial pathogens is triggered by the recognition of conserved motifs in infectious microorganisms mediated by TLRs, surface molecules able to recognize distinct structural components of pathogens, and activation of signal transduction pathways by TLRs leads to up-regulation of different genes that operate in host defense, including costimulatory molecules, cytokines, and chemokines (18). Using real-time RT-PCR, we have observed constitutive expression by human BPH cells of mRNA transcripts encoding TLR1 through TLR10 (Fig. 2A). All of the TLRs analyzed were found to be expressed, with a relatively higher abundance of transcripts encoding TLR3, 4, 5, 6, and 9.

To determine whether TLRs expressed by BPH cells were functional, we analyzed chemokine and cytokine production by BPH cells stimulated with human TLR agonists activating TLRs 1–9. Data in Fig. 2B show a strong induction of cytokine and chemokine production by BPH cells stimulated with poly(I:C) (TLR3 agonist) and LPS E. coli K12 (TLR4), weaker induction by Pam3CSK4 (TLR1/2), heat-killed L. monocytogenes (TLR2), flagellin S. typhimurium (TLR5), FSL1 (TLR6/2), Imiquimod (TLR7), ssRNA40 (TLR8), and virtually no induction by oligodeoxynucleotide 2006 (TLR9). Conversely, production of IL-2, IL-4, IL-10, IFN-γ, TNF-α, and CCL22 was not induced in the same culture supernatants by agonists stimulating TLR3, 4, and 7 (data not shown).

BPH cells express MHC class II and costimulatory molecules, up-regulate IL-12 and IL-23 subunits, and present alloantigen to CD4+ T cells

To analyze the capacity of BPH cells to act as APCs, we first examined their expression of MHC molecules. BPH cells constitutively express both MHC class I and class II molecules, which are strongly up-regulated by a 48-h incubation with IFN-γ, as detected by cytofluorometry (Fig. 3A). Markedly up-regulated expression of class II MHC molecules following treatment with IFN-γ was also observed by confocal microscopy (Fig. 3B). Expression of MHC class II molecules by epithelial and stromal BPH cells can be detected also in situ in prostate specimens obtained by TURP from BPH patients (Fig. 3C). To assess the capacity of BPH cells to act as APCs, we examined their ability to present alloantigen to alloreactive CD4+ T cells. Constitutive expression of MHC class II molecules by BPH cells, albeit relatively low, is already sufficient to induce proliferation of CFSE-labeled alloreactive CD4+ T cells, which is increased following IFN-γ treatment of BPH cells (Fig. 4A). BPH cells are also able to induce IFN-γ...
secretion by alloreactive CD4+ T cells (Fig. 4B). Maximal accumulation of IFN-γ produced by alloreactive CD4+ cells is induced after 2–3 days of incubation with IFN-γ-treated BPH cells and after 6 days of incubation with untreated BPH cells (Fig. 4B). Functional expression of MHC class II molecules is required for alloreactive CD4+ T cell activation, as shown by abrogation of the response in the presence of a neutralizing anti-MHC class II mAb recognizing HLA-DR, DP, and DQ molecules (Fig. 4C). Alloreactive CD4+ T cells cocultured with IFN-γ-stimulated BPH cells produce not only IFN-γ, the IL-12p75-dependent signature cytokine of Th1 cells, but also IL-17, a cytokine secreted by pathogenic T cells under the control of IL-23 (Fig. 4D).

Expression of CD40, CD80, CD86, and CD134 costimulatory molecules was determined in untreated and IFN-γ-treated BPH cells by confocal microscopy. Results in Fig. 5A show a faint cytoplasmic and membrane staining of these costimulatory molecules in untreated BPH cells, which is markedly enhanced by incubation for 48 h with IFN-γ. Expression of these costimulatory molecules was also confirmed in a functional assay by adding neutralizing mAbs specific for the individual molecules to cocultures of IFN-γ-treated BPH cells and alloreactive CD4+ T cells using secretion of IFN-γ as readout for T cell activation (Fig. 5B). The results show that CD40, CD80, CD86, and CD134 are all functionally expressed in alloantigen presentation by BPH cells. Consistent with data shown in Fig. 4C, the addition of anti-class II MHC mAb abrogates alloreactive CD4+ T cell activation, as revealed by the nearly complete inhibition of IFN-γ production (Fig. 5B). Complete inhibition of IFN-γ production by alloreactive CD4+ T cells is also induced by anti-IL-12p75 mAb (Fig. 5B).

Stimulation of BPH cells with a cytokine mixture comprising IFN-γ, IL-17, and TNF-α significantly up-regulates the inducible IL-12 family subunits p40 and p19, but has no effect on the constitutive expression of IL-12p35 transcripts (Fig. 6A). IL-12p75 and IL-12p75 secretion is induced in BPH cells by MHC class II-dependent interaction with alloreactive CD4+ T cells, with higher levels secreted by IFN-γ-treated compared with untreated BPH cells, although the difference is not statistically significant (Fig. 6B). In both cases, secretion of IL-12/IL-23p40 and IL-12p75 was nearly completely inhibited by addition to cocultures of anti-class II MHC mAb (Fig. 6C), indicating the requirement for alloreactive CD4+ T cell activation to induce efficient secretion of these molecules by BPH cells.

IFN-γ and IL-17 induce production of proinflammatory cytokines and chemokines by BPH cells

As described above, BPH cells can present alloantigen to CD4+ T cells, inducing secretion of IFN-γ and IL-17. Interestingly, these two cytokines markedly up-regulate production of IL-8, CXCL10, and IL-6 concentrations in the supernatants were measured by ELISA. Data are from one representative experiment of four performed.
and IL-6 by BPH cells (Fig. 7). IL-8 is strongly up-regulated by IL-17 and less markedly by IFN-γ. Conversely, CXCL10 is induced only by IFN-γ and not by IL-17. In the production of IL-6, a synergistic effect appears to be exerted by the combination of IFN-γ and IL-17. These results demonstrate that IFN-γ and IL-17, proinflammatory cytokines secreted by BPH-activated CD4 T cells specific for alloantigen, can induce in BPH cells elevated levels of IL-8 and IL-6, growth factors for epithelial and stromal prostate cells (19, 20), as well as robust secretion of CXCL10, a chemokine able to recruit CXCR3 Th1 cells.

IL-8, produced by BPH cells and by prostate cells in situ, is associated with presence of intraprostatic neutrophils and stimulates BPH cell proliferation

To confirm the capacity of BPH cells to produce IL-8, they were analyzed by confocal microscopy following staining with anti-IL-8 mAb. Results in Fig. 8A demonstrate constitutive production of IL-8 by BPH cells, which is up-regulated by treatment with IFN-γ. The IL-8 cognate receptors, CXCR1 and CXCR2, are also expressed by BPH cells, both constitutively and after IFN-γ treatment. To analyze the capacity of prostate cells to produce IL-8 in situ, prostate tissue samples obtained from BPH patients by TURP were stained by immunohistochemistry. Prostate sections from a representative BPH patient are shown in Fig. 7B. Staining with H&E demonstrates a florid lymphomononuclear cell infiltrate with a typical nodular appearance, exemplifying the inflammatory component of BPH. The proliferative component of BPH is illustrated by the strong expression in epithelial and stromal prostate cells of Ki-67, a cell proliferation marker. Both stromal and epithelial prostate cells produce IL-8 and express the cognate receptors CXCR1 and CXCR2. Production of IL-8 is functional, as shown by CD15 neutrophil polymorphonucleated cells, the major cell type recruited by IL-8, which are conspicuously present in the prostate inflammatory infiltrate (Fig. 8B).

We next tested the capacity of IL-8 to induce BPH cell proliferation. BPH cells were stimulated in culture with increasing concentrations of IL-8, from 0.1 to 100 ng/ml, and BPH cell numbers were recorded 48 h after culture initiation. Results in Fig. 9A show a dose-dependent enhancement of BPH cell proliferation induced by IL-8. The growth-promoting properties of IL-8 on BPH cells are comparable to the proliferation induced by optimal concentrations of classical proliferative stimuli for prostate cells.
like testosterone, KGF, and IGF (Des(1–3) IGF-1) (15). IL-8 induces autocrine/paracrine proliferation of BPH cells, as shown by the marked inhibition induced by addition of the neutralizing anti-CXCR1 mAb (Fig. 9B). Conversely, anti-CXCR2 mAb has an inferior inhibitory effect on IL-8-induced BPH cell proliferation, which does not reach statistical significance, indicating that the autocrine/paracrine proliferative effect of IL-8 is mostly mediated by binding to the IL-8 receptor CXCR1 (Fig. 9B).

**IFN-γ and IL-17 stimulate stromal BPH cell proliferation via production of IL-8**

Having demonstrated that IFN-γ and IL-17, secreted by BPH-stimulated CD4+ cells, are able to strongly enhance production of IL-8 by BPH cells, we examined the capacity of this cytokine combination to stimulate stromal BPH cell proliferation via production of IL-8. Results in Fig. 9C show that the combination of IFN-γ and IL-17 stimulates BPH cell proliferation. BPH cell proliferation is completely inhibited by addition to cultures of a neutralizing anti-IL-8 mAb, indicating the involvement of IL-8 produced by BPH cells in response to IFN-γ/IL-17 stimulation in the induction of BPH cell proliferation (Fig. 9C). The involvement of an IL-8-dependent loop in the autocrine/paracrine proliferation of BPH cells is further supported by the abrogation of IFN-γ/IL-17-induced proliferation in the presence of a neutralizing mAb specific for CXCR1. A lesser effect, although statistically significant, was induced by a mAb specific for CXCR2, whereas no inhibition of BPH cell proliferation was induced by isotype controls (Fig. 9C).

**Discussion**

Results in this study demonstrate, for the first time that human stromal prostate cells obtained from surgical samples of BPH patients can act as APCs, activating alloantigen-specific CD4+ T cells to produce IFN-γ and IL-17. Thus, prostate stromal cells have the potential to induce and sustain an autoimmune response. IFN-γ and IL-17, as well as TLR triggering, induce in BPH cells production of IL-8 and IL-6, key growth factors for epithelial and stromal prostate cells, providing a potential link between the T cell response induced by BPH cells and prostatic hyperproliferation.

Our data show that stromal BPH cells exhibit key features of APCs: expression of class II MHC molecules, expression of costimulatory molecules, and production of high levels of IL-12/IL-23p40 and IL-12p75. Expression of MHC class II molecules has been previously observed in BPH specimens (10, 21), although not consistently (22), but expression of costimulatory molecules by BPH has never been reported so far. Many cytokines have been shown to be produced by BPH cells (7), but not cytokines directing or amplifying the differentiation of pathogenic T cells such as IL-12p75 and IL-23, although BPH-derived T cells have been demonstrated to produce IL-17 (13). Cells producing IL-12p75 belong to two overlapping groups, cells involved in innate immunity, such as neutrophils, mast cells, and keratinocytes, and APCs also involved in adaptive immunity, in particular monocytes/macrophages, Langherans cells, and dendritic cells (23). Many cell types in the first group behave as nonprofessional APCs, suggesting that BPH cells could also exert this function similarly to thyroid epithelial cells shown to express MHC class II and costimulatory molecules and to present thyroid autoantigens to autoreactive CD4+ T cells (24, 25).

We have demonstrated the ability of BPH stromal cells to function as APCs by showing their capacity to present alloantigen and activate autoreactive CD4+ cells via MHC class II-dependent presentation. In turn, autoreactive CD4+ T cells markedly up-regulate IL-12/IL-23p40 and IL-12p75 secretion by BPH cells. Activation of alloantigen-specific CD4+ T cells results in secretion of IFN-γ and IL-17, signature cytokines of Th1 and Th17 cells, respectively, the major pathogenic effector T cell subsets in autoimmune diseases (26).

The induction of pathogenic T cell responses by BPH cells suggests common mechanisms promoting and sustaining BPH and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). The pathogenesis of CP/CPPS is still poorly understood, but evidence indicating an autoimmune component has begun to emerge (27). Prostate-specific antigenic peptide determinants are recognized by human CD4 and CD8 T cells, revealing lack of tolerance to prostatic self-proteins (28). PBMC and CD4+ T cells from CP/CPPS patients proliferate in response to seminal plasma (29, 30) and to specific prostate Ags (31, 32), indicating expression of the autoimmune T cell repertoire in disease pathogenesis. High-titer IgG autoantibodies to prostate-associated proteins are found in patients with CP/CPPS (33), further indicating a T cell-dependent autoimmune process. In addition, patients with a clinical diagnosis of CP/CPPS show higher levels, compared with controls, of proinflammatory cytokines, like IL-6, IL-8, IL-1β, and TNF-α, in seminal plasma (34–37). We have recently shown a concomitant increase of these and other proinflammatory cytokines and chemokines in the seminal plasma of BPH and CP/CPPS patients, consistent with the involvement of an important chronic inflammatory component in the pathogenesis of both diseases (38).

In particular, IL-8 has been found to represent a reliable biomarker of BPH and CP/CPPS and to discriminate between inflammatory and noninflammatory CP/CPPS (38). The potential value of IL-8 as a surrogate marker of BPH was further supported by the marked positive correlation of seminal IL-8 levels with symptom scores and serum concentration of prostate-specific Ag in BPH patients (38). IL-8 has been implicated in the pathogenesis of BPH via induction of FGF-2, a potent stromal and epithelial growth factor (19), and a growth-promoting activity of IL-8 has been observed in senescent prostate epithelial cells (39). Induction of prostate growth factors like KGF and FGF-2 in cultured BPH cells by IL-1α and IL-8 (19, 40) suggests that an activated immune system might sustain prostate overgrowth through locally produced growth factors, a situation similar to wound healing (2). Our present data show that IL-8 can directly promote autocrine/paracrine proliferation of BPH cells, which express both IL-8 cognate receptors CXCR1 and CXCR2. In addition, IL-8 production by prostate epithelial and stromal cells in situ, as documented by immunohistological analysis of BPH specimens, is associated with the presence of CD15+ neutrophils, suggesting the capacity of locally produced IL-8 to recruit lymphomononuclear cells into the prostate. Interestingly, our results demonstrate that IL-8-mediated BPH cell growth can be induced by a combination of IFN-γ and IL-17, thus establishing a possible relationship between the autoimmune response induced by BPH cells and prostate cell growth. These results are consistent with an IL-8-dependent link bridging inflammatory response and cell growth in BPH cells, which can be targeted by the vitamin D receptor agonist elocalcitol through multiple mechanisms of action (41).

IL-8 as well as IL-6, another autocrine growth factor for normal and cancer prostate cells (42), are induced at high levels also following triggering of TLRs expressed by BPH cells. We demonstrate expression of all TLRs by BPH cells, extending previous observations documenting selected TLR expression (43, 44). TLRs expressed by BPH cells are functional, with the apparent exception of TLR9, and their triggering by viral or bacterial products, such as poly(I:C) and LPS, induces production of proinflammatory chemokines like IL-8 and CXCL10 and cytokines like IL-6. In addition to the growth-promoting properties of IL-8 and IL-6 on
prostate cells, the capacity of IL-8 and CXCL10 to recruit inflammatory cells could play a role in inducing and maintaining chronic inflammatory conditions of the prostate, such as those observed in BPH and CP/CPPS patients.

In conclusion, our results show that human prostate cells can act as APCs, able to stimulate alloreactive CD4+ T cells to produce IFN-γ and IL-17. The induction of a BPH cell-driven autoimmune response, as well as triggering of TLRs expressed by BPH cells, up-regulate production of IL-8, IL-6, and CXCL10, key factors sustaining prostate inflammation, recruiting inflammatory leukocytes, and promoting prostate cell hyperplasia. Thus, BPH and CP/CPPS could share common pathogenetic mechanisms, based on the capacity of human stromal prostate cells to act as inducers and targets of chronic inflammation. Critically important for the development of both diseases could be the capacity of prostate cells to act as APCs and to respond to TLR agonists producing proinflammatory cytokines and chemokines able to recruit infiltrating cells and to promote prostate cell growth.

Disclosures

The authors have no financial conflict of interest.

References