Use of Aspirin and Statins in Relation to Inflammation in Benign Prostate Tissue in the Placebo Arm of the Prostate Cancer Prevention Trial

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ABSTRACT

Aspirin and statin use may lower the risk of advanced/fatal prostate cancer, possibly by reducing intraprostatic inflammation. To test this hypothesis, we investigated the association of aspirin and statin use with the presence and extent of intraprostatic inflammation, and the abundance of specific immune cell types, in benign prostate tissue from a subset of men from the placebo arm of the Prostate Cancer Prevention Trial. Men were classified as aspirin or statin users if they reported use at baseline or during the 7-year trial. Presence and extent of inflammation were assessed, and markers of specific immune cell types (CD4, CD8, FoxP3, CD68, and c-KIT) were scored, in slides from end-of-study prostate biopsies taken irrespective of clinical indication, per trial protocol. Logistic regression was used to estimate associations between medication use and inflammation measures, adjusted for potential confounders. Of 357 men included, 61% reported aspirin use and 32% reported statin use. Prevalence and extent of inflammation were not associated with medication use. However, aspirin users were more likely to have low FoxP3, a T regulatory cell marker [OR, 5.60; 95% confidence interval (CI), 1.16–27.07], and statin users were more likely to have low CD68, a macrophage marker (OR, 1.63; 95% CI, 0.81–3.27). If confirmed, these results suggest that these medications may alter the immune milieu of the prostate, which could potentially mediate effects of these medications on advanced/fatal prostate cancer risk.

Introduction

A growing body of evidence indicates that chronic inflammation contributes to prostate carcinogenesis (1). Intraprostatic inflammation is highly prevalent in older men with elevated PSA, abnormal digital-rectal examination (DRE), and benign prostatic hyperplasia (BPH; refs. 2–4), as well as in older men without clinical indication for prostate tissue removal (5–7). Chronic inflammation in the prostate could arise through exposure to infectious agents, environmental toxins, dietary factors, hormones, or possibly aging-associated factors, and could contribute to carcinogenesis via release of mutagenic reactive oxygen species or pro-proliferative and angiogenic cytokines (1).

However, despite biological plausibility, establishing a direct link between chronic intraprostatic inflammation and prostate cancer risk has been challenging. Inflammation can be assessed in tissue collected for indication (i.e., elevated PSA), but intraprostatic inflammation may also contribute to rising PSA levels (8). As a result, tissue collected for indication is enriched for inflammation regardless of prostate cancer status. Our team previously conducted the only two studies that have examined inflammation in men without indication for biopsy in relation to prostate cancer risk: a case–control study in the Prostate Cancer Prevention Trial (PCPT) that found a positive association between inflammation in at least one biopsy core and overall and high-grade prostate cancer (6), and a prospective study of men who participated in both PCPT and the Selenium and Vitamin E PCPT that found a positive trend between increasing mean percent of tissue with inflammation and odds of subsequent prostate cancer diagnosis (7).

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If intraprostatic inflammation is causally associated with prostate cancer, then strategies to combat inflammation could plausibly reduce prostate cancer risk. Aspirin and statins are medications commonly used by older adults that are known to have anti-inflammatory properties (9, 10). With respect to prostate cancer prevention, meta-analyses of observational studies support that regular use of aspirin and other NSAIDS (11, 12) and statins (13) are modestly inversely associated with prostate cancer risk, and more strongly inversely associated with risk of advanced or fatal disease. Evidence of a relationship between anti-inflammatory medication use and intraprostatic inflammation would enhance the biological plausibility of these findings; however, to our knowledge, this association has not yet been examined in prostate tissue collected without clinical indication.

The purpose of this study was to investigate the association between aspirin, nonaspirin NSAID, and statin use and the prevalence and extent of inflammation, as well as the abundance of specific immune cell types, in benign prostate tissue collected irrespective of indication from men in the placebo arm of the PCPT. We hypothesized that use of these medications would be associated with a decreased prevalence and extent of intraprostatic inflammation and a differing abundance of specific immune cells.

Materials and Methods

Study sample

This study included a subset of men from the PCPT, a phase III, randomized, double-blinded, placebo-controlled trial designed to evaluate finasteride for the primary prevention of prostate cancer (14). Between 1993 and 1997, the trial recruited 18,882 men ages 55 years and older with no evidence of prostate cancer at enrollment [normal DRE, PSA ≤3 ng/mL, and International Prostate Symptom Score (IPSS) <20] from 221 study sites across the United States. Participants underwent annual prostate cancer screening for up to 7 years and were recommended for biopsy if their PSA was elevated or their DRE was abnormal. At the end of 7 years, all participants not diagnosed with prostate cancer were asked to undergo an end-of-study biopsy, irrespective of indication.

This study included men from the placebo arm of the PCPT who underwent an end-of-study biopsy and were selected for a nested case–control study of lower urinary tract symptoms (LUTS) incidence and progression (5). Case–control sets for LUTSs incidence and progression were selected from men who had IPSS <15 at baseline, were not taking LUTS medications, and had no history of BPH surgery or physician-diagnosed BPH/LUTS, and were developed on the basis of the IPSS at baseline and 7 years. Participants who had prostate cancer detected at the end-of-study biopsy were not excluded, to minimize potential for selection bias. For this study, LUTS cases and controls were combined, as LUTS case–control status was only weakly associated with intraprostatic inflammation in biopsies from the periphery of the prostate in the prior study (5). These LUTS cases and controls were used for this study because data on inflammation and immune cell types were already generated.

This study was conducted in accordance with the U.S. Common Rule. The PCPT was approved by the institutional review boards (IRB) at all participating study sites; this study was approved by the Johns Hopkins Bloomberg School of Public Health IRB (Baltimore, MD) and the Colorado Multiple IRBs. All participants provided written informed consent to participate in the PCPT.

Measurement of medication use and other covariates

At enrollment, baseline demographics, medical, and lifestyle factors were collected via questionnaire. Current medication use was assessed with both closed (e.g., "do you use aspirin?") and open-ended questions. Participants were also asked to report any new use of medications at each in-person or telephone follow-up, occurring every 3 months postbaseline. Participants were categorized as users of each medication if they reported use at baseline or any point during the 7-year follow-up.

Baseline weight and height were measured using standardized protocols, and weight was remeasured annually. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Men were asked to complete a food frequency questionnaire at the first annual follow-up. Serum PSA was measured in samples collected from baseline and at annual follow-up visits at a central laboratory.

Measurement of intraprostatic inflammation and immune cell markers

We used published (5) and unpublished data previously collected in the LUTS nested case–control study. Briefly, to assess presence and extent of inflammation, an average of two (range: 1–6) randomly selected hematoxylin and eosin–stained slides containing one or more prostate biopsy cores were digitized and reviewed using Aperio ImageScope Viewer Software. In biopsy cores with both tumor and benign tissue, only benign tissue was reviewed. The study pathologists recorded the presence of any inflammatory cells in each biopsy core and the approximate percentage of total biopsy core area with inflammatory cell involvement.

To assess the abundance of specific immune cell types, an average of two (range: 1–3) randomly selected unstained slides containing one or more biopsy cores were IHC stained for (i) CD4 ($CD4^+$ T cells), (ii) CD8 ($CD8^+$ T cells), (iii) FoxP3 [T regulatory cells (Tregs)], (iv) $CD68^+$ cells (macrophages), and (v) c-KIT (mast cells). These immune cell types were chosen as cell types that were expected to be observed within the prostate on the basis of prior studies from tissue collected for indication (15). One of two study pathologists visually reviewed and scored each slide on a scale of 0–4, with 0 indicating no cells identified and 4 indicating an extensive number of cells.

Statistical analysis

Characteristics of users and nonusers of each medication were described using medians for continuous variables and proportions for categorical variables. In univariate analyses, proportions, χ^2 tests, and Cochran–Armitage trend tests were used to compare each outcome of interest by aspirin, nonaspirin NSAID, and statin use. These outcomes included (i) the presence of intraprostatic inflammation, defined as having at least one biopsy core with inflammation (yes and no), (ii) the extent of intraprostatic inflammation, defined as the percentage of biopsy cores with inflammation (categorized as 0%, >0% but <100%, and 100%) and the mean percentage of tissue area with inflammation (categorized as 0%, <3%, and ≥3%, because 3% was the median of the nonzero values), and (iii) the abundance of markers of each immune cell type. Because multiple slides per person were reviewed, and because each slide had varying numbers of biopsy cores, a weighted average score for each immune cell marker was calculated on the basis of the number of cores per scored slide. Using this weighted score, the abundance of each immune cell marker was categorized as low (less than the median, i.e., <1), medium (1), or high (>1).

Multivariate regression models were used to examine the association between aspirin and statin use and inflammation/immune cells after adjusting for potential confounders. Multivariate models were not run for nonaspirin NSAID use given the null univariate results. The presence of inflammation was modeled using logistic regression, and the percentage of biopsy cores with inflammation [none (reference), some, and high], the mean percentage of tissue area with inflammation [0% (reference), <3%, and ≥3%], and abundance of immune cell markers [low, medium (reference), and high] were modeled using polytomous logistic regression. Ordinal logistic regression was also attempted, but the proportional odds assumption did not hold. Multivariate models simultaneously included both aspirin and statin use and were adjusted for other potential confounders, including age at biopsy (continuous), race (white and non-white), baseline BMI (continuous), cigarette smoking status (current, former, and never), physical activity (sedentary, light, moderate, and active), education (college and no college), and diabetes (yes and no).

As a sensitivity analysis, univariate analyses were repeated after restricting to the LUTS controls ($N = 86$); these were men with IPSS <8 at baseline and at year 7, and men with IPSS <8 at baseline and baseline to year 7 slope <25th percentile. Analyses were also repeated after restricting to men who were not diagnosed with prostate cancer on the end-of-study biopsy ($N = 295$). While in the primary analysis, men with a PSA ≥4 ng/mL could have been included if they had a prior negative biopsy during trial follow-up, and were consequently not clinically indicated for biopsy at the end of the trial despite elevated PSA, in a sensitivity analysis, we restricted to men with a PSA <4 ng/mL immediately prior to the end-of-study biopsy ($N = 317$). These sensitivity analyses were conducted to ensure that the case–control sampling procedure for LUTS, the inclusion of men with prostate cancer, and the inclusion of who may have been clinically indicated for biopsy under conventional protocols did not meaningfully alter the results. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Analyses were conducted in SAS version 9.4.

Results

There were 357 men from the placebo arm of PCPT included in this analysis. The median age at the end-of-study biopsy was 70 years old, and the median PSA was 1.50 ng/mL. Of these men, 218 (61%) reported aspirin use and 115 (32%) reported statin use during the trial period. Eighty-six men (24%) reported use of both aspirin and statins. Other unadjusted characteristics of the study sample are displayed in **Table 1**. Both aspirin and statin users were less likely than nonusers to have a college education and were more likely to be former smokers. PSA concentration at the end-of-study biopsy was slightly lower among men who used aspirin or statins. At the

Table 1. Characteristics of a subset[a] of men from the placebo arm of PCPT, by aspirin use[b] and statins use[b].

	Aspirin use		Statins use	
	No	Yes	No	Yes
N	139	218	242	115
Age at end-of-study biopsy, median	69	70	69	71
Race[c], %				
White	96	96	95	97
Non-white	4	4	5	3
College education[c], %	60	48	54	50
Diabetes[c], %	6	6	4	9
BMI[c] (kg/m^2), median	26.7	27.4	26.9	27.3
Smoking status[c], %				
Current	8	8	10	3
Former	48	66	56	64
Never	44	27	34	32
Pack-years of smoking[c], median	21.5	23.1	21.5	23.1
Physical activity[c], %				
Sedentary	19	24	21	23
Light	12	17	14	16
Moderate	58	46	53	45
Very active	11	13	11	16
Missing	1	0	0	0
Daily intake[c], median				
Energy (kcal)	1,930	2,094	2,115.1	1,860.5
Vegetables (servings/day)	1.9	2.0	1.8	2.1
Total fat (g)	67.6	74.5	77.9	62.3
Polyunsaturated fatty acids (g)	14.0	15.4	15.4	13.0
Total protein (g)	78.6	87.9	88.0	78.0
Red meat (servings/week)	3.5	3.9	4.0	3.4
Alcoholic beverages (drinks/day)	0.3	0.2	0.4	0.1
PSA at biopsy (ng/mL), median	1.7	1.4	1.6	1.3
Prostate cancer diagnosis at end-of-study biopsy, %	15	19	16	21

[a]From a case–control study of LUTSs nested in the placebo arm of PCPT (5). The men did not have a clinical indication for biopsy.
[b]Reported at trial entry or during the 7 years of the trial.
[c]Reported at trial entry.

Table 2. Presence and extent of intraprostatic inflammation and abundance of immune cell markers[a] by aspirin use and statins use, in a subset[b] of men from the placebo arm of PCPT.

	Aspirin use			Statins use		
	No, %	Yes, %	P^c	No, %	Yes, %	P^c
≥1 core with inflammation	64	68	0.5	66	68	0.7
Percent of cores with inflammation						
None	37	32	0.9	35	33	0.9
Some	53	62		57	61	
All	10	6		8	6	
Mean percent tissue area with inflammation						
None	36	32	0.7	34	32	0.8
<3%	30	34		31	37	
≥3%	34	33		35	30	
CD4						
Low	15	20	0.3	19	18	0.8
Medium	43	41		41	44	
High	42	39		41	38	
CD8						
Low	8	12	0.2	9	14	0.1
Medium	69	69		69	70	
High	23	19		22	17	
FoxP3						
Low	2	8	0.1	5	5	0.8
Medium	70	68		68	70	
High	29	25		27	25	
CD68						
Low	13	14	0.4	11	20	**0.02**
Medium	75	76		77	72	
High	13	10		12	8	
c-KIT						
Low	11	8	0.4	9	10	0.2
Medium	73	74		72	77	
High	16	18		20	13	

[a]Abundance was scored on a scale of 0–4. When multiple slides per individual were scored, a weighted average was calculated using the number of cores per slide. Abundance was categorized on the basis of the median value of 1 (low: <1, medium: 1, and high: >1).

[b]From a case–control study of LUTSs nested in the placebo arm of PCPT (5). The men did not have a clinical indication for biopsy.

[c]*P* value from the χ^2 test (for dichotomous variables) or Cochran–Armitage trend test (for ordinal variables). Bolded values are statistically significant.

end-of-study biopsy, 19% of aspirin users (vs. 15% of nonaspirin users) and 21% of statin users (vs. 16% of nonstatin users) were diagnosed with prostate cancer.

A median of four biopsy cores per man were assessed for inflammation (range: 1–11). The prevalence of having at least one biopsy core with inflammation was similar among aspirin users and nonusers (68% vs. 64%) and statin users and nonusers (68% vs. 66%; **Table 2**). The extent of inflammation also did not differ by medication use, both when assessed as the percentage of biopsy cores with inflammation and the mean percentage tissue area with inflammation (**Table 2**). Consistent with these univariate results, aspirin and statin use were not associated with either the presence or extent of inflammation after multivariate adjustment (**Table 3**). The presence and extent of inflammation also did not differ for men who reported use of both aspirin and statins (Supplementary Table S1).

There were 321, 326, 315, 325, and 297 men with data on abundance of CD4-, CD8-, FoxP3-, CD68-, and c-Kit-positive cells, respectively. A median of four biopsy cores per person (range: 1–14) were stained for CD4, CD8, FoxP3, and CD68 cells, and a median of 2.5 cores per person (range: 1–10) were stained for c-Kit cells. For all markers, the median and mode weighted scores were 1. In univariate analyses, aspirin users appeared to have lower abundance (i.e., scores <1) of CD4, CD8, and FoxP3 cells compared with nonaspirin users (**Table 2**). The difference between aspirin users and nonusers in FoxP3 cells was statistically significant after multivariate adjustment [OR, 5.60, 95% confidence interval (CI), 1.16–27.07 for low vs. medium staining for FoxP3; **Table 4**]. Statin use appeared to be associated with lower abundance of CD8 and CD68 cells. Associations with CD68 were statistically significant in both univariate and multivariate models (OR, 1.92; 95% CI, 1.00–3.71 for low vs. medium staining for CD68 in the model adjusted for age and race), although further adjustment for lifestyle factors attenuated this result (**Table 4**). Use of both aspirin and statins was also associated with low CD68 (Supplementary Table S1).

Similar patterns were observed for all outcomes in sensitivity analyses restricted to LUTS controls (Supplementary Table S2), men without prostate cancer (Supplementary Table S3), and men with a PSA <4 ng/mL at the end of the trial (Supplementary Table S4).

Thirty-three percent of the study sample reported use of nonaspirin NSAIDs. Nonaspirin NSAID use was not associated with any of the outcomes examined (Supplementary Table S5).

Discussion

This study examined associations between aspirin, nonaspirin NSAID, and statin use and the overall presence and extent of inflammation, as well as markers of specific immune cells, in benign prostate tissue to inform a mechanistic link between use of the medications and prostate cancer prevention. We found that the presence and extent of intraprostatic inflammation was similar among users and nonusers of these medications. However, slight differences were observed in the abundance of specific immune cell markers. Specifically, FoxP3, a marker of Tregs, was less abundant in benign prostate tissue of aspirin users compared with nonusers, while CD68, a marker of macrophages, was less abundant among statin users.

To our knowledge, this is the first study to examine the relationship between anti-inflammatory medication use and intraprostatic inflammation in men without biopsy indication or other clinical reason for prostate tissue removal. Prior studies have examined statin use in relation to inflammation in negative prostate biopsies that were clinically indicated (16) and in prostatectomy specimens from men with prostate cancer (17), but these tissue specimens may have been enriched for inflammation due to the clinical indication and presence of prostate cancer, respectively. Other studies have examined aspirin (18–28) and statin (29) use in relation to circulating

Table 3. Associations between aspirin use and statins use and the presence and extent of intraprostatic inflammation in a subset[a] of men from the placebo arm of PCPT.

	Aspirin use		Statins use	
	At least one core with inflammation		At least one core with inflammation	
	No	Yes	No	Yes
# Users/nonusers	70/50	148/89	37/83	78/159
Model 1 OR (95% CI)	Reference	1.19 (0.76–1.86)	Reference	1.10 (0.69–1.77)
Model 2 OR (95% CI)	Reference	1.11 (0.70–1.75)	Reference	1.02 (0.63–1.65)
Model 3 OR (95% CI)	Reference	1.16 (0.71–1.89)	Reference	1.04 (0.62–1.73)

	Percent of cores with inflammation			Percent of cores with inflammation		
	None	Some	All	None	Some	All
# Users/nonusers	70/52	135/73	13/14	35/73	63/124	6/16
Model 1 OR (95% CI)	Reference	1.14 (0.87–2.17)	0.69 (0.30–1.59)	Reference	1.12 (0.69–1.81)	0.77 (0.30–1.99)
Model 2 OR (95% CI)	Reference	1.29 (0.81–2.06)	0.61 (0.26–1.43)	Reference	1.04 (0.63–1.70)	0.67 (0.26–1.75)
Model 3 OR (95% CI)	Reference	1.33 (0.81–2.19)	0.67 (0.26–1.70)	Reference	1.01 (0.60–1.70)	0.92 (0.33–2.56)

	Mean percent tissue area with inflammation			Mean percent tissue area with inflammation		
	None	<3%	≥3%	None	<3%	≥3%
# Users/nonusers	70/50	75/42	73/47	37/83	43/74	35/85
Model 1 OR (95% CI)	Reference	1.28 (0.76–2.15)	1.11 (0.66–1.86)	Reference	1.30 (0.76–2.24)	0.92 (0.53–1.61)
Model 2 OR (95% CI)	Reference	1.21 (0.70–2.06)	1.02 (0.61–1.73)	Reference	1.23 (0.71–2.15)	0.84 (0.48–1.47)
Model 3 OR (95% CI)	Reference	1.21 (0.68–2.13)	1.12 (0.64–1.95)	Reference	1.23 (0.67–2.20)	0.87 (0.48–1.58)

Note: Model 1, unadjusted; model 2, adjusted for age and race; model 3, adjusted for age (continuous), race (white and non-white), BMI (continuous), smoking status (current, former, and never), physical activity (sedentary, light, moderate, and active), education (college and no college), diabetes (yes and no), and statins use (for aspirin model) or aspirin use (for statins model; yes and no).

[a]From a case–control study of LUTSs nested in the placebo arm of PCPT (5). The men did not have a clinical indication for biopsy.

markers of inflammation, but circulating markers are not necessarily indicative of inflammation within the prostate, which is most etiologically relevant for prostate cancer. There is evidence that these drugs have general immune modulatory effects (30, 31), but effects on specific immune cells in the prostate have not been examined to date.

Our study observed a slightly lower abundance of Tregs in benign prostate tissue in aspirin users as compared with nonusers. This finding is plausible given that aspirin, via inhibition of the cyclooxygenase enzymes, inhibits synthesis of prostaglandin E_2 (PGE_2), which has been shown to promote development of Tregs (32). Inhibition of COX-2/PGE_2 has also been shown to reduce Treg activity in murine lung cancer models (33). Tregs downregulate the immune system and may block T cells from mounting an effective antitumor response (34–36). In accordance with this proposed protumorigenic role, studies have found Tregs to be more prevalent in tumor versus benign prostate tissue from the same patients, and in peripheral blood of prostate cancer versus non-prostate cancer donors (37). Greater numbers of epithelial Tregs have also been positively associated with Gleason sum and pathologic stage (38). On the other hand, Tregs may also inhibit cancer development by restraining cancer-promoting inflammation (39). Thus, while our study suggests that aspirin use may lower the number of Tregs in the prostate, additional studies of Tregs and prostate cancer incidence and progression are needed to better understand the implications of this finding.

We also observed a lower abundance of macrophages in benign prostate tissue of men who reported using a statin. Macrophages are one of the most abundant immune cells in the tumor microenvironment and can promote tumor growth and progression via promotion of inflammation, immunosuppression, angiogenesis, invasion, and metastasis (40). M2 macrophages, in particular, are thought to suppress the antitumor immune response and have been associated with poorer prostate cancer prognosis (41–43). Statins may influence macrophage function via the mevalonate biosynthetic pathway and the associated protein farnesylation and small G protein signaling activity (44, 45). There is biological evidence for such a link, as statins have been shown to regulate gene expression in human macrophages treated with oxidized low-density lipoprotein (44), and animal studies have found statins to reduce the proliferation and activation of macrophages within atherosclerotic plaques (46). Further research is needed to understand why macrophages, but not other immune cells, appeared influenced by statins use, to quantify the abundance of specific macrophage phenotypes (i.e., M1 vs. M2), and to determine whether the difference in macrophage abundance observed in this study is clinically meaningful.

In this study, a higher proportion of aspirin users (19%) than nonusers (15%) and statin users (21%) than nonusers (16%) were diagnosed with prostate cancer at the end-of-study biopsy ($n = 62$ end-of-study prostate cancers diagnosed total), although neither of these differences was statistically significant ($P = 0.37$ for aspirin; $P = 0.23$ for statins). These results are consistent with studies of the full placebo arm of PCPT, which

Table 4. Associations between aspirin use and statins use and the abundance[a] of immune cell markers in a subset[b] of men from the placebo arm of PCPT.

	Aspirin use			Statins Use		
	CD4			CD4		
	Low	Medium	High	Low	Medium	High
# Users/nonusers	42/17	84/50	79/49	18/41	44/90	38/90
Model 1 OR (95% CI)	1.47 (0.76–2.85)	Reference	0.96 (0.58–1.58)	0.90 (0.46–1.74)	Reference	0.86 (0.51–1.46)
Model 2 OR (95% CI)	1.50 (0.77–2.91)	Reference	0.94 (0.57–1.55)	0.94 (0.48–1.84)	Reference	0.82 (0.48–1.40)
Model 3 OR (95% CI)	1.66 (0.81–3.40)	Reference	1.06 (0.61–1.82)	0.91 (0.45–1.85)	Reference	0.85 (0.49–1.50)
	CD8			CD8		
	Low	Medium	High	Low	Medium	High
# Users/nonusers	25/9	144/82	39/27	14/20	71/155	17/49
Model 1 OR (95% CI)	1.58 (0.71–3.55)	Reference	0.82 (0.47–1.44)	1.53 (0.73–3.20)	Reference	0.76 (0.41–1.41)
Model 2 OR (95% CI)	1.60 (0.71–3.60)	Reference	0.80 (0.45–1.40)	1.58 (0.75–3.36)	Reference	0.71 (0.38–1.33)
Model 3 OR (95% CI)	1.33 (0.56–3.13)	Reference	0.76 (0.41–1.40)	1.60 (0.72–3.57)	Reference	0.77 (0.40–1.50)
	FoxP3			FoxP3		
	Low	Medium	High	Low	Medium	High
# Users/nonusers	15/2	135/80	50/33	5/12	67/148	24/59
Model 1 OR (95% CI)	4.44 (0.99–19.94)	Reference	0.90 (0.53–1.51)	0.92 (0.31–2.72)	Reference	0.90 (0.52–1.57)
Model 2 OR (95% CI)	**4.98 (1.10–22.58)**	Reference	0.86 (0.51–1.45)	1.15 (0.38–3.48)	Reference	0.84 (0.48–1.48)
Model 3 OR (95% CI)	**5.60 (1.16–27.07)**	Reference	0.94 (0.53–1.67)	1.01 (0.31–3.23)	Reference	0.97 (0.54–1.77)
	CD68			CD68		
	Low	Medium	High	Low	Medium	High
# Users/non-users	30/15	157/88	20/15	21/24	74/171	8/27
Model 1 OR (95% CI)	1.12 (0.57–2.20)	Reference	0.75 (0.36–1.53)	**2.02 (1.06–3.86)**	Reference	0.69 (0.30–1.58)
Model 2 OR (95% CI)	1.09 (0.55–2.14)	Reference	0.73 (0.36–1.51)	**1.92 (1.00–3.71)**	Reference	0.65 (0.28–1.51)
Model 3 OR (95% CI)	0.83 (0.40–1.73)	Reference	0.73 (0.33–1.60)	1.63 (0.81–3.27)	Reference	0.72 (0.30–1.75)
	c-Kit			c-Kit		
	Low	Medium	High	Low	Medium	High
# Users/nonusers	15/12	137/81	34/18	9/18	72/146	12/40
Model 1 OR (95% CI)	0.74 (0.33–1.66)	Reference	1.12 (0.59–2.11)	1.01 (0.43–2.37)	Reference	0.61 (0.30–1.23)
Model 2 OR (95% CI)	0.71 (0.32–1.60)	Reference	1.10 (0.58–2.09)	0.95 (0.40–2.25)	Reference	0.58 (0.28–1.19)
Model 3 OR (95% CI)	0.48 (0.19–1.22)	Reference	1.18 (0.60–2.32)	1.23 (0.48–3.19)	Reference	0.62 (0.30–1.31)

Note: Model 1, unadjusted; model 2, adjusted for age and race; model 3, adjusted for age (continuous), race (white and black), BMI (continuous), smoking status (current, former, and never), physical activity (sedentary, light, moderate, and active), education (college and no college), diabetes (yes and no), and statins use (for aspirin model) or aspirin use (for statins model; yes and no). Bolded values are statistically significant.

[a]Abundance was scored on a scale of 0–4. When multiple slides per individual were scored, a weighted average was calculated using the number of cores per slide. Abundance was categorized on the basis of the median value of 1 (low: <1, medium: 1, and high: >1).

[b]From a case–control study of LUTSs nested in the placebo arm of the PCPT (5). The men did not have a clinical indication for biopsy. Sample size for analyses of each marker was as follows: CD4, $n = 321$; CD8, $n = 326$; FoxP3, $n = 315$; CD68, $n = 325$; and c-Kit, $n = 297$.

reported no protective associations between aspirin or statin use and total prostate cancer in this cohort (47, 48). While these findings may seem surprising given that aspirin and statins are purported to have anticarcinogenic effects, they are not in direct conflict with the existing literature, which has shown weaker associations for total prostate cancer risk, but much more consistent and robust inverse associations between aspirin and statin use and advanced, lethal, or fatal prostate cancer (11–13). PCPT participants were also screened annually for prostate cancer, and the lack of inverse associations observed in PCPT could indicate that these medications do not lower prostate cancer risk in highly screened populations, where risk, and particularly risk of advanced disease, may be mitigated more strongly by screening and early detection. Nevertheless, our results were consistent when we excluded the men with prostate cancer detected on the end-of-study biopsy, and our biological findings should apply equally to screened and unscreened populations.

For each immune cell marker, data were not available for 9%–17% of the men due to unavailability of slides, insufficient tissue on slides, or problems with IHC staining. Rates of missingness for the immune cell markers were lower among aspirin users, but missingness was not associated with any other demographic or clinical variables and reasons for missingness did not differ by aspirin use, suggesting that lower rates of missing data among aspirin users occurred by chance.

Limitations of this study include the small sample size and cross-sectional study design. Because we tested multiple hypotheses, we also cannot rule out the possibility that our findings related to Tregs and macrophages were false positives, particularly given that null associations were observed for other immune cell types regulated by similar biochemical pathways. Conversely, the null findings for the other immune cell types and for inflammation overall may have been false negatives due to nondifferential misclassification of our exposure or outcome. Misclassification of medication use may have occurred due to our lack of data on the duration of medication use, and specifically on whether men stopped taking aspirin or statins before the end-of-study biopsy. Misclassification of inflammation and immune cell measurements may have occurred as each outcome was visually assessed by pathologists as opposed to quantitatively measured. Study pathologists included multiple genitourinary pathologists trained for the review of inflammation and immune cell markers, but we cannot rule out nondifferential misclassification due to differences in pathologists' scoring. As technology is rapidly advancing, future studies will be able to utilize more precise methods for quantifying the extent of inflammation and profiling immune cells in prostate tissue; such studies will be key for confirming both our positive and null results.

This study also has several notable strengths. The study included the use of multiple measures, including both the presence and extent of inflammation, and the abundance of markers of innate and adaptive immune cells. Such detailed assessment allowed us to not only assess the extent of inflammation within prostate tissue, but to understand the specific immune cells that might be modulating the inflammatory response. IHC staining was performed by a single laboratory with trained pathologists using validated, standardized protocols, thereby minimizing opportunities for error. Importantly, for the majority of men, inflammation and immune cell markers were measured in prostate tissue collected without indication for biopsy, thus avoiding the selection bias that arises when only men with suspected or diagnosed prostate cancer because of elevated serum PSA are included.

This study provides preliminary population-based evidence that aspirin and statin use may influence certain immune cells within the prostate of men without indication for biopsy. Additional research utilizing increasingly precise methodologies is needed to confirm these observational findings and further interrogate the hypothesis that aspirin and statin use may influence advanced/fatal prostate cancer risk via immune modulation. However, given our observed lack of association for aspirin and statins use and the overall presence and extent of intraprostatic inflammation, other potential mechanisms linking aspirin and statins use to prostate cancer should also be explored.

Disclosure of Potential Conflicts of Interest

M.T. Marrone reports grants from NCI (K99CA246097) during the conduct of the study. K.B. Arnold reports grants from Fred Hutchinson Cancer Research Center during the conduct of the study. C.G. Drake reports personal fees from AZ Medimmune, Bayer, BMS, Compugen, Ferring, F-Star, Genocea, Janssen, Kleo, Merck, Merck-Serono, Pfizer, Pierre Fabre, Roche/Genentech, Shattuck Labs, Tizona, UroGen, Werewolf, and Harpoon outside the submitted work as well as has a patent to BMS licensed. E.A. Platz reports grants from NIH and Johns Hopkins University during the conduct of the study. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Authors' Contributions

L.M. Hurwitz: Conceptualization, formal analysis, investigation, methodology, writing-original draft. **I. Kulac:** Data curation, investigation, writing-review and editing. **B. Gumuskaya:** Data curation, investigation, writing-review and editing. **J.A.B. Del Valle:** Data curation, investigation, writing-review and editing. **I. Benedetti:** Data curation, investigation, writing-review and editing. **F. Pan:** Writing-review and editing. **J.O. Liu:** Writing-review and editing. **M.T. Marrone:** Writing-review and editing. **K.B. Arnold:** Writing-review and editing. **P.J. Goodman:** Writing-review and editing. **C.M. Tangen:** Writing-review and editing. **M.S. Lucia:** Writing-review and editing. **I.M. Thompson:** Writing-review and editing. **C.G. Drake:** Investigation, methodology, writing-review and editing. **W.B. Isaacs:** Investigation, methodology, writing-review and editing. **W.G. Nelson:** Investigation, methodology, writing-review and editing. **A.M. De Marzo:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing-review and editing. **E.A. Platz:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing-review and editing.

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