The Invisible Arm of Immunity in Common Cancer Chemoprevention Agents

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Abstract

Immunoprevention refers to a strategy of preventing pathogen-associated and spontaneous cancers through the use of vaccines, antibodies, and immune modulators. Immune modulators function by enhancing the endogenous ability of the immune system to monitor for malignancy, so-called "immunosurveillance." There is growing evidence that many of the most promising cancer chemoprevention agents including aspirin, COX-2 inhibitors, aromatase inhibitors, and bisphosphonates mediate their effects, in part, by enhancing immunosurveillance and reversing the immune evasive mechanisms that premalignant lesions employ. In the following review, we introduce critical components of the human immune surveillance system—dendritic cells, T cells, and immune suppressive cells—and discuss the emerging data suggesting that common chemoprevention agents may modulate the function of these immunologic cells.

Introduction

Cancer prevention strategies include (1) risk reduction through elimination of environmental factors (asbestos, tobacco, and alcohol); (2) chemoprevention in high-risk populations with agents such as cyclo-oxygenase-2 (COX-2) inhibitors and aromatase inhibitors (AIs); and (3) immunoprevention with vaccines, antibodies, and immune modulators. The most successful application of immunoprevention to date has been vaccination against the infectious causative agents of hepatocellular carcinoma (HCC) and cervical cancer, two of the most common cancers worldwide. In prevention of HCC, a nationwide hepatitis B vaccination program in Taiwan was shown to reduce the average annual incidence of HCC in children over several years from 0.7 per 100,000 children (1981-1986) to 0.57 (1986-1990) and 0.36 (1990-1994) with a decrease in corresponding rates of mortality (1). In the prevention of cervical and other gynecologic cancers related to human papillomavirus (HPV), two international, double blind, placebo controlled randomized trials (FUTURE I/II) evaluated the efficacy of the quadrivalent HPV vaccine (serotypes 6, 11, 16, and 18). In lesions caused by virus corresponding to the specific serotypes included in the quadrivalent vaccine, efficacy at preventing cervical intraepithelial neoplasia grade I was 96% and for vulvar and vaginal intraepithelial neoplasia reached 100% (2). Vaccines designed to stop

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infection associated cancers have been one of the most successful prevention strategies to date.

Most cancers, however, have not been shown to be caused by infectious agents, instead arising from genetic alterations, and the immune system may inhibit tumor growth even in this setting. Thomas and Burnet developed the "tumor surveillance" theory in the 1950s in which they hypothesized that the immune system protects against nascent cancers by destroying abnormal cells before evolution to invasive malignancy. Burnet predicted that "if there were tumor immunity, it would be invisible," anticipating the difficulty of providing evidence in humans to support immune surveillance (3). Evidence for the role of the immune system in modulating the growth of common cancers now exists. First, many cancer patients across a variety of tumor types spontaneously develop significant levels of antibodies and/or T cells specific for antigens expressed on their tumors, which, in some cases, are associated with prognosis including the occasional spontaneous regression (4). Second, the composition of tumor infiltrating lymphocytes (TILs) has been shown to have prognostic implications in a variety of different malignancies (5). Indeed, for colon cancer an immune scoring system based on enumerating CD8+ T cells and memory T cells can predict prognosis with greater accuracy than standard TNM staging (6). Third, immunodeficiency has been associated with cancer risk. Patients with impaired immunity, e.g. HIV infection or the use of antirejection drugs for transplantation, have a higher risk of both virally associated and non-virally induced tumors, suggesting a cancer protective effect via an intact immune system (7).

Standard cytotoxic chemotherapy has long been thought to work primarily by selectively causing death of rapidly proliferating tumor cells. Recently, however, many chemotherapeutic agents have been shown to stimulate tumor-specific immune responses by inducing immunogenic cell death or stimulating/activating immune effector cells which contribute to drug efficacy (8). Within the nascent field of cancer immunoprevention, similar data is emerging that many of the most promising chemoprevention agents under study may exert their effects, in part, by enhancing immune surveillance. As with cytotoxic drugs, chemoprevention agents have been shown to increase antigen processing by potent antigen presenting cells (APC), stimulate the proliferation and anti-tumor capabilities of CD8+ T cells, and inhibit the function or decrease the number of immune suppressive cells. In the following review, we discuss relevant elements of cancer destructive immunity and explain how chemoprevention agents may have immunomodulatory effects.

Cancer chemoprevention agents may enhance the immunologic activity of antigen presenting cells

Dendritic cells (DCs) are the most effective of the APCs in presenting immunogenic proteins to T cells. DCs sample antigens in peripheral tissues and process them into small peptides as they mature and migrate to lymphoid organs. After antigen uptake, APCs must receive suitable activation or stimulatory signals that result in sufficient maturation so that they differentiate to promote immunity rather than tolerance, as most immunogenic cancer associated proteins are self-antigens. Once activated, APCs present processed peptides to naïve T cells, stimulating a cellular immune response composed of CD4+ T helper cells (Th) and cytotoxic effector CD8+ T cells that are critical for destruction of pre-invasive and invasive lesions (Fig. 1A, B) (9).

Aspirin and the COX-2 inhibitors are well studied chemoprevention agents. Aspirin and the COX-2 inhibitor celecoxib have both been shown to reduce the risk of colorectal cancer (10-14). Additionally, systematic reviews of the results of aspirin in cardiovascular studies have suggested that low-dose aspirin reduces overall cancer incidence and mortality (15-17).

NSAIDs may limit carcinogenesis by preventing prostaglandin E2 (PGE$_2$)-mediated inhibition of DCs. PGE$_2$ is a product of COX enzymes and, normally, mediates physiologic functions such as maintenance of gastric mucosal integrity and renal blood flow when constitutively expressed. However, components of the tumor microenvironment can also produce PGE$_2$ through COX-2 expression during oncogenesis. PGE$_2$ alters the balance and function of DCs in different ways dependent on their maturation state at the time of exposure to the prostaglandin. Early in development, PGE$_2$ has been shown to suppress differentiation of human monocytes into functional Th1-inducing APC (Fig. 1A), instead redirecting monocytes to become immunosuppressive MDSCs (Fig. 1B). Th1 are critical in mediating tumor regression (9). When monocyte-derived immature DCs are matured with IL-1 and tumor necrosis factor alpha (TNF-) in the presence of PGE$_2$ in vitro, resultant DCs are phenotypically identical but have reduced capacity to produce IL-12 when stimulated with CD40L and IFN-g (Fig. 1A). IL-12 is necessary for efficient generation of Th1 and cytotoxic T cells (Type I cells). While naïve Th cells primed with PGE$_2$-DCs have similar expansion kinetics as compared to controls, the cells also have an enhanced ability to produce Th2-type cytokines, IL-4 and IL-5, and a reduced ability to produce the Th1 cytokine IFN-g. Th2 cells can contribute to tumor growth by dampening the generation of cytotoxic T cells and support cancer proliferation. The effects of PGE$_2$ on DCs may be most important early in oncogenesis, as cervical, breast, head and neck, and colorectal precursor lesions have been shown to overexpress COX-2 (18-21). In a cervical model, COX-2 expression was inversely correlated with density of DCs and ability to stimulate T cells (20). NSAIDs and COX-2 inhibitors may be particularly suited to cancer prevention by protecting the integrity of DCs in mediating immunosurveillance by allowing selective induction and proliferation of Type I T cells (Fig. 1).

Cancer chemoprevention agents may stimulate the adaptive immune system

APCs must induce protective T cell responses through antigen recognition (9). This adaptive T cell response is largely composed of Th1 CD4+ T cells and CD8+ T cells. Th1 cells are critical for propagation of the acute tissue destructive inflammatory response, secreting cytokines such as IFN-g, TNF- , and IL-2 that support CD8+ T cells and tumor destruction. This is in contrast to Th2 cells that secrete cytokines such as IL-4, IL-5, and IL-10, which limit CD8+ T cell proliferation and promote tumor growth (Fig. 2A). CD8+ T cells are activated after binding antigen presented by MHC class I molecules on APCs and some tumor cells and can deliver cytokines and cytotoxic enzymes that result in tumor cell lysis (9). Lesions that escape cell-mediated death do so by subverting this arm of the immune system, shifting the tumor environment to a Th2 type and inhibiting proliferation of CD8+ T cells. Cancer escapes immune surveillance in a myriad of ways including down regulation of MHC class I molecules, rendering them invisible to CD8+ T cells; production of factors that inhibit CD8+ T cell survival and expansion; and production of cytokines and chemokines that attract immune suppressive cells (22).

After antigenic stimulation, CD8+ T cells undergo expansion of antigen-specific effector populations followed by persistence of long-lived central and effector T_M cells. The number of tumor infiltrating central and effector T_M cells is inversely correlated with tumor invasion including vascular emboli, lymphatic invasion, and perineural invasion (23). In addition, the presence of T_M cells is associated with reduced risk of tumor recurrence, suggesting that these cells are important for secondary prevention (23, 24). The anti-diabetic drug metformin has been suggested as a chemoprevention agent that may enhance the generation of the T_M cell compartment (Fig. 2B). Preclinical and epidemiologic studies have suggested a cancer prevention role of metformin with a recent meta-analysis showing a 30% overall reduction in cancer incidence in diabetics on metformin compared to other diabetics (25-27).

Metformin significantly decreased both aberrant crypt foci and proliferating cell nuclear antigen index over a single month compared to controls in a small, randomized pilot study (28). Metformin may enhance T_M cell numbers via modulation of fatty acid metabolism. Preclinical experiments with mice deficient in tumor necrosis factor (TNF) receptor associated factor 6 (TRAF-6) demonstrated that, while mice mounted normal effector CD8+ T cell responses to infections, they had compromised CD8+ T_M cell generation. Microarray data revealed altered expression of genes that regulate fatty acid metabolism with defective AMP-activated kinase (AMPK) activation and mitochondrial fatty acid oxidation. Metformin, which has been shown to promote AMPK activation, when administered restored the ability to generate T_M cells (Fig. 2B) (29). Furthermore, metformin promoted survival of CD8+ T cells in wild type mice, resulting in enhanced generation of T_M cells (Fig. 2B).

Effective CD8+ T cell immunosurveillance is dependent on MHC I-mediated antigen presentation. Immunohistochemical staining across a spectrum of premalignant and malignant lesions has demonstrated an association between malignant transformation of cells and HLA class I antigen defects, suggesting that loss of Class I expression is an early, critical step in selection and outgrowth of malignant lesions (30, 31). Strategies that increase MHC I expression on transformed cells may restore immunosurveillance and prevent the development of overt malignancy (32). In a preclinical study, metformin increased MHC I expression on cancer cells (Fig. 2C). Most cancer cells shift from oxidative phosphorylation to glycolysis to generate energy. In vitro studies with leukemic cells demonstrated culture conditions that forced respiration also had the effect of upregulating MHC I transcription and protein levels at the cell surface, suggesting a link between the bioenergetic signature of cancer cells and their visibility to the immune system (33). In the SKBR3 breast cancer cell line, metformin enhanced oxidative phosphorylation resulting in a 25-fold increase in cell surface associated MHC-I protein levels (Fig. 2C) (34). Since MHC-I down regulation has been well documented in premalignant lesions, metformin could serve to increase the immunogenicity of pre-invasive disease. The RXR agonist bexarotene also has the potential to enhance T cell numbers and function. Bexarotene has been extensively studied in pre-clinical models and has been shown to be a potent anti-proliferative agent (35). Indeed, clinical trials assessing the potential for bexarotene as a chemoprevention agent are ongoing (36). Bexarotene has also been shown to upregulate the expression of high affinity IL-2 receptor on the surface of immune cells, when cultured together in vitro, potentially allowing an enhanced proliferation when exposed to an environment rich in IL-2 (37). In addition, the use of bexarotene may increase the lifespan of T cells as the agent has been shown to increase BCL2 expression and inhibit the development of apoptosis in T cells (Fig. 2D) (38).

Curcumin is a potential cancer chemoprevention agent currently being tested in clinical trials that may promote a Th1 environment that increases the number of CD4+ and CD8+ T cells. Curcumin inhibits targets important in oncogenesis including COX-2, tumor growth factor beta (TGF-), and indoleamine 2,3-dixoygenase (IDO), which suppresses the adaptive T cell immune response (39). Preclinical studies have demonstrated chemoprevention effects of curcumin across multiple tumor types (39). Results of a phase IIa trial revealed that curcumin treatment significantly reduced aberrant crypt foci (40). In the Apc$^{Min/+}$ mouse, curcumin administration reduced colonic tumor formation by approximately 70%. Immunohistochemistry of the mucosal lymphoid population of curcumin treated mice revealed a 30% increase in CD4+ T cells compared to controls, although these cells were not further characterized (41). In a mouse model of mammary carcinoma, curcumin inhibited tumor growth by (1) reversing tumor-induced depletion of CD4+ and CD8+ T cells (Fig. 2B,C) and potentiating CD8+ T cell cytotoxicity (Fig. 2C); (2) restoring memory T cell

(T_M) populations to levels comparable to controls (Fig. 2B); and (3) shifting the cytokine signature from Th2 to Th1 (Fig. 2A) (42).

Gamma-delta T cells are T lymphocytes with attributes of both the innate and adaptive immune system and account for 1-10% of all peripheral blood T cells. Gamma delta T cells exhibit many qualities of the innate immune system such as the capability of being activated by non-self ligands and phosphoantigens generated by the isoprenoid pathway used by microorganisms or mevalonate pathway in infected or transformed cells. Once activated, gamma delta T cells can expand, exhibit cytotoxicity in both a MHC-dependent and independent fashion, and release Th1 cytokines that further support the adaptive immune system (43). In mouse models of prostate and carcinogen-induced cutaneous malignancy, mice lacking gamma delta T cells developed higher disease burdens and progression of premalignant lesions to overt malignancy than controls and adoptive transfer of gamma delta cells could abrogate this effect (44, 45). In a longitudinal case-control study of renal transplant patients, 18 patients who developed cancer 2-6 years after transplantation were compared to a control group of 45 transplant recipients with similar demographics. Interestingly, patients who developed cancer had significantly fewer gamma delta T cells (<4%) measured in blood at 6, 12, and 18 months before their diagnosis of cancer compared to control patients (46). These findings have increased interest in the potential utility of gamma delta T cells in cancer prevention.

Nitrogen-bisphosphonates (N-BP) such as zoledronic acid (ZA) are being studied as chemoprevention agents that may stimulate the proliferation of gamma delta T cells (Fig. 2E) (47). N-BPs are primarily used for osteoporosis therapy and to reduce skeletal-related events in patients with bone metastases in solid tumors (48). Preclinical evidence for a cancer preventative effect was seen with the bisphosphonate ibadronate, which reduced colorectal dysplasia induced in an experimental mouse model of ulcerative colitis (49). Multiple observational studies have suggested that bisphosphonates are associated with reductions in the incidence of both breast and colon cancer (50, 51). N-BPs stimulate gamma delta T cells indirectly by increasing concentrations of isopentenyl pyrophosphate (IPP), a precursor in the mevalonate pathway, in peripheral blood mononuclear cells, which subsequently activates gamma delta T cell receptors (TCRs). There is also evidence that N-BPs can increase IPP in tumor cells themselves, resulting in a chemotactic and stimulatory signal for gamma delta T cells (Fig. 2E) (52). Activated gamma delta T cells release Th1 cytokines such as TNF-a, IL-6, and IFN-g that are important in immune surveillance (53, 54). Both preclinical data and phase I studies have shown N-BPs can activate tumoricidal gamma delta T cells in a broad range of tumors including breast, prostate, and renal cell carcinoma (55-58).

Cancer chemoprevention agents may inhibit the function of immune suppressor cells

Foxp3 regulatory T cells (Tregs) constitute 5% of all peripheral CD4+ T cells in healthy adults and are important in the regulation of immune responses to both self and foreign antigens and maintenance of immune homeostasis. Tumor-infiltrating Tregs have been shown to correlate with poor prognosis across a spectrum of different cancers. Studies have shown that Tregs suppress both proliferation and activity of effector T cells. In cancer prevention, the strongest support for an important role of Tregs comes from preclinical rodent studies with carcinogen-induced tumors. In studies with methylcholanthrene (MCA)-induced fibrosarcomas, depletion of 50-70% the total number of Tregs with specific antibodies prevented fibrosarcoma development compared to control mice, suggesting that Tregs interfere with immune surveillance (59, 60). Modulation of Tregs could be a means of cancer prevention.

PGE_2 increases the inhibitory potential of Tregs and there is evidence that COX-2 inhibitors reverse this effect (Fig. 3A). In the cancer prevention setting, a mouse model of azoxymethane-induced colon cancer was studied to assess effects of PGE_2 reduction. Mice underwent genetic deletion of mPGES-1, an inducible terminal synthase that produces PGE_2, resulting in 40% reduction of premalignant aberrant crypt foci in the distant colon; 85% suppression of tumor number; and a 90% reduction in tumor load. Evaluation of colon histology of mPGES-1 knockout mice revealed presence of macroscopically inflamed mesenteric lymph nodes with markedly elevated CD4+ and CD8+ lymphocytes and 55% reduction of CD4+ Foxp3+ cells (61). Further support comes from the cancer literature where multiple studies have demonstrated that PGE_2 enhances the ability of Tregs to suppress effector T cell proliferation and that COX-2 inhibition abrogated this effect (62, 63). Curcumin has also been shown to have similar effects, preventing cancer-induced Treg expansion and reducing Th2 cytokine release (Fig. 3B) (42). These findings suggest that both COX-2 inhibition and curcumin may decrease Treg function and contribute to enhanced immune surveillance (Fig. 3A,B).

Aromatase inhibitors (AI) are breast cancer chemoprevention agents associated with up to a 65% relative reduction in annual incidence of invasive breast cancer (64). AIs may mediate this effect in part by decreasing Treg populations (Fig. 3B) (47). AIs function by decreasing the peripheral conversion of androgenic precursors into estrogen. Estrogen has been shown to promote a Th2 cytokine profile and expand Tregs, raising the possibility that AIs could shift this balance to Th1 and resolution of aberrant lesions. Consistent with this observation, in a preclinical mouse study of inflammatory arthritis, AI-treatment resulted in a lower percentage of splenic and lymph node Tregs and increased Th1-cytokine release in response to lymphocyte stimulation compared to untreated mice (Fig. 3B) (65). In a randomized phase II trial, patients with locally advanced ER+ breast cancer received the AI letrozole or letrozole plus cyclophosphamide. There was a significant reduction in Treg number for all patients after treatment with a nonsignificant trend toward the letrozole and cyclophosphamide arm and Treg number at residual histology showed a significant, inverse relationship with response (Fig. 3B) (66).

MDSCs are regulatory cells that suppress tumor immune surveillance. In healthy individuals, these immature myeloid cells generally differentiate into mature cells of the myeloid lineage. In cancer patients, MDSCs accumulate and can contribute to oncogenesis through inhibition of Type I immunity (67). MDSCs can inhibit IL-2 production by activated intratumoral T cells as well as activation and proliferation of CD4+ and CD8+ T cells. In addition, MDSC have the capacity to stimulate Treg recruitment and proliferation. MDSCs act via the depletion of environmental arginine (Arg), an essential amino acid for T cell function. MDSC stimulate the secretion of inducible nitric oxide synthase (iNOS) and the reactive oxygen species (ROS), which promote mutagenesis and inhibit T cells (Fig. 3C) (67). Studies modulating MDSC through inhibition of function or selective depletion have resulted in prevention of carcinogen-mediated neoplasia and restoration of immune surveillance (68, 69).

PGE_2 influences differentiation of monocytes, promoting the MDSC phenotype, and COX-2 inhibition may prevent this effect (Fig. 1B). In preclinical studies, PGE_2 shifted development of monocytes from immature DCs to MDSCs when added to a standard preparative regimen of GM-CSF and IL-4 (Fig. 1B) (70). In addition, PGE_2 exposure promoted COX-2 expression in MDSCs, suggesting that PGE_2 initiates a COX2-mediated positive feedback loop, perpetuating the immunosuppressive signal (71). PGE_2 has also been shown to be responsible for chemotaxis of MDSCs to tumor sites through chemokine induction and COX-2 inhibition reversed this effect (72). In a carcinogen induced mouse model of intestinal cancer, the COX-2 inhibitor celecoxib administration delayed tumor

development and reduced number of tumors at autopsy compared to controls. Coinciding with this, there was a significant reduction in tumor infiltrating MDSCs and splenic MDSCs and a decrease in NOS and Arg mRNA levels from splenic cells (73). In a mouse model of glioma prevention, treatment with aspirin or celecoxib reduced systemic PGE_2 production, MDSC number, and consequently significantly delayed glioma development (74).

Retinoids are promising chemoprevention agents that may function by redirecting development of MDSCs to immature DCs (Fig. 3C). Initial studies suggested that retinoids were important in myeloid development, as vitamin A deficient mice had significant myeloid expansion in bone marrow, spleen, and peripheral blood, and this effect was reversed with introduction of vitamin A (75). In prevention, 13-cis-retinoic acid decreased leukoplakia lesion size in 67% of patients compared to 10% in placebo and reversed dysplasia in 54% of patients compared to 10% in the placebo group (76). In vitro studies using MDSCs isolated from patients with a variety of solid tumors demonstrated that all-trans retinoic acid (ATRA) could reverse their suppressive effects on CD8+ T cells (Fig. 3C). ATRA was shown to mediate this effect by re-differentiating MDSCs into immature DCs (77-79). In a therapeutic vaccine trial, a recombinant HPV protein vaccine inhibited HPV-related tumor growth by 85% when combined with ATRA compared to 42% with the vaccine alone and this coincided with a significant decrease in the number of MDSCs, an increase in mature DCs, and enhanced HPV-specific CD8+ T cell response (80). In addition to reducing MDSCs, there is evidence that ATRA can enhance observed proliferation of effector CD8+ T cells by increasing IL-2 release and can augment T_M cells when given in combination with a viral vaccine (81). These studies suggest retinoids can reduce suppressive MDSCs and, as a consequence, enhance proliferation and effector function of CD8+ T cells (Fig. 3C). Curcumin has been shown to have similar effects, reducing percentages of MDSCs in peripheral tissues of mice and may actually repolarize them toward a Type 1 (M1) macrophage phenotype (Fig. 3C) (82).

Tumor associated macrophages (TAMs) are recruited early in dysplastic or premalignant lesions and contribute significantly to oncogenesis (83-86). M1 macrophages are tumoricidal while type 2 (M2) macrophages support tumorigenesis and immunosuppression. M2 macrophages express a host of tumorigenic factors including vascular endothelial growth factor (VEGF), COX-2, epidermal growth factor receptor (EGFR) and matrix metalloproteinases (MMP), which support angiogenesis, tissue repair, and remodeling in tumors (Fig. 3D). M2 macrophages also induce immunosuppression through elaboration of cytokines such as IL-10 and TGF- and inhibit T cell proliferation through expression of Arg and IDO (Fig. 3D) (87). The presence of M2 macrophages correlates with poor prognosis in a number of different tumor types (88). There is evidence that TAMs are recruited early in preneoplasia (89). In a transgenic mouse of mammary cancer (PyMT mice), mice progress through four stages from benign hyperplasia to overt malignancy with metastases. There was a significant correlation between low density of macrophages in primary tumors and a delay in vascular development and malignant transition. Macrophage depletion resulted in delayed progression of premalignant lesions (90).

In addition to their other anti-tumor effects, COX-2 inhibitors inhibit M2 macrophage accumulation (Fig. 3A). PGE_2 overexpression increases M2 macrophage density in models of dysplastic and premalignant gastric, esophageal, and colon lesions, which is associated with progression to overt malignancy (83-85). In a rodent model of prevention, surgically induced duodenal reflux resulted in inflammation-induced dysplasia that progressed to squamous cell carcinoma in the forestomach. In control mice, 90% developed dysplasia and 38% of mice developed SCC at week 60 compared to 20% and 0%, respectively, in mice given the COX-2 inhibitor meloxicam. COX-2 was predominantly detected in infiltrating macrophages, suggesting that these cells mediated inflammation-induced COX-2 expression

and oncogenesis (91). In another study, $Apc^{Min/+}$ mice developed premalignant polyps heavily infiltrated by M2 macrophages with a Th2 cytokine profile. Celecoxib administration for 8 weeks (1) reduced the size and number of polyps compared to controls; (2) shifted the TAM phenotype from M2 to M1 macrophage predominant infiltrate; and (3) increased the Th1 signature in the environment (Fig. 3A) (92).

Bisphosphonates have been shown to have inhibitory effects on TAMs as well, which is not unexpected given the shared lineage between macrophages and osteoclasts (Fig. 3D). Macrophages endocytose bisphosphonates and release them into the cytosol where they can induce apoptosis by inhibiting the mevalonate pathway (93). Bisphosphphonates decrease release of protumorigenic factors such as MMP-9 by activated macrophages and decrease other proangiogenic factors like VEGF associated with activated macrophages (Fig. 3D) (93). In the transgenic BALB-neuT mouse, mice develop spontaneous mammary tumors in a stepwise manner similar to human breast cancer. In one study, mice received either control drug or cycles of ZA mimicking standard dosing schedules. Mice treated with ZA had significant extension of median tumor-free survival, delayed growth kinetics, and overall survival compared to control. Tumor stroma of control mice exhibited heavy infiltration of VEGF-expressing macrophages, while ZA-treated mice had a marked reduction. Interestingly, macrophages from control mice exhibited a Th2 cytokine pattern, while macrophages from ZA-treated mice were strongly positive for IFN-g, suggesting that in addition to reducing total macrophage numbers, ZA repolarized M2 macrophages to M1 type (94). In a murine model expressing HPV-16 genes (HPV/E_2 mouse) of cervical carcinogenesis, mice develop cervical intraepithelial neoplasia (CIN) lesions that progress to invasive SCCs. In a trial comparing effect of ZA on mice with CIN-3 lesions to controls, at 5 months, controls had SCC incidence of 85%, while ZA-treated mice had an incidence of 30%, suggesting a strong preventative effect. In addition to inducing apoptosis of tumor cells themselves, ZA reduced infiltrating macrophages by 10% and MMP-9 expression by 73% in remaining macrophages relative to control again suggesting a repolarizing effect of the drug (Fig. 3D) (95).

Conclusion

The immune system is a powerful sentinel against cancer with several types of cells surveying the environment and eliminating pre-invasive lesions before the development of overt malignancy. For immunocompetent individuals, a hallmark of cancer involves evolution of the mechanisms for the tumor to evade the immune system (96). In the field of immunoprevention, an emerging strategy involves enhancing immune surveillance via pharmacologic means. Tumor vaccines and antibodies hold promise in terms of bolstering the adaptive immune system for tumor prevention. However, many of the most promising chemoprevention agents may exert their effects, in part, by enhancing function of both DCs and effector T cells and decreasing the functional impact of immunosuppressive cells. As the immune effects of chemoprevention agents are further delineated, the exciting possibility of using them in combination to elicit effective tumor immune surveillance will be within reach.

Acknowledgments

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Abbreviation

AI	aromatase inhibitors
AMPK	adenosine monophosphate kinase-activated kinase
APC	antigen presenting cells
Arg	arginine
ATRA	all-trans retinoic acid
CIN	cervical intraepithelial neoplasia
COX-2	cyclo-oxygenase 2
DC	dendritic cells
EGFR	epidermal growth factor receptor
FOXP3	forkhead box protein P3
FPP	farnesyl disphosphate synthase
HCC	hepatocellular carcinoma
HLA	human leukocyte antigen
HPV	human papillomavirus
IDO	indoleamine 2,3-dixoygenase
IFN-	interferon gamma
IL	interleukin
IPP	isopentenyl pyrophosphate
MCA	methylcholanthrene
MDSC	myeloid-derived suppressor cell
MMP	matrix metalloprotein

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mPGES-1	terminal prostaglandin synthase 1
N-BP	nitrogen-bisphosphonate
NOS	nitrogen oxide synthetease
NSAIDs	non-steroidal anti-inflammatory drugs
PGE$_2$	prostaglandin E$_2$
ROS	reactive oxygen species
RXR	Rexinoid receptor
SCC	squamous cell carcinoma
SERMS	selective estrogen receptor modulators
SIL	squamous intraepithelial lesions
TAM	tumor-associated macrophage
TCR	T cell receptor
TGF-	tumor growth factor
Th	T-helper
TILs	tumor infiltrating lymphocyte
T$_M$	memory T cells
TNF-	tumor necrosis factor
TRAF	tumor necrosis factor receptor associated factor 6
Treg	regulatory T cells
VEGF	vascular endothelial growth factor
ZA	zoledronic acid
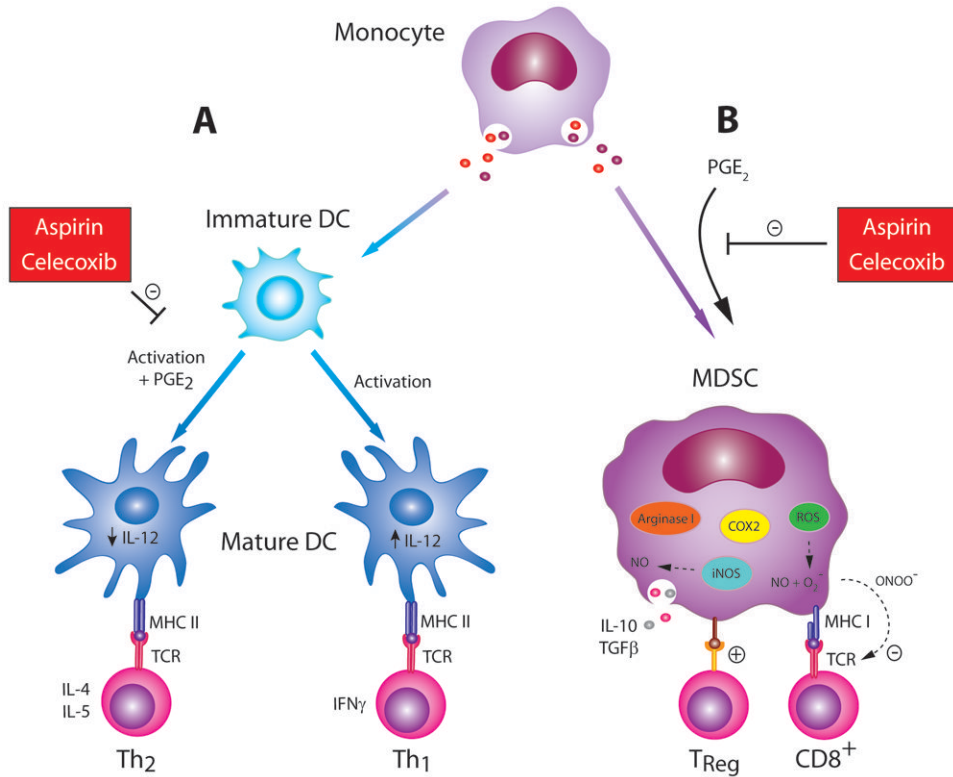
Figure 1. Cancer chemoprevention agents enhance the immunologic activity of antigen presenting cells

(A) PGE$_2$ can inhibit the ability of maturing DCs to produce IL-12 during priming, biasing the resulting adaptive immune response toward a Th2 profile. COX-2 inhibitors such as aspirin and celecoxib can reverse this effect, allowing generation of Th1; (B) PGE$_2$ facilitates differentiation of monocytes into immunosuppressive MDSCs, which function to inhibit the adaptive immune response and promote Treg populations through depletion of environmental arginine, expression of nitric oxide synthase, production of reactive oxygen species, and elaboration of Th2 cytokines IL-10 and TGF-B. COX-2 inhibitors aspirin and celecoxib can reverse this effect.
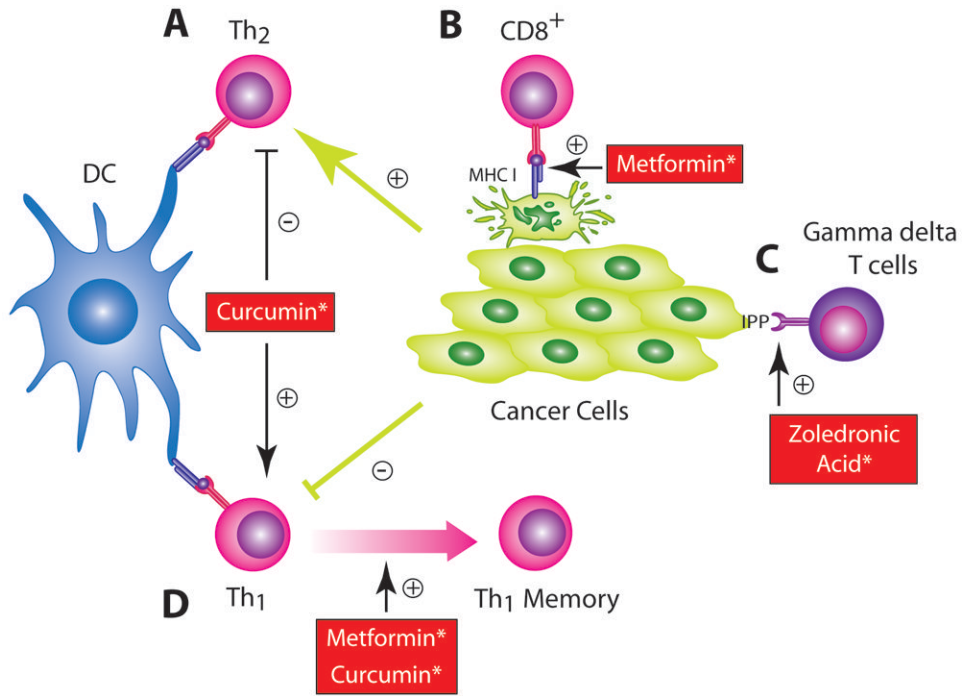
Figure 2. Cancer chemoprevention agents stimulate the adaptive immune system
(A) Curcumin administration can enhance the Th1 and decrease Th2 immune response; (B) Metformin and curcumin increase effector CD8+ T cell populations and resulting memory cells, both of which are critical for an effective adaptive immune response; (C) Metformin may increase MHC-I expression on tumor cells, increasing visibility to effector CD8+ T cells; (D)Bexarotene inhibits apoptosis in T cells by increasing expression of BCL2; (E) ZA and other bisphosphonates increase phosphoantigens in PBMCs and on cancer cells themselves, resulting in activation of anti-tumor gamma delta T cells. * Only preclinical data exists to support concept at this time.
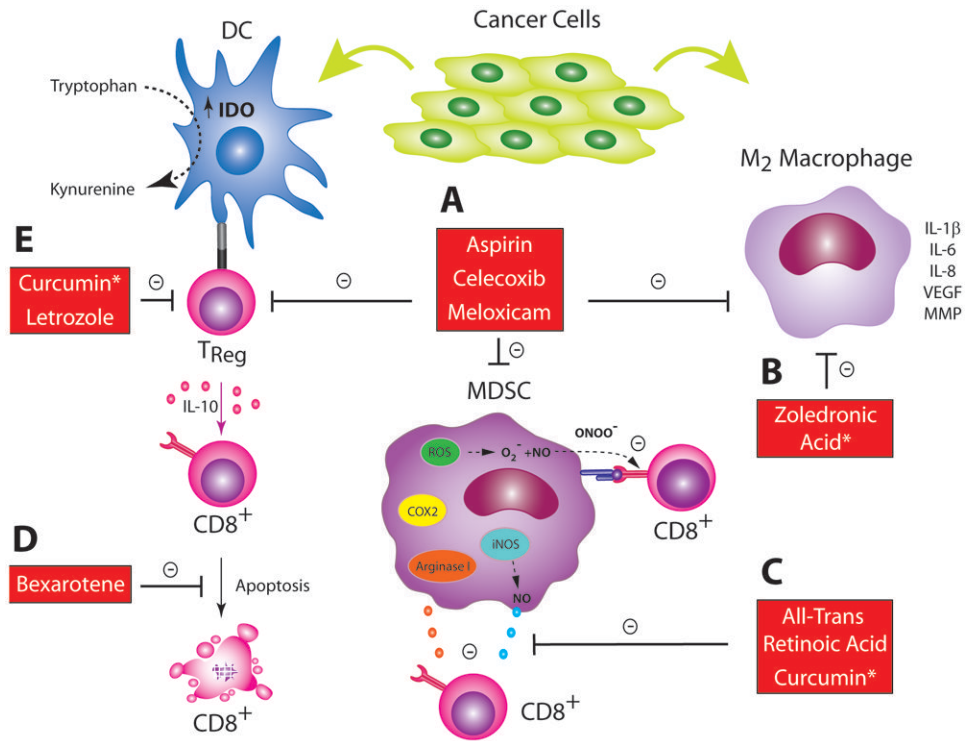
Figure 3. Cancer chemoprevention agents inhibit the function of immune suppressor cells
(A) PGE$_2$ can increase immunosuppressive Treg, MDSC, and M2 macrophage populations. COX inhibitors aspirin, celecoxib, and meloxicam can reverse this effect; (B) Both the AI letrozole and curcumin have been shown to reduce Treg populations; (C) The retinoid ATRA can differentiate MDSCs into immature DCs, which may account for its ability to enhance proliferation of both effector and memory CD8+ T cells. Curcumin can inhibit MDSCs and differentiate them toward a M1-like phenotype; (D)ZA decreases populations of M2 macrophages and may re-polarize them to the anti-tumor M1 phenotype. * Only preclinical data exists to support concept at this time.