FOCUS: NEW PARADIGMS IN IMMUNE SYSTEM REGULATION

The Suppression and Promotion of Cancer: The Dichotomy of Tumor Immunology

KRISTIN R. LANDIS-PIWOWAR, THOMAS LOSS

LEARNING OBJECTIVES
1. Compare and contrast normal wound healing with invasive tumor growth.
2. Detail the process of immunoediting.
3. Correlate the inflammatory response used in wound healing with that seen in tumor tissue.
4. Explain the role of myeloid-derived suppressor cells in cancer.
5. Identify the inflammatory molecules that promote cancer survival, genomic instability, and metastasis.

ABSTRACT
A disruption in tissue homeostasis requires repair mechanisms that commence with acute inflammation. While this type of response is beneficial to the host tissue in short duration, as is the case with wound healing, numerous lines of evidence support a link between chronic inflammation and a predisposition for cancer development. In fact, neoplastic cells have been shown to arise in tissues that endure prolonged infection, irritation, or inflammation. Inflammatory molecules have also been implicated in conferring the oncogenic changes that both initiate and promote carcinogenesis. Moreover, as a tumor develops, the normal architecture of the primary tissue becomes disordered and further provokes an inflammatory response. The immune cells that infiltrate the tissue protect the body from cancer while simultaneously shaping the immunogenicity of tumor cells in an extensive and dynamic crosstalk between normal, primary tissue and cancer cells. Remarkably, tumor cells also possess the ability to promote an inflammatory response for survival. The role of inflammation in the tumor microenvironment is discussed.


INDEX TERMS: Tumor microenvironment, Immunoediting, Immunosuppression, Myeloid-derived suppressor cells


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INTRODUCTION
Carcinogenesis, or the creation of cancer, is a process by which normal cells are transformed into cancer cells. This transformation is a multistep, progressive phenomenon by which a cell accumulates a series of genetic alterations that cause its growth pattern to become progressively abnormal. The process requires a succession of DNA changes that initiate carcinogenesis,
an environment that promotes growth, and additional mutations/stimuli that advance the progression of pre-malignant cells to full malignancy. The immediate surroundings of a cancer cell are referred to as the tumor microenvironment and are crucial to the development of a malignancy. It is within the tumor microenvironment that the initiating somatic mutations and the survival signals that allow the clonal expansion of initiated cells occur. While there are a myriad of molecular events that contribute to the development of a cancer, understanding the dynamic crosstalk between tumor cells and other nearby cells within the tumor microenvironment is increasingly important in understanding carcinogenesis.

Evidence for the importance of the tumor microenvironment in carcinogenesis stems from findings that cancers arise from sites of infection (Helicobacter pylori is linked to gastric cancer and mucosa-associated lymphoid tissue [MALT] lymphoma), result from autoimmune diseases (inflammatory bowel disease increases the risk of colon cancer) and are associated with conditions of prolonged inflammation (prostatitis is linked to the development of prostate cancer). Inflammatory molecules are therefore postulated to contribute to the initiation and the promotion phases of carcinogenesis by co-opting inflammatory signaling molecules and receptors for proliferation, survival, and migration. Yet cancer cells can also arise in the absence of chronic inflammation while still manipulating the inflammatory response as a survival mechanism. Two pathways have been described to connect inflammation and cancer: an extrinsic pathway that is driven by inflammatory molecules, thereby increasing cancer risk (chronic inflammation) and an intrinsic pathway that is driven by molecular alterations (such as oncogenes) originating within the cancer cell and that cause inflammation. In either case, tissue homeostasis is disrupted leading to the suggestion that tumors are similar to a wound that fails to heal.

Wound Healing
A normal inflammatory response accompanies all types of non-lethal cellular and tissue injury. Destruction of tissue is followed by a period of healing that begins during acute inflammation but that may not be complete for as long as two years. The healing process that follows acute inflammation is the sum of responses from hemostasis, inflammation, proliferation (also referred to as reconstruction), and finally, maturation and remodeling.

In brief, hemostasis occurs within seconds to minutes following an injurious event and functions to create a barrier to bacterial invasion, unite the wound edges, and provide a framework for collagen or regenerated tissue cells that will fill the damaged space. The inflammatory response occurs within minutes to hours and is an ordered process that begins with a vascular response of vasoconstriction (thromboxanes and prostaglandins) followed by vasodilation (histamine). The outcome of this vascular response is an exudate that functions to dilute microorganisms and contains sentinel white blood cells (WBCs). The vascular events are accompanied by a cellular response in which granulocytes, monocytes, and resident mast cells are recruited in order to debride and engulf potential pathogens. This cellular response is promoted by various chemokines, kinins, and fibronectin.

Within 48 hours of an injurious event, the process of proliferation commences. This process entails capillary sprouting (angiogenesis), the production of new extracellular matrix ([ECM], a process called granulation), the action of myofibroblasts that pull wound margins together (contraction), and finally, the movement of epidermal cells from the wound edges (epithelialization). Proliferation functions to encourage rapid cell growth, produce ECM to fill the wound space, and ultimately to resurface the wound. The final stage of a healing wound, maturation and remodeling, can last a few days but may take years to complete. During this phase, monocytes outnumber neutrophils, collagen is synthesized at a rapid rate, and epidermal cells continue to migrate, all in order to strengthen and reorganize the new granulated tissue to fit the surrounding tissue.

While numerous cells are involved in the wound healing process, macrophages and fibroblasts are of particular importance. Besides acting as the primary phagocyte of debridement, macrophages secrete biochemical mediators that promote healing (Table 1). The outcome of these mediators is the recruitment of fibroblasts and the production of collagen that is initially deposited randomly, but during remodeling of the tissue the collagen fibers are dissolved by collagenase and reformed, reoriented along the lines of mechanical stress
**Table 1.** Cytokines and their Functions in Wound Healing and Immunoediting

<table>
<thead>
<tr>
<th>Cytokines produced</th>
<th>Function in Wound healing</th>
<th>Function in Immunoediting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase</td>
<td>Debrides collagen fibers in the wound</td>
<td>Tumor cell invasion of the extracellular matrix</td>
</tr>
<tr>
<td>Epidermal growth factor (EGF)</td>
<td>Epidermal regeneration (motility and proliferation)</td>
<td>Modulates cell migration, adhesion, and proliferation</td>
</tr>
<tr>
<td>Fibroblast growth factor (β-FGF)</td>
<td>Stimulates fibroblasts to enter the lesion</td>
<td>Drives cancer cell survival; Supports proliferation and tumor angiogenesis</td>
</tr>
<tr>
<td>Insulin-like growth factor (IGF-1)</td>
<td>Re-epithelialization</td>
<td>Tumor cell proliferation</td>
</tr>
<tr>
<td>Platelet derived growth factor (PDGF)</td>
<td>Fibroblast proliferation and chemoattraction; Macrophage chemoattraction and activation</td>
<td>Triggers stromal recruitment; Involved in epithelial–mesenchymal transition; Promotes tumor growth, angiogenesis, invasion, and metastasis</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>Stimulates endothelial cells to form capillary buds (angiogenesis)</td>
<td>Modulates cell migration and proliferation; stimulates angiogenesis</td>
</tr>
<tr>
<td>Tumor necrosis factor (TNF-α)</td>
<td>Activates fibroblasts; collagen synthesis</td>
<td>Cytotoxic to tumor cells; chronic low concentration promotes tumor growth and angiogenesis</td>
</tr>
<tr>
<td>IL-1, IL-6, Transforming growth factor (TGF-α and β)</td>
<td>Re-epithelialization; recruit fibroblasts</td>
<td>Promotes angiogenesis, tumor growth, and metastasis</td>
</tr>
<tr>
<td>IL-10</td>
<td>Anti-inflammatory functions</td>
<td>May support immune attack against malignant cells; May be immunosuppressive to promote tumor immune escape</td>
</tr>
<tr>
<td>IL-12</td>
<td>Promotes differentiation of CD4+ cells; Stimulates T cells, NK cells, to produce IFN-γ</td>
<td>Same function as wound healing; Plays a significant role in the ‘elimination’ phase of immunoediting</td>
</tr>
<tr>
<td>Interferon (IFN)</td>
<td>Inhibits fibroblast proliferation</td>
<td>Antiproliferative, pro-apoptotic, and antiangiogenic</td>
</tr>
</tbody>
</table>

All together, the “normal” inflammatory response associated with wound healing is self-limiting. However, dysregulation of a “normal” inflammatory response can lead to abnormalities such as neoplasia.

**Cancer Immunoediting**

The immune system and tumor tissue have been shown to partake in a dichotomous relationship. On the one hand, an intact immune system destroys abnormal cells including cancer cells and in a mechanism similar to wound healing, there is tissue resolution and inflammation-mediated signaling pathways are resolved. On the other hand, the growth factors and cytokines that support an inflammatory response can also drive
the proliferation and survival of cancer cells. This ability to prevent/control and shape/promote cancer is a process referred to as ‘cancer immunoediting’.

Cancer immunoediting can be viewed as three processes that include elimination, equilibrium, and escape. The immune system is proposed to monitor and survey the cells and tissues of the body and to be responsible for recognizing and eliminating cells whose phenotype deviate from normal, as is the case with early neoplastic cells (and is also the case with cells that have been damaged in a wound). If this notion of immune surveillance holds true, then tumors that survive to a detectable mass of cells have adopted a means to avoid both the innate and adaptive arms of the immune system. In fact, it appears that highly immunogenic cancer cells are eliminated in hosts with an intact immune system while weakly immunogenic cancer cell variants are left to proliferate. The clinical evidence for cancer cell immunogenicity has been observed in numerous studies that have shown that over time, immunocompromised transplant patients treated with immunosuppressive drugs are at increased risk for the development of many different types of cancers, especially those of viral origin. Additionally, tumors have also been shown to grow more frequently and rapidly in carcinogen-exposed immunocompromised animal models. The increased potential for the development of cancer in a host with diminished immune capacity suggests that tumor cells are immunogenic and would normally be eliminated in a host with an intact immune system.

**Cancer Cells Avoid Elimination**

An immunogenic mass of cells is the impetus for the elimination phase of immunoediting. Cell surface markers (antigens) are expressed by both normal and tumor cells and can be immunogenic when they are expressed in an altered form, quantity, or at a time that is different from what the immune system recognizes as ‘normal’. Such is the case with tumor cells that aberrantly express oncoogenic viral and oncofetal antigens (only expressed normally during development), various glycolipids/proteins hyaluronic acid receptor (CD44), and several other molecules that are categorized as danger associated molecular patterns or DAMPs. Stressed cells (those that experience starvation, hypoxia, chemo-, and radio-therapy) release immunogenic DAMPs that contribute to a disordered tumor microenvironment. The high mobility group B1 molecule (HMGB1) is one such DAMP that is passively released from necrotic tumor cells (imparting immunogenicity) but that also participates in pathways leading to cell proliferation, angiogenesis, and chemotaxis in a setting akin to wound healing. Finally, growing tumors must remodel the stroma during processes of angiogenesis and invasive growth and this remodeling is likely to release numerous pro-inflammatory molecules that further impart immunogenicity to the tumor cells.

Natural killer (NK) cells begin elimination by recognizing immunogenic tumor cells and releasing interferon-γ (IFN-γ). The IFN-γ recruits and activates macrophages that, together with NK cells, destroy and scavenge tumor cells. This leads to the activation of a tumor-specific adaptive immune response by which recruited dendritic cells acquire tumor antigens and present them to and activate CD4+ T cells in the draining lymph node and leads to the development and activation of CD8+ cytotoxic T lymphocytes (CTLs). At this latest stage, the tumor cell specific CD4+ and CD8+ cells can migrate to and contribute in the elimination of immunogenic tumor cells.

Since there are numerous indicators that cancer cells possess immunogenicity, that a growing tumor disrupts the local tissue as a result of stromal remodeling, and that immune mediators can destroy cancer cells, then why aren’t all tumors simply eliminated by the host with an intact immune system? Immunologic failure to reject a developing tumor likely requires an integrated innate and adaptive immune response failure and is proposed to occur when immunosurveillance declines and when cells within a tumor mass acquire mutations for resistance to immune attack.

**Cancer Cells Persist in Equilibrium**

During elimination, lymphocytes and their secretion of IFN-γ, exert selective pressure on the tumor tissue in order to contain the tumor cells. Any tumor cell that survives elimination by the immune system is thought to persist in a state of host immune system equilibrium. To achieve equilibrium, tumor cells must adopt a phenotype that allows them to survive, an event that is likely only possible upon the tumor cell acquiring chromosomal instability. The equilibrium process is probably the longest phase of the immunoediting...
process. It may transpire over the course of several years to result in a bed of tumor cell clones with reduced immunogenicity. The cells that possess a lessened immunogenic phenotype may exhibit an enhanced capacity to grow even when they exist in an immune-eliminating environment.\textsuperscript{18}

Clinical evidence for the equilibrium phase has been demonstrated in patients who develop cancer from transplant donors who were classified as ‘cancer-free’ and in cancer remission prior to transplantation. It is proposed that the donor’s intact and competent immune system had maintained occult tumors in the equilibrium phase and that pharmacologic immune suppression in transplant recipients lead to the growth of the ‘dormant’ tumor cells.\textsuperscript{18} For this reason, organ donation is contraindicated in some situations and the U.S. Donor Transmitted Assessment Committee has developed guidelines to help determine risk of donor-transmitted disease.\textsuperscript{19}

**Cancer Cells Escape Immunity**

When tumor cell variants that have been selected in the equilibrium phase grow in an immunologically intact environment, they have circumvented both innate and adaptive immune systems and are considered to enter into the escape phase of immunoediting. Cancer cells can acquire mutations that confer resistance to immune detection (e.g. reduced antigen expression and IFN-γ sensitivity) and resistance to elimination (e.g. faulty death-receptor signaling or constitutive anti-apoptotic signaling). Tumor cells may also participate in the secretion of transforming growth factor beta (TGF-β) and other immunosuppressive factors such as IL-10 that can impede CTLs and IFN-γ secreting NK cell function.\textsuperscript{20,21}

A final means that cancer cells use to facilitate escape is through the recruitment of immunosuppressive cells including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) to a nascent tumor which can additionally suppress the actions of tumor antigen-specific CTLs.\textsuperscript{22,23} Interestingly, while Tregs are beneficial to a healing wound and are important for tissue regeneration they have also been described to frequent tumor tissues that include breast, lung, liver, malignant melanoma, pancreatic, and gastrointestinal malignancies. Their presence has been linked to a poor prognosis in some cancers.\textsuperscript{24,25,26} The chemokine (C–C motif) ligand 22 (CCL22) that is secreted by the tumor cells and/or tumor infiltrating macrophages is a likely chemoattractant to recruit Tregs to these tumor tissues.\textsuperscript{27}

Myeloid derived suppressor cells (MDSCs) may also have a role in wound healing and tissue repair. Tumor cells appear to be able to harness these characteristics for antitumor immunity and tolerance. They are a heterogeneous population of immature, myeloid progenitor cells that have the capacity to suppress CTL and NK cell-mediated antitumor functions and increased numbers have been observed in tumor tissues.\textsuperscript{28} MDSCs are characterized as Lin-HLA-DR-CD33+ or CD11b+CD14-CD33+. MDSCs exert their immunosuppressive activities in the tumor microenvironment by inhibition of T cell proliferation and other T cell functions (e.g. T cell receptor zeta chain suppression and can induce T cell apoptosis.\textsuperscript{29} Refer to Figure 1.

**Tumor Promoting Inflammation**

Thus far, it is apparent that a prolonged inflammatory response increases the potential for neoplasia and that cancer cells are adept at circumventing immune detection and destruction. Remarkably, tumor cells have an additional ability to promote an inflammatory response that enables their proliferative and survival capacity, genomic instability, and metastasis.

**Proliferation and Survival**

As the normal tissue architecture is modified by tumor cell growth, soluble growth factors that should remain embedded in the extracellular matrix are released. Furthermore, the pro-inflammatory tumor microenvironment recruits immune cells that supply additional bioactive molecules such as growth factors and pro-angiogenic factors such as vascular endothelial growth factor (VEGF). Cancer cells may exploit growth factor signaling through varied strategies but seem to converge on two major pathways 1) signaling through signal transducer and activator of transcription 3 (STAT3) and 2) activation of nuclear factor-kB (NF-kB). As cells of the innate immune system react to a disruption in tissue homeostasis induced by tumor growth, a key cytokine, interleukin-6 (IL-6) is released from tumor infiltrating macrophages (Table 1). While
IL-6 serves to further stimulate an immune response, it has also been shown to activate the STAT pathway (in particular, STAT3), leading to increased expression of cell cycle regulatory proteins such as D and B cyclins and the anti-apoptotic protein, B-cell lymphoma-extra large (BCL-XL). IL-6 signaling to STAT3 has also been shown to activate NF-kB, a transcription factor that regulates expression of survival genes and those required for cell proliferation. Moreover, NF-kB is routinely activated by the cytokine tumor necrosis factor-α (TNF-α) that, like IL-6, is secreted by tumor infiltrating macrophages.

Genomic Instability
Inflammation appears to play a prominent role in neoplastic processes by increasing the rate of DNA damage (mutations) while simultaneously compromising DNA repair mechanisms. Inflammatory carcinogens are derived from various sources. First, reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are released by macrophages and neutrophils in the tumor microenvironment, lead to DNA double strand breaks, point mutations, and other more complex DNA aberrations. Second, the expression of DNA deaminases may be up-regulated by signaling from several cytokines including TNF-α, IL-1, and TGF-β and may introduce mutations into tumor suppressor genes such as p53 and oncogenes such as MYC (myelocytomatosis viral oncogene). The inactivation of p53-mediated genomic surveillance leads to an accelerated mutation rate in cancer cells. Finally, cytokines such as TNF-α and IL-1 and ROS can signal to repress the ability of DNA repair through mismatch repair proteins (e.g., prevent microsatellite instability). The reduced ability to undergo DNA repair contributes to increased rates of DNA replication errors throughout the tumor cell genome.

Metastasis
Metastasis is a multistage process. In brief, cancer cells acquire the ability to invade local tissue followed by intravasation of the cancer cells into proximal blood and lymphatic vessels, survival of cancer cells in the circulation, and ultimately, extravasation and colonization of cancer cells at distant sites. In order for the metastatic process to begin, cancer cells acquire mesenchymal cell characteristics such as an increased ability to migrate and invade through basal membranes; this process is known as the epithelial-mesenchymal transition (EMT). Notably, monocytes and...
macrophages facilitate the EMT through secretion of TGF-β, TNF-α, IL-6, and IL-1. The subsequent downstream activation of STAT3 and NF-kB reduce the expression of cell adhesion molecules such as E-cadherin and up-regulate the expression of matrix metalloproteinases, both of which contribute to the migratory capacity of tumor cells. Finally, TNF-α promotes vascular permeability and recruits inflammatory monocytes to promote extravasation and seeding of tumor cells at distant sites.

CONCLUSIONS

Chronic inflammation is linked to the development of several types of cancers. Wound healing/repair and tumor growth are analogous with respect to tissue remodeling, cell proliferation, angiogenesis, infiltration of pro- and anti-inflammatory cells, and the regulation of cytokines and growth factors. The inflammatory processes observed in wound healing are evaded by subtle modifications in the tumor microenvironment in which cancer cells are immunoedited to a population of rogue cells with reduced immunogenicity that then proliferate and metastasize.

Because of the various mechanisms of crosstalk between cancer cells and an inflammatory response, inflammation has become a target for cancer prevention and therapy. In fact, nonsteroidal anti-inflammatory drugs (NSAIDs) are observed to prevent colon and other cancers. Numerous drugs have been assessed for their abilities to target cancer-related inflammation and include antagonists of IL-6, the IL-6 receptor, various chemokines and their receptors (e.g. CCL2), and TNF-α. Theoretically, pharmacologic inhibition of the immune response should deplete immune and inflammatory cells in the tumor microenvironment, inhibit signal transduction and activation of survival molecules such as NF-kB, sequester chemokines and cytokines, and even reduce inflammation following anticancer therapy. This could lead to a re-alignment from a tumor-promoting to a tumor-inhibiting microenvironment and possibly inhibit metastatic spread. From the clinical laboratory perspective, individualized care will likely demand new molecular assays that detect polymorphisms associated with anti-inflammatory pharmaceutical metabolism.

REFERENCES


