Review

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 "immuno-surveillance." 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 use. 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.

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Introduction

Cancer prevention strategies include (i) risk reduction through elimination of environmental factors (asbestos, tobacco, and alcohol); (ii) chemoprevention in high-risk populations with agents such as COX-2 inhibitors and aromatase inhibitors; and (iii) 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, 2 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 papilloma virus (HPV), 2 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 1 was 96% and for vulvar and vaginal intraepithelial neoplasia reached 100% (2). Vaccines designed to stop 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 (TIL) 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 tumor-node-metastasis staging (6). Third, immunodeficiency has been associated with cancer risk. Patients with impaired immunity, for example HIV infection or the use of antirejection drugs for transplantation, have a higher risk of both virally associated and nonvirally 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 are 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 antitumor 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 APCs

Dendritic cells are the most effective of the APCs in presenting immunogenic proteins to T cells. Dendritic cells 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 (Th1) and cytotoxic effector CD8⁺ T cells that are critical for destruction of preinvasive and invasive lesions (Fig. 1A and B; ref. 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). In addition, systematic reviews of the results of aspirin in cardiovascular studies have suggested that low-dose aspirin reduces overall cancer incidence and mortality (15–17). Nonsteroidal anti-inflammatory drugs (NSAID) may limit carcinogenesis by preventing prostaglandin E₂ (PGE₂)-mediated inhibition of dendritic cells. PGE₂ 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 PGE₂ through COX-2 expression during oncogenesis. PGE₂ alters the balance and function of dendritic cells in different ways dependent on their maturation state at the time of exposure to the prostaglandin. Early in development, PGE₂ 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 dendritic cells are matured with interleukin (IL)-1β and TNF-α in the presence of PGE₂ in vitro, resultant dendritic cells are phenotypically identical but have reduced capacity to produce IL-12 when stimulated with CD40L and IFN-γ (Fig. 1A). IL-12 is necessary for efficient generation of Th1 and cytotoxic T cells.
Although naive T H cells primed with PGE2–dendritic cells 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-γ. TH2 cells can contribute to tumor growth by dampening the generation of cytotoxic T cells and support cancer proliferation. The effects of PGE2 on dendritic cells 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 dendritic cells and ability to stimulate T cells (20). NSAIDs and COX-2 inhibitors may be particularly suited to cancer prevention by protecting the integrity of dendritic cells 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-γ, TNF-α, 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 Cytotoxic T cells. The number of tumor-infiltrating central and effector T cells is inversely correlated with tumor invasion including vascular emboli, lymphatic invasion, and perineural invasion (23). In addition, the presence of T 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 TH1-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 CD8+ T-cell numbers via modulation of fatty acid metabolism. Preclinical experiments with mice deficient in TNF receptor-associated factor 6 (TRAF-6) showed that, although mice mounted normal effector CD8+ T-cell

![Figure 2. Cancer chemoprevention agents stimulate the adaptive immune system. A, curcumin administration can enhance the TH1 and decrease TH2 immune response. B, metformin may increase MHC-I expression on tumor cells, increasing visibility to effector CD8+ T cells. C, ZA and other bisphosphonates increase phosphoantigens in PBMCs and on cancer cells themselves, resulting in activation of anti-tumor γδ T cells. D, metformin and curcumin increase effector CD8+ T-cell populations and resulting memory cells, both of which are critical for an effective adaptive immune response.](image-url)
responses to infections, they had compromised CD8+ Tm-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 Tm cells (Fig. 2B; ref. 29). Furthermore, metformin promoted survival of CD8+ T cells in wild-type mice, resulting in enhanced generation of Tm 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 shown 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 leukemia cells showed 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; ref. 34). Because MHC-I downregulation has been well documented in premalignant lesions, metformin could serve to increase the immunogenicity of preinvasive disease. The retinoid X receptor agonist bexarotene also has the potential to enhance T-cell numbers and function. Bexarotene has been extensively studied in preclinical models and has been shown to be a potent antiproliferative 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; ref. 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 β (TGF-β), and indoleamine 2,3-dioxigenase (IDO), which suppresses the adaptive T-cell immune response (39). Preclinical studies have shown chemoprevention effects of curcumin across multiple tumor types (39). Results of a phase Ia trial revealed that curcumin treatment significantly reduced aberrant crypt foci (40). In the ApcMin/+ 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 (i) reversing tumor-induced depletion of CD4+ and CD8+ T cells (Fig. 2B and C) and potentiating CD8+ T-cell cytotoxicity (Fig. 2C); (ii) restoring memory T-cell (Tm) populations to levels comparable to controls (Fig. 2B); and (iii) shifting the cytokine signature from T1/2 to T1/1 (Fig. 2A; ref. 42).

γδ T cells are T lymphocytes with attributes of both the innate and adaptive immune system and account for 1% to 10% of all peripheral blood T cells. γδ T cells exhibit many qualities of the innate immune system such as the capability of being activated by nonself ligands and phosphoantigens generated by the isoprenoid pathway used by microorganisms or mevalonate pathway in infected or transformed cells. Once activated, γδ T cells can expand, exhibit cytotoxicity in both a MHC-dependent and independent fashion, and release T1 cytokines that further support the adaptive immune system (43). In mouse models of prostate and carcinogen-induced cutaneous malignancy, mice lacking γδ T cells developed higher disease burdens and progression of premalignant lesions to overt malignancy than controls and adoptive transfer of γδ cells could abrogate this effect (44, 45). In a longitudinal case–control study of renal transplant patients, 18 patients who developed cancer 2 to 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 γδ 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 γδ T cells in cancer prevention.

Nitrogen-bisphosphonates (N-BP) such as zoledronic acid are being studied as chemoprevention agents that may stimulate the proliferation of γδ T cells (Fig. 2E; ref. 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 ibandronate, 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 γδ T cells indirectly by increasing concentrations of isopentenyl pyrophosphate (IPP), a precursor in the mevalonate pathway, in peripheral blood mononuclear cells, which subsequently activates γδ T-cell receptors (TCR). There is also evidence that N-BPs can increase IPP in tumor cells themselves, resulting in a chemotactic and stimulatory signal for γδ T cells (Fig. 2E; ref. 52). Activated γδ T cells release T1 cytokines such as TNF-α, IL-6, and IFN-γ that are important in immune surveillance (53, 54). Both preclinical data and
phase I studies have shown N-BPs can activate tumoricidal γδ 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-induced fibrosarcomas, depletion of 50% to 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.

PGE2 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 PGE2 reduction. Mice underwent genetic deletion of mPGES-1, an inducible terminal synthase that produces PGE2, 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 showed that PGE2 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; ref. 42). These findings suggest that both COX-2 inhibition and curcumin may decrease Treg function and contribute to enhanced immune surveillance (Fig. 3A and B).

Aromatase inhibitors are breast cancer chemoprevention agents associated with up to a 65% relative reduction in annual incidence of invasive breast cancer (64). Aromatase inhibitors may mediate this effect in part by decreasing Treg populations (Fig. 3B; ref. 47). Aromatase inhibitors 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 aromatase inhibitors could shift this balance to Th1 and resolution of aberrant lesions. Consistent with this observation, in a preclinical mouse study of inflammatory arthritis, aromatase inhibitor treatment resulted in a lower percentage of splenic and lymph node Tregs and increased Th1 cytokine release (Fig. 3B; ref. 65). In a randomized phase II trial, patients with locally advanced
ER+ breast cancer received the aromatase inhibitor 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; ref. 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, MDSCs 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, which promote mutagenesis and inhibit T cells (Fig. 3C; ref. 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).

PGE2 influences differentiation of monocytes, promoting the MDSC phenotype, and COX-2 inhibition may prevent this effect (Fig. 1B). In preclinical studies, PGE2 shifted development of monocytes from immature dendritic cells to MDSCs when added to a standard preparative regimen of GM-CSF and IL-4 (Fig. 1B; ref. 70). In addition, PGE2 exposure promoted COX-2 expression in MDSCs, suggesting that PGE2 initiates a COX2–mediated positive feedback loop, perpetuating the immunosuppressive signal (71). PGE2 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 PGE2 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 dendritic cells (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 showed 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 redifferentiating MDSCs into immature dendritic cells (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 dendritic cells, 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 Treg 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; ref. 82).

Tumor-associated macrophages (TAM) are recruited early in dysplastic or premalignant lesions and contribute significantly to oncogenesis (83–86). M1 macrophages are tumoricidal whereas type 2 (M2) macrophages support tumorigenesis and immunosuppression. M2 macrophages express a host of tumorigenic factors including VEGF, COX-2, epidermal growth factor receptor, 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-β and inhibit T-cell proliferation through expression of Arg and IDO (Fig. 3D; ref. 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 4 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 antitumor effects, COX-2 inhibitors inhibit M2 macrophage accumulation (Fig. 3A). PGE2 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 squamous cell carcinoma (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, ApcMin/1 mice developed premalignant polyps heavily infiltrated by M2 macrophages with a T\textsubscript{H}2 cytokine profile. Celecoxib administration for 8 weeks (i) reduced the size and number of polyps compared to controls; (ii) shifted the TAM phenotype from M2 to M1 macrophage predominant infiltrate; and (iii) increased the T\textsubscript{H}1 signature in the environment (Fig. 3A; ref. 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). Bisphosphonates decrease release of protumorigenic factors such as MMP-9 by activated macrophages and decrease other proangiogenic factors such as VEGF associated with activated macrophages (Fig. 3D; ref. 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 zoledronic acid mimicking standard dosing schedules. Mice treated with zoledronic acid 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, whereas zoledronic acid–treated mice had a marked reduction. Interestingly, macrophages from control mice exhibited a T\textsubscript{H}2 cytokine pattern, whereas macrophages from zoledronic acid–treated mice were strongly positive for IFN-\textgamma, suggesting that in addition to reducing total macrophage numbers, zoledronic acid repolarized M2 macrophages to M1 type (94). In a murine model expressing HPV-16 genes (HPV/E\textgamma; mouse) of cervical carcinogenesis, mice develop cervical intraepithelial neoplasia (CIN) lesions that progress to invasive SCCs. In a trial comparing effect of zoledronic acid on mice with CIN-3 lesions to controls, at 5 months, controls had SCC incidence of 85%, whereas zoledronic acid–treated mice had an incidence of 30%, suggesting a strong preventative effect. In addition to inducing apoptosis of tumor cells themselves, zoledronic acid 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; ref. 95).

**Conclusion**

The immune system is a powerful sentinel against cancer with several types of cells surveying the environment and eliminating preinvasive 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 dendritic cells 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.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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