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Immunotherapy for cancer: promoting innate immunity

Ramin Lotfi[1,2], Hubert Schrezenmeier[1], Michael Thomas Lotze[2]

[1]Institut fur klinische Transfusionsmedizin und Immungenetik Ulm, Institut fur Transfusionsmedizin Universitat Ulm, Helmholtzstrasse 10, 89081 Ulm, Germany, [2]G.27A Hillman Cancer Center of the University of Pittsburgh Cancer Institute 5117 Centre Avenue, Pittsburgh, PA 15213 USA

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1. ABSTRACT

Development of tumor over many years leads to reciprocal alterations in the host immune response and the tumor, enabling tumor growth seemingly paradoxically in the setting of necrosis and inflammation. Innate immune cells, granulocytes - neutrophils, eosinophils, basophils - and mast cells belong to the first line of defense sensing pathogen and damage associated molecular pattern (PAMPs, DAMPs) signals, initiating and modulating the subsequent inflammatory response. Nontheless, the prevailing contemporary strategies of immunotherapy for cancer have focused on the second line of the immune response, the adaptive immune response. We have determined that most highly evolved tumors in adults undergo necrosis, releasing DAMPs, promoting reactive angiogenesis, stromagenesis and reparative epithelial proliferation of the tumor cell. Means to aerobically eliminate such DAMPs by peroxidases released by innate immune effectors allows us to consider novel strategies for limiting tumor progression. Summarized here is our current understanding of acute and chronic inflammation and its impact on tumor development, the pathophysiology of immunity in cancer, and the influence of granulocytes and mast cells in this setting.

2. INTRODUCTION

It was in 1863 that Rudolf Virchow noted leukocytes within neoplastic tissue and suggested a relationship between inflammation and cancer. He suggested that the "lymphoreticular infiltrate" reflected the origin of cancer at sites of chronic inflammation. Our understanding of the inflammatory microenvironment of malignant tissues has supported Virchow's hypothesis, and the links between cancer and inflammation have substantial implications for emerging strategies for prevention and treatment. Cancers occurring in adults most frequently arise in the setting of chronic inflammation (1), distinguishing these neoplasms from embryonic/pediatric tumors that more frequently arise from inherited mutations and genetic alterations that are acquired during the rapid cellular expansion that occurs during embryogenesis (2). This discrimination is important, since an inflammatory response may not itself necessarily impute a good prognosis.

Chronic infection and inflammation contributes to an estimated 25% of all cancer cases worldwide (3). Indeed, leukocyte infiltration into cancer tissues may also be associated with a poor prognosis for patients when

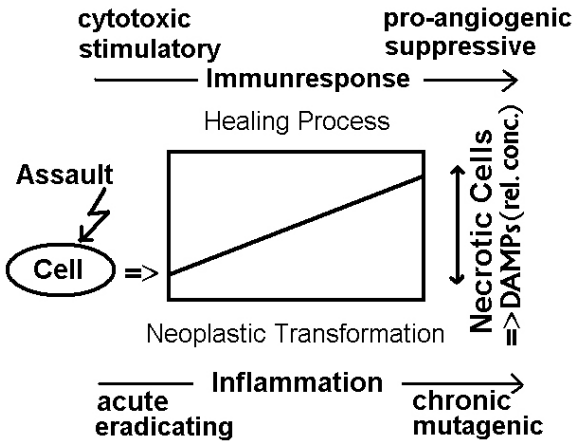

Figure 1. Cross-Roads Between Tissue Healing and Neoplastic Transformation. Damage associated molecular pattern molecules (DAMPs) are released from necrotic cells experiencing an unscheduled cell death. Tissue healing is initiated once an acute inflammatory response has eradicated the remnants of necrotic cells. Chronic exposure of tissue to cytotoxic factors released by activated leukocytes or an inflammatory response insufficient toeradicate the pathogen, induces chronic release of DAMPs promoting a carcinogenic microenvironment being both pro-angiogenic and immunosuppressive.

considering the emergent role of T (4-6), or other immune regulatory cells such as eosinophilic granulocytes (7, 8) or mast cells (9). Even released factors from inflammatory cells may be cytotoxic for tumor or promote carcinogenesis. Reactive oxygen species (ROS) generated by eosinophils, neutrophils and macrophages, for instance, can promote or respond to tumor necrosis of cells by both direct cytotoxic effect and indirectly by contributing to desorption of cytotoxic neutrophil proteases from the proteoglycan matrix in the granules (10). Older premises that ROS serve as promoters of neoplastic growth (11), are belied by recent observations in human clinical trials suggesting that antioxidants may promote tumor growth (12). A question of balance and concentration of released factors within inflammatory tissue as well as its chronicity may induce tumor death or proliferation.

The development of tumor over many years typically leads to reciprocal alternations in the host and the tumor, enabling tumor growth, paradoxically in the setting of substantial necrosis and (chronic) inflammation. In these circumstances genomically unstable cells harness collaborative capabilities (see below) of immune cells and local non-mutated but injured tissues to favor their own survival and proliferation in part, by releasing immune suppressive factors such as TGFβ and IL-10. This way tumor cells escape eradication and release tissue healing factors providing neo-vascularization with subsequent nutrition supply to tumor cells. Wound healing and tumor stroma formation share many important properties (13). Nevertheless, wound healing is itself a self-limiting process, whereas tumors "addicted to continuous necrotic death" (1) release damange associated molecular pattern molecules (DAMPs) sustaining tissue proliferation, angiogenesis and continuous leukocyte recruitment (Figure 1).

The tumor microenvironment is characterized by both secreted and released factors from the tumor, associated with qualitative differences in the infiltrating leukocytes, recruited stem cells, and promotion of reparative angiogenesis and stromagenesis. Tumors are almost always totally dependent on the host-created microenvironment. This makes it difficult to cultivate tumor cells *in vitro* but on the other hand opens unique opportunities for cancer therapy. Thus when evaluating a tumor, it is important to assess three elements within the microenvironment: 1. Factors released by tumor cells themselves and their microenvironment consisting of specialized local mesenchymal cells, fibroblasts, epithelial and endothelial cells, as well as infiltrating leukocytes; 2. The quantity and quality of tumor-associated leukocytes; and 3. Their state of activation.

The mammalian immune system is reciprocally composed of dynamic networks of immune cells and non-immune cells, enabling metabolic homeostasis, timely eradication of effete cells, and protection against pathogens. Simultaneous tolerance towards self-antigens and reciprocal reactivity to new or occult antigens often occurs in settings of tissue damage and wound healing. When tissue homeostasis is perturbed, mast cells, granulocytes, and macrophages immediately release mediators such as cytokines, chemokines, matrix remodeling proteases and ROS, and bioactive mediators such as histamine, that induce mobilization and infiltration of additional leukocytes into damaged tissue (inflammation). Subsequently, the process of wound healing begins, characterized by phagocytosis of cell debris and apoptotic cells, immune suppression, enabling re-epithelialization and synthesis of extracellular matrix (ECM), thus inflammation resolves, restoring tissue homeostasis.

The unique characteristic of innate immune cells – their intrinsic ability to rapidly respond when tissue injury occurs, without memory of previous assaults or antigen specificity – is a defining feature that distinguishes them from adaptive immune cells. Either failure in the precise control of immune components, persistence of pathogen, or tumor itself can lead to chronic inflammation. This is the pathologically conductive microenvironment favoring the initiation and progression of cancer (3). Prolonged (chronic) inflammation itself is mutagenic by virtue of prolonged exposure of normal tissue to DAMPs (Figure 1). ROS generated largely intracellularly can also promote mutagenic changes in cells when aerobic denaturation of extracellular DAMPs is ineffective (11). TNF-alpha (14) and matrix metalloproteinases promote recruitment of inflammatory cells and tissue remodeling. The tumor protective effect of long-term usage of anti-inflammatory drugs, such as aspirin and selected cyclooxygenase-2 (COX-2) inhibitors (15) supports the notion that chronic inflammation is mutagenic.

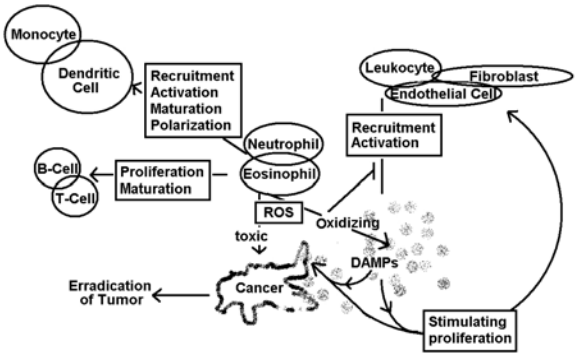

Figure 2. Impact of Eosinophilic and Neutrophilic Granulocytes in Eradication of Neoplastic Tissue, Degrading DAMPs. Sensing DAMPs released by (necrotic) neoplastic cells, eosinophils and neutrophils produce and release reactive oxygen species (ROS) inducing both, tumor cell death and oxidation, thus inactivation, of DAMPs. In addition, they initiate an acute inflammatory response recruiting, activating and polarizing DCs, monocytes, cytotoxic lymphocytes and B-cells. Chronic exposure to active DAMPs promotes angiogenesis and immunosuppression by recruiting and activating endothelial cells, fibroblasts and immunosuppressive leukocytes such as T-regulatory cells, and myeloid derived suppressor cells (MDSCs).

3. NEUTROPHILS AND CANCER

Neutrophilic polymorphonuclear leukocytes (neutrophils) are derived from hematopoietic stem cells in the bone marrow and are the most abundant type of blood white cells in humans. As a first line of defense, neutrophils are recruited within 15-45 minutes to sites of injury and infection (16) recruited by so called signal 0, pathogen associated molecular pattern molecules (PAMPs) and DAMPs (17). DAMPs are released from injured host cells and include S100, uric acid, ATP, hyaluronan, heat shock proteins (HSPs), high mobility group box 1 (HMGB1), heparan, and syndecan while PAMPs are essential polysaccharides and polynucleotides that differ little from one pathogen to another but are not found in the host. PAMPs comprise bacterial lipopolysaccharide (LPS) bacterial flagellin, lipoteichoic acid from Gram positive bacteria, peptidoglycan, and nucleic acid variants normally associated with viruses, such as double-stranded RNA (dsRNA) or unmethylated CpG motifs.

Neutrophils can act not only as phagocytes, but also as killer cells releasing cytotoxic factors into tissues, as stimulatory cells releasing factors which enhance the resultant immune response, and as immunoregulatory cells promoting the TH1-TH4 adaptive responses during the process of tissue healing. Neutrophils that sense tissue damage but fail to encounter a pathogen still release granular contents into the ECM (18), inducing tissue permeability and swelling. Local formation of purulent material increases tissue turgor and thus pressure on surrounding tissue leading to collapse of lymphatics and capillaries, thereby limiting egress of pathogens and possibly neoplastic cells. The neutrophil respiratory burst is the rapid, non-mitochondrial reduction of oxygen to form ROS (Figure 2). The respiratory burst products and its derivates include superoxide, singlet oxygen, ozone, hydrogen peroxide, hypohalous acids, such as hypochlorous acid, chloramines and hydroxyl radicals (19). Patients with impaired oxidative burst of their granulocytes, suffer from recurrent, severe bacterial infections, supporting the notion that the primary role of the neutrophilic oxidative burst is its cytotoxic activity (19).

As key components of the inflammatory response, neutrophils recruit, activate, and program antigen presenting cells (APCs) (16). Releasing chemotactic factors neutrophils not only induce recruitment of monocytes and dendritic cells (DCs) but also influence their polarization (20-22). Neutrophil activation of DCs is fostered by cell-cell contact, in which the specific carbohydrates on CD11b engage DC-specific ICAM1-grabbing non-integrin (DC-SIGN) (23). Moreover, neutrophils secrete the TNF family ligand B-lymphocyte-stimulator (BlyS) (24) and respond to IFNgamma (25) that drives proliferation and maturation of B cells, differentiation of T cells and activation of macrophages (26), respectively. Even though neutrophils make fewer molecules of a given cytokine than do macrophages or lymphocytes on a per-cell-basis, neutrophils outnumber mononuclear cells at inflammatory tissue by up to two orders of magnitude (16). Local deprivation of iron is another cytotoxic mechanism of neutrophils. Following activation, neutrophils release the iron-binding protein lactoferrin into the phagosome and ECM (16). As neutrophils migrate along a concentration gradient they progressively extrude their nuclear proteins and DNA (27), leading to a capture and killing at the plasma membrane of target cells.

Granulocyte colony stimulating factor (G-CSF) is an essential regulator of neutrophil maturation and release from bone marrow. The rate of neutrophil production is influenced by macrophages and T-lymphocytes, through secretion of Interleukin- (IL) 23 and IL-17, respectively. IL-17 released from gamma-delta- and alpha-beta- T cells stimulates bone marrow stroma cells to produce G-CSF. Non-activated neutrophils undergo an apoptotic death, having a lifespan of about 8 hours in blood. Following phagocytosis of apopototic neutrophils, macrophages fail to release IL-23 which normally promotes IL-17 release from T-cells (28).

As far as immunosuppression and wound healing is concerned, it is well demonstrated that in patients with advanced cancer, activated neutrophils can impair T-cell-receptor (TCR) ζ–chain expression and cytokine production (29). Another factor released from neutrophils in the setting of wound healing is secretory leukocyte protease inhibitor (SLPI) (30). SLPI inhibits neutrophil elastase and protects itself from inactivation by suppressing the neutrophil oxidative burst (31). The epithelial-cell-growth-promoting cytokine proepithelin (PEPI, also known as progranulin) is protected from degradation by SLPI, thereby inhibiting neutrophil elastase. PEPI synergizes with SLPI in inhibiting neutrophil activation and it also signals to epithelial cells to proliferate and close wounds (32), a condition which favors tumor proliferation.

Macrophages are well-established sources for TGF-beta1 in inflammation and wound healing (33, 34) but TGF-beta1 is released only from macrophages which have engulfed apoptotic granulocytes within injured tissue (35). This observation puts emphasis on the central role of granulocytes in the induction of TGF-beta1-dependent myofibroblast differentiation and wound healing as well as their possible participation in tumor proliferation and local immunosuppression.

Neutrophils are capable of effectively degrading laminin, collagen, proteoglycans and fibronectin by virtue of their three major matrix metalloproteases (MMPs), MMP8, MMP9, and MMP25 (36). Enzymatically active serprocidins (cathepsin G, neutrophil elastase, and protease 3) also degrade most of the components of the ECM. MMPs together with serprocidins facilitate neutrophil recruitment and tissue break down, but may also induce metastasis of yet adherent tumor cells. Additionally, nonadherent neutrophils are less efficacious in terms of ROS production. Adherent neutrophils release about two orders of magnitude more H_2O_2 than neutrophils in suspension (18).

The main message from neutrophils in inflammatory sites is best paraphrased by Carl Nathan in his review on neutrophils and immunity (16): "To microorganisms and host cells that stand in the way: die. To themselves: if the site still seems infected, summon reinforcements until the crucial concentration of neutrophils that is required to clear a given tissue volume of bacteria is attained; and if infection has been brought under control, wrap yourself in a phosphatidylserine flag and wait for macrophages to remove your apoptotic corpse for the safe disposal of your unexploded weapons." (16) Intratumoral activation of neutrophils by low dose TNF-alpha in end-stage melanoma patients treated with neutrophil recruiting tumor-specific-monoclonal antibody could induce within hours after treatment almost complete necrosis of bulky tumors in multiple visceral sites (37) demonstrating the impressive ability of neutrophils to alter tissue homeostasis.

4. EOSINOPHILS AND CANCER

Eosinophilic granulocytes are also derived from CD34+ hematopoietic progenitor cells and mature in the bone marrow. The development of multi-potential cells into eosinophil progenitors occurs as a stochastic process and survival following pathway entry is supported by SCF, IL-3, IL-4, IL-5, GM-CSF, and eotaxin (38, 39) sustaining the terminal stages of maturation and release into the blood stream (40-42). Once in the circulation, eosinophils have a half-life of about 18h and a mean blood transit time similar to neutrophils (26 hrs) (43). The low numbers of circulating eosinophils belies the extraordinary prevalence of these granulocytes residing in mucosal (gut and lung) tissue compartments (44). Interestingly, compartments with abundant resident populations of eosinophils include tissues with substantial cellular turnover and regenerative capacity such as the bone marrow, the primary and secondary lymphoid tissues (e.g., spleen, lymph nodes, and thymus) (45), the uterus (46), and nearly the entire gastrointestinal tract (with the exception of the esophagus (45, 47)). This linkage with cell turnover and tissue repair also may explain the presence of eosinophils at sites of wound repair (48) and the commonality of an eosinophil infiltrate among solid tumors (49). A systematic survey of biopsy and autopsy specimens revealed that the only organ displaying both eosinophilic infiltration and significant degranulation was the gastrointestinal tract (50). At baseline (healthy conditions), most eosinophils reside within the lamina propria in the stomach and small bowel (51), whereas eosinophils are scarce within intestinal lymphoid tissues (e.g., Peyer's patches) (45, 51). Eosinophil homing in gastrointestinal tissue occurs during embryonic development and their levels in perinatal mice are comparable to those observed in adults (51), indicating that their homing is not dependent upon the presence of intestinal flora. Indeed germ-free animals, with demonstrably fewer lymphocytes within the lamina propria showed similar eosinophilic tissue infiltration when compared with control mice (51-53). Eosinophil localization to the lamina propria at baseline is critically regulated by eotaxin, a chemokine constitutively expressed throughout the gastrointestinal tract (54). Although eotaxin is required for eosinophil homing to the gut submucosa, its expression within the gastrointestinal tract (e.g., the esophagus) is by itself not sufficient to induce eosinophil accumulation, because the esophagus is normally devoid of these granulocytes. This suggests the potential involvement of other eosinophil chemoattractants and activating factors which contribute to tissue specific accumulation and degranulation. In particular, the correlation of eosinophil recruitment/activities with the concomitant tissue damage and cell death associated with these inflammatory responses suggests that damage associated molecular pattern molecules (DAMPs) may represent previously overlooked signaling molecules which elicit eosinophil agonist activities (Figure 2). Our findings (*Human Eosinophils Respond To Damage Associated Molecular Pattern Molecules (DAMPs); Implications For Immune Response to Necrosis. Lotfi R., Beer Stolz D., Rubartelli A., Schrezenmeier H., and Lotze M.T., submitted)* demonstrate that HMGB1 (a prototypic DAMP member) serves as a chemoattractant and survival factor for eosinophilic granulocytes.

Eosinophilic granulocytes are found within necrotic tissues and the surrounding pseudocapsule of tumors (55). These immune cells contain, and can release several cationic proteins which in addition to their toxic tissue damaging character are also potentially important for tissue remodeling and clearance of cellular debris (56). The inventory of secondary granule proteins from several species have been defined; humans and the mouse are particularly well studied. These analyses have revealed that the proteins stored in eosinophil secondary granules are evolutionarily conserved. Secondary granule proteins from both humans and mice subdivide into three major groups: 1) eosinophil major basic proteins-1 and -2 (MBP-1, MBP-2 (57-60)); 2) eosinophil peroxidase (EPO (61, 62)); and 3) eosinophil associated granule ribonucleases (Humans: eosinophil cationic protein (ECP (63)) and eosinophil

derived neurotoxin (EDN (64)); Mouse: eosinophil associated ribonucleases (EARs-1, -2, -3, -5, -6, -7 (65)). The common perception is that eosinophils are, in part, responsible for the cell death and tissue damage commonly observed in disease states associated with increased numbers and tissue-specific recruitment (66, 67).

Since the mid-1980's eosinophils have been found to mediate their effects via at least three independent mechanisms in addition to the release of cytotoxic granule proteins. These mechanisms enable eosinophils to modulate the intensity of inflammation as well as to elicit cell death leading to the loss of tissue integrity: 1) Eosinophils are potent regulators of local inflammatory responses (68); 2) Recruited eosinophils are a source of reactive oxygenated species (69) and established small molecule lipid mediators of inflammation. In particular, eosinophils generate cysteinyl leukotrienes (i.e., LTC4, LTD4, LTE4 and LTB4) (70, 71), 5-HETE (72), PGE_2 (73), and platelet-activating factor (PAF) (74). The capability of cysteinyl leukotrienes to mediate primary inflammatory responses such as edema (75), the recruitment of other proinflammatory leukocytes (76), and the induction of tissue histopathology (77) uniquely positions these molecules as mediators of inflammation. 3) Eosinophils are a prodigious source of cytokines associated with tissue repair and remodeling.

A growing body of literature suggests that both eosinophil-mediated immunoregulation and tissue repair/remodeling in particular may represent important non-overlapping eosinophil effector functions (56). A quantitative assessment of eosinophil recruitment/accumulation in solid tumors showed that the tissue eosinophilia is apparently mediated by one or more factors released directly from necrotic tissues within the tumor (56). Studies linking eosinophil recruitment and activations with cell death and necrosis abound. In particular, Stenfeldt and colleagues (78) discovered that damaged epithelial cell lines (e.g., genital (HeLa), respiratory (HEp-2), and intestinal (HT29) cells) induce eosinophil migration, the release of putative tissue-damaging factors, such as eosinophil secondary granule proteins, and secretion of eosinophil-derived pro-fibrotic factors, such as fibroblast growth factors (FGF-1 and -2) and transforming growth factor (TGF-beta1). Thus, DAMPs released from damaged/dying epithelial cells may represent a previously underappreciated signaling event capable of mediating both eosinophil recruitment and the execution of effector functions leading (and/or promoting) tissue repair and remodeling. In addition to their capacity to synthesize and release a variety of immunoregulatory molecules (79), some studies have suggested that eosinophils may function as antigen presenting cells (APCs).

Lucy and colleagues demonstrated induction of HLA-DR (80) expression on eosinophils and four years later Weller *et al.*, found that antigen-pulsed HLA-DR+ eosinophils induced lymphocyte proliferation (81). Adoptive transfer of antigen-pulsed eosinophils also induced antigen-specific T-cell responses *in vivo* (82, 83). Furthermore, mouse models of parasite infection also suggested that eosinophils display similar antigen presentation activities as part of pathogen defense (84). Recently, Duez and colleagues showed that thoracic lymph node (TLN) eosinophils following allergen sensitization/aerosol challenge expressed higher levels of MHC class II and CD86 when compared with blood and pulmonary eosinophils. Most of the TLN eosinophils also expressed the costimulatory molecule, CD80, and the adhesion molecule, CD54 (85). Recently, we demonstrated that eosinophil are capable of enhancing DC maturation (68). Eosinophils may also affect local T cell responses by modulating the balance of Th1-Th2 immune responses (e.g., through eosinophil-derived indoleamine 2,3-dioxygenase (IDO) production of kynurenine (8)). Importantly, IDO appears to be essential for the induction of tolerance by tissue recruited T-cells (7, 8, 86). Thus, similar to other eosinophil-mediated immunosupressive activities (e.g., the potential induction of T*regs* through TGF-beta production (87)), eosinophil-derived IDO may also play a crucial role in immunosuppression and potentially facilitate tumor growth. Eosinophils are capable of both synthesizing and releasing cytokines characterized as either Th1 (e.g., IFN-gamma), Th2 (e.g., IL-4, -5, and IL-13), Th3 (TGF-beta, IL-10), and possibly TH4 (IL-17 (88-90) also known as TH17) cytokines, or acute phase responses (e.g., TNF-alpha, IL-1, IL-6, and VEGF).

4.1. Eosinophils and the immunotherapy of patients with cancer

Eosinophils are frequently observed in the setting of immunotherapy with IL-2 (91, 92), IL-4 (93, 94), GM-CSF (95), and antibodies to CTLA-4 but their appearance has been an interesting side-note whose significance has remained largely unknown. In particular, the anti-tumor effects of successful cytokine therapy of cancer with IL-2 has been associated with the identification of degranulating eosinophils within the tumor (91, 92), suggesting that eosinophil effector functions (e.g., direct (91) or antibody-dependent (96) tumor cell lysis or the immunuoregulative capacity of eosinophils modulating the local tumor microenvironment) may play a role in the anti-cancer activities mediated by systemic IL-2 administration. However, despite the promises of these potential eosinophil-mediated anti-tumor activities, the presence of eosinophils as a prognostic indicator has not been found to be prognostically important for high dose IL-2 treated patients.

Mouse studies suggesting a link between eosinophils and the therapeutic value of the anti-tumor responses associated with IL-4 administration (93, 97) have also led to clinical trials evaluating these responses in cancer patients. In a Phase I clinical trial of IL-4 administered to cancer patients, Sosman and colleagues (94) showed that IL-4 therapy induced systemic eosinophil degranulation with increases in serum and urine MBP levels. The increase in serum MBP was IL-4 dose dependent. Unfortunately, the linkage of anti-tumor activities in these patients to eosinophils is only correlative and similar to the observations in patients following IL-2 administration, no definitive conclusions as to if and how eosinophils modulate tumor growth can be made.

Efforts to demonstrate experimentally a role for eosinophils in tumor immunity have also been fraught with complicating variables that yielded qualified interpretations. Most notably, considerable excitement was generated by data from the elegant studies of Tepper and colleagues (93, 98) which demonstrated in athymic nude mice that malignant cell lines transfected for constitutive expression of Interleukin 4 (IL-4) elicited a tumor associated macrophage and eosinophil infiltrate that led to the attenuation of tumor growth. This provoked a series of studies, all done with transplantable tumors (see for example (99, 100) in an attempt to define the cellular and molecular mechanisms of this apparent IL-4 mediated anti-tumor effect. Although these studies have shown that even spontaneous tumors evidenced tumor regression associated with tumor infiltrating eosinophils (99), none of these studies has resolved the role (s) of eosinophils in tumor rejection reactions.

Within several tumor types including gastrointestinal tumors, tumor associated tissue eosinophilia (TATE) is associated with a significantly better prognosis (79). The converse is true in other tumor types such as differentiated oral squamous cell carcinoma (79) or Hodgkin lymphoma (101). The mechanism by which eosinophils in particular are recruited into tumor tissue is largely unknown. Candidates factors eliciting eosinophil chemotaxis into tumor tissue are DAMPs including the nuclear protein HMGB1. HMGB1 is released upon necrotic cell death and secreted by many cells, particularly during periods of nutrient, hypoxic, or oxidant stress.

Thus, eosinophil activities are likely to have multiple roles, dictated by specific circumstances, which were adapted to maintain tissue homeostasis. Eosinophils are not only able to destroy tissue but are also attracted and activated by stressed and damaged cells (78). It is likely that stressed cells attract and activate eosinophils by expression of molecules such as major histocompatibility complex class I chain related A (MIC-A), MIC-B, Letal (102) as well as UL16 binding proteins (ULBP). These stress associated molecules serve as ligands for NKG2D, described first on NK cells (103) and subsequently on eotaxin-activated eosinophils (104) and T-cells (105). Thus, tumor-associated eosinophils appear to have at least two dominant non-overlapping activities: 1) Destructive effector functions which may limit tumor growth and cause recruitment and activation of other leukocytes and 2) Immunoregulative and remodeling activities which suppress immune response and release cytokines, promoting wound healing and resultant tumor proliferation.

Consistent with the hypothesis that DAMPs initiate innate immune cell activation when encountering microbes or parasites (106), eosinophils are often first responders to tissue damage and likely mediate some aspects of tissue remodeling and repair. The presence of DAMPs such as HMGB1 in the necrotic areas of tumors may, in part, elicit both eosinophil tissue recruitment and localized execution of effector functions such as degranulation. The available data, however, suggests that while all "threatened" epithelial cells, including cancer cells, release DAMPs, not all eosinophil tissue infiltration is associated with tumor eradication. This conclusion again suggests that the relationship of eosinophils with the modulation of tumor onset/growth is complex and that the expression of DAMPs is likely only one of several inflammatory mechanisms necessarily capable of eliciting eosinophil effector functions. The dramatic rise in organ transplantation makes this a case of particular significance as eosinophil infiltration is a commonly used prognostic factor suggesting transplant rejection (107-109).

In summary, while the role of eosinophils in tumor onset/growth is unresolved, recent studies suggest that eosinophils are a common and robust tumor infiltrate and that much interesting biology remains to be explored. Specifically, do eosinophil activities limit tumor growth through destructive effector functions or do eosinophil-derived immunoregulation and tissue repair/remodeling promote tumor growth and metastasis? The resolution of these issues could inform the intiation of eosinophil-based modalities and, in turn, novel therapeutic approaches to treat cancer patients.

5. MAST CELLS AND CANCER

Mast cells are derived from CD34+ hematopoietic progenitor cells within the bone marrow, but unlike other leukocytes they do not mature at that site. Rather, immature CD34+c-Kit+CD13+ progenitors circulate in the blood and are recruited into vascularized tissue where they undergo final differentiation under the influence of factors found in the microenvironment (110, 111). The primary regulator of mast cell extravasation, proliferation, and differentiation is stem cell factor (SCF), the ligand for the c-kit tyrosine kinase receptor constitutively expressed on the mast cell surface. Although SCF is by far the most important promoter of mast cell development, many other factors, such as IL-3, IL-4, IL-5, IL-6, IL-9, nerve growth factor (NGF), chemokines, and retinoids, contribute to mast cell differentiation either synergistically or antagonistically in a complex network. During their maturation process, mast cells acquire their typical granular morphology and begin to express the high-affinity receptor for IgE (FcRepsilonI), which is one of the classic markers for these cells. The tissue microenvironment has a profound impact on the development and subsequent phenotypic expression of mast cells. Depending on differences in the cytokine milieu and cell interactions occurring in various tissues, mast cells develop heterogeneous phenotypes and functional characteristics. Even so, two major categories can be distinguished. One population contains the proteases tryptase and chymase in the granules and is thus designated ^{MC}TC, whereas the remaining population stores only tryptase and is abbreviated accordingly as ^{MC}T (112). In tissue localization, ^{MC}TC predominate in the skin and intestinal submucosa, whereas ^{MC}T is the predominant subtype in the lung and intestinal lamina propria. Characteristic features of mast cells are the abundance of electron-dense granules and the capacity to release a great variety of inflammatory mediators.

Mast cell mediators can cause vascular effects (histamine, PGD2, LTC4, VEGF); stimulation of angiogenesis (IL-8, proteases, VEGF); tissue remodeling, such as fibrosis (proteases, TGF-beta); inflammatory cell recruitment (chemokines, TNF-alpha, PGD2, LTB4); immunomodulation (IL-4, IL-13, CD40 L/CD154, CD30 L/CD153); and stimulation of granulocytes (GM-CSF, IL-3, IL-5). An association between mast cells and many human tumors is clear, but the relevance of this relationship is not. There is a growing body of data indicating that mast cells promote tumor growth and metastasis following the release of various mediators rather than by providing active defense against tumors (9). Direct evidence for the contribution of mast cell–derived proteases during experimental skin carcinogenesis in mice has been reported (113). Mast cells can stimulate tumor growth either directly through cell–cell interactions and release of cytokines and growth factors, or indirectly by facilitating angiogenesis and tissue remodeling.. Mast cells are associated with angiogenesis in tumors, such as hemangioma, carcinomas, lymphoma, and multiple myeloma (114), but not in Hodgkin's lymphoma (115).

6. BASOPHILS AND CANCER

One year after he had found mast cells (116), Paul Ehrlich described basophils (117). Basophilic granulocytes have been suggested to arise from CD34+/IL-3Ralpha+/IL-5+ eosinophils/basophil progenitor cells in the bone marrow (118). The occurrence of granulocytes displaying a hybrid eosinophil/basophil phenotype in patients with CML or acute myelogenous leukemia supports the idea of a common eosinophil/basophil progenitor. Nevertheless, the new antibody 97A6 has been described as specific for mature mast cells and basophils and their progenitors (119), but not reacting with any other hematopoietic or nonhematopoietic cell type. Thus, the epitope recognized by 97A6 may therefore be associated with a commitment of the CD34+ precursor to a mast cell or basophil lineage distinct from other lineages. The possibility of a common mast cell or basophil lineage also arises from the surprising observation that basophils with phenotypic features characteristic of mast cells (presence of typtase, chymase, c-kit, carboxypeptidase A) can be found in patients with asthma, allergy, or allergic drug-reactions (120). Mature basophils can be distinguished phenotypically from mast cells by their differential surface expression of IL-3 alpha chain (CD123, not found on mast cells) and SCF receptor (c-kit/CD117, strongly expressed on mast cells but not, or only weakly expressed on mature basophils) (121). Unlike mast cells which mature in tissue, mature basophils are found in the circulatory system.

Recent studies on human leukemic cell lines have led to the hypothesis that a common basophil/megakaryocyte precursor (CFU-Baso/Mega) might exist. Indeed, most megakaryocytic cell lines (UT7 and its subclones D1, HEL, CMK, LAMA84, and MTT95) express mRNA for HDC and synthesize histamine (122). The existence of a common basophilic/megakaryocytic precursor is also in agreement with the fact that antibodies recognizing basophils are produced upon immunization with megakaryocytic cell lines. Finally, it has been demonstrated that in certain conditions, the cell line UT7, whose megakaryocytic potential is clearly established, can exhibit features of basophilic as well as of eosinophilic differentiation, thus raising the question of a common baso/eosino/mega progenitor (123).

Basophils, together with eosinophils and Th2 lymphocytes, are rapidly recruited to the skin (124), lung (125), and nose (126) after allergen challenge. Human basophils respond to chemokines as eotaxin (127) (CCL11), eotaxin-2 (CCL24) , eotaxin-3 (CCL26) (128), RANTES, MCP-3 (CCL7), MCP-1, and MIP1-alpha (129, 130).

The growth factor for *in vitro* cultivaton of basophils from progenitor cells are IL-3, IL-5, GM-CSF, transforming growth factor-beta (TGF-beta), and nerve growth factor (NGF), with IL-3 beeing the main growth and differentiation factor (121). Basophils are a very potent source of IL-4 and IL-13 (131). IL-4, especially in combination with TNF-alpha, induces the producton and release os eotaxin by human dermal fibroblasts (132). IL-13 on the other hand prolongs the survival of eosinophils (133). Due to their constitutive expression of CD40-L and CCR3 the may play an important role in atopic disorders as well as DC maturation within gastrointestinal tissue.

A short preincubation or coculture of basophils with IL-3 causes significant enhancement of histamine and LTC4 release to a number of stimuli such as IgE, C5a, C3a, MBP, PAF (134-137). C5a is a highly potent and efficacious inducer of histamine release from basophils following binding to its receptor, CD88 (138). A good overview on expression of surface antigens on basophils is given by Arock and colleagues (139).

Of note, several nonantigen-specific stimuli, derived from various organisms, have been shown to induce mediator or cytokine release from basophils. For example, protein Fv, an endogenous Ig-binding protein released in the intestine of patients affected by viral hepatitis, as well as the HIV-1 glycoprotein gp 120 have been shown to induce IL-4 and IL-13 production from basophils by binding to the VH 3 region of IgE (140, 141). Similarly, soluble egg antigens derived from the parasite Schistosoma mansoni induce the release of IL-4 and other mediators from basophils of nonimmune donors (142). Thus, because basophils rapidly release considerable amounts of IL-4 upon various stimulations, they may have a critical impact on the outcome of a primary infection by inducing T-cell differentiation to the T-helper cell type 2 (Th2) phenotype.

Consistent with their ability to function as cytotoxic cells basophils express and release of granzyme B (GzmB) within 6 to 24 hours by exocytosis following IgE-dependent and -independent (ie. C5a, eotaxin, MCP-1, fMLP, IL-3) activation (143). Of note, constitutive or inducible expression of GzmB is neither detected in eosinophils nor neutrophils. The function of GzmB has been thoroughly studied for its involvement in granule-

mediated cytotoxicity. In cytotoxic lymphocytes, perforin is needed to deliver GzmB into the cytosol thereby inducing apoptosis of target cells (144). Although basophils do not express detectable levels of perforin they show an NK-like activity that is enhanced by IL-3 and diminished by GzmB inhibition, indicating a perforin-independent unknown killing mechanism (143) that needs further investigation. GzmB is less inhibited by antiproteases in bodily fluids (145) than other cell-derived enzymes. Additionally, extracellular matrix remodeling by GzmB with induction of death in adherent cells by cell detachment (anoikis) (146) has been demonstrated. Thus the impact of basophils and specifically their GzmB in tumor eradication needs more study.

Bone marrow basophilia is significantly present in chronic myeloproliferative disorders, idiopathic myelodysplasia , and aplastic anemia. The incidence of marrow basophilia in patients with lymphoma, acute leukemia, or solid carcinoma is not significantly different from what it would be as a chance occurrence (147). In patients with de novo myelodysplastic syndrome (MDS) bone marrow basophilia is an independent risk factor for evolution to AML (148).

7. SUMMARY AND PERSPECTIVE

Chronic inflammation is associated with tumorigenesis and tumor progression. Although some speculate that our immune response was not selected to recognize neoplastic transformation of tissues, a more hopeful and in our estimation, more accurate sense of cancer, is that the inflammatory response mediated by innate effectors are quite sensitive to unscheduled cell death, characteristic of most adult neoplasms. In some instances, indeed the adaptive immune response to tumor can enhance tumor progression by promoting nonapoptotic, nonautophagic cell death. Thus in some instances, immunosuppressive strategies such as the use of steroid administration may be considered for transient use to benefit patients with cancer. Sadly, the consequences of immunosuppression are short-lived even with a microenvironment of tumors containing cytokines as TGF-beta and cells such as CD4+CD25+FOXP3+ T regulatory cells. Understanding the role of nonimmune leukocytes may indeed be the helpful in understanding and treating patients with cancer. The fact that some tumor patients survive and reject tumor apparently without treatment suggests that there are effective means to limit cancer progression. In case of dysplastic transformation of cells, it could be reasonable to modify the local microenvironment that would limit inflammation using immunosuppression, anti-angiogenic agents and apoptosis-inducing drugs, since. phagocytosis of apoptotic cells triggers powerful anti-inflammatory signals (149, 150). In case of advanced tumors which have acquired the ability to invade and die unscheduled cell deaths, reintroducing both a potent innate immune response mediated by granulocytes, specifically eosinophils and neutrophils, as well as a subsequent strong adaptive immune response may elicit a more effective means to control and eradicate tumors.

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Abbreviations: PAMPs: pathogen associated molecular pattern; DAMPs: damage associated molecular pattern; DC: dendritic cell; ROS: reactive oxygen species; ECM: extracellular matrix; COX-2: cyclooxygenase-2; HSP: heat shock protein; LPS: lipopolysaccharide; dsRNA: double-stranded RNA; DC-SIGN: DC-specific ICAM1-grabbing non-integrin; BlyS: B-lymphocyte-stimulator; SLPI: secretory leukocyte protease inhibitor; MMP: matrix metalloproteases: MBP: eosinophil major basic protein; EPO: eosinophil peroxidase; EAR: eosinophil associated granule ribonuclease; ECP: eosinophil cationic protein; EDN: eosinophil derived neurotoxin; PAF: platelet-activating factor; TLN: thoracic lymph node; IDO: indoleamine 2,3-dioxygenase; MIC-A: major histocompatibility complex class I chain related A; ULBP: UL16 binding protein; HMGB1: high mobility group box 1; GzmB: granzyme B

Key Words : Cancer, Neoplasia, Necrosis, Apoptosis, Healing, Damage, Eosinophil, Neutrophil, Basophil, Basophil, Mast Cell, Granulocyte, Innate, Adaptive, Pathogen, Immunotherapy, Therapy, Oxidation, Oxidative Burst, Microenvironment, Tumor, Review

Send correspondence to: Michael Thomas Lotze, G.27A Hillman Cancer Center of the University of Pittsburgh Cancer Institute, 5117 Centre Avenue, Pittsburgh, PA 15213 USA, Tel: 412-623-1211, Fax: 412-692-2520, E-mail: lotzemt@upmc.edu

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