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Innate Immunity and Cancer Pathophysiology

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Abstract

Chronic inflammation increases the risk of a number of cancers, including gastric, colon, and hepatic cancers. Conversely, tumors, similar to tissue injury, trigger an inflammatory response coordinated by the innate immune system. Cellular and molecular mediators of inflammation modulate tumor growth both directly and by influencing the adaptive immune response. Depending on the balance of immune cell types and signals within the tumor microenvironment, inflammation can support or restrain the tumor. Adding to the complexity, research from the past two decades has revealed that innate immune cells are highly heterogeneous and plastic, with variable phenotypes depending on tumor type, stage, and treatment.

The field is now on the cusp of being able to harness this wealth of data to a) classify tumors based on their immune makeup, with implications for prognosis, treatment choice, and clinical outcome; and b) design therapeutic strategies that activate anti-tumor immune responses by targeting innate immune cells.

Keywords

inflammation; innate immunity; immuno-oncology; cancer immunology; tumor microenvironment; plasticity

Introduction

Tumor development elicits a host response that resembles inflammation and is similarly coordinated by the innate immune system. Cellular and extracellular components engaged in this process shape tumor growth and progression by modifying the abundance and functions of one another, interacting with cancer cells, and modulating the adaptive immune response. Inflammation is a plastic process and ultimately, whether inflammation promotes or inhibits cancer depends on the balance of a complex and still incompletely understood cellular and molecular circuit [Fig. 1].

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One of the earliest indications of a connection between immunity and cancer was the detection of immune cells in histological sections of tumors (1). Another clue came from clinical practice. In the 19th century, the German physicians Wilhelm Busch and Friedrich Fehleisen independently recognized that patients could experience cancer regression after contracting post-operative infections (2). This observation was further expanded upon by William B. Coley, an American surgeon, who, in the 1890s, began inoculating patients with bacteria, with the idea that the response to the pathogen could clear both the infection and the cancer. Eventually, he settled on “Coley’s toxins”, a formulation comprising heat-killed gram-positive *Streptococcus pyogenes* and gram-negative *Serratia marcescens* bacteria. The therapy had severe side effects, required daily inoculations, and beneficial responses were mostly limited to sarcoma, a rare cancer. However, Coley’s was the first concerted and broadly recognized effort to elicit an immune response against cancer (3, 4).

Early research focused mostly on inducing inflammation to elicit anti-cancer immunity; yet, the possibility that inflammation could also drive cancer was already suggested in 1828 by Jean-Nicolas Marjolin, a French surgeon, who reported the occurrence of squamous cell carcinoma in chronically inflamed wounds (5). In 1863, the German pathologist Rudolf Virchow proposed that cancer originates at sites of chronic inflammation (1). Starting in the 1990s, seminal work using genetically engineered mouse models demonstrated that innate immune cells such as neutrophils, macrophages, and mast cells contribute to cancer progression (6–13).

During the past decade, the clinical success of cancer immunotherapy has sparked renewed interest in the role of innate immunity in cancer. Although most approved strategies are aimed at boosting tumor-specific cytotoxic T cell responses, strategies to engage the innate immune system in anti-cancer responses are being developed. Moreover, sophisticated techniques to resolve gene expression and proteomic profiles at the single-cell level have allowed researchers to classify cancers based on their immune makeup: the type of immune cells present in the tumor, their phenotypes, and their location within the microenvironment. These studies have also highlighted the plasticity of innate immune cells, *i.e.*, how their phenotype and function change in response to the microenvironment. Along with its genotype, the cancer immune makeup is refining our capacity to predict clinical outcome. Here, we review the contrasting roles of innate immunity in cancer and discuss the clinical application of these findings.

1. Inflammation and cancer are highly interconnected

Inflammation is a biological reaction the body mounts in response to infections, wounds, and chemical exposure, to restore homeostasis and prevent loss of tissue function (14, 15). The cells and molecules responsible for triggering and coordinating inflammation make up the innate immune system. Tissue-resident macrophages and mast cells are the first to recognize the insult. They secrete a variety of soluble mediators, cytokines and chemokines, to recruit other innate immune cells to the infection (or injury) site. Neutrophils are the first cells to respond to such signals, becoming activated and killing invading bacteria (16, 17). If this acute inflammatory response is not able to eliminate the insult, macrophages and T cells are next attracted to and activated by increased expression of chemokines, growth

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factors, and cytokines. This process is followed by a resolution and repair phase in which the local release of signals, *e.g.*, resolvins, protectins, and transforming growth factor β (TGF-β), inhibits further neutrophil recruitment (18). Instead, monocytes are attracted to the site, where they differentiate into macrophages, remove dead cells, and initiate tissue repair mechanisms. This orchestrated immune response mediates neutralization of the offending agent. However, when the body is unable to resolve this acute inflammatory response, the result is chronic inflammation (19). In 1986, Howard Dvorak published an essay where he drew a parallel between tumors and "wounds that do not heal" (20). He argued that tumors invoke an inflammatory wound healing response similar to the one described above, creating favorable conditions for survival and growth [Fig. 2].

Epidemiological data support the notion that chronic inflammation drives tumor development. Prospective studies have shown that patients displaying elevated levels of circulating inflammatory markers (*e.g.*, C-reactive protein) at routine checkups have more than twice the risk of developing cancer within one year than those with normal levels (21). A 2018 study estimated that 42% of adult cancers in the United States are caused by modifiable risk factors (22), all of which cause either local or systemic inflammation. For example, cigarette smoking accounts for 19% of cancers, obesity is linked to 7.8%, alcohol intake explains 5.6% of cases, and chronic infections are the cause of 3.3% of cancers in the United States and 13% of cases worldwide (23).

Local inflammation can promote cancer development and progression within the same tissue or organ site (24, 25). Several infections can result in cancer: i) *Helicobacter pylori*-induced gastritis can progress to gastric cancers, ii) chronic hepatitis B or C virus infections can lead to liver cancers, and iii) unresolved infection with human papillomavirus can result in cervical cancers. Besides direct carcinogenic mechanisms associated with the infectious agents, the persistent inflammatory environment resulting from the failure to clear the infection contributes to the development of these cancers (26). Chronic inflammatory diseases in the absence of infections can also forge a local microenvironment that is primed for tumor development. For instance, inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, increase the risk of developing colorectal cancer (27). Similarly, chronic pancreatitis carries an elevated risk of developing pancreatic cancer (28). Lastly, environmental factors can predispose patients to and promote cancer by causing local inflammation. Most notably, exposure to particulate or tobacco smoke has a well-defined relationship with chronic obstructive pulmonary disease development, which increases the risk of lung cancer (29).

In addition to changing the local inflammatory microenvironment, some insults such as tobacco smoke and obesity drive systemic inflammation. As a result, levels of circulating pro-inflammatory mediators are chronically elevated, leading to an increased risk of developing cancer in several organs (30). For instance, in a diet-induced mouse model of obesity, high levels of serum interleukin (IL)-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) cause lung inflammation and subsequent metastasis to this site (31). Consistent with inflammation's pro-tumorigenic role, long-term treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with lower cancer incidence (32), including a notable decline in the incidence of lung cancer in chronic smokers (33).

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Moreover, a phase III trial (CANTOS; NCT01327846) of an inhibitory antibody targeting the pro-inflammatory molecule IL-1β for atherosclerosis also found that it significantly reduced lung cancer incidence (34), while a subsequent trial testing the anti-IL-1β antibody together with chemotherapy in established lung cancer found no effect (35). However, not all chronic inflammatory diseases increase the risk of cancer. For instance, psoriasis and rheumatoid arthritis do not appear to promote cancer, although it is possible that the drugs used to keep these inflammatory diseases in check also modify cancer risk.

Cancers not directly associated with inflammation still recruit innate immune cells, release cytokines, and exhibit angiogenesis and tissue remodeling—essentially driving the establishment of "tumor-intrinsic" inflammation (36) [Fig. 3a]. NSAIDs can also reduce mortality from some of these cancer types, *e.g.*, prostate and brain cancers (32, 37).

Drivers of tumor-intrinsic inflammation include genetic and epigenetic alterations in tumor-suppressor genes and oncogenes. The tumor-suppressor *TP53* is a good example. In several cancer models and in clinical studies, immune cells are attracted to the primary tumor in response to P53 loss. For instance, in prostate cancer, loss of P53 triggers C-X-C chemokine motif ligand 17 (CXCL17) upregulation and the subsequent recruitment of immunosuppressive innate immune cells in the tumor microenvironment (TME) (38). Beyond this local effect, P53 loss in breast cancer stimulates tumor-associated macrophages (TAMs) to release high levels of IL-1β, driving systemic inflammation and ultimately supporting metastasis (39).

Another important cause of tumor-intrinsic inflammation is necrotic cell death, occurring when, *e.g.*, rapidly growing tumors outpace the blood supply, resulting in hypoxia and necrosis. Necrotic cells release potent danger-associated molecular patterns (DAMPs), endogenous "danger signals" recognized by innate immune system cells via germline-encoded pattern recognition receptors (PRRs), *e.g.*, Toll-like receptors (TLRs) (40). DAMP sensing increases phagocytosis of the necrotic debris and amplifies the inflammatory response so antigen-presenting cells (*e.g.*, dendritic cells [DCs] and macrophages) can activate the adaptive immune response. High mobility group box 1 (HMGB1) is a nuclear non-histone-binding protein that serves as a DAMP by signaling through TLR3, -4, and -9, as well as the scavenger receptor RAGE (receptor for advanced glycation end products) [Fig. 3a]. HMGB1 binding to its receptors ultimately leads to inflammatory cell recruitment and induces the release of pro-inflammatory cytokines (41, 42). DAMPs are also important in eliciting the antigen-specific adaptive immune response evoked by immunogenic cell death of tumor cells. This is a unique form of cell death defined by its ability to elicit protective immunity and mainly caused by cytotoxic agents like anthracyclines and radiotherapy. Following immunogenic cell death, the chronic exposure of DCs to DAMPs, particularly calreticulin, ATP, and HMGB1, favors DC maturation and priming of protective T cell responses that can kill tumor cells and establish anti-tumor immunological memory (43). Supporting the importance of this mechanism for anti-tumor immunity, germline mutations in PRRs affect cancer risk and response to therapy. For example, patients with breast cancer who carry a *TLR4* allele displaying reduced binding to HMGB1 relapse faster after chemotherapy (41) [Fig. 3b].

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In summary, inflammation is a hallmark of cancer, whether it originated before or is driven by the cancer.

2. Innate immune cells in the tumor are heterogeneous and plastic

The innate immune system comprises immune cells of either myeloid or lymphoid lineage, and several classes of proteins, including cytokines, chemokines, receptors, and proteins of the complement system. These components are not inherently tumor-supportive or tumor-opposing. Rather, their activity depends on the relative abundance of each cell type in the specific tissue, the balance of signals within the TME, and the tumor progression stage [Fig. 1]. For instance, lung cancer cells engineered to express a strong antigen are rejected by cytotoxic T cells in the lung, but expression of the same antigen and a very similar genetic makeup in pancreatic cancer cells instead exacerbates the disease, as this site has fewer DCs capable of activating T cells (44). In addition, the phenotype and function of innate immune cells are plastic and change as their local environment changes. We present key findings that illustrate this context-dependence of innate immune cell types and how it affects cancer.

2.1 Myeloid cells modulate tumor-associated inflammation—

Myeloid cells comprise heterogeneous cell populations derived from a common myeloid progenitor in the bone marrow. These cells are recruited to the tumor and can regulate the tumorigenic process, from initiation to invasion and metastasis. The most well-studied myeloid cells are macrophages/monocytes, neutrophils, myeloid-derived suppressor cells (MDSCs), and DCs.

2.1.1 Macrophages and monocytes: Macrophages are large phagocytic cells critical for host defense, especially against bacteria, but are also necessary for tissue homeostasis, *e.g.*, by clearing cell debris and dysfunctional cells. Tissue-resident macrophages originate from cells seeded to tissues during embryogenesis, but macrophages can also expand from bone marrow-derived blood monocytes, which are recruited in large numbers in response to injury or infection (45).

Macrophages and their monocytic precursors constitute the largest fraction of leukocytes in most solid tumors and are critical drivers of cancer-associated inflammation (46, 47). Activated inflammatory macrophages produce potentially mutagenic reactive nitrogen species (RNS) and reactive oxygen species (ROS) and secrete cytokines, including tumor necrosis factor α (TNF-α) and interleukins (*e.g.*, IL-1β, IL-6, IL-12), providing fertile soil for initiation and progression of chronic inflammation-associated cancers. In a model of colitis-associated cancer, constitutive genetic inactivation of the canonical nuclear factor κB (NF-κB) pathway, a master regulatory pathway of inflammation, specifically in myeloid cells (macrophages and neutrophils), results in downregulated inflammatory cytokine secretion and reduced tumor incidence (12). Furthermore, when stimulated by interferon-γ (IFN-γ) and TLR ligands, macrophages can directly kill tumor cells by generating nitric oxide (NO) (48). However, as the tumor progresses, cues in the microenvironment drive TAMs to become tumor-supportive. Early findings showed that this phenotypic shift could occur in response to IL-4/IL-13 derived from *e.g.*, T lymphocytes (49). Yet, other cytokines (*e.g.*, IL-10 and TGF-β), tumor cell-derived metabolic products, hypoxia, and immune complexes can also induce pro-tumorigenic polarization of TAMs (50–52).

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Much of the early literature on macrophages categorized anti-tumorigenic macrophages as classically activated/M1 and pro-tumorigenic macrophages as alternatively activated/M2. This dichotomy reconciled the antithetical roles that macrophages display in cancer, but it is now clear that a spectrum of phenotypes exists beyond the two extremes. Accordingly, the field has moved away from the binary nomenclature, favoring a more precise definition of populations based on how the cells are isolated and which markers define them (53). In 2018, high-dimensional profiling techniques like single-cell RNA sequencing and mass cytometry have allowed us to granularly characterize the heterogeneous macrophage populations in tumors and map their plastic evolution during disease progression or treatment (54).

TAMs' ability to directly sustain tumor progression has been widely documented and reviewed [*e.g.*, (47)]. In brief, TAMs are key players driving the acquisition of a tumor vasculature, the so-called "angiogenetic switch". $Tie2^+$ monocyte-derived macrophages are an essential source of vascular endothelial growth factor (VEGF) and support angiogenesis in several mouse models (13). In addition, intravital imaging of mammary tumors showed that perivascular TAMs aid cancer cells in entering blood vessels (55). But TAMs can also promote invasion by remodeling the extracellular matrix (ECM) through expression of proteases such as matrix metalloproteinases (MMPs) and by promoting the epithelial-mesenchymal transition of cancer cells (9, 56). At the metastatic site, macrophages can aid cancer cells to adapt to the new environment. For instance, in the lungs, α4 integrin on macrophages can serve as a receptor for vascular cell adhesion molecule 1 (VCAM-1) expressed on breast cancer cells and can activate pro-survival signaling (57). In addition, both monocytes (or monocytic MDSCs, see below) and macrophages reinforce the immunosuppressive TME by secreting cytokines, *e.g.*, IL-10 and TGF-β, which inhibit the anti-tumor immune response (58).

A clinically relevant aspect of macrophage biology is its profound effect on treatment outcome. Depending on the treatment and their phenotype, TAMs can either contribute to or interfere with the therapeutic mechanism (59). For instance, in mice, macrophages capture therapeutic anti-programmed cell death protein 1 (PD-1) monoclonal antibodies (mAbs) from the surface of T cells, the intended target, thus blunting therapeutic efficacy (60). However, PD-1 is also expressed on TAMs, and PD-1 blockade in *NOD scid gamma* mice, which lack T, B, and natural killer (NK) cells, leads to a TAM-dependent decrease in tumor burden, suggesting that TAMs can be pharmacologically re-educated (61) and again highlighting the plasticity of these innate immune cells. Indeed, a colony stimulating factor 1 receptor (CSF-1R) inhibitor causes tumor regression by re-polarizing macrophages rather than simply depleting them in a mouse model of glioma (62). This inherent plasticity of macrophages represents both an obstacle in trying to untangle their complex biology and an opportunity for therapeutic targeting.

2.1.2 Neutrophils: Neutrophils are some of the first immune cells to be recruited to damaged tissues. They can eliminate pathogens by phagocytosis, by the release of antibacterial proteins and proteases, and by the formation of neutrophil extracellular traps (NETs) (63). Granulocyte-colony stimulating factor (G-CSF) and other cytokines that promote the differentiation and release of neutrophils from the bone marrow are

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often elevated locally in the tumor and systemically in patients with cancer, leading to the mobilization of high numbers of both mature and immature neutrophils (39). High levels of tumor-associated neutrophils and neutrophils in blood (neutrophilia) and high neutrophil-to-lymphocyte ratios are associated with poor prognosis in cancer (46, 64, 65). Like macrophages, neutrophils also have pro- or anti-tumor activities (66), depending on context. Yet, the stimuli leading to these opposite activities are much less defined than they are for macrophages.

Tumor-associated neutrophils can support cancer cell proliferation, angiogenesis, and immunosuppression in the TME (67). However, by harnessing their plasticity, they can become anti-tumor through TGF-β blockade (68). Similarly, IFN-β suppresses genes encoding homing and angiogenic factors in neutrophils, delaying tumor growth in melanoma and fibrosarcoma mouse models (69).

Early research showed that neutrophils from tumor-bearing animals could increase the invasive potential of cancer cells (70). For example, UV-damaged keratinocytes release HMGB1, resulting in a neutrophilic skin inflammatory response. In turn, neutrophil-derived TNF-α increases melanoma cells' migration along blood vessels and consequently lung metastasis (71). Besides aiding cancer cells to escape the primary tumor, neutrophils can assist cancer cells in leaving blood vessels, *e.g.,* by tethering cancer cells to liver sinusoids (72). During early stages of tumor progression, neutrophils are mobilized and accumulate at metastatic sites, where they help establish a premetastatic niche before cancer cells infiltrate (73–75). In the lungs of mouse mammary tumor virus–polyoma middle T antigen (MMTV-PyMT) tumor-bearing mice, neutrophil-derived leukotrienes support the preferential expansion of a highly metastatic subpopulation of cancer cells, and targeting this mechanism is sufficient to decrease metastasis (76). Lastly, at both the primary and metastatic sites, neutrophils (or the possible overlapping cell population sometimes referred to as polymorphonuclear MDSCs, see below) can drive immunosuppression (77). Contrasting these findings, in experimental metastasis models, neutrophils activated by inflammatory stimuli inhibit liver metastasis by releasing cytotoxic NO, and thrombospondin-1 released by neutrophils in the lung establishes a metastasis-resistant niche (78, 79).

In the last decade, neutrophil-expelled NETs were found to promote metastasis. NETs are extracellular networks comprising chromatin and granule-derived antimicrobial peptides and proteases, such as neutrophil elastase, cathepsin G, myeloperoxidase, and MMP9. NETs were first described as contributors to the innate immune response, with the ability to immobilize and eliminate large pathogens that could not be engulfed by phagocytosis (80). However, since 2013, NETs in the TME have been found to be associated with tumor progression both in animal models of cancer and in patients with cancer, including breast, ovarian, colorectal, or lung cancer (81–83). NETs can promote metastasis through multiple means. Our group and others reported that NETs can be chemotactic for metastatic cancer cells (82, 84). NET-associated DNA was later shown to serve as a chemotactic factor for cancer cells expressing the transmembrane protein CCDC25, which upon sensing DNA, enhances cell motility and facilitates metastasis to the liver (85). Besides increasing cancer cell migration/invasion, NETs can also trap cancer cells in the vasculature and facilitate extravasation (81). The presence of an early-stage ovarian tumor in the abdominal

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cavity induces neutrophils to accumulate in the omentum and release NETs in response to cell-derived factors (IL-8, G-CSF, CXCL1, and CXCL2), promoting the formation of a favorable premetastatic niche (83). Furthermore, we have shown that in the lungs, NET-associated proteases can induce cell proliferation through ECM remodeling (86). Specifically, neutrophil elastase and MMP9 cleave the basement membrane protein laminin, producing a cryptic epitope that activates integrin signaling and re-awakens dormant cancer cells. Lastly, NETs may physically shield cancer cells from cytotoxic immune cells (87). Based on these diverse pro-metastatic mechanisms, inhibiting NETs is thus a potential therapeutic strategy. Notably, targeting NETs *in vivo* with DNase I particles or a PAD4 inhibitor reduces metastatic burden in breast and ovarian cancers and inhibits the NET-activated awakening of dormant cancer cells (83, 84, 86).

2.1.3 Immature myeloid cells and myeloid-derived suppressor cells (MDSCs): MDSCs are a heterogeneous population of immature myeloid cells that greatly expand during pathological conditions such as cancer. They are usually divided into two groups based on surface marker expression: monocytic MDSCs (M-MDSCs) and granulocytic or polymorphonuclear MDSCs (PMN-MDSCs). Morphologically and phenotypically, M-MDSCs and PMN-MDSCs are difficult to distinguish from monocytes and neutrophils, respectively, but they are functionally defined by their ability to restrain T cell activities. MDSCs expand in response to stem cell factors and cytokines like GM-CSF, G-CSF, M-CSF, IL-6, and VEGF. However, MDSCs' immunosuppressive activity also requires activation by IL-4, IL-13, or TGF-β (88).

Unlike classical monocytes and neutrophils, MDSCs express high levels of molecules that inhibit T cell responses, including L-arginase, inducible nitric oxide synthase (iNOS), TGF-β, IL-10, cyclooxygenase-2 (COX2), and indoleamine 2, 3-dioxygenase (IDO). L-arginase and IDO are important suppressing factors that catabolize essential metabolites and/or produce toxic metabolites that accumulate in the TME, inhibiting T cell proliferation (89). NO production suppresses T cell function by inhibiting major histocompatibility complex (MHC) class II expression or inducing T cell apoptosis (90). Moreover, MDSCs have a dormant metabolic phenotype characterized by repressed glycolysis, which they can "pass on" to T cells by transferring the metabolite methylglyoxal, ultimately causing metabolic and functional paralysis of activated $CD8^+$ T cells (91). A preclinical study found that depleting MDSCs after primary tumor resection delayed lung metastasis and extended survival, implicating them in metastatic progression as well. In this setting, low-dose adjuvant epigenetic therapy (5-azacytidine and entinostat) decreases MDSC trafficking, reducing metastasis (92). Consistent with the ability of MDSCs to repress T cell function, MDSC level is negatively associated with patient responses to immunotherapy, including to cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-1 inhibition (93, 94). Targeting MDSCs may therefore represent an attractive therapeutic opportunity, but with the caveat that there is currently no clear method to distinguish MDSCs from neutrophils and monocytes—cell types with many complex functions.

2.1.4 Dendritic cells (DCs): DCs are innate immune cells with a crucial role in bridging innate and adaptive immunity. DCs constantly scan their surroundings and detect antigens,

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danger signals, and invading pathogens. Once they take up antigens, they migrate to lymphoid tissues (thymus, spleen, and lymph node), where they present antigens via MHC class I and II complexes to stimulate antigen-reactive effector immune cells, primarily T cells and B cells (95). Traditionally, DCs have been divided into two major populations: conventional (or myeloid) DCs (cDCs) and plasmacytoid DCs (pDCs) (96).

cDCs are professional antigen-presenting cells, further classified as cDC1s and cDC2s, priming $CD8^+$ or $CD4^+$ T cells, respectively. They have the ability to "cross-present", *i.e.*, present tumor-derived MHC-I antigens to $CD8^+$ T cells. In humans, cDC1s express CD141 and BDCA3. In mouse, the development of these cross-presenting DCs depends on the transcription factors *Batf3* and *Irf8*, and tumor growth is increased both in $Batf3^{-/-}$ and $Irf8^{-/-}$ mice (97, 98). In lymphoid tissues, cDC1s are $CD8\alpha^+$, while in the periphery, they are identified by the expression of integrin αE, also known as CD103. Though they are a relatively rare population (often <1% of tumor-infiltrating immune cells), $CD103^+$ DCs play a prominent role in recruiting and activating cytotoxic T cells. As antigen-presenting cells, cDC1s influence the breadth of the immune response, *i.e.*, how many antigens are targeted. Adoptive cellular therapy with T cells expressing FMS-like tyrosine kinase 3 ligand (FLT3L), a DC growth factor, leads to cDC1-dependent expansion of the anti-tumor T cell repertoire (99). In a model of melanoma, $CD103^+$ DCs were shown to be necessary for recruiting effector T cells intratumorally through CXCL9/10 expression (100). Accordingly, ablating $CD103^+$ DCs thwarts tumor rejection after adoptive transfer of activated tumor-specific T cells in combination with immune checkpoint blockade (98, 101). cDC1s also influence chemotherapy response. For example, in the MMTV-PyMT breast cancer model, macrophages inhibit the secretion of IL-12 by $CD103^+$ DCs and ablating macrophages restores DCs' IL-12 production, increases T cell influx, and improves outcome (58). Exposure to chronic stress can inhibit the immunostimulatory activity of DCs, compromising the response to lung and colon cancer in mice. Stress causes elevated glucocorticoid levels, which repress the response to the important pro-inflammatory type I IFN cytokines in tumor-infiltrating DCs and curtail the immune response (102).

In contrast to cDCs, pDCs have limited antigen-presentating ability but are immunomodulatory via *e.g.*, the production of type I IFNs (103). pDCs are largely immunoinhibitory in cancer, and pDC recruitment is associated with poor prognosis in several tumors, including ovarian and breast cancers (104, 105). However, $OX40^+$ pDCs from patients with head and neck cancer can stimulate tumor-specific T cell responses (106). Dysfunction of pDCs or their acquisition of immunosuppressive properties are a result of *e.g.*, tumor-derived IL-10, TGF-β, and TNF-α, which directly suppress pDCs' IFN-α production (107, 108). In addition, by expressing IDO and inducible T cell costimulator ligand (ICOSL), pDCs support tolerogenic regulatory T cell expansion (109, 110).

DCs can tune the response between immune control or immune tolerance, whereby the tumor is recognized but not attacked. For instance, in inflammatory conditions, cDC2s activate anti-tumor $CD4^+$ T cells, whereas in non-inflamed lymph nodes, they induce ineffective priming of $CD4^+$ cells and a tolerogenic response (111). Single cell RNA sequencing (scRNA-Seq) of murine and human lung cancers revealed that upon antigen uptake, cDCs in the TME activate a transcriptional program that limits their

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immunostimulatory function and T cell priming. IL-4 signaling drives this program and IL-4 blockade abolishes its effects, supporting the notion that the cytokine milieu serves as a rheostat for DC activation and determines the outcome of the immune response (112).

2.2 Lymphoid cells and lymphocytes straddle immunomodulation and effector functions—Innate lymphoid cells and unconventional T lymphocytes [γδ T cells and natural killer T [NKT] cells) share attributes of innate and adaptive immunity. Besides contributing to cytokine secretion in the TME, they display effector functions, such as cytotoxic killing of cancer cells.

2.2.1 Innate lymphoid cells (ILCs): ILCs have a lymphoid progenitor in common with T cells. However, they lack antigen-specific receptors, and their cytotoxic activity is instead regulated by soluble ligands that bind either activating or inhibitory surface receptors [Fig. 3]. Based on their cytokine-production pattern and the transcription factors required for their development, ILCs are categorized into five major groups: natural killer (NK) cells, group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), group 3 ILCs (ILC3s), and lymphoid tissue-inducer cells (113, 114).

Like other immune cells, tissue type and cytokine milieu strongly influence whether ILCs are pro- or anti-tumor. For instance, administering IL-33 to 4T1 breast cancer-bearing mice results in accelerated tumor progression mediated by the accumulation of MDSCs and IL-13 producing ILC2 (115). In contrast, in pancreatic adenocarcinoma, IL-33-dependent expansion of ILC2s leads to therapeutic tumor immunity by recruiting $CD103^+$ DCs and activating $CD8^+$ T cells (116). IL-33 also triggers ILC1-dependent anti-tumor activity. In a mouse model of metastatic melanoma, IL-33 triggers ILC1 expansion and IL-5 upregulation in the lung, which in turn suppress lung metastasis via a mechanism that depends on eosinophil recruitment (117). ILC1s can also exert immune surveillance of early-stage tumors. In the MMTV-PyMT model of breast cancer, tumor initiation triggers an IL-15-dependent expansion of tissue-resident ILC1-like cells with cytotoxic activity against cancer (118). However, an ILC1-like phenotype can also curb anti-tumor immunity. For example, owing to the plasticity of NK cells, TGF-β can convert them into ILC1s, resulting in a decreased ability to restrain tumor growth (119, 120). Contrasting roles have been reported for ILC3s as well. In a murine model of melanoma, expression of C-C motif chemokine ligand 21 (CCL21) recruits C-C chemokine receptor 7^+ ($CCR7^+$) ILC3s with lymphoid tissue-inducing ability, which promotes lymphoid stroma formation and the establishment of an immunosuppressive, pro-tumorigenic milieu (121). In contrast, ILC3s from human lung cancer specimens can instead be polarized toward an inflammatory phenotype, which correlates with better clinical outcome. Interaction with tumor cells or tumor-associated fibroblasts triggers these ILC3s to produce inflammatory (TNF-α, IL-22) and chemotactic (IL-8, IL-2) cytokines, causing activation of the endothelium and potentially recruiting anti-tumor leukocytes (122).

2.2.1.1 NK cells: The role of NK cells in cancer was recognized decades before that of the other ILCs, when in 1980, Talmadge et al. showed that tumor cells transplanted to beige mice, deficient in NK cell activity, grew faster than in control mice (123). In humans, the presence of NK cell infiltrate or overexpression of NK-activating ligands by cancer

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cells correlates with better prognosis (124). In contrast, NK cell dysfunction, measured by flow cytometry analysis of cell-surface receptor expression and functional assays, predicts metastatic progression (125, 126).

NK cells can directly eliminate tumor cells by releasing cytotoxic granules or engaging death receptors (127). MHC-I molecules, expressed by most cells, serve as ligands for inhibitory receptors on NK cells, protecting healthy cells from being targeted. Cancer cells often lose MHC-I, especially when subjected to selective pressure by cytotoxic T cells, which rely on MHC-I to recognize their target. In such cases, the ability of NK cells to kill MHC-I-negative cells is an important fail-safe mechanism against immune escape (128). However, MHC-I downregulation is not the only signal that targets cancer cells for NK killing. Cancer cells also present ligands for NK cell-activating receptors (129). For instance, activating the DNA damage response in transformed cells leads to increased expression of ligands for the NK cell-activating receptor natural killer group 2D (NKG2D) (130). Genetic models lacking NK cell-activating receptors display impaired tumor immunosurveillance, supporting their important role in making cancer cells susceptible to NK killing. For instance, crossing NKG2D-deficient mice with a transgenic model of prostate cancer or a transgenic model of B cell lymphoma leads to the development of highly malignant, early arising cancer (131). Similarly, genetically ablating the natural cytotoxicity receptor NKp46 increases lung metastasis following transplantation of B16F10.9 melanoma or Lewis lung carcinoma cells (132). Under selective pressure, cancer cells can lose NK-activating ligand expression, leading to immune escape [*e.g.*, (131, 133)].

Besides directly killing tumor cells, NK cells also amplify the anti-tumor immune response by secreting FLT3L, CCL5, and X-C motif chemokine ligand 1 (XCL1/2), which recruit cDC1s to the TME (134, 135). Activating cDC1s, in turn, can potentiate the NK cell response to the tumor. For instance, in the lungs of tumor-bearing mice, cDC1-derived IL-12 suppresses metastasis via an NK cell- and IFN-γ-dependent mechanism (136).

Similar to cytotoxic T cells, activated NK cells are also susceptible to so-called functional exhaustion, a state of decreased effector function. The activating cytokine IL-15 induces expression of the intracellular inhibitory molecule cytokine-inducible SH2-containing protein (CIS), which ultimately renders NK cells unresponsive to IL-15 in a negative feedback loop (137). In preclinical mouse models of metastasis, lung NK cells are activated by IL-12 but are also induced to upregulate checkpoint inhibitory receptors, *e.g.*, PD-1, lymphocyte activating 3 (Lag-3), and T cell immunoreceptor with Ig and ITIM domains (TIGIT) (138). TIGIT expression was detected on tumor-infiltrating NK cells from patients with colon cancer and in several mouse models of cancer and was associated with dysfunction and reduced anti-tumor potential of these cells (139). In addition, NK cell activity is also restricted by modulated interleukin signaling. For instance, IL-1R8 negatively regulates ILR and TLR downstream signaling. IL-1R8-deficient mice display enhanced NK cell maturation and effector functions and are protected from liver cancer development and metastasis in the liver and lungs (140).

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2.2.2 Unconventional T cells

2.2.2.1 γδ T cells: γδ T cells comprise 0.5–5% of all circulating T cells in healthy individuals and are most abundant in the gut mucosa. Instead of the classical T cell receptor (TCR) with α and β chains, these cells have a distinct TCR consisting of γ and δ chains. This TCR confers non-MHC-restricted antigen recognition [Fig. 3c]. The presence of intratumoral γδ T cells is associated with a favorable prognosis across many cancer types (46). Accordingly, *in vitro* studies have shown that γδ T cells are able to kill cancer cells via a) the granzyme-perforin pathway (141); b) expression of the death receptor ligand TNF-related apoptosis-inducing ligand (TRAIL) (142); and c) TNF-α and IFN-γ secretion (143). γδ T cells also induce enhanced cytotoxic activation of NK cells by engaging the co-stimulatory molecule 4–1BB (144). However, like other innate immune cells, γδ T cells are plastic and can acquire a pro-tumorigenic phenotype in response to soluble cues. Stimulation with IL-1β, IL-6, and IL-23 induces γδ T cell enrichment, producing the major immunosuppressive cytokine IL-17, with effects ranging from angiogenesis to immune escape (77, 145, 146). In pancreatic cancer, intratumoral γδ T cells express high levels of exhaustion-inducing ligands, leading to a curtailed adaptive response (146). In a mouse model of breast cancer, γδ T cell-secreted IL-17 and IL-1β stimulate neutrophil expansion and polarization (77). These neutrophils suppress $CD8^+$ T cell function, which in turn facilitates the establishment of metastasis.

2.2.2.2 Natural Killer T (NKT) cells: NKT cells are a small subset of T lymphocytes that recognize lipid antigens presented on the non-polymorphic MHC-I-like molecule CD1d. This family is subdivided into Type I NKT cells or iNKT cells, presenting a semi-invariant TCR, and Type II NKT cells, with a variable TCR [Fig. 3c]. Most studies have found that type I and type II NKT cells play contrasting roles in cancer immunity, with the former promoting and the latter suppressing anti-tumor responses.

Much like NK cells, iNKT cells can directly kill cancer cells through non-antigen-specific cytotoxic mechanisms (147). iNKT cells can also target other cells expressing CD1d in the TME, including TAMs (148). In addition, iNKT cells support the efficient cross-priming of cytotoxic T cells by inducing DC maturation. For instance, iNKT stimulation with α-galactosylceramide, a high-affinity ligand for CD1d, leads to the upregulation of co-stimulatory molecules on DCs via CD40-CD40L interaction (149). DCs cross-primed by iNKT cells uniquely recruit T cells via the CCL17-CCR4 axis, potentiating anti-tumor immunity (150). Lastly, iNKT cell activation directly boosts cytotoxic cells, as exhausted NK cells and T cells can be rescued via iNKT cell-dependent production of IL-21, IL-12, and IL-2 (151). Conversely, an elegant genetic approach comparing CD1d-deficient mice (lacking all NKT cells) to $J\alpha18^{-/-}$ mice (deficient in iNKT cells only) showed that type II NKT cells suppress anti-tumor immunity in several mouse models (152). In fact, type I and type II NKT cells can be mutually antagonistic: when stimulated by specific ligands, type II NKT cells suppress the proliferation and cytokine production of type I NKT cells (153).

2.3 The complement system bridges innate and adaptive immunity—The complement system is a central part of the humoral arm of innate immunity. It comprises more than 50 plasma proteins, regulators, and receptors that serve as a first defense

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against pathogens and unwanted host molecules, besides mediating the effects of antibodies involved in a variety of activities, from regulating cytotoxicity to adaptive immunity and tissue homeostasis (154, 155). The complement system is activated by three major pathways (the classical, alternative, and lectin pathways) that converge into the cleavage of C3 into C3b. C3b binds to the surface of cells and marks them for phagocytosis by macrophages or neutrophils. After C3 is activated, C5 is cleaved and initiates assembly of the membrane attack complex, which accumulates on cell membranes and induces cell lysis. Furthermore, the cleavage products of C3 and C5—C3a and C5a—are chemokines with important inflammatory and chemoattractant functions [Fig. 3d].

The complement system's activation can induce tumor cell lysis and phagocytosis by immune cells. In fact, the complement system mediates tumor cytolysis induced by rituximab, a chimeric anti-CD20 mAb developed to treat B cell lymphomas (156). Chemotherapy-induced immunogenic cell death (in breast cancer) activates signaling through the complement system, leading to a switch in B cell phenotype, which ultimately boosts anti-tumor immunity by increasing the ratio of effector T cells to regulatory T cells (157). However, in some settings, components of the complement system impair anti-tumor immunity instead. C5a generation suppresses $CD8^+$ T cell responses by recruiting regulatory T cells and MDSCs and producing immunosuppressive molecules, *e.g.*, L-arginase, IL-10, IL-6, CTLA-4, and PD-L1 (158, 159). Furthermore, C3a and/or C5a change the TME by recruiting TAMs, decreasing NK cell infiltration, and promoting NET formation (160–162). Another complement factor, iC3b, has been shown to promote IL-10 and TGF-β2 expression and MDSC generation (163, 164) to induce immunosuppression. Lastly, genetically or pharmacologically ablating the long pentraxin 3 (PTX3), which restrains complement activation, results in increased susceptibility to mesenchymal or epithelial carcinogenesis due to unleashing macrophage-mediated inflammation (165).

3. The TME modulates innate immunity

Besides cancer cells and immune cells in the TME, innate immune cell plasticity can be triggered by other cellular and non-cellular components, including the ECM, fibroblasts, and the microbiome.

3.1 Extracellular matrix (ECM)—

The ECM is a three-dimensional scaffold of extracellular macromolecules, proteins, and polysaccharides that provides structural and biochemical support to cells. Several cell types, including fibroblasts, immune cells, and cancer cells, cooperate to produce, assemble, and modify the ECM.

Tumor progression is often accompanied by the deposition of a tumor-specific ECM, a typically stiffer and more fibrotic matrix characterized by higher levels of remodeled and cross-linked proteins (166) than regular ECM. The tumor-specific ECM can augment many hallmarks of cancer, such as resistance to cell death (167), induction of angiogenesis (168), and metastasis (169). In addition, the ECM modulates immune cell activation, polarization, and survival (170). A first layer of regulation comes from the physical and mechanical properties of the ECM. In human breast cancers, more aggressive subtypes display stiffer ECM and higher TGF-β, coinciding with higher macrophage infiltration,

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especially at the invasive front (171). Moreover, ECM stiffness and density regulate several immunoregulatory genes in macrophages. For example, when macrophage-like RAW 264.7 cells are cultured on high-density collagen matrix, their ability to inhibit T cell proliferation increases, while their ability to attract cytotoxic $CD8^+$ T cells decreases (172). As a second layer of regulation, ECM components can be functional ligands of receptors on innate immune cells. As do many human tumors, Lewis lung carcinoma cells overexpress the matrix proteoglycan versican, which signals through TLR2 and stimulates macrophages to produce TNF-α, ultimately sustaining metastatic spread in mice (173). Collagen activates the immune-inhibitory receptor leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1), and overexpression of collagen by tumor cells impairs NK cell cytotoxic activity through LAIR-1 signaling (174).

Matrix remodeling is another layer of the ECM-mediated regulation of innate immune cells. Enzymes such as MMPs, members of a disintegrin and metalloproteinase (ADAM), and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) families cleave ECM proteins, producing peptide fragments, called matrikines, with immunomodulatory activity. For example, versikine, derived from versican proteolysis, enhances the differentiation of cross-presenting cDC1s from the bone marrow-derived precursor, thus promoting T cell anti-tumor immunity (175).

3.2 Fibroblasts—Within the TME, fibroblasts are a major producer of ECM and soluble factors. Normal fibroblasts can suppress tumor initiation and progression via direct cell-cell contact, secreting soluble factors and maintaining ECM integrity. However, with tumor progression, fibroblasts' tumor-suppressive functions are lost. When normal fibroblasts turn into cancer-associated fibroblasts (CAFs) it triggers a range of tumor-supporting signals. As reviewed elsewhere, CAFs promote tumor growth by multiple mechanisms (176). One of these is driving an immunosuppressive microenvironment by secreting cytokines and chemokines that regulate the recruitment and functional differentiation of tumor-infiltrating immune cells. For instance, CAF-produced IL-6 and GM-CSF induce monocyte differentiation toward alternatively activated macrophage (M2)-like TAMs and promote metastasis in a colon cancer mouse model (177). Human colorectal cancer-derived CAFs can attract monocytes by secreting IL-8 and subsequently enhance the pro-tumor polarization of macrophages, and these macrophages in turn suppress NK cell cytotoxicity and activation (178). Tumor-derived CSF-1 can repress CAFs' expression of chemokines that attract PMN-MDSCs. Therefore, an unwanted effect of CSF-1R inhibitors, used to block recruitment of macrophages into tumors, is the expression of PMN-MDSC-recruiting cytokines by CAFs, resulting in PMN-MDSC accumulation, and thus reducing the therapeutic benefit of CSF-1R inhibitors (179). Furthermore, CAFs induce infiltration of IDO-producing DCs, $PDL1^+$ neutrophils, and regulatory T cells, while decreasing the infiltration and abrogating the functions of NK cells (180–183). In pancreatic adenocarcinoma, besides inflammatory fibroblasts ($\alpha SMA^{low}IL\text{-}6^{high}$) with a secretory phenotype and myofibroblasts ($FAP^+\alpha SMA^{high}$) that secrete ECM, a novel population of CAFs was shown to express MHC-II and CD74, adding a potential immunomodulatory role as antigen-presenting cells (184). FAP^+ fibroblasts inhibit immunological control of tumors: CXCL12 derived from FAP^+ CAFs can be captured on the surface of cancer cells and mediates the exclusion of

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cytotoxic lymphocytes from the tumor bed (185, 186). Inhibiting CXCR4, the CXCL12 receptor, sensitizes the tumors to checkpoint inhibition and in patients with cancer produces an integrated response, recruiting adaptive and innate immune cells (185, 187).

3.3 Microbiome—As exemplified by Coley's toxin, bacterial components can activate immunity and restrict cancer development. Microbes within a human organism interact with the host at numerous sites, like skin and mucosal surfaces. These microbes are not inert, but regulate innate and adaptive immunity, thus exerting a major influence on health and disease (188). Dysbiosis—a disruption in the homeostasis of microbial communities—has been linked to carcinogenesis. Indeed, large case-control studies have demonstrated that prolonged antibiotic use is an independent risk factor for cancer occurrence (189). Besides antibiotics, eating a high-fat diet also induces changes in the intestinal microbiome, favoring tumor progression in gastrointestinal cancer (190).

Microbiota can promote distinct inflammatory responses, thereby indirectly supporting tumor development. For instance, in a mouse model of colorectal cancer, erosion of the intestinal epithelial barrier favors entry of microbial products into the TME of early-stage lesions. This entry leads to the activation of IL-23-producing myeloid cells and enrichment of tumor-promoting cytokines, including IL-17 and IL-6 (191). Immunoregulatory effects of the intestinal microbiota, however, extend beyond the local environment, since other organs, *e.g.*, the liver, can be exposed to the gut microbiome and its metabolites through the circulation. In mouse models of liver cancer, bile acids metabolized by gram-positive bacteria in the gut circulate back to the liver and downregulate expression of CXCL16 on endothelial cells, inhibiting the CXCL16-mediated recruitment of NKT cells and tumor control (192).

Besides the gut, bacteria and even yeast can reside within the TME of other tissues, *e.g.,* lung and pancreas (145, 193, 194). In a genetically engineered mouse model, lung cancer development is decreased in germ-free or antibiotic-treated mice vs. specific pathogen-free mice (145). At the molecular level, the lung microbiota activates myeloid cells to release IL-1β and IL-23, thereby inducing IL-17 production from γδ T cells and ultimately inflammation and tumor cell proliferation (145). Analogously, the pancreas microbiota generates a tolerogenic environment by inducing an immunosuppressive phenotype in macrophages and monocytes in a TLR signaling-dependent fashion (193).

Aside from contributing to cancer development, pioneering studies in mice revealed that response to immunotherapy in mouse models of melanoma depends on the composition of the intestinal microbiota. Modifying the microbiome, *e.g.,* by administering specific bacteria or performing fecal transplant, re-sensitizes non-responders, leading to increased DC function and enhanced $CD8^+$ T cell priming (195, 196). These findings spurred clinical trials in which patients with previously immunotherapy-refractory melanoma experienced partial and complete tumor regression when the treatment was repeated after fecal transplant from responsive patients (197, 198). Further studies will need to define the distinct mechanisms responsible for the microbiota's effect on anti-tumor immunity, as well as define more scalable treatment options to modulate the microbiome.

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4. Multidimensional approaches to studying innate immune cells in the TME

While the first decades of TME research, including on innate immune cells, relied heavily on mouse genetics and cell biology to dissect the role of single cell types or pathways, recent advances have also been made using system-level approaches. The field has benefitted from -*omics* analyses, which produce large-scale biological datasets capturing *e.g.*, the entire complexity of a sample's mRNAs, proteins, or lipids. Initially focused on cancer cells, these approaches have now been extended to study the complexity of the immune infiltrate: the relative abundance of cell populations, diversity of phenotypes, and activation status. A crucial advance was the development of deconvolution techniques capable of estimating the abundance of different immune cell types in a sample from bulk transcriptomics data (199). An early large-scale analysis applied one such computational tool, CIBERSORT, to data from >27,000 patients across 25 cancers and revealed that intratumoral neutrophils are the leukocyte population most significantly associated with an adverse prognosis (46). Another group used RNA-Seq and flow cytometry to identify immune subtypes of triple negative breast cancer (TNBC) from mouse models and clinical datasets. Focusing on two myeloid compartments, they discovered that tumors with a macrophage-enriched microenvironment respond to checkpoint blockade, especially upon macrophage depletion. In contrast, the absence of immune infiltrate or the local and systemic accumulation of neutrophils correlates with a lack of immunotherapy response (200).

Additional improvements in high-throughput techniques have led to characterizing the TME at single cell resolution: capturing the heterogeneity within one cell type, including innate immune cells. Compared to scRNA-Seq, which informs on thousands of genes per cell, single cell proteomics protocols are not yet quite as powerful. Immune cells have historically been cataloged by extracellular markers; however, techniques like mass cytometry, which can probe tens of protein markers, including intracellular markers, have proven particularly informative in the field of cancer immunology. Integrating these approaches in multi-omics studies that yield single cell-level measurements for both proteins and transcripts promises to further amplify our ability to characterize the TME. Paired scRNA-Seq and mass cytometry of the immune compartment in early-stage lung tumor specimens revealed that cross-presenting $CD141^+$ DCs (cDC1s) and NK cells are already depleted in early lesions compared to normal lungs. Stressing the importance of the single cell resolution, macrophages were as abundant as in normal tissue, but had a more pro-tumorigenic phenotype in tumors, with high expressions of IL-6 and the immune suppression-associated transcription factor peroxisome proliferator-activated receptor γ (PPARγ) and low expression of the co-stimulatory molecule CD86 (201). Later, the same group used scRNA-Seq to discover a cluster of DCs that had gone undetected by bulk methods, named "mature DCs enriched in immunoregulatory molecules" (mregDCs). The transcriptional program associated with mregDCs is activated upon antigen uptake and, depending on IL-4 signaling, can either enhance or inhibit DC immunostimulatory activity, making it an attractive candidate for clinical intervention (112).

Immunophenotyping tumors is further aided by topological techniques that collect complex data on tumor tissue sections, thus preserving spatial information. A major advantage of this analysis is that it can be applied to archival tissues, such that immune phenotype can

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be retrospectively correlated with outcome. For instance, a highly multiplexed version of traditional immunohistochemistry techniques was optimized to probe 12-antibody biomarker panels for lymphoid and myeloid cells on specimens from patients with pancreatic ductal adenocarcinoma. Analyzing the leukocyte infiltrates in intratumoral regions revealed that therapeutic response to neoadjuvant GVAX therapy correlates with a rich myeloid infiltrate, but not with lymphoid infiltrate (202). Methods that further increase multiplexing or dimensionality are also available. A good example is Multiplexed Ion Beam Imaging by Time of Flight (MIBI-TOF), a method that leverages mass spectrometry to image at subcellular resolution large samples (up to 1 mm^2) labeled with isotope-conjugated antibodies. By integrating this technique with sophisticated digital segmentation of the images, one group was able to spatially locate 36 proteins and reconstruct the microenvironmental architecture of TNBC samples. One important takeaway from the study is that tumors with equally abundant immune infiltrates can vary dramatically in the compartmentalization between tumor cells and immune cells (203). A similar conclusion was reached in another TNBC study, where researchers identified four immune subtypes based on the spatial distribution of $CD8^+$ T cells and gene expression profiles from laser capture microdissection of stroma and epithelium. The subtype associated with the poorest outcome had unexpectedly high infiltration of $CD8^+$ T cells, but these were restricted to the stroma and accompanied by elevated levels of IL-17-producing cells and neutrophils (204).

As the field moves forward, we must integrate immune phenotyping obtained from multi-dimensional approaches with the other parameters that guide decision-making in the clinic to improve outcomes. Initiatives such as The Human Tumor Atlas Network, which will link multiparametric analysis of tumor samples with patients' clinical information, promise to greatly accelerate progress (205).

5. Innate immune cells can be targeted for cancer therapy

Immunotherapies boost the immune system's ability to fight diseases like cancer. They include antibody-based approaches, adoptive cellular therapies (including engineered immune cells), cancer vaccines, cytokine therapy, and small molecules targeting signaling pathways in immune cells. Although the approach of activating the immune system traces back to Coley's work more than 100 years ago, it took off in earnest with the FDA approval of immune checkpoint inhibitors targeting CTLA-4 in 2011 (206). The majority of approved immunotherapies activate adaptive immune cells, *i.e.*, B cells and $CD4^+$ and $CD8^+$ T cells, which are excellent targets because of their "memory" function. Nonetheless, innate immune cells can greatly influence adaptive immune responses. This potential is demonstrated by Bacillus Calmette-Guérin (BCG), a vaccine containing an attenuated *Mycobacterium* strain that has been used to treat non-muscle invasive bladder cancer for over 40 years. Intravesical administration of BCG activates innate immune cells through PRR signaling and subsequently leads to potent cytotoxic responses against the tumor (207). Approaches to reprogram, deplete, or reduce the recruitment of innate immune cells with immunosuppressive functions are therefore areas of very active investigation.

5.1 Macrophages: removed or reprogrammed—

One strategy to target macrophages is to block their recruitment to tumors. CCL2 and the cytokine CSF-1 promote

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tumor infiltration of monocytes and their maturation to TAMs. Genetically ablating CCL2 or CSF-1 in mouse models limits tumor progression (11, 208). Several antibodies (carlumab, RG7155, AMG 820) and small molecules (PF-04136309, PLX3397) targeting CCL2 and CSF-1, or their receptors CCR2 and CSF-1R, have been studied in clinical trials. CCL2 inhibition correlates with reduced tumor progression and metastasis in breast cancer mouse models (209). A phase II study of carlumab (CNTO 888; a humanized antibody that binds CCL2) in metastatic castration-resistant prostate cancer did not show anti-tumor activity as a single agent (210). In contrast, the CCR2 small inhibitor PF-04136309 combined with the chemotherapeutic regime FOLFIRINOX resulted in an objective tumor response in 16 of 33 patients with pancreatic cancer (211). However, results of randomized, double-blinded trials targeting CCR2 have not yet been reported. PLX3397, a CSF-1R inhibitor, was tested in phase I and II trials in advanced tenosynovial giant cell tumors, where it was well tolerated, and 12 of 23 patients showed an anti-tumor response after treatment (212). Yet, when PLX3397 was tested in a phase II study in patients with recurrent glioblastoma, it showed no efficacy (213). The antibody RG7155 (emactuzumab) blocks CSF-1R dimerization and showed promising results in a phase I trial: 86% of the 28 patients achieved an objective response and 7% achieved a complete response (214). AMG 820, an anti-CSF-1R antibody, was recently tested in combination with pembrolizumab in patients with colorectal, pancreatic, and non-small cell lung cancer. The combination therapy showed on-target pharmacodynamic effects, such as CSF-1 accumulation in the serum, and had an acceptable safety profile, but no efficient anti-tumor responses were observed (215).

A recent study took advantage of myeloid cells' propensity to home to metastatic niches and genetically manipulated myeloid cells to deliver IL-12. Upon homing to the metastatic niches, these IL-12-genetically engineered myeloid cells (IL-12-GEMys) elicited a strong anti-tumor immune response by activating endogenous T and NK cells and modulating the metastatic TME, resulting in greatly reduced lung and liver metastasis in mice (215a).

Strategies exploiting the plasticity of macrophages—attempting to reprogram or re-polarize them from pro-tumor to anti-tumor—are also being explored (45). Re-polarizing macrophages theoretically has the advantage of leading to the activation of cytotoxic effector immune cells (*e.g.*, NK and T cells) by producing cytokines (including TNF-α, IL-6, and IL-12). The most straightforward method to re-polarize macrophages toward an anti-tumor phenotype is by activating PRRs, like TLRs. Synthetic ligands for diverse PRRs, many initially developed as vaccine adjuvants, are now being tested as cancer immunotherapies (216). The topical administration of the TLR7 ligand imiquimod has anti-tumor activity in superficial basal cell carcinoma and breast cancer skin metastasis (217, 218). The TLR9 ligand IMO-2055 combined with targeted and anti-angiogenic therapy in patients with non-small cell lung cancer showed good tolerability and possible anti-tumor activity (219). Motolimod, a small molecule targeting TLR8, is in clinical trials for advanced cancers (220). We are exploring how the combination of monophosphoryl lipid A (MPLA, a TLR4 agonist) with IFN-γ can reprogram TAMs to upregulate TNF-α, IL-12, and iNOS expression; decrease CD206 expression; and activate T cells in breast and ovarian cancer mouse models. The result is significantly reduced primary tumor growth and metastasis (48).

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COX2 inhibitors, histone deacetylase (HDAC) inhibitors, phosphoinositide 3-kinase γ (PI3Kγ) inhibitors, and stimulator of interferon genes (STING) agonists are also used to reprogram TAMs. COX2 inhibition increases TNF-α, IL-12, and iNOS expression, shifting the macrophages to an anti-tumor phenotype (221, 222). In a phase I clinical trial for the treatment of docetaxel-resistant prostate cancer, patients treated with a COX2 inhibitor (celecoxib) and an epidermal growth factor receptor (EGFR) inhibitor (gefitinib) showed reduced tumor growth and invasion (223). HDAC inhibitors modify the epigenetic profile of monocytes and macrophages, resulting in altered gene expression and polarization toward an anti-tumor phenotype. In a mouse model of breast cancer, the HDAC inhibitor TMP195 induced the recruitment and differentiation of monocytes/macrophages into highly phagocytic and immunostimulatory cells, resulting in reduced tumor burden and metastasis and increased response to chemotherapy and immunotherapy with anti-PD1 antibodies (224). In mouse models of head and neck squamous cell carcinoma and breast cancer, selective inactivation of PI3Kγ in macrophages promoted an immunostimulatory transcriptional program in TAMs and in turn restored $CD8^+$ T cell activation and cytotoxicity, leading to increased survival (225). A PI3Kγ inhibitor IPI-549 (eganelisib) is currently being tested in multiple phase I/II clinical trials (NCT03719326, NCT03980041, NCT03961698, NCT03795610). Finally, targeting STING induced TAM re-polarization *in vitro*; increased IFN-γ, iNOS, and IL-12 production; and led to promising results in mouse models (226). Different STING ligands are currently in clinical trials as sensitizers for multiple immunotherapies (227).

5.2 DCs: boosting presentation of tumor antigens—DC-based therapies utilize patient-derived DCs, generally produced by isolating circulating DCs or monocytes from the patient's blood. Monocytes are then differentiated into monocyte-derived DCs by culturing them with GM-CSF and IL-4, and these DCs are further matured with a cocktail of substances, *e.g.*, IL-6, TNF-α, IL-1β, prostaglandin E2 (PGE2), and polyinosinic:polycytidylic acid [poly(I:C)] (228). Maturation—involving enhanced expression of MHC-I, MHC-II, and co-stimulatory molecules and increased cytokine production—is essential, as incompletely matured DCs can induce tolerance rather than immunity (229). DCs have also been engineered using gene-editing technologies, like RNA interference, viral transduction, and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9, to promote their maturation (230, 231). Sipuleucel-T, a DC-based therapy approved in 2010 for castration-resistant prostate cancer (232), consists of matured and tumor antigen-loaded patient-derived DCs. Patients with melanoma have also been treated with *ex vivo* activated cDCs loaded with the tumor-associated antigens of tyrosinase and glycoprotein 100 (gp100) (233).

Various approaches have also been used to boost cDC mobilization to tumors, *e.g.*, administering FLT3L (234) or modifying DC activities within the TME. The latter approaches include using TLR ligands, such as intra-tumoral injection of TriMix mRNA (three mRNA molecules encoding for CD70, CD40L, and constitutively active TLR4) (235), oncolytic viruses (236), self-replicating IL-12 RNA encapsulated in oncolytic nanoparticles (237), CD40-TLR4 agonists (238), or STING agonists (239). Such approaches have resulted in lasting systemic antigen-specific T cell immunity and tumor regression in many

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preclinical models, including of melanoma, breast carcinoma, colon cancer, and lung cancer (235, 237–239).

5.3 NK cells: a new frontier of cytotoxic immunotherapy—Different approaches have been investigated to engage NK cell function to treat cancer, including immunomodulatory cytokines, mAbs, and adoptive transfer of engineered NK cells. The most prominent cytokines used in NK cell activation are IL-2 and IL-15, both known to induce the expansion and increased cytotoxicity of NK cells (240). IL-2 treatment, the first approved immunotherapy, was approved to treat patients with metastatic renal cell carcinoma and melanoma (241). Moreover, the adoptive transfer of autologous or allogeneic NK cells is also used to improve NK cell tumor surveillance. mAbs have been designed to either block the interaction between the inhibitory NK cell receptor and its corresponding ligand on the cancer cells or engage activating receptors on NK cells. For example, lirilumab targets the inhibitory killer cell immunoglobulin-like (KIR) receptor and is in clinical trials for treating acute myeloid leukemia, multiple myeloma, lymphoma, chronic lymphocytic leukemia, and Hodgkin lymphoma (242). Another inhibitory receptor on NK cells, NKG2A, can be targeted by monalizumab, resulting in both enhanced NK cell activation and T cell function (243, 244). Recombinant reagents that increase the specificity and efficacy of NK cell activation—named bispecific and trispecific killer cell engagers (BiKEs and TriKEs, respectively)—are also in development. BiKEs bind to a surface tumor antigen, *e.g.*, the highly expressed CD30 on Hodgkin lymphoma, and to an NK cell receptor, *e.g.*, CD16, to trigger NK cell-mediated toxicity of the cancer cells (245). A TriKE has been designed to target CD33-positive hematological malignancies: it engages CD33 on the cancer cells; contains a CD16-heavy chain to activate NK cells; and also contains an IL-15 molecule to drive NK cell priming, expansion, and survival. This TriKE is currently in phase I clinical trials (NCT03214666) (246). Recently, NK cells have also been engineered to contain chimeric antigen receptors (CARs), which enables them to be directed against specific targets (247), much like similarly engineered CAR-T cells. Adaptive transfer with CAR-NK cells has shown promising results in preclinical models of B cell lymphoma and is currently being evaluated in a phase I/II study (248).

5.4 Neutrophils: an underrated target—There are currently no approved therapeutics to inhibit neutrophil activity in cancer. However, strategies to limit neutrophils' pro-tumorigenic functions are being tested in early stage trials. These strategies include blocking CXC chemokine receptor 2 (CXCR2), which is critical for neutrophil recruitment (249). The CXCR2 antagonist AZD5069 markedly reduced neutrophils in a phase II clinical trial in patients with asthma and is now being evaluated in patients with advanced tumors (NCT02583477, NCT02499328, NCT03177187). In addition, the small molecule inhibitor reparixin, a non-competitive CXCR1/CXCR2 antagonist, was safe and tolerable in a phase Ib trial for HER-2-negative metastatic breast cancer (NCT02001974) (250). Neutrophils and granulocytic MDSCs can suppress anti-cancer immune responses, in part via arginase-1 activity (251). The combination of arginase-1 inhibitors with various chemotherapies or anti-PD-1 is in phase I and II clinical trials (NCT02903914, NCT03361228, NCT03314935).

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6. Conclusion

Inflammation is an integral part of cancer pathology. Inflammation can favor cancer development, and cancer, in turn, elicits an inflammatory response. In fact, even cancers that are considered *immunologically cold* for lack of an adaptive immune response are often populated by innate immune cells. As described above, targeting the innate immune system therefore offers opportunities to a) prevent cancer development, b) stratify patients for specific treatments, and c) elicit an anti-tumor immune response.

Some chronic inflammatory conditions are well known to favor cancer development, but more research is needed to identify opportunities to interrupt the signaling that supports tumorigenesis and prevent tumor progression. In addition, we are just starting to appreciate how genetic factors [*e.g.*, polymorphisms in immune-related genes (41)] and environmental factors [*e.g.*, diet (31, 190), stress (102)] influence the immune system and contribute to tipping the scale towards tumorigenesis.

Technological advances now allow us to profile the composition and phenotype of the tumor and systemic immune microenvironment. Soon, we should be able to harness this knowledge to better identify patients at high risk of recurrence and to assign patients to the therapy they are most likely to benefit from (39, 200, 202, 204).

From a therapeutic standpoint, targeting inflammatory cells provides an opportunity to influence cancer growth and improve adaptive immune responses. The outcome of the inflammatory response is highly context-dependent. In fact, almost universally, components of the immune system display both pro-tumor and anti-tumor functions. This duality implies that the innate immune system has an inherent degree of plasticity that can be leveraged. More work needs to be done to define which molecular players reprogram inflammatory cells to an anti-tumor phenotype. Moreover, as innate immune cells influence both one another and adaptive immune cells, understanding the hierarchy of signals that can switch an immunosuppressive microenvironment to an inflammatory one can allow us to act on the upstream components.

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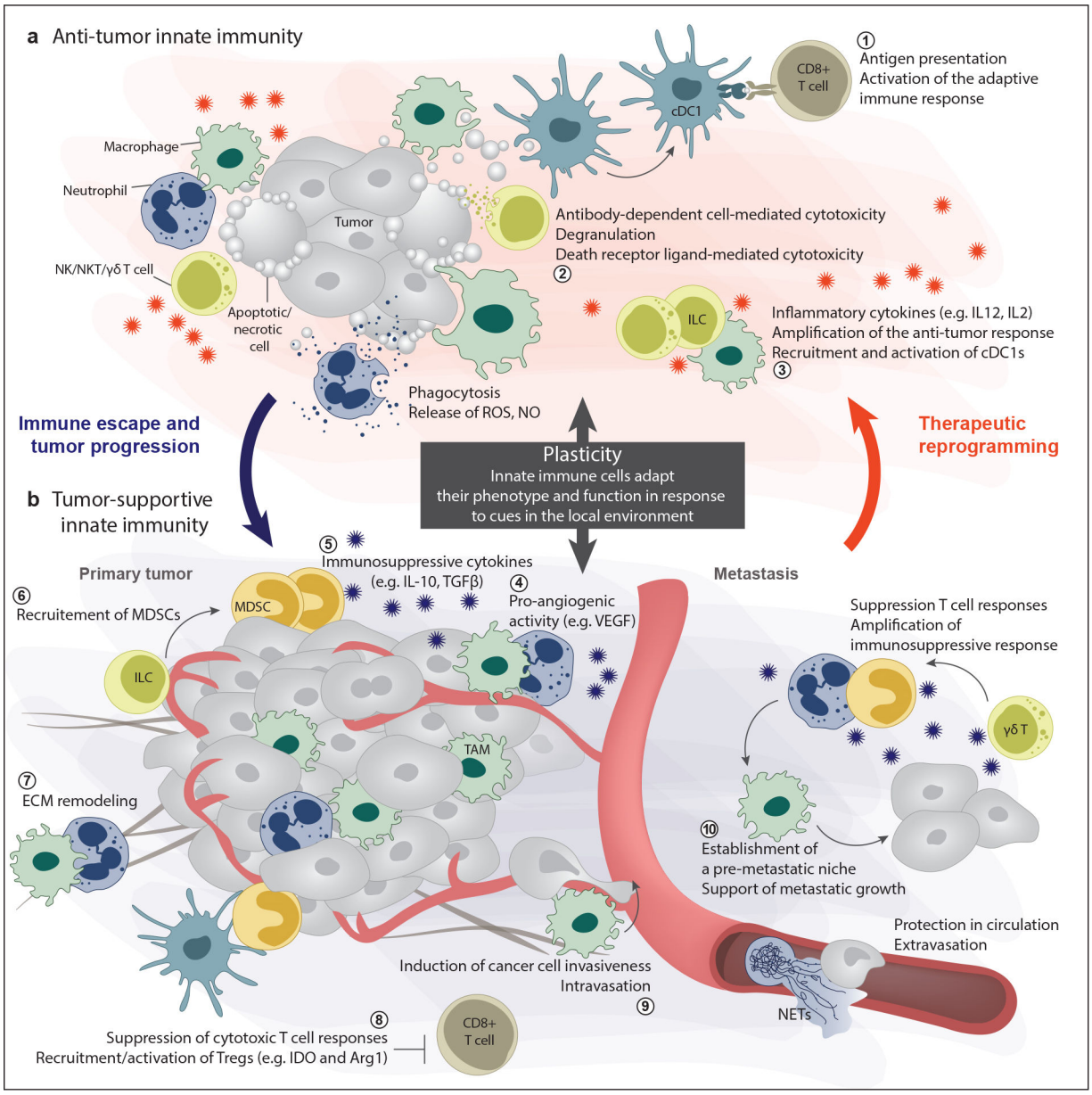

Fig. 1. Plasticity of the innate immune system

The components of the innate immune system are not inherently tumor-supportive or tumor-opposing. Rather, cells of the innate immune system are highly plastic and their phenotype and activity depend on the balance of signals within the tumor. (a) The anti-tumorigenic functions of the innate immune system include 1) antigen presentation and activation of the adaptive response, 2) direct killing of cancer cells, and 3) amplification of the anti-tumor immune response through cytokine secretion. (b) During tumor progression signals from tumor cells and other cells in the microenvironment can polarize innate immune cells towards supporting the tumor, e.g., through 4) angiogenesis, 7) ECM remodeling, 5, 6, 8) immunosuppression, and 9, 10) pro-metastatic activities. Thus, because of plasticity, the innate immune system has tumor-promoting potential. However, plasticity also affords us the opportunity to therapeutically reprogram the innate immune system to fight the tumor.

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Abbreviations: DC, dendritic cell; ECM, extracellular matrix; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; ILC, innate lymphoid cell; MDSC, myeloid-derived suppressor cell; NET, neutrophil extracellular trap; NK, natural killer; NKT, natural killer T; NO, nitric oxide; ROS, reactive oxygen species; TAM, tumor-associated macrophage; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

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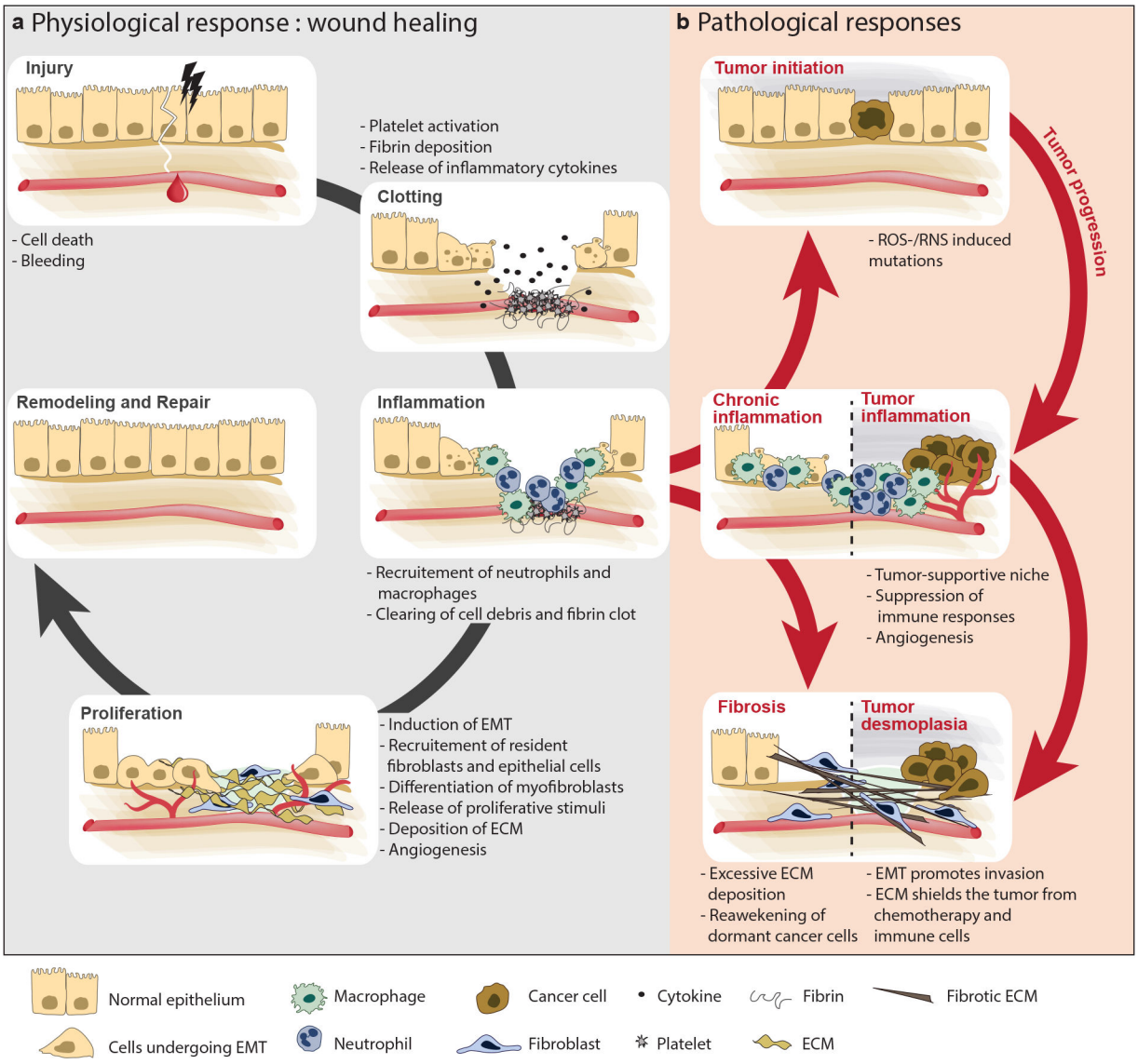

Fig. 2. Aberrant wound healing response, fibrosis, and cancer

Partly owing to the activation of the innate immune system, features of the tumor microenvironment resemble an aberrant wound healing response. Wound healing consists of overlapping phases (left). Injury of adult tissue results in local hemorrhage, immediately followed by clotting. A temporary matrix of fibrin is deposited locally, which serves as a scaffold for migrating immune cells, epithelial cells, fibroblasts, and endothelial cells. During wound healing, neutrophils and macrophages kill bacteria, degrade the fibrin clot, and remove cellular debris. Neutrophils also secrete mediators such as TNF-α, IL-1β, and IL-6, amplifying the innate response. Macrophages produce VEGF and other growth factors, such as TGF-β, that stimulate the next phase: migration and proliferation of cells within the wound. In this phase blood supply is restored, new connective tissue is produced, and the wound re-epithelializes. Lastly, during the repair phase, the extracellular matrix is remodeled and new blood vessels are culled. Failure to clear the rich inflammatory infiltrate results in chronic inflammation (right). The persistence of inflammatory cells results in

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the accumulation of toxic compounds such as reactive oxygen and nitrogen species, as well as cytokines, which support tumor initiation and progression and sustain myofibroblast activation and fibrosis. Inflammation, fibrosis, and cancer are tightly linked in a vicious cycle, in which they can each trigger and aggravate the other.

Abbreviations: ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; IL, interleukin; RNS, reactive nitrogen species; ROS, reactive oxygen species; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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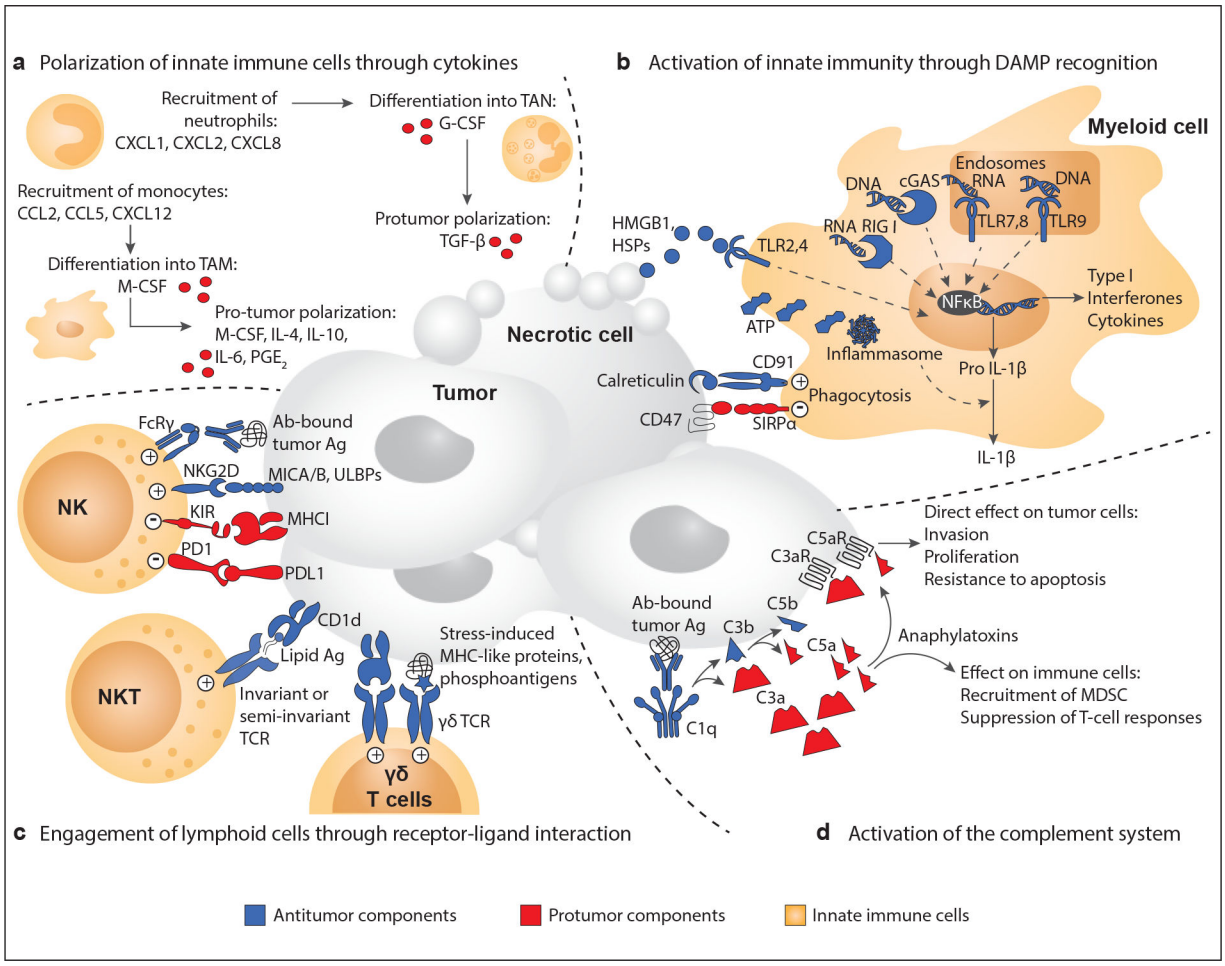

Fig. 3. Interactions between cancer and the innate immune system

Cellular and molecular components of innate immunity interact with cancer cells through a variety of mechanisms that can support or restrain tumor growth. (a) By virtue of the high degree of plasticity of innate immune cells, cytokines secreted in the tumor microenvironment can polarize innate immune cells toward tumor-supportive phenotypes. (b) Necrotic cells release DAMPs, endogenous “danger signals” recognized by pattern recognition receptors (PRRs), *e.g.*, Toll-like receptors (TLRs), on innate immune cells. DAMP sensing increases phagocytosis of the necrotic debris and amplifies the inflammatory response, leading to activation of the adaptive immune response. Opposite mechanisms also exist. For example, the binding of CD47 to SIRPα helps cancer cells escape phagocytosis, by transmitting a “don’t eat me” signal. (c) Innate lymphoid cells (ILCs and NK cells) and unconventional T lymphocytes (γδ T cells and NKT cells) can directly eliminate tumor cells by releasing cytotoxic granules or engaging death receptors. In NK cells, this cytotoxic activity is regulated by cancer cell ligands that bind either activating or inhibitory surface receptors. Unconventional T lymphocytes recognize cancer cells through their TCR. The γδ TCR allows for non-MHC-restricted recognition of *e.g.*, phosphoantigens. The invariant or semi-invariant TCR on NKT cells binds lipid antigens presented on the non-polymorphic MHC-I-like molecule CD1d. (d) The complement system can mediate tumor cell lysis and phagocytosis by immune cells, *e.g.,* by binding anti-cancer antibodies on the

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surface of cancer cells. By contrast, cleavage products of complement activation (C3a and C5a) can support tumor growth, either by directly affecting cancer cells or by recruiting immunosuppressive cells.

Abbreviations: DAMP, danger-associated molecular pattern; G-CSF, granulocyte colony–stimulating factor; HSP, heat shock protein; IL, interleukin; ILC, innate lymphoid cell; M-MDSC, monocytic myeloid-derived suppressor cell; M-CSF, macrophage colony stimulating factor; MHC, major histocompatibility complex; NK, natural killer; NKT, natural killer T; PGE_2, prostaglandin E2; PMN-MDSC, polymorphonuclear myeloid-derived suppressor cell; PRR, pattern recognition receptor; SIRPα, signal-regulatory protein α; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil; TCR, T cell receptor; TLR, Toll-like receptor.