

Consumption of Soy Isoflavone Enriched Bread in Men with Prostate Cancer Is Associated with Reduced Proinflammatory Cytokines and Immunosuppressive Cells

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Abstract

We hypothesized that soy phytochemicals may have immunomodulatory properties that may affect prostate carcinogenesis and progression. A randomized, phase II trial was conducted in 32 patients with prostate cancer with asymptomatic biochemical recurrence but no measurable disease on standard staging studies. Patients were randomized to two slices of soy bread (34 mg isoflavones/slice) or soy bread containing almond powder daily as a source of β -glucosidase. Flow cytometry and bioplex assays were used to measure cytokines or immune cell phenotype in blood at baseline (day 0) and following intervention (day 56). Adequate blood samples were available at enrollment and day 56 and evaluated. Multiple plasma cytokines and chemokines were significantly decreased on day 56 versus baseline. Subgroup analysis indicated reduced T_H1 ($P = 0.028$) and myeloid-derived

suppressor cell (MDSC)-associated cytokines ($P = 0.035$). T_H2 and T_H17 cytokines were not significantly altered. Phenotypic analysis revealed no change in $CD8^+$ or $CD4^+$ T cells but showed increased $CD56^+$ natural killer (NK) cells ($P = 0.038$). The percentage of cells with a T regulatory cell phenotype ($CD4^+CD25^+FoxP3^+$) was significantly decreased after 56 days of soy bread ($P = 0.0136$). Significantly decreased monocytic ($CD33^+HLADR^{neg}CD14^+$) MDSC were observed in patients consuming soy bread ($P = 0.0056$). These data suggest that soy bread modulates systemic soluble and cellular biomarkers consistent with limiting inflammation and suppression of MDSCs. Additional studies to elucidate impact on the carcinogenic process or as a complement to immune-based therapy are required. *Cancer Prev Res*; 8(11); 1036–44. ©2015 AACR.

Introduction

Soybeans and the foods derived from them are a rich source of bioactive phytochemicals. Among the phytochemicals, the isoflavones (primarily genistein and daidzein) have received the greatest attention regarding putative health promoting properties, ranging from bone health to cancer prevention. The concept of soy as an intervention for prostate cancer was initially founded upon prior epidemiologic studies demonstrating a lower risk of prostate cancer in populations consuming considerable dietary soy (1, 2).

Soy-enriched diets and pure phytochemicals from soy have subsequently shown anticancer activity in experimental models of prostate carcinogenesis (3–6).

On the basis of these data, human clinical trials testing multiple types, schedules and doses of dietary soy, or soy components have been conducted in humans with prostate cancer in both the pre- and post-prostatectomy setting. These studies have produced very interesting, yet sometimes seemingly divergent results given the heterogeneity in study population and design, as well as concerns for study size and statistical power (7–11). Our group and others are actively investigating the potential of diets rich in soy components to prevent or slow human prostate cancer progression, particularly in hormone-sensitive disease (4, 5, 12–14). Soy isoflavones are hypothesized to act through various pathways to impact prostate cancer progression, such as inhibition of tumor growth factor signaling (15), antiangiogenesis (12, 16), cell-cycle inhibition (4), and metastasis (17).

One emerging area in prostate carcinogenesis, progression, and therapy concerns the role of the host immune response. Indeed, activation of inflammatory pathways has been linked to early carcinogenesis and progression (18–20). More recently, novel strategies to harness the host immune response for therapeutic benefit are the focus of extensive preclinical and clinical research. For example, multiple phase III studies of novel strategies (e.g., PSA-TRICOM vaccine, antibodies targeting CTLA4 and PD1/PDL1) are building upon the 2011 approval of sipuleucel-T/Provenge

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immune therapy for prostate cancer (18–21). Yet, how dietary components, such as soy, may impact the host to potentiate or possibly antagonize immune therapy remains unknown.

Complex networks of white blood cells and soluble factors control the type, magnitude, and duration of immune responses to pathogens, various environmental insults, or emerging cancer cells. Following an acute inflammatory response, the host orchestrates negative regulatory cells and soluble factors to limit residual tissue injury and promote healing. In contrast, chronic inflammation is associated with repeated tissue damage, elevated levels of proinflammatory cytokines (TNF α , IL6, IL1 β), and aberrant expansion of immunoregulatory cells in a futile attempt to control smoldering immune responses (19). Myeloid-derived suppressor cells (MDSC) and T regulatory cells (Tregs) have emerged as key cellular subsets that regulate inflammation, cancer development, and progression.

Although heterogeneity in the phenotype of human MDSC and Tregs is appreciated, both cell populations can promote the ability of cancers to evade CD8⁺ T and natural killer (NK) cell-mediated immune responses (22). These cells therefore represent a potential barrier to maximizing benefit from immunotherapy regimens for prostate cancer. MDSC, present systemically and in the prostate microenvironment, act through a variety of mechanisms to suppress survival, signaling, and cytotoxic function by immune effector cells (22). Several reports have shown that MDSC and Tregs are relevant biomarkers that are elevated and, in some cases, associated with reduced overall survival in patients with advanced prostate cancer (23–29). Despite our increasing understanding as to the role of immunosuppression in cancer, no studies to date have explored dietary intervention with soy phytochemicals as a means by which to modulate immunosuppressive cell populations in patients.

We hypothesized that dietary soy may benefit patients with prostate cancer by modulating the balance of proinflammatory cytokines and immunosuppressive cells. To address this hypothesis, peripheral blood was obtained from patients with prostate cancer with asymptomatic biochemical recurrence who were enrolled in a phase II clinical trial evaluating the safety, compliance, and pharmacokinetic profile of a novel bread enriched with soy phytochemicals. In the present study, we demonstrate that the peripheral blood of patients with prostate cancer had significantly reduced proinflammatory cytokines, MDSC, and Tregs following soy bread intervention as compared with baseline. These data represent the first report suggesting that dietary soy may modulate immune parameters in favor of anticancer immune responses in humans.

Materials and Methods

Clinical trial design

All studies were conducted according to guidelines under Institutional Review Board Approval at The Ohio State University (Columbus, OH). A phase II clinical trial of soy phytochemical enriched bread was conducted in men ($n = 32$) with prostate cancer who were experiencing asymptomatic biochemical recurrence (increasing prostate-specific antigen) with no evidence of measurable disease on staging studies. All patients had a good performance status (ECOG 0 and 1). The majority of patients (24 of 32 total patients) had undergone radical prostatectomy prior to entry into this study. Detailed eligibility criteria, response criteria, dietary constraints, and other clinical parameters are

described in our accompanying publication (30). The study protocol was approved by The Ohio State University Comprehensive Cancer Center's Clinical Scientific Review Committee and the Biomedical Sciences Cancer Institutional Review Board (NCT:01682941). Briefly, a crossover design was used in which men were randomized to receive 2 slices per day of either a soy bread or soy almond bread. Men received one bread formulation for 8 weeks (56 days) following an initial 2-week legume-free washout. After a second 2-week washout, men received an additional 8 weeks of the alternate bread formulation. The primary objective of this trial was to characterize the safety and compliance, pharmacokinetics of the soy phytochemicals (30). This study describes results from a series of immunologic correlative studies conducted in a retrospective manner, as a secondary objective using only data from the first 56 days of the study, prior to cross-over.

Soy bread formulation

A novel soy bread formulation was developed that delivers 34-mg total soy isoflavones per 50-g slice. Two slices of this bread provided a dose of soy phytochemicals similar to the daily intake consumed by Asian populations in which lower cancer incidence has been noted in epidemiologic studies (1, 2). A second bread was formulated to accelerate the conversion of glucosides to aglycones and possibly alter pharmacokinetic properties by incorporating ground almonds (which are naturally high in β -glucosidase) and steaming the soy ingredients (to aid in conversion of the malonyls; ref. 31). These soy isoflavone delivery vehicles are described in further detail as previously reported by our group (31).

Procurement of peripheral blood

Approximately 8 to 10 mL of blood was drawn from patients with prostate cancer into sodium heparin tubes following a 2-week, legume-free washout (day 0) and following 8 weeks of daily soy bread intervention (day 56). Peripheral blood mononuclear cells (PBMC) were separated using Ficoll-Paque and density gradient centrifugation and cryopreserved prior to batch analysis as previously described (32). Plasma was snap-frozen and stored at -80°C prior to analysis.

Analysis of plasma cytokines

A panel of 54 cytokines and chemokines was analyzed in plasma from each patient at day 0 and day 56 using the commercially available, high-throughput Luminex Multiplex Cytokine Kits (Procarta Cytokine Assay Kit, Affymetrix) as described previously (33). All samples were batch run in duplicate and quantified on the basis of a unique standard curve for each analyte.

Flow cytometric analysis

Cryopreserved PBMCs from each patient obtained at days 0 and 56 were assayed for phenotypic markers consistent with NK cells, T lymphocytes, MDSC, and Tregs as previously described (26, 32). Briefly, PBMCs from each patient were suspended at a concentration of $1 \times 10^7/\text{mL}$ in flow staining buffer (PBS containing 1% FBS). Cells were incubated with fluorochrome-labeled antibodies for 1 hour at 4°C . Specific antibodies include CD4-APC (Beckman Coulter), CD8-APC (Beckman Coulter), NKRD1 (Beckman Coulter), CD33-PE (BD Biosciences), HLA-DR PERCP-Cy5.5 (eBioscience), and CD14-Pacific Blue (BD Biosciences). PBMCs

were also labeled with the appropriate isotype control antibodies for each fluorochrome to use as negative controls. Cells were then washed with flow buffer, fixed with 1% formalin, and stored at 4°C until analysis. All samples were run on a BD LSR II flow cytometer and were subsequently analyzed with FlowJo software (Tree Star, Inc.). Monocytic MDSC were defined as cells with a CD33⁺HLA-DR^{neg}CD14⁺ phenotype. Tregs were defined as CD4⁺CD25⁺FoxP3⁺ and assessed using the commercially available Human T regulatory cell staining Kit per manufacturer's recommendations (eBioscience).

CD33⁺ cell depletion studies

Patient PBMCs from $n = 5$ representative patients were depleted of MDSC by using anti-CD33/66b magnetic microbeads (STEM-CELL Technologies) and negatively selected using an Easy Sep magnet. Isolated cells were washed twice prior to further studies. Patient PBMCs (\pm CD33-positive cells) were labeled with 1 μ mol/L carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen) and cultured with CD3/CD28 beads (Invitrogen) for 3 days. Cells were collected, stained for CD4⁺ or CD8⁺ T cell markers (Beckman Coulter), and fixed for flow cytometric analysis on a FACS Calibur. Cells were gated on CD4⁺ or CD8⁺ T cells, and the percentage of T-cell proliferation was determined on the basis of CFSE dilution.

Statistical analysis

A global testing approach (34) was used to compare change from baseline to day 56 for groups of cytokines defined *a priori* according to putative function. Within patient, differences in individual cytokine and chemokine levels and cellular data were compared using paired *t* tests. Using the approach of Gordon and colleagues (35), the mean number of false discoveries in the cytokine/chemokine panel was set to 2 to address the issue of multiplicity. Comparisons between treatment groups were made using two-sample *t* tests. Where necessary, data were log-transformed to meet test assumptions of homoscedasticity and normality.

Results

Compliance with soy bread and soy metabolism

The details of the clinical study and extensive examination of soy isoflavone pharmacokinetics and metabolism are presented in a parallel publication (30). In brief, patients with prostate cancer ($n = 32$) with asymptomatic biochemical recurrence were recruited, and detailed patient characteristics and dietary composition are presented in an accompanying publication (30). Both soy and soy almond breads were without grade 2 or higher toxicity, and self-reported compliance was >92% of the targeted dose. Of the 32 patients enrolled, 25 remained on the clinical protocol for at least 56 days, provided adequate blood samples, and were therefore eligible for assessment of immunologic biomarkers, the primary endpoint of this report. Four of the ineligible patients were excluded because of cancer progression requiring intervention that arose during the course of the study.

Soy bread intervention is associated with decreased proinflammatory cytokines and chemokines in peripheral blood of prostate cancer patients

The concentrations of 54 cytokines and chemokines were profiled in plasma from patients obtained at baseline (following

the 2-week washout) and after 56 days of soy bread intervention. Significant changes were evident when comparing the plasma concentrations of soluble immunologic mediators following soy bread consumption as compared with the baseline values (Fig. 1A). Global tests of hypotheses were performed in which individual cytokines were grouped into well-characterized subclasses on the basis of putative function. For example, cytokines associated with T_H1 (IL1 β , IL2, IL12, IFN γ , TNF α), T_H2 (IL4, IL5, IL10), or T_H17 (IL17A, IL17F) immune responses were grouped together. Similar groupings were devised for cytokines associated with differentiation and function of MDSC [IL6, granulocyte macrophage colony-stimulating factor (GM-CSF), G-CSF, macrophage colony-stimulating factor (M-CSF)] or Tregs (TGF β , IL10). There were significant reductions in the level of both T_H1-type cytokines ($P = 0.028$) and MDSC-associated cytokines ($P = 0.035$) when comparing day 56 with baseline values across all patients (Table 1). Although modest alterations were evident in the level of T_H2-associated cytokines, T_H17-associated cytokines, and Tregs, these data did not reach statistical significance (Table 1). In addition to these biologically grouped analyses, paired *t* tests were used to analyze differences of individual cytokines across all patient samples. These data revealed significant reductions in individual factors implicated in a variety of immunologic functions, including IL6, IL12, MIP1 β , IL4, TRAIL, NGF-38, and IFN α (Fig. 1B and Supplementary Table S1). Setting the mean number of false discoveries at 2 for the 54-factor panel, significant differences at the 0.037 level were detected in 11 factors. Finally, the mean fold change was elevated across patients for a subset of individual soluble factors, including MIG, LEPTIN, RANTES, IL2, MIP3 α , and Resistin at the day 56 time point, although significant variability across individual patients for these biomarkers precluded statistical significance. Importantly, the observed cytokine changes were consistent regardless of whether men consumed soy bread or soy almond bread (all between-group $P > 0.5$ for cytokine subsets; Supplementary Table S2A).

Soy bread intervention is associated with alterations in the percentage of NK and T cells in patient peripheral blood

On the basis of the significant reduction in numerous plasma cytokines following the soy bread intervention, we postulated that this intervention may also alter immune cell phenotype within the peripheral blood of these patients. Therefore, phenotypic analysis of cryopreserved PBMCs obtained at baseline and day 56 (following soy intervention) was conducted by flow cytometry. As compared with baseline, there was a modest but statistically significant increase in the percentage of CD56⁺ NK cells ($P = 0.038$), but no significant change in the percentage of CD8⁺ or CD4⁺ T cells ($P = 0.75$ and $P = 0.065$, respectively; Fig. 2A).

Soy bread reduced the ratio of Tregs to CD8⁺ cells and MDSC in peripheral blood

Consistent with the cytokine data, the total percentage of CD4⁺CD25⁺FoxP3⁺ Tregs and the ratio of Treg:CD8⁺ T cells were significantly reduced at day 56 as compared with baseline ($P = 0.0136$ and $P = 0.03$ respectively; Fig. 2B and C). Compared with other cell subsets within the peripheral blood, we observed noticeable heterogeneity in the percentage of cells with phenotypic properties consistent with monocytic (CD33⁺HLA-DR^{neg}CD14⁺)

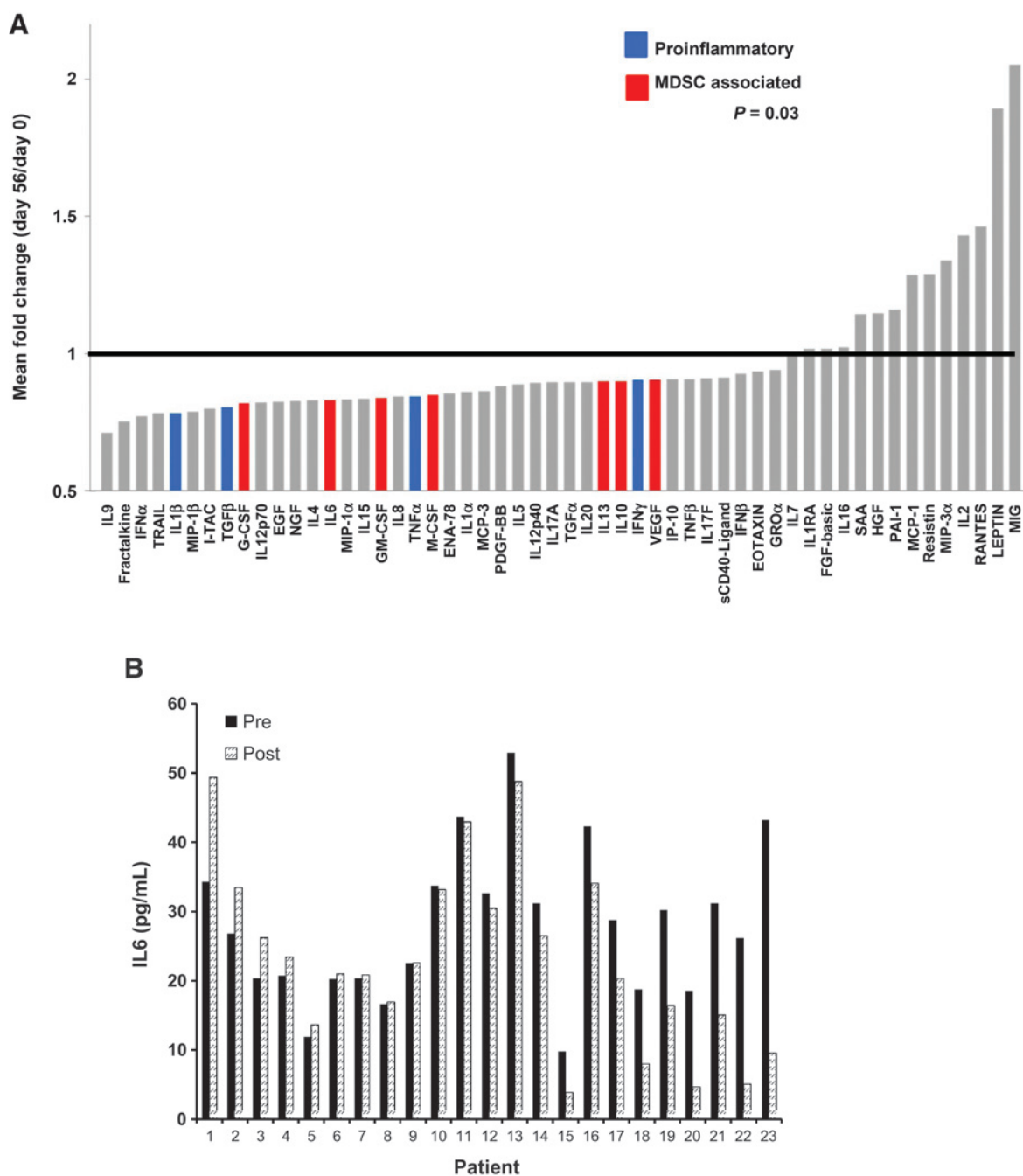


Figure 1. Soy bread consumption is associated with modulation of cytokines in plasma from patients with prostate cancer. Plasma from patients obtained on days 0 and 56 were analyzed via bioplex analysis for the expression of 54 soluble cytokine and chemokine mediators. A, data are presented as the mean fold change in expression for each factor across the 23 patients who were evaluable on study with adequate samples for analysis. Significantly reduced levels of both canonical proinflammatory cytokines (IL1 β , TGF β , TNF α , IFN γ ; shown in blue) and MDSC-associated cytokines (G-CSF, IL6, GM-CSF, M-CSF, IL10, IL13, VEGF; shown in red) were observed following dietary soy intervention. B, representative raw data for plasma IL6 (in pg/mL) from patients prior to (day 0) and following soy intervention (day 56).

MDSC (Fig. 3A). As expected for a population with excellent performance status (ECOG 0 and 1; ref. 30) and low tumor burden, the circulating percentage of these myeloid cell populations was modest. However, the percentages of the monocytic MDSC were also significantly lower in patients at day 56 as compared with

baseline ($P = 0.0056$; Fig. 3A). No significant differences were detected between patients consuming soy bread versus soy almond bread for any of these outcomes (all $P > 0.2$; Supplementary Table S2B). Importantly, *ex vivo* depletion of CD33⁺ cells from the PBMCs of representative patients significantly improved CD4⁺

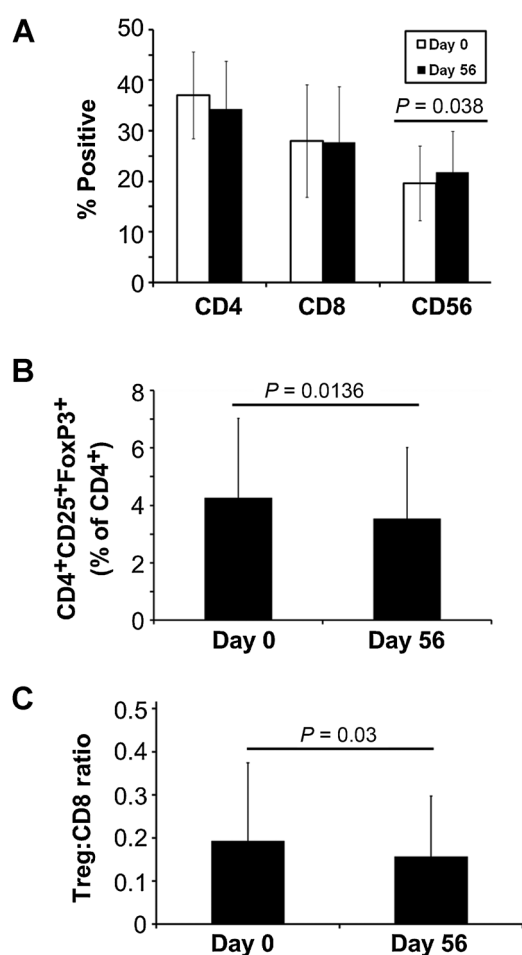
Table 1. Changes in cytokines by groups in plasma from patients with prostate cancer following eight weeks of soy bread consumption

Cytokine group	Mean difference (z-score)	Lower 95% CL	Upper 95% CL	Pr > t
MDSC	-1.7442	-3.3527	-0.1357	0.035
Treg	-0.7412	-1.5748	0.0924	0.079
T _{H1}	-2.0844	-3.9275	-0.2414	0.028
T _{H17}	-0.6364	-1.4361	0.1632	0.113
T _{H2}	-1.0983	-2.362	0.1654	0.085

NOTE: A global testing approach was used and differences were in terms of z-scores.

Abbreviation: CL, confidence limits.

($P = 0.0048$) and CD8⁺ ($P = 0.0009$) T-cell proliferation following stimulation with CD3/CD28-targeted beads (Fig. 3B–D). These data support the concept that cells with an MDSC phenotype were in fact, functionally suppressive.

**Figure 2.**

Soy bread consumption and phenotypic changes in circulating NK and T-cell populations in patients with prostate cancer. Percentages of circulating CD4⁺, CD8⁺, and CD56⁺ lymphocytes (A) or CD4⁺CD25⁺FoxP3⁺ Tregs (B) were evaluated at days 0 and 56 by flow cytometry. Data are presented as the mean % positive cells for each phenotypic subset across the 25 patients who were evaluable on study. C, the ratio of CD4⁺CD25⁺FoxP3⁺ Tregs to CD8⁺ cells was significantly reduced at day 56 after soy bread intervention as compared with day 0. Data are presented as the mean ratio of Treg:CD8⁺ cells across the 25 evaluable patients.

Discussion

Our study demonstrates significant reductions in circulating proinflammatory cytokines, Tregs, and MDSC after a soy bread intervention. These data represent the first evidence that dietary soy can modulate human immune markers relevant to inflammation and cancer progression. Collectively, these findings and preclinical studies (3–5) support the hypothesis that dietary soy may influence the human immune system in a manner that reduces inflammatory processes associated with early carcinogenesis and promote immunosurveillance during cancer progression.

A key consideration in conducting informative human studies of foods and immune function is a fully characterized intervention agent. Studies of dietary patterns or individual foods provide layers of complexity that go far beyond pure drugs or even pure nutrients. First, we controlled the participant's background diet to limit exposure to soy phytochemicals (30). In parallel, we standardized the vitamin and mineral supplement used to further control potential confounding exposures. Second, we developed and fully characterized the soy bread products that are easily incorporated into the daily diet with high compliance while providing quantifiable exposure to the array of potentially bioactive phytochemicals found in a food product (31). In this study, we also compared two variations of the soy bread that modestly alters the pharmacokinetics of isoflavones (30). However, with regards to the immunologic markers examined, we saw no significant difference in men consuming the soy or soy almond bread (enriched in β -glucosidase to increase aglycones). Overall, this whole food-based approach offers the added advantage of safety and in our ability to deliver a defined dose of bioactives in the context of a food matrix which may positively impact absorption and downstream biologic effects (36). This bread provided soy isoflavones to our study participants in amounts similar to intake consumed by Asian populations and earlier linked to a lower risk of prostate cancer in epidemiologic studies (1, 2). Importantly, the sensory properties (taste, texture etc.) of this soy bread formulation were such that remarkably high self-reported compliance (>92%) was observed among patients.

The significant reduction in specific cytokines observed after soy bread intervention was consistent with alterations in the percentage of circulating MDSC and Tregs:CD8⁺ ratios observed in these patients. Our finding is just the beginning of an effort to understand the mechanisms whereby soy phytochemicals act on the immune system and impact health. For example, most *in vitro* and rodent studies investigating immune readouts have been largely restricted to pure genistein, although a small number of reports have been published with equol or daidzein (37, 38). In these provocative reports, genistein has been shown to possess anti-inflammatory effects by virtue of its ability to suppress delayed-type hypersensitivity (DTH) responses to various stimuli, decrease thymocyte maturation, and decrease specific Ab titers in keyhole limpet hemocyanin (KLH) or ovalbumin-immunized mice (39–43). Interestingly, reports suggest that genistein increases the activity levels of cytotoxic T cells and natural killer cells, conferring resistance to tumor challenge (39, 44, 45). However, these data should be interpreted with the following considerations in mind. First, many studies showing an immune altering effect

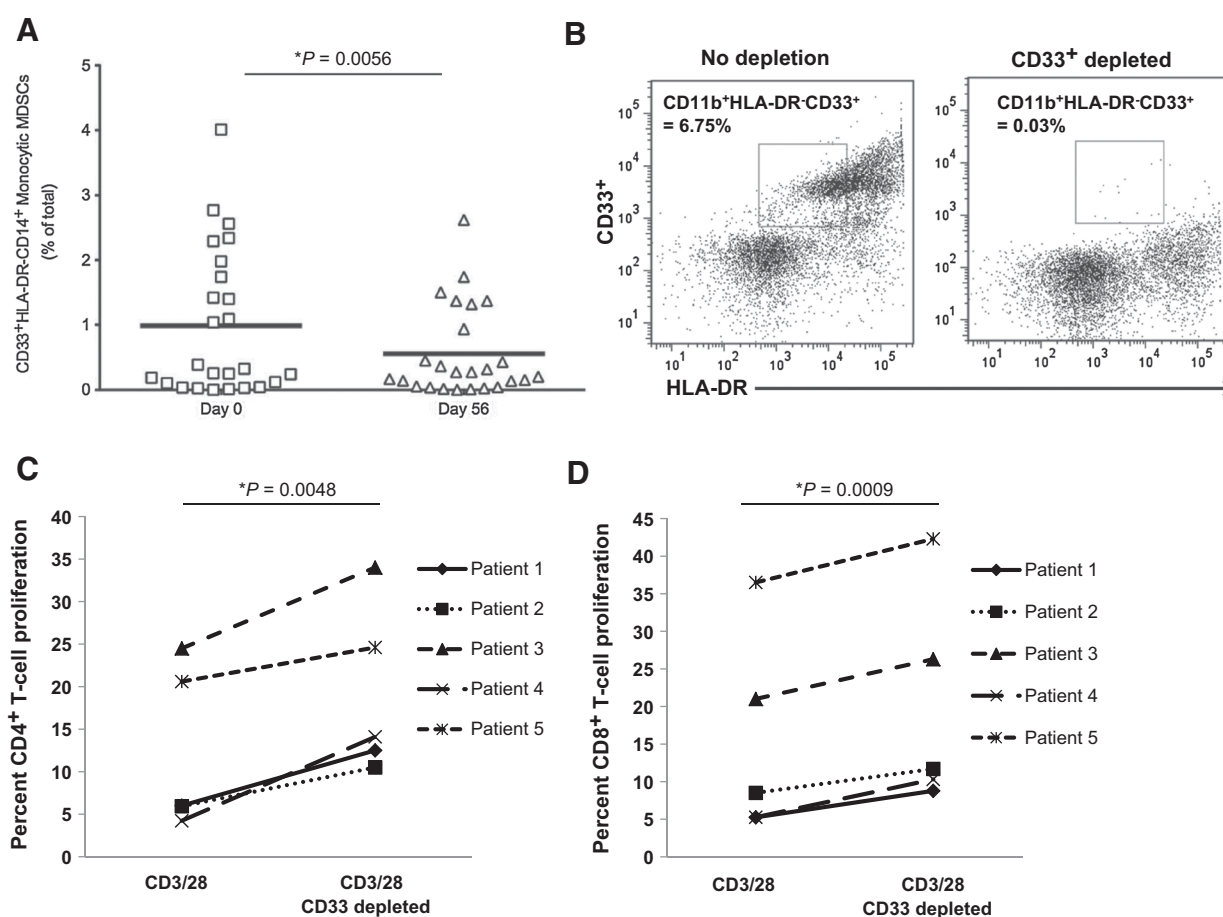


Figure 3.

Soy bread consumption and phenotypic changes in circulating MDSC populations in patients with prostate cancer. PBMCs were obtained from the 25 evaluable patients with cancer on days 0 and 56 and stained for MDSC using fluochrome-labeled antibodies targeting CD33, HLA-DR, and CD14 or the appropriate isotype controls. MDSC levels were evaluated by flow cytometry on the basis of a minimum of 20,000 live events and presented as the percentage of total cells. Data were analyzed and presented as the total percentage of (A) CD33⁺HLA-DR⁺CD14⁺ monocytic MDSC. Each symbol represents data from an individual patient, whereas the horizontal lines indicate the mean within each group. B, representative flow cytometric dot plots validating effective depletion of MDSC from patient peripheral blood samples. Depletion of CD33⁺ cells results in enhanced proliferation following a 72-hour stimulation with CD3/CD28 beads in CFSE-labeled (C) CD4⁺ and D, CD8⁺ T cells from peripheral blood of patients. Data are presented as the percentage of T-cell proliferation from blood taken on day 0 from 5 representative patients on study. Cryopreserved cells were thawed, CFSE-labeled, stimulated, stained for CD4 or CD8 markers, and analyzed by flow cytometry.

of genistein have used pharmacologic concentrations, much higher than what would be obtained *in vivo* through chronic dietary exposure (46). Second, the effects of genistein or other pure isoflavones on immunity may not necessarily correspond to the effects produced by exposure to the more complex phytochemical profiles at lower dosages within a food matrix that people actually consume (46). Third, most available data are derived from *in vitro* studies and some rodent models rather than human cells or subjects. Finally, the mechanisms by which dietary soy modulate immune function deserves further investigation. It is possible that the immunologic changes observed may be a direct consequence of soy components acting upon immune cells. Alternatively, immune effects may be secondarily influenced by soy-mediated alterations in host metabolism. This is an interesting possibility given our observations that a significant reduction in body mass index (BMI) was observed in the study, whereas a subset of men with hypercholesterolemia had decreased low-density lipoprotein (LDL) and cholesterol

(30). The potential interactions between these physiologic events and immune modulation deserve future attention in subsequent studies.

Numerous studies have suggested a potential benefit of dietary soy for patients with prostate cancer, but these efforts have been compromised by their limited power and scope. Soy isoflavones are reported to augment the direct effect of radiation therapy on prostate cancer cells while limiting damage to normal tissues (47). However, the clinical results across all human studies have been heterogeneous and are truly difficult to compare due to variation in study design, populations examined, compliance, and the soy products provided. For example, a recent study was completed in patients post-prostatectomy, comparing a milk protein with a soy protein drink showed no impact on risk of biochemical progression (7). Together these data highlight the complexity of dietary intervention studies and demonstrate the need for further definitive research using highly defined food delivery systems, such as those described in this study (8, 11). The present study does

provide unique insight into the potential for dietary soy as a novel strategy to alter immunologic processes that may be relevant to prostate cancer at multiple stages of disease progression. For example, we can hypothesize that reduced inflammatory processes may inhibit the early progression of prostatic intraepithelial neoplasia to cancer. Optimizing anticancer immunity by dietary means may potentiate the impact of immunotherapy, including vaccines, for this disease.

Although our results highlight the potential for dietary soy as a novel immunomodulatory intervention, there are a number of caveats to this phase II study that deserve mention. First, there was no placebo group per se as all men served as their own controls, and our team is now supported to pursue such studies in the near future. Second, we have not yet determined whether the systemic immune changes are reflected in the tumor microenvironment and are the focus of a future trial. This study was also not designed to determine whether the effects of a soy diet are lasting or are reversible upon returning to a usual diet. On the basis of these initial data, we are in the process of conducting a clinical trial of soy bread intervention to assess local and systemic immunologic biomarkers in the pre-prostatectomy setting. This study design will use a placebo group and allow for acquisition of tissue upon surgery to address this limitation. Third, because of limitations in sample quantity and the unavoidable use of cryopreserved cells in this retrospective study, our phenotypic analysis of PBMCs was limited to CD4⁺CD25⁺FoxP3⁺Tregs and the monocytic subset of MDSC. Certainly, we are interested in understanding the impact of dietary soy on more precisely defined, freshly isolated, immune cell populations such as inducible Tregs or other MDSC subsets (i.e., CD15⁺ granulocytic MDSC) that may be altered via cryopreservation. However, in representative patients with sufficient quantities of cells, we did demonstrate that CD33⁺ cell depletion led to significantly improved T-cell proliferation upon *in vitro* stimulation. These data confirm the functional properties of cells with an MDSC phenotype in the present study. Finally, it was also interesting to note the strong concordance between reduced cytokines in the PBMC compartment and circulating levels of these cells. Although not previously reported for soy isoflavones, other dietary agents with putative chemopreventative properties, including 25-hydroxyvitamin D₃, curcumin, and EGCG, have been shown to modulate aberrant myeloid cell expansion (48–50).

Our initial observations documenting an impact of diet on biomarkers of human immune function have important implications. First, this finding supports the concept that immune-based mechanisms may underlie dietary associations with human cancer risk. As the role of immunologic processes in carcinogenesis is further elucidated for specific cancers, informative studies can be undertaken. Perhaps more critically, our human study implicates diet as one of the variables that may impact the efficacy of our rapidly expanding portfolio of immunologic therapies for cancer. Ongoing and future clinical trials of novel immune therapies may benefit from considering diet and nutrition as potential modulators of outcome that contribute to heterogeneous responses.

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Indeed the value of immunotherapeutic studies may be enhanced by controlling for the multitude of supplements and dietary strategies used by patients with cancer to thwart disease progression. Many of these are unsubstantiated, yet may impact relevant processes such as immune function.

In summary, these data indicate that dietary soy can influence the composition of specific types of immune cell populations and the orchestration of the cytokine network impacting immune responses. Together these research findings suggest that dietary interventions represent a novel means to tune the immune system in a manner that benefits cancer therapy and prevention.

Disclosure of Potential Conflicts of Interest

Y. Vodovotz has ownership interest in U.S. patent 7,592,028. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

Any opinions, findings, and conclusions expressed in this material are those of the authors and do not necessarily reflect those of the Pelotonia Fellowship Program.

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