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Cancer Epidemiology, Biomarkers & Prevention

Review

A Review of the Application of Inflammatory Biomarkers in Epidemiologic Cancer Research

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Abstract

Inflammation is a facilitating process for multiple cancer types. It is believed to affect cancer development and progression through several etiologic pathways, including increased levels of DNA adduct formation, increased angiogenesis, and altered antiapoptotic signaling. This review highlights the application of inflammatory biomarkers in epidemiologic studies and discusses the various cellular mediators of inflammation characterizing the innate immune system response to infection and chronic insult from environmental factors. Included is a review of six classes of inflammation-related biomarkers: cytokines/chemokines, immune-related effectors, acute-phase proteins, reactive oxygen and nitrogen species, prostaglandins and cyclooxygenase-related factors, and mediators such as transcription factors and growth factors. For each of these biomarkers, we provide a brief overview of the etiologic role in the inflammation response and how they have been related to cancer etiology and progression within the literature. We provide a discussion of the common techniques available for quantification of each marker, including strengths, weaknesses, and potential pitfalls. Subsequently, we highlight a few under-studied measures to characterize the inflammatory response and their potential utility in epidemiologic studies of cancer. Finally, we suggest integrative methods for future studies to apply multifaceted approaches to examine the relationship between inflammatory markers and their roles in cancer development. *Cancer Epidemiol Biomarkers Prev; 23(9); 1729–51. ©2014 AACR.*

Introduction

The role of inflammation in the development and progression of cancer is of great scientific and public health interest and has drawn much attention of late. Several excellent reviews have described the likely cellular and molecular roles of inflammation in the development of cancer (1–13) and have outlined the consistent associations between chronic inflammatory conditions (14–19) and inflammation-inducing risk factors (such as tobacco; refs. 20–22) in the development of cancer at various sites. As the burden of cancer increases globally (23, 24), so does the value of identifying therapeutic targets. The role of inflammation in carcinogenesis requires additional research to clarify the mediators, pathways, and steps through which increased or altered inflammation leads to neoplastic development or progression. Ultimately, continued research is required to identify the key points of potential intervention to successfully improve outcomes. To proceed, epidemiologic studies will require integrative techniques across many platforms to elucidate meaningful mechanisms and improve outcomes.

In this review, we provide an overview of several effectors of inflammation involving the response of the immune system to infection and to chronic insult from environmental factors. We summarize the commonly used measurements to evaluate inflammatory status or alteration in the development of cancer, including the strengths and weaknesses of the common techniques available for each marker. We have provided recent evidence and findings to date about associations with cancer etiology and progression. Our literature summaries are not meant to be exhaustive due to the extent of this field and, where possible, we refer readers to relevant meta-analyses and literature reviews for concision. Subsequently, we highlight several under-studied measures

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of the inflammatory response. A general framework for the biomarkers of interest and their inter-relationships with cancer risk is depicted in Fig. 1. We suggest integrative multifaceted approaches for future studies seeking to examine the relationship between the markers and their roles in cancer development. Per definition, we refer to all of the biologic measures of the immune and inflammation responses included in this article as "inflammation biomarkers" for simplicity, although there is certainly great variety in the measures discussed.

Methodologic issues in epidemiologic studies on inflammation and cancer

In the evaluation of the associations between inflammation and cancer risk, careful consideration must be taken to address the potential for biased associations due to the well-known proinflammatory potential of tumors and, thus, their microenvironment (6). Prospective studies are, therefore, preferable due to lower risk of presenting temporally biased associations. Prospective designs also allow for latency analyses to determine whether the inflammatory marker associations are causal drivers of carcinogenesis or simply prediagnosis manifestation of tumor-related inflammation. Therefore, ideal prospective research designs investigating inflammatory markers should be conducted on samples taken many years, perhaps even decades, before diagnosis. Repeat sampling is useful because of the different mechanisms by which inflammation can drive carcinogenesis, for example, DNA damage (in earlier years) versus enhancement of angiogenesis (later). Samples collected only a few years before diagnosis may no longer reflect an evaluation of causality of the biomarker, instead becoming an evaluation of an early disease prediction marker, which although still clinically relevant, reflects a different hypothesis. In this context, large population-based cohorts with biobanking initiatives are extremely valuable in evaluating associations of inflammatory biomarkers. In our presentation of the literature, we emphasized large, prospective studies over retrospective designs. We also emphasize evidence from biomarkers measured in blood and to a lesser degree urine samples in large epidemiologic studies. Although several protocols exist for measuring target organ-specific inflammation-related compounds in several media, including exhaled breath and its condensate (25), sputum (26, 27), brochioalveolar lavage (28), and feces (29) among others (30), at present, these biospecimens are prohibitively expensive to feasibly collect in large population-based initiatives.

The inflammation responses under investigation may be due to a multiplicity of factors that have been consistently linked to cancer risk including, but not limited to, tobacco consumption (31), overweight and obesity (32, 33), physical inactivity (34, 35), persistent and/or transient infection (36), and immunosuppression (37–39). Thus, in a well-designed epidemiologic evaluation of the causality of inflammatory biomarkers during carcinogenesis, these factors should be accounted for, depending on which specific biomarkers are being evaluated, in both the design and analysis stages to best isolate the causal associations being examined.

In studies of inflammation and subsequent cancer-related clinical outcomes and survival, presurgical or pretreatment blood samples should be collected to avoid the

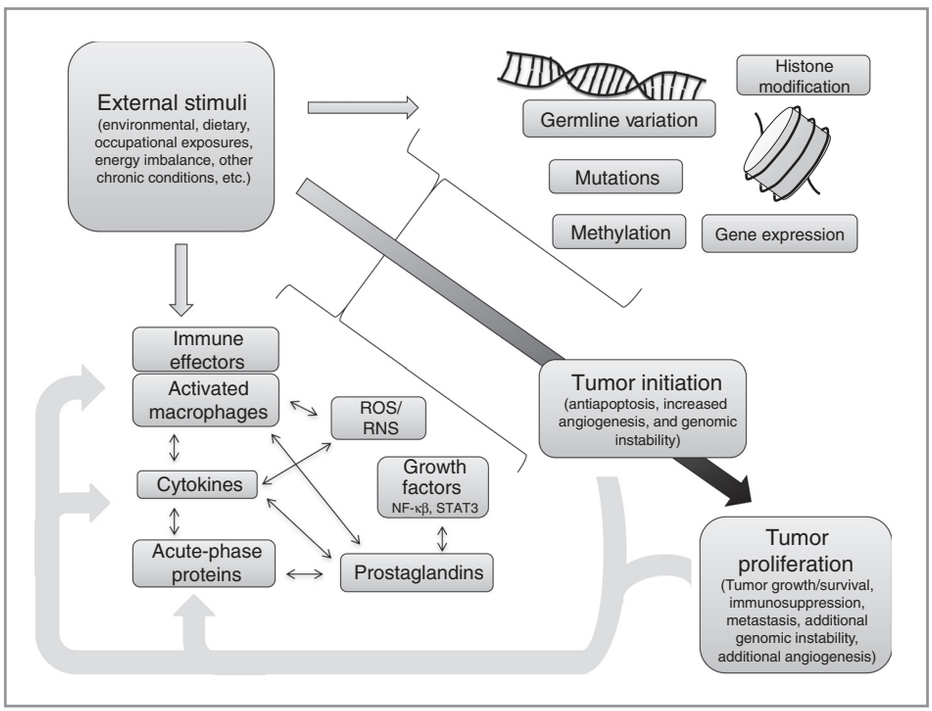

Figure 1. The complex interactions involved in the role of inflammation in the cancer progression spectrum.

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impact of treatment on levels of inflammatory and immune markers. In population-based studies examining inflammatory biomarkers on survival outcomes through active follow-up or passive cancer registry linkage, attention must be taken to collect detailed staging information for adjustment in analysis. Failure to do so invites the possibility of confounding due to a third factor related to inflammation and advanced stage at diagnosis, therefore affecting survival. Below we review six main classes of inflammation-related biomarkers: cytokines/chemokines, immune-related effectors, acute-phase proteins [C-reactive protein (CRP) and serum amyloid A (SAA)], reactive oxygen species (ROS) and reactive nitrogen species (RNS), prostaglandins and cyclooxygenase (COX)-related factors, and mediators such as transcription factors and growth factors.

Inflammation Biomarkers

Cytokines/chemokines

Background. During both acute and chronic inflammatory processes, a variety of soluble factors known as cytokines are involved in leukocyte recruitment through increased expression of cellular adhesion molecules and chemoattraction (40–43). To a large extent, they orchestrate the inflammatory response, that is, they are major determinants of the make-up of the cellular infiltrate, the state of cellular activation, and the systemic responses to inflammation (44). Cytokines are central in extensive networks that involve synergistic as well as antagonistic interactions and exhibit both negative and positive regulatory effects on various target cells (42). Although produced by a wide variety of cell types, macrophages and T lymphocytes (T cells) are the primary producers of cytokines, which may have predominantly proinflammatory (inflammation-promoting; IL1α, IL1β, IL2, IL6, IL8, IL12, TNFα, IFNγ; ref. 45) or anti-inflammatory (inflammation-suppressive; IL4, IL5, IL10, TGFβ) abilities.

Measurement. The measurement of cytokines as an indicator of inflammatory status in population-based initiatives is an area of great promise; yet, it provides several challenges due to the biochemistry of the molecules, particularly their short half-life (46, 47). Considering the immediate response of the body to injury, it can be advisable to draw the blood tube that is dedicated for cytokine measurements first during a blood draw. Cytokines can be measured in serum and plasma samples; however, measurements from the different sample types cannot be used interchangeably (48, 49). They can also be measured in tissues or as supernatant from cultured peripheral blood mononuclear cell (PBMC) preparations 50)). Cytokine measurements can be multiplexed to simultaneously assess multiple targets (51), presenting the opportunity to broaden the scope of investigation or test for possible interactions between the mediators. These techniques are, however, limited by differential concentrations of the varying cytokines. In addition, cytokine quantification can be affected by degradation through freeze/thaw cycles over longitudinal storage (52). Also, issues of standardized sample collection, processing, and study design must be carefully considered or sensitivities in the protein measurements may create artifactual associations if care is not taken (46). Concentrations of cytokines are known to vary in different tissues and a standard blood draw may not adequately reflect tissue-specific levels of inflammation (53). However, measurements of circulating cytokines may provide a general sense of an individual's inflammatory state. Additional advantages and disadvantages to measurement of cytokines are summarized in Table 1.

Cancer associations. *Risk.* Systemic cytokine concentrations have been associated with both cancer risk (54–57) and cancer progression (58–62), suggesting a pivotal role in carcinogenesis. For example, in the Health, Aging, and Body Composition cohort, circulating IL6 and TNFα were associated with lung cancer, IL6 was also associated with colorectal cancer; however, neither were associated with breast and prostate cancer (62). Investigation of serum IL6 and IL8 levels in the Prostate Lung Colon and Ovarian (PLCO) Cancer Screening Trial showed associations with lung cancer [IL6: odds ratio (OR), 1.48; 95% confidence interval (CI), 1.04–2.10; IL8: OR, 1.57; 95% CI, 1.10–2.24], compared with the lowest quartile. However, increased IL6 levels were only associated with cancers diagnosed within 2 years of blood collection, whereas increased IL8 levels were associated with cancers diagnosed more than 2 years after blood collection (OR, 1.57; 95% CI, 1.15–2.13; ref. 54). Whether this difference in association is due to cytokine degradation over time or due to a real association remains to be determined.

IL10 has been investigated in the development of non-Hodgkin lymphoma (NHL) in a prospective study with a significant positive association observed (63) as well as with prediagnostic levels of IL10, TNFα, and sTNF-R2 in a separate prospective investigation (64).

Progression. Several studies have observed negative prognosis of various cancers associated with IL6 level, including prostate cancer (65), renal cell carcinoma (RCC; ref. 66), non–small cell lung cancer (67), ovarian cancer (68), lymphoma (69, 70), chronic lymphocytic leukemia (CLL; ref. 71), esophageal cancer (72), colorectal cancer, and breast cancer (73).

Investigation of prognosis with IL6 serum concentrations (≥4.0 pg/mL) in the Multiethnic Cohort Study showed associations with significantly poorer survival in both African Americans [hazard ratio (HR), 2.71; 95% CI, 1.26–5.80] and Caucasians (HR, 1.71; 95% CI, 1.22–2.40). IL10 (HR, 2.62; 95% CI, 1.33–5.15) and IL12 (HR, 1.98; 95% CI, 1.14–3.44) were associated with lung cancer survival only in African Americans (74). Serum levels of IL6 have also been associated with tumor-proliferative activity among patients with colorectal cancer (75).

An examination of clinical outcomes among patients with hepatocellular carcinoma (HCC) after potentially curative hepatectomy reported that higher pretherapy serum levels of IL17 and lower levels of IL1 were

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Table 1. Summary of inflammatory markers, associated techniques, and tissue requirements with corresponding advantages and disadvantages of their application

Inflammatory marker	Explanation	Techniques[a]	Advantages	Disadvantages	Tissue requirement[b]	Current evidence of cancer association[c]
Cytokines and chemokines						
Cytokines/chemokines	Small secreted proteins that mediate as well as regulate immunity, inflammation, and hematopoiesis. Cytokines generally act at very low concentrations over short distances and short time spans.	ELISA, multiplex bead assays	Simultaneous measurement of several cytokines possible	No strong evidence to predict progression or survival. Blood draw and processing conditions can affect levels. Degradation over time when samples stored improperly or over multiple freeze/thaw cycles. Costly Tumors create an inflammatory milieu and can produce cytokines. Difficult to assess reverse causality without longitudinal data Lack of standardization of assays Function in multiple pathways (lack of specificity)	Serum/plasma/tissue/cell culture supernatant Depends on assay used (5–100 μL)	Direct measurement in several cancers with correlation with tumor stage and disease extent. Related to cancer risk in prospective collected data.

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Table 1. Summary of inflammatory markers, associated techniques, and tissue requirements with corresponding advantages and disadvantages of their application (Cont'd)

Inflammatory marker	Explanation	Techniques[a]	Advantages	Disadvantages	Tissue requirement[b]	Current evidence of cancer association[c]
Immune-related effectors						
WBC count	A measure of the total white blood content, generally indicative of infection (neutrophils and monocytes—bacteria, lymphocytes—viral, eosinophils—parasitic).	FACS	Routinely measured and used in clinical practice (useful for prediction and maximization of currently available data and used clinical algorithms).	Levels may be altered because of transient infection not-related to chronic inflammation.	Serum	Associated with lung cancer risk in prospectively collected data.
			Stable over time when frozen.		Portable kits available that need only 10 μL	Associated with cancer-related mortality in prospective studies.
			Blood neutrophilia and thrombocytosis established as indicators of systemic inflammatory response.			
mGPS	A combination of albumin and CRP measurements into a 3-level predictive score. 2 when both CRP >10 mg/L and albumin <35 g/L. 1 if only one abnormality present. 0 if both not.	Combined CRP and albumin tests.	Inexpensive if CRP and albumin already measured.	Levels may be altered because of transient infection not-related to chronic inflammation same issues for CRP.	Serum	Not related to risk of cancer development.
			Standardized score			Evidence suggests use as a prognostic score independent of tumor stage and treatment.
NLR	The ratio of neutrophils to lymphocytes, where higher values reflect states of dramatic inflammation.	Same as WBC count	Potential as a simple, cost effective, and readily available test.	Different cutoff levels reported across studies.	Serum	Shown to be related to survival in many cancer sites after diagnosis and various treatment modalities.

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Inflammatory marker	Explanation	Techniques[a]	Advantages	Disadvantages	Tissue requirement[b]	Current evidence of cancer association[c]
				Additional value obtained beyond normal white cell counts is of question		
PLR	The ratio of platelets to lymphocytes, where higher values reflect states of dramatic inflammation.	Same as WBC count	Routinely measured and used in clinical practice (useful for prediction and maximization of currently available data and used clinical algorithms).	Levels may be altered because of transient infection not-related to chronic inflammation.	Serum	Predicts outcomes in colorectal cancer.
			Potential as a simple, cost-effective, and readily available test.	Not well studied with risk of disease.		
Th17 lymphocytes	Recently discovered inflammatory T-cell subset with associations to autoimmune diseases and potential role in cancer risk and progression.	IHC, FACS	All three cell types give insight into the T-cell functional status, and thus immune responses. A combined analysis can potentially advance the current analysis focusing on one of the cell types.	Heterogeneous results are assumable between cancer entities. Material acquisition might be problematic.	Tissue (TMAs), peripheral blood cells (require fresh cells for flow cytometry)	Not as of yet extensively studied with risk.
Acute-phase proteins						
CRP	An acute-phase protein produced by hepatocytes in response to proinflammatory cytokines. Produced in times of inflammation to age damaged cells for excretion by the liver.	Fluorescence polarization-immunoassay, nephelometry, ELISA	Quantitative and sensitive measurement	Nonspecific marker of inflammation	Serum or plasma	Large meta-analysis indicates poor evidence to support use as a diagnostic marker; may be useful in colorectal and lung cancers.

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Table 1. Summary of inflammatory markers, associated techniques, and tissue requirements with corresponding advantages and disadvantages of their application (Cont'd)

Inflammatory marker	Explanation	Techniques[a]	Advantages	Disadvantages	Tissue requirement[b]	Current evidence of cancer association[c]
			Easily measured	Levels may be altered because of transient infection not related to chronic inflammation (CRP rises drastically in acute inflammation, such as infection, therefore several measurements over time are encouraged for a better characterization of chronic states).		
SAA	Similar to CRP an acute-phase protein, but levels may be even more responsive to inflammation.	Fluorescence polarization-immunoassay, nephelometry, ELISA	Easily measured	Limited evidence for use to predict treatment response.	Serum or plasma	Strongly associated with worse long-term survival from breast cancer.
			Rather novel marker			
ROS and RNS						
ROS and RNS	Chemically reactive molecules produced as byproducts of normal metabolic processes in all aerobic organisms. Characterized by the presence of unpaired electrons.		Would provide a direct estimate of ROS burden and would be beneficial for prediction of risk and carcinogenicity of lifestyle patterns.	No standardized methods to capture actual ROS levels in humans to date	Tissue	Direct evidence and measurement in prospective studies lacking
				Compounds have very short half-life in systems.		Signaling pathways regulated by ROS in cancer models.

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Inflammatory marker	Explanation	Techniques[a]	Advantages	Disadvantages	Tissue requirement[b]	Current evidence of cancer association[c]
				Questionable quality due to the volatility of compounds.	Serum	Association with expression/enzyme activity of ROS/RNS producers shown in various cancers.
				Levels affected by lifestyle factors, i.e., nutritional status		Weak evidence of variants in ROS/RNS production genes and cancer risk.
Oxidatively/nitrosatively modified DNA, or proteins	The product of excess ROS/RNS in tissue					
3-Nitrotyrosine	The product of nitrosylated proteins	HPLC		Not well standardized	Healthy or tumor tissue	Higher level observed in the tumors of never smoking lung cancer cases suggesting a marker for inflammation-related carcinogenesis.
8-oxodg or 8-OHdG	8-oxodG is a sensitive surrogate biomarker for *in vivo* oxidative stress.	ELISA, HPLC methods	Provides a measure of DNA damage due to ROS.	Not tissue specific	Plasma, urine	Elevated levels observed in several cancers included esophageal, colon, and breast
8-Iso-PGF_{2_α}	LPO product.	ELISA, HPLC methods	Provides a measure of DNA damage due to ROS.	Not tissue specific	Plasma, urine	Related to breast and colon cancer risk
MDA	LPO product.	ELISA, HPLC methods	Provides a measure of DNA damage due to ROS.	Not tissue specific	Plasma, urine	Associated with lung and colon cancer risk
HNE	LPO product.	ELISA, HPLC methods	Provides a measure of DNA damage due to ROS.	Not tissue specific	Plasma, urine	Not as well studied as other peroxidation products

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Table 1. Summary of inflammatory markers, associated techniques, and tissue requirements with corresponding advantages and disadvantages of their application (Cont'd)

Inflammatory marker	Explanation	Techniques[a]	Advantages	Disadvantages	Tissue requirement[b]	Current evidence of cancer association[c]
Prostaglandins, COX, lipoxygenases, and related factors						
Prostaglandin levels	Lipid compounds containing 20 carbon ring including a 5-carbon group. Produced by the sequential oxidation of prostaglandin. By COX1 and COX2. COX1 is believed to control baseline levels of prostaglandins, whereas COX2 increases levels of PGE by response to stimulation	ELISA		Levels may be tissue-specific difficult to measure.	Saliva, urine, serum, EDTA and heparin plasma	
		Liquid chromatography/ tandem mass spectrometry		Affected by several pharmacologic interventions which could complicate association modeling. Some prostaglandins are rapidly degraded		
COX2 expression	Enzymes integral to prostaglandin synthesis.	IHC	Interest as target for chemoprevention	Levels may be tissue-specific difficult to measure.	Tissue culture media	COX2 expression observed in nearly every tumor type examined.
				Affected by several pharmacologic interventions that could complicate association modeling.	TMA	

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Table 1. Summary of inflammatory markers, associated techniques, and tissue requirements with corresponding advantages and disadvantages of their application (Cont'd)

Inflammatory marker	Explanation	Techniques[a]	Advantages	Disadvantages	Tissue requirement[b]	Current evidence of cancer association[c]
Transcription factors and growth factors						
NF-κB activation	A transcription factor that functions in inflammatory pathways by inducing the expression of inflammatory cytokines, adhesion molecules, COX, NOS, and angiogenic factors.	ELISA RT-PCR to measure mRNA	Can measure quantity, activation, translocation and transcriptional potential.	Clinical evaluation of NF-κB requires cell culture.	Serum, plasma, peripheral blood lymphocytes	Predictive of outcomes in breast cancer and colorectal cancer.
STAT3 activation	A transcription factor activated in response to various factors, including inflammatory cytokines. Mediates the expression of several key cell growth and apoptosis genes.	ELISA		Also regulated by other non-inflammatory growth factors.	Serum or plasma	Less well studied than NF-κB.

[a]Most commonly cited techniques although others may exist.
[b]Quantity of sample will depend on quality of material extraction, processing, and type.
[c]For additional details see text.

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associated with early recurrence. After adjustment for general tumor clinicopathologic factors, elevated serum levels of IL17 ($\geq$0.9 pg/mL) were found to be an independent risk factor for HCC early recurrence with an HR of 2.46 (95% CI, 1.34–4.51). Patients with larger tumors (>5 cm in diameter) and elevated serum levels of IL17 had the highest risk of early recurrence as compared with those with only one of these factors ($P = 0.009$) or without any ($P < 0.001$). The authors suggest that these factors showed similar effects on the overall survival (OS) of patients with HCC (76).

Immune-related effectors

Background. Leukocytes comprise an integral portion of the innate, as well as of the adaptive, immune system and include granulocytes (neutrophils, basophils, and eosinophils), monocytes, macrophages, dendritic cells, and lymphocytes (B&T cells), which can exert immune-stimulating or immunosuppressive functions (77). In patients with cancer, several pathways can be activated to suppress the effective adaptive immune response, triggered to avoid the destruction of the tumor by immune cells (78). Leukocytes also activate to release cytokines and growth factors, which support tumor growth. The activities of the immune system lead to a change of blood leukocytes profile, which serves as a marker of the systemic inflammatory response. On the basis of this principle, several measurements of inflammation and a shift in number or ratios of immune cells have been investigated for the association with cancer risk or outcome of the disease such as the modified Glasgow Prognostic Score (mGPS; ref. 79), neutrophil to lymphocyte ratio (NLR; ref. 80), and platelet to lymphocyte ratio (PLR; ref. 81).

Tumor-infiltrating lymphocytes (TIL) are white blood cells (WBC) found within the tumor that presumably reflect an immune response against the tumor (82). It is thought that TILs work in combination with chemotherapies that can promote cytotoxic T lymphocytes that can produce antitumor immunity and thus lead to improved outcomes (83). However, a role in supporting tumor growth cannot be excluded. T helper 17 (Th17) cells are a $CD4^+$ T-cell subset in addition to Th1 and Th2 that lead to increased levels of IL17, IL22, and IL21 production (84–86). IL6 and other cytokines, including IL23, are thought to play a key role in the production of the Th17 cells (84, 87, 88). They mediate host-defensive mechanisms to various infections to provide antimicrobial immunity at epithelial–mucosal barriers and are involved in the pathogenesis of many autoimmune diseases (86).

Measurement. The measurement of lymphocytes can be performed using tissue or peripheral blood samples and is based on standard clinical routines (WBC counts). Flow cytometry has also become a widely used tool to quantify phenotypic subsets of immune cells and thus provides a snapshot that allows for some understanding of the current immune response (89–91). Flow cytometry can also be used to quantify T-cell proliferation using dyes (92).

Despite great promise, flow cytometry is limited in its epidemiologic application as the experiments are sensitive to issues of standardization particularly from differences in reagents, sample handling, instrument setup, and data analysis. These differences across study sites are known to affect outcome measurements (91, 93, 94) and have been shown to affect results in multicentered projects (95). Attention to standardization of procedures along the project pipeline may aid in alleviating these concerns and promote cross-project collaborations, which is one of the goals of the human immunology project (96). In addition, the quantification of immune cells generally requires fresh biospecimen, which limits its use in epidemiologic studies.

TILs can be measured by immunohistochemistry (IHC; ref. 97) using different stains, including hematoxylin and eosin, and through the use of multicolor flow cytometry (98). TILs can be quantified using tissue microarrays (TMA) and whole tissue sections (99). Th17 can be measured by multicolor flow cytometry (100) and can be evaluated in peripheral blood and other body fluids (101, 102).

Cancer associations. *Etiology.* A comprehensive review of the associations between these immunologic markers and outcomes is beyond the scope of this review. Briefly, various measures of leukocyte quantities, such as WBC count, PLR, and NLR, have been associated not only with increased risk of several types of cancer, including breast cancer, colorectal cancer and endometrial Cancer, but also with tumor progression (81, 103). The Women's Health Initiative (WHI) observed a significant association between WBC count and increased risk of invasive breast cancer, colorectal cancer, endometrial Cancer, and lung cancer in more than 140,000 postmenopausal women (103).

Prognosis. Likewise, a study investigating the association between several inflammation-based prognostic scores, such as mGPS, NLR, and PLR, and cancer survival observed strong prognostic values of all three scores for cancer survival independent of tumor site (breast cancer, bladder cancer, ovarian cancer, prostate cancer, gastroesophageal, hematological, RCC, colorectal cancer, NHC, hepatopancreaticobiliary, and lung cancer) in more than 27,000 patients (104).

TILs have been the focus of many studies and were shown to be positively associated with improved survival among patients with cancer, including colorectal cancer (105), lung cancer (106), and others sites (107–109). In addition, the assessments of TIL densities at the margin of liver metastasis in patients with colorectal cancer were predictive for chemotherapy response (110). $CD8^+$ TILs were independently predictive of improved breast cancer survival; however, results vary by molecular subtype (improved in basal, but not in triple-negative; ref. 111) and by estrogen receptor status and histologic grade (112).

The presence of Th17 cells in ovarian cancer (113), prostate cancer (114), lung cancer (115), and pancreatic

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cancer (116) as well as in melanoma (117) were repeatedly associated with better survival of patients (118).

Acute-phase proteins

CRP. *Background.* CRP is an acute-phase protein found in blood, which is synthesized in the liver in response to inflammation. Physiologically the protein activates the complement system via the Q1 complex (119). Once activated, the complement system aids in clearing the injured or dead cells from tissues. CRP has been related to systemic levels of inflammation in various inflammatory conditions as well as chronic diseases such as cardiovascular disease and type II diabetes (120). CRP is also highly related to obesity [generally measured using body mass index (BMI) in population studies] across genders and study populations (121), although most research has been done on Caucasian populations, as obesity is a chronic inflammatory state/condition (122). Obesity has been associated with cancer risk and progression at various sites with one of the suggested mechanisms to be operating through chronic altered inflammation (32, 33, 123–127). Therefore, CRP may act not as a causal protein but as a marker of systemic inflammation. Elevated CRP levels have also been correlated with other elevated inflammatory markers (128).

Measurement. CRP measurements can be performed in whole blood, plasma, and serum using various immunoassays (129) with high-sensitivity nephelometry being the gold standard. As with other inflammatory markers, CRP has a relatively short half-life, and thus proper sample processing is essential (130). Transient conditions, such as a common cold or mild injury/trauma, can drastically alter individual CRP levels (131). Thus, variability of CRP levels may lead to issues in analyses and/or biased statistical estimates. It is possible to recognize and eliminate very high infection-induced values by reviewing CRP levels against age- and BMI-standardized rates and excluding individuals with a certain level of variability above. Nevertheless, single studies with single measurements can be affected by transient conditions. One investigation into the effects of a single CRP measurement in epidemiologic studies suggested that conducting a single measurement could largely attenuate observed effect sizes from true effect sizes (132). Multiple measurements would therefore be optimal to track changes in levels over time. However, an analysis with repeated measurements has shown that a small index of individuality was observed in healthy individuals with relative rankings over a 6-month interval differing minimally (133).

Cancer associations. Etiology. Several prospective analyses have shown that CRP is associated with risk of cancer at various sites (54, 134, 135). An investigation of risk at multiple sites in the Healthy, Aging, and Body Composition Study showed that baseline levels were associated with lung cancer, colorectal cancer, and breast cancer risk. A nested case–control study also suggested associations for HCC, lung cancer, skin cancer, RCC, and bladder cancer (136). Prospective investigations have observed null associations for breast cancer (137, 138) but increased risk for ovarian cancer (139).

The association results for CRP and colorectal cancer risk, however, are contradictory, as a previous meta-analysis of eight prospective studies suggested that increased CRP levels collected at baseline was related to a modest increase in colorectal cancer risk [relative risk (RR), 1.12; 95% CI, 1.01–1.25; ref. 134], whereas a recent nested case–control conducted in the PLCO Cancer Screening Trial observed a 15% reduction in risk of developing colorectal adenoma (OR, 0.85; 95% CI, 0.75–0.98; $P_{trend} = 0.01$; ref. 140). A study by Toriola and colleagues (141) using repeat assessments of CRP in the WHI Observational Study Cohort among 980 women and controls demonstrated that CRP was associated with an increased risk of colorectal cancer; however, the change in CRP over time was not predictive, thus suggesting little value as an early detection marker.

For lung cancer, the associations seem to be consistent across studies. A meta-analysis of 10 studies involving 1,918 lung cancer cases showed a pooled RR of 1.28 (95% CI, 1.17–1.41) for one unit change in natural logarithm (ln) CRP (142).

Prognosis. CRP has also been shown to be associated with cancer progression (143) and survival (144, 145). Clinical investigations have shown that CRP levels of patients with pancreatic cancer (146), esophageal cancer (147), prostate cancer (148), and NHL had advanced staging (149), higher disease recurrence (150, 151), and shorter survival, which was also observed for colorectal cancer (152).

A meta-analysis of 10 breast cancer studies that involved 4,502 patients observed significantly decreased OS (HR, 1.62; 95% CI, 1.20–2.18) and disease-free survival (HR, 1.81; 95% CI, 1.44–2.26) when CRP levels were elevated. For cancer-specific survival, the pooled HR in higher CRP expression in breast cancer was 2.08 (95% CI, 1.48–2.94), which could strongly predict poorer survival in breast cancer (153).

SAA. *Background.* SAA is another acute-phase protein similar to CRP. However, circulating SAA levels are thought to be more responsive to inflammation as levels drop off more rapidly following an inflammatory stimulus (154). Unlike CRP, which activates the complement system, to eliminate target cells and induce inflammatory cytokines and tissue factor in monocytes (155, 156), the physiologic effects of SAA are far less understood.

Measurement. SAA is measured in serum, similarly to CRP, using high-sensitivity nephelometry often with micro-latex agglutination tests as the gold standard. Levels can also be detected in saliva using different techniques, including fluorescent immunoassays (157).

Cancer associations. Etiology. SAA has been related to risk at several cancer sites, including colon (OR, 1.5; 95% CI, 1.12–2.00), among women (141). Elevated SAA levels have also been highly related to lung cancer risk in the PLCO study as well as gastric cancer in the Japan Public Health Center–based prospective study (158). These

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analyses have also shown a strong correlation between SAA and CRP, suggesting that measurement of both is essential to control for possible confounded associations and that any independent predictive ability remains to be determined.

Prognosis. SAA is related to stage of disease (159) and strongly associated with reduced long-term survival of breast cancer (160), lung cancer (161), and esophageal squamous cell carcinoma (162). SAA may represent a link between inflammation and metastasis, thereby reducing survival outcomes in colorectal cancer (163).

ROS and RNS

Background. ROS and RNS are free radicals that are produced as part of the normal metabolic cycle. ROS generation is based on the reduction of molecular oxygen, catalyzed by NAD(P)H oxidases and xanthine oxidase or in a nonenzymatic reaction by redox–reactive compounds of the mitochondrial electron transport chain (164). RNS are produced as byproducts of the conversion of arginine to citrulline by nitric oxide synthase (NOS). Both ROS and RNS are important signaling molecules and involved in metabolism, cell-cycle signaling cascades, and intercellular signaling cascades, especially in inflammation processes (41), as their formation is stimulated by cytokines and chemokines through activation of protein kinase signaling cascades (165). In a vicious cycle, ROS and RNS recruit additional inflammatory cells, leading to further generation of free radicals. An overproduction of ROS or RNS and limited antioxidative capacities can result in unbalanced metabolism and consequently lead to oxidative or nitrosative stress (166). This is accompanied by damage of DNA, protein, lipids, carbohydrates, and small metabolites and can be deleterious for cells, tissues, and organisms (14, 165, 167). DNA damage through nitrosative deamination of nucleobases or guanosine peroxidation results in 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), the addition of a hydroxyl radical to the c8 position of the guanine ring. This alteration can subsequently lead to single- or double-stranded breaks, deoxynucleotide or deoxyribose modifications, and DNA cross-links (168). These genomic alterations can exert oncogenic effects through altered replication, transcription, and translation (169, 170). Oxidation of the guanine base is the most abundant DNA lesion and can be a highly mutagenic miscoding lesion (171). Measurement of oxidatively generated DNA damage products in urine has been shown to be useful for epidemiologic studies to quantify inflammatory exposures (172). 8-oxodG is often referred to as 8-hydroxy-2′deoxyguanosine (8-OHdG); however, for consistency in the review, we henceforth refer only to 8-oxodG even for those studies who have used the term 8-OHdG. For a discussion of this nomenclature, see Cooke and colleagues (173), who recommend this term as it conforms with the International Union of Pure and Applied Chemistry. Furthermore, this is the more appropriate term as the oxidized nucleobase (8-oxoGua) is a tautomer that at physiologic pH is mainly present in the oxo form and not in the hydroxy form.

ROS also leads to lipid peroxidation (LPO) whose products are genotoxic and mutagenic and can react with protein and DNA (174). Two LPO products generated by ROS that have been investigated in cancer etiology are DNA-reactive aldehyde byproducts *trans*-4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA). These molecules react with DNA bases to form exocyclic DNA adducts (175, 176). Reaction of DNA bases with these LPO end products yields five-membered rings (etheno-DNA adducts) attached to DNA, including 1,N^6-etheno-2′-deoxyadenosine (εdA) and 3,N^4-etheno-2′-deoxycytidine (εdC; ref. 177). εdA and εdC seem to be promising tools for quantifying promutagenic DNA damage in early, premalignant stages of the carcinogenesis process (178). These etheno-DNA adducts can be directly quantified in tissues and urine. They have been implicated in clinical studies (179) and may serve as potential risk markers for associations between inflammatory diseases and cancer (180).

A F2-isoprostane isomer, 8-isoprostaglandin $F_{2\alpha}$ (8-Iso-$PGF_{2\alpha}$), has also been found to be a sensitive and reliable index of *in vivo* oxidative stress reflective of DNA damage through LPO (181).

In addition, ROS play a crucial role in angiogenesis by triggering the release of angiogenic factors such as vascular endothelial growth factor (VEGF). Thus, it is hypothesized that ROS are involved not only in developing cancer, but also in cancer progression (13, 182).

Measurement. The measurement of ROS presents an interesting and challenging possibility to directly quantify the oxidative burden within tissues. ROS/RNS can be measured either directly in several different tissue types (182–184) or indirectly by measuring the product of ROS/RNS reactions. The main limitation of the direct measurement of ROS/RNS is the extremely short half-life with an estimated lifespan of OH component of <1 nanosecond in blood (185). Consequently, most blood and tissue storage protocols used in observational study designs are not feasible. Measurement of H_2O_2 can be measured directly in urine as a proxy of whole body oxidative stress (186); however, as dietary factors can also raise urinary H_2O_2 (187), associations may be confounded. H_2O_2 can also be measured in exhaled air and breath condensate (188), this, however, may not be feasible on a large scale for population-based studies. Probes such as dichloro-dihydrofluorescein diacetate (DCFH-DA) can also be used to detect "cellular peroxides" in cells (189).

Methods have been developed that assess oxidative DNA and protein damage that results from ROS/RNS using tissue-specific measures of protein residues (190). Oxidative DNA damage can be measured by gas chromatography–mass spectrometry (GC-MS), high-performance liquid chromatography coupled to electrochemical detection (HPLC-ECD), HPLC–mass spectrometry (MS) as well as immunoassays and enzymatic assays among others (167, 191).

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8-oxodG in urine serves as a reliable measure of "whole-body" oxidative stress (192, 193) and can be quantified using HPLC-MS (194–196) or HPLC-ECD (197). Both 8-oxodG and 8-Iso-PGF2_α can also be measured in plasma using commercially available ELISA protocols. Of concern for epidemiologic studies is the poor agreement between ELISA and tandem mass spectrometric HPLC-MS in methodologic comparisons of measurements from urine (198–200).

MDA can be quantified in plasma, urine, and tissue using several methods, including HPL-ECD, GC-MS, and liquid chromatography–electrospray with tandem mass spectrometry (LC-ES-MS/MS; ref. 201). MDA quantification by HPLC has shown good interlaboratory validity in replicate human EDTA-treated plasma samples sent to multiple laboratories (202). HNE can also be reliably detected in plasma and urine using both HPLC (203) methods and ELISAs (204).

Etheno-DNA adducts can be directly quantified in tissues and urine (179). εdA can be quantified using immunoprecipitation/HPLC/fluorescence detection methods (205) and εdC can be quantified using modified thin-layer chromatographic protocols (206). HPLC-MS protocols have also been developed to quantify εdA and εdC from a single DNA sample using purified DNA from cells or tissues (207). A recent population-based application of immunoaffinity/32P-postlabeling (208) successfully quantified εdA and εdC from buffy coat collected in a population-based study [European Prospective Investigation into Cancer and Nutrition (EPIC) Heidelberg], suggesting potential utility in larger population-based investigations as a direct measure of exposure-related DNA alterations from oxidative stress (209).

Nitrotyrosine, a byproduct of reactions with nitrogen radicals and RNS, can be measured in various tissues. 3-Nitrotyrosine can be assayed in serum or from tissue sample using commercially available ELISA kits; however, commercially available kits have provided low reproducibility and conflicting results (210). It can also be measured using electron spin resonance (211), polychromatic flow cytometry (FACS; ref. 212), and GC-MS (213). These techniques have been limited in their application in population-based studies; however, these biomarkers present as an interesting avenue for inflammation quantification projects.

Cancer associations. *Etiology.* Epidemiologic studies have shown that serum 8-oxodG levels were significantly increased in patients with colorectal cancer compared with controls. A Japanese study suggested that levels of 8-oxodG and fibrosis were significant risk factors for HCC, especially in patients with hepatitis C virus infection (214). Several studies have observed either elevated blood (215), urinary, or salivary levels of 8-oxodG in oral cancer compared with controls. For example, an investigation of salivary 8-oxodG levels in patients with oral squamous cell carcinoma showed a 65% increase compared with controls (216). Urinary 8-oxodG levels were also significantly higher among patients with breast (217, 218) and colorectal (219) cancer than among control subjects in adjusted analyses. Elevated 8-oxodG levels have also been observed in blood from patients with squamous cell carcinoma of the esophagus (220–222). Elevated 8-oxodG levels have been associated with a modestly increased risk of breast cancer [incidence rate ratio (IRR), 1.08 (1.00–1.17 per nmol/mmol creatinine excretion) increase; ref. 223] and lung cancer among never smokers [IRR, 1.17 (1.03–1.31); ref. 224].

Epidemiologic data examining DNA damage using LPO suggest that increased 8-Iso-$PGF_{2\alpha}$ is positively associated with risk of breast cancer (225, 226) and colorectal cancer (227). MDA levels have been associated with lung cancer (228, 229). In a prospective investigation of prediagnostic serum levels of reactive oxygen metabolites (ROM), specifically hydroperoxides, in the EPIC, ROM were associated with overall colorectal cancer risk when comparing highest tertile with lowest tertile (adjusted IRR, 1.91; 95% CI, 1.47–2.48). This association was, however, seen only in subjects with relatively short follow-up, suggesting that the association results from production of ROS by preclinical tumors (230). In a study of patients with oral cavity cancer, LPO products, such as lipid hydroperoxide (LHP) and MDA, and nitric oxide products, such as nitrite (NO_2^-), nitrate (NO_3^-), and total nitrite (TNO_2^-), were significantly elevated, whereas enzymatic and nonenzymatic antioxidants were significantly lowered in patients with cancer when compared with healthy subjects (231).

Progression. ROS have been much less well studied in regard to disease progression, likely because of the related difficulties in collecting appropriate materials for measurement and the influence of cancer treatment modalities on ROS generation and subsequent byproducts. Expression of nitrotyrosine and inducible NOS has, however, been associated with poor survival in patients with stage III melanoma (232).

Prostaglandins, cyclooxygenases, lipoxygenases, and related factors

Background. Prostaglandins have a wide range of strong physiologic effects and can be found in most tissues and organs (233). Prostaglandins constitute a group of lipid compounds that are enzymatically derived from essential fatty acids (EFA) and have important functions in different cell types (234). EFAs are modified by either of two pathways: the prostaglandin H synthase–COX pathway or the lipoxygenase (ALOX) pathway. The COX pathway produces thromboxane, prostacyclin, and prostaglandins D, E, and F. The COX pathway includes two rate-limiting enzymes, COX1 and COX2 (235). COX1 has been traditionally characterized as constitutionally expressed, and thus responsible for baseline prostaglandin levels, whereas COX2 is more easily inducible, including through IL6 and peroxides. The ALOX pathway is inactive in leukocytes and synthesizes leukotrienes in macrophages (236). Both of these pathways, their intermediates, or end products are involved in the

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inflammation response, although COX2 has been given more attention in the investigation of cancer etiology in population-based research (235).

Measurement. The role of disruption in prostaglandin synthesis in cancer development can be evaluated at several points in the various pathways using several techniques. The most frequently used methods to measure levels of prostaglandins in a variety of liquid biospecimens are chromatography-based methods, such as GC-MS, and antibody-based methods, such as ELISAs and RIAs (237, 238). Although GC-MS provides high sensitivity and specificity, the method also involves labor-intensive sample preparation and is not suitable for high-throughput analysis. In contrast, antibody-based methods enable the measurements of multiple samples simultaneously; however, these assays frequently lack specificity (238). At present, the most precise, informative, and reliable method with a reasonable throughput is LC/MS-MS (239), which was recently optimized for the measurement of PGE_2 and PGD_2, by incorporating special standards in the samples (240). However, this approach is not high throughput. COX2 expression can also be measured by quantitative IHC in tissue.

Cancer associations. *Etiology.* Direct measurement of urinary PGE metabolites (PGE-M) has been associated with increased cancer risk for breast cancer among postmenopausal women who did not regularly use nonsteroidal anti-inflammatory drugs [NSAID; HR, 2.1 (95% CI, 1.0–4.3), 2.0 (95% CI, 1.0–3.9), and 2.2 (95% CI, 1.1–4.3)] for the second, third, and highest quartiles of PGE-M (241). Increasing quartiles of urinary PGE-M levels were also associated with risk of gastric cancer [statistically significant test for trend ($P = 0.04$); ref. 242].

Prognosis. A meta-analysis of 23 studies evaluating COX2 expression from IHC suggested that COX2 overexpression in tumor tissues had an unfavorable impact on OS in patients with colorectal cancer (HR, 1.19; 95% CI, 1.02–1.37; ref. 243). COX2 correlates with poor prognostic markers in breast cancer (large tumor size and high tumor grade), but not with outcome (244) and with reduced survival in cervical and ovarian cancer (245–247). In an investigation of COX2 expression in bladder cancer, a weak association with recurrence in non–muscle-invasive bladder tumors was observed ($P = 0.048$). In the multivariable analyses, COX2 expression did not independently predict any of the considered outcomes (248).

Transcription factors and growth factors as mediators of an inflammation and cancer association

Background. Several substances created by the cellular mediators of inflammation propagate the inflammation response and their actions elicit a cellular growth response. These growth factors/transcription factors are proteins that bind to cellular and nuclear receptors to elicit a downstream response. NF-κB one such transcription factor that has been suggested to play a strong molecular role linking inflammation and cancer development (249, 250). NF-κB is activated downstream through (i) the Toll-like receptor (TLR)–MYD88 pathway responsible for sensing microbes and tissue damage, as well as the inflammatory cytokines TNFα and IL1B (251). NF-κB activation can also be the result of cell-autonomous genetic alterations (252). NF-κB functions in inflammatory pathways by inducing the expression of inflammatory cytokines, adhesion molecules, COX, NOS, and angiogenic factors, all propagating an exacerbated inflammation response (253). It also promotes tumor survival by inducing antiapoptotic genes (*BCL2*; ref. 254). A lack of checkpoint for growth factors, such as NF-κB activation, leads to increased proinflammatory cytokine and chemokine secretion as well prostaglandin release downstream of NF-κB signaling, which were shown to promote neoplasia (255).

Another transcription factor also believed to play a pivotal role in linking inflammation and cytokines to cancer development and progression is STAT3. Most inflammatory signals affect tumorigenesis by activating STAT3 in a similar method to those described for NF-κB (256, 257). Persistent STAT3 activation in malignant cells stimulates proliferation, survival, angiogenesis, invasion, and tumor-promoting inflammation (258, 259).

Measurement. Transcription factors, such as NF-κB activity, can be measured on various levels, including (i) quantity in fluids, (ii) levels of activation, and (iii) translocation. Quantification can be completed using ELISA and other high-sensitivity assays; however, stability in blood samples is an issue. Levels of NF-κB activation in stimulated normal peripheral blood lymphocytes can be completed using a real-time PCR to measure of Iκβα mRNA levels as a rapid, sensitive, and powerful method to quantify the transcriptional power of NF-κB. It can be used for clinical evaluation of NF-κB status, but requires cell culture and is thus not easily adaptable in epidemiologic studies (260). NF-κB translocation to the nucleus, where it regulates cytokine and immunoglobulin expression, can be measured by both confocal microscopy and flow cytometry (261).

Cancer associations. In comparison with the other markers discussed in this review, comparatively fewer studies directly quantifying cancer risk and prognosis related to changes in NF-κB and STAT3 quantity in fluids, levels of activation, and translocation have been completed, perhaps due to the difficulty of appropriate biospecimen collection. Several investigations have examined polymorphisms in *NF-κB* genes in the development of ovarian cancer (262) and colorectal cancer (263, 264). Examination of NF-κB activation has suggested an association with a high-risk subset of hormone-dependent breast cancer (263) with increased expansion of cancer stem cells in basal-like breast cancers (265). Results also suggest that NF-κB activation may be predictive of response to treatment (266) and survival (267) in colorectal cancer. Proteasome

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inhibitors used for treatment of various cancers, including multiple myeloma and NHL (268), elicit their effects partially reducing NF-κB activity (269).

Discussion

The potential for prevention and therapeutic intervention

Prevention. Several of the biomarkers discussed in this review presently have the potential to be used for cancer prevention. From a primary and tertiary prevention perspective, the use of NSAIDs has been related to reduced cancer risk at several sites, including breast cancer, colorectal cancer, ovarian cancer, gastric cancer, and lung cancer (270–276), and improved disease outcomes (277). These data suggest that blocking inflammatory pathways, in this case the prostaglandin-related and subsequent downstream pathways (278), can prevent the development of cancer at the population level. Evaluations of whether intervention at different points across the inflammation spectrum can prevent cancer are an interesting area of developing research (279–281) that could yield great impact in the prevention of cancer.

From a secondary prevention perspective, several research groups have and continue to evaluate the utility of inflammatory biomarkers in the development of risk prediction models. For example, Pine and colleagues (54) observed that 10-year predicted risk for lung cancer was highest among those smokers with elevated CRP and IL8 in the PLCO study. Extensions of these methods and models with other types of inflammatory markers and other cancer sites may help to identify those at greatest risk for developing cancer, and therefore refine the population that would most benefit from increased screening based on their inflammatory profiles.

Therapeutic intervention. Several developing avenues of immune-based therapies, including tumor vaccine approaches, immune-checkpoint inhibitors, and antagonists of immunosuppressive molecules such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1), among others, seem promising in early stages of development and trials (282). This topic is beyond the scope of this review, but it is a rapidly emerging area in cancer therapeutics and early results seem quite promising with cancer immunotherapy heralded as the scientific breakthrough of 2013 (283).

The need for integrative targeted approaches to examine inflammation in cancer

As the inflammation response and its role in carcinogenesis is vastly complex in nature, novel approaches to characterize the roles of inflammatory markers in cancer development and progression to identify elevated risk profiles and subsequent intervention targets are needed. An approach limited to single markers will yield ineffective and fragmented results suboptimal for the reduction of cancer burden. Three overarching principles should be the goal of future research in this area.

First, investigations should aim to be as comprehensive as possible to examine multiple exposures as well as their interactions (Fig. 1). For example, assessing a comprehensive set of biomarkers will provide a better picture and will enable more pathway-based analyses. In addition, evaluating germline variation, epigenetic modification, expression, and protein product levels in a comprehensive pathway-based analytic approach would provide a more resolute image of the relevant associations. This will, however, require substantial funding and access to a diverse set of biospecimens.

Second, the use of existing data platforms, such as large prospective studies and bio-repositories, should be targeted for the evaluation of these integrative hypotheses to be able to better address the issue of causality. This approach will require focusing on analytes that are less sensitive to degradation over time. Integration with lifestyle factors in these data platform would be also important as several of them (per Introduction) are correlated with the inflammatory markers of interest. Mediation analyses or structural equation modeling/path analyses may be necessary to adequately unravel the complex associations of interest.

Finally, etiologic and prognostic associations should be evaluated across cancer sites where possible. As discussed, imbalances in the markers of altered inflammation have been associated differently with cancers at multiple sites, yet it is clear that inflammatory imbalances play a role to some degree across the majority of solid tumor sites. Comprehensively evaluating associations similarly across cancer sites where sample availability permits, for example, in a large cohort setting, dramatically increases the potential benefit of identifying chemopreventive or therapeutic targets. Several initiatives are under way to advance this cross-cancer inflammation hypothesis, yet more research is needed.

Disclosure of Potential Conflicts of Interest

C.M. Ulrich is a consultant/advisory board member for Bayer. No potential conflicts of interest were disclosed by the other authors.

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A Review of the Application of Inflammatory Biomarkers in Epidemiologic Cancer Research

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