

The Immune Microenvironment: A Major Player in Human Cancers

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Abstract

Cancer is a major public health issue and figures among the leading causes of death in the world. Cancer development is a long process, involving the mutation, amplification or deletion of genes and chromosomal rearrangements. The transformed cells change morphologically, enlarge, become invasive and finally detach from the primary tumor to metastasize in other organs through the blood and/or lymph. During this process, the tumor cells interact with their microenvironment, which is complex and composed of stromal and immune cells that penetrate the tumor site via blood vessels and lymphoid capillaries. All subsets of immune cells can be found in tumors, but their respective density, functionality and organization vary from one type of tumor to another. Whereas inflammatory cells play a protumoral role, there is a large body of evidence of effector memory T cells controlling tumor invasion and metastasis. Thus, high densities of memory Th1/CD8 cytotoxic T cells in the primary tumors correlate with good prognosis in most tumor types. Tertiary lymphoid structures, which contain mature dendritic cells (DC) in a T cell zone, proliferating B cells and follicular DC, are

found in the tumor stroma and they correlate with intratumoral Th1/CD8 T cell and B cell infiltration. Eventually, tumors undergo genetic and epigenetic modifications that allow them to escape being controlled by the immune system. This comprehensive review describes the immune contexture of human primary and metastatic tumors, how it impacts on patient outcomes and how it could be used as a predictive biomarker and guide immunotherapies.

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Introduction

Cancer is a major public health issue and figures among the leading causes of death in the world. The most recent reports have estimated that, each year, over 12 million new cases are diagnosed worldwide and almost 8 million people will die of related causes [1].

Although progress has been made in cancer management during the last decade, the clinical outcome for patients is often poor. For most solid tumors, early-stage curative surgery may result in cure, and therefore large efforts for the early detection of cancer have been made. However, once tumor cells have disseminated from the primary tumor site, they form metastases in distant or-

gans. In fact, most cancer patients die due to metastatic cancer, and the identification of new markers is mandatory to determine the prognosis of patients and to guide their clinical management. Currently, clinical prognosis is based on the Union for International Cancer Control TNM classification, which describes the severity of cancer based on the size and extent of the tumor and whether or not it has spread to distant organs. Stage I refers to a local tumor, stage II to a local tumor that largely invades the tumor microenvironment, stage III to tumors with metastases in the draining lymph nodes and stage IV to tumors disseminated in distant organs [2]. Life expectancy decreases according to stage. This internationally accepted standard for cancer staging is useful, but it does not take into account the fact that although disease progression in cancer is a multistage process, metastases may begin as a clinically silent event early in the development of a primary tumor. More refined and dynamic prognostic markers are therefore mandatory and should be actively sought out.

It is well established that cancer is a genetic disease in which modifications of the genes controlling cell proliferation, such as the mutation of oncogenes and suppressor genes, the amplification of antiapoptotic genes and genes involved in invasion, angiogenesis or cellular migration, render cells autonomous from control signals and this allows the formation of an invasive tumor [3]. Several prognostic gene signatures, based on these modifications, have been proposed in breast [4], colorectal [5], renal cell [6] and other cancers and their clinical value is actively being tested.

Having been underestimated for a long time, it is now recognized that the cellular microenvironment of a tumor also plays a major role in controlling cancer development [7]. The tumor microenvironment includes the immune cells comprising myeloid and lymphoid cells, blood and lymphatic endothelial cells as well as fibroblasts. Whereas nutrients and oxygen brought through the rich vascularization network induced by the tumor cells exert a positive influence on tumor cell proliferation, the presence of immune and inflammatory cells contribute to controlling tumor growth and invasion [8, 9]. Inflammation caused by bacterial (e.g. *Helicobacter pylori* or *Escherichia coli*) [10, 11], viral (e.g. Epstein-Barr or hepatitis B and C viruses) [12, 13] or parasitic (e.g. *Schistosoma haematobium*) infections [14], by chemicals (e.g. cigarette-smoke components or asbestos) [15, 16] or by the developing tumor itself, promotes transition from a pre-cancerous state to an invasive cancer, fuels an established tumor through cell-proliferating cytokines such as inter-

leukin (IL-1) and IL-6 and increases neovascularization via the production of vascular endothelial growth factor (VEGF) [17]. This transition also results in the recruitment of monocytes by chemoattractants such as colony-stimulating factor-1 (CSF-1) and chemokine (C-C motif) ligand 2 (CCL-2) [18]. Monocytes differentiate into macrophages within the tumor. These macrophages with an 'inflammatory' phenotype are similar to the trophic, immunomodulatory macrophages that promote tissue formation during development [19]. Macrophages are educated in the tumor microenvironment. The tumor cells produce mediators that allow their differentiation into different subpopulations (e.g. immunosuppressive or angiogenic macrophages) that promote tumor progression [20]. It has also been reported that macrophages may facilitate tumor invasion and the entry of tumor cells into the circulation [21], with the epidermal growth factor (EGF)-CSF-1 paracrine loop playing an important role in this metastatic process [22]. In addition, macrophages can facilitate the extravasation, survival and growth of tumor cells [23, 24] and may contribute to the formation of a metastatic niche that protects metastatic cells and helps their nidation in distant organs [25] (fig. 1).

In contrast, a strong infiltration of Th1/cytotoxic cells may prevent tumors from growing locally [9, 26, 27]. The differentiation of these effector-memory T cells takes place either in the draining lymph nodes or in ectopic lymphoid structures also known as tertiary lymphoid structures (TLS) that are present locally in the tumor microenvironment [28, 29]. Once locally educated, central-memory T cells also generated in situ may circulate and additionally control the disseminating and potentially metastatic cancer cells [30] (fig. 2).

TLS exist transiently because they disappear after removal of the inflammation, in contrast to secondary lymphoid organs (SLO). TLS arise in non-lymphoid tissues in response to chronic inflammation that can occur in autoimmunity, chronic allograft rejection and microbial infection [31]. Because of their plasticity and close proximity to antigenic sites, TLS are considered to be a privileged site for the generation of anti-infectious [32, 33], anti-allogenic [34] and anti-self reactions [35–39]. However, the TLS impact is unique for each situation. Whereas TLS are associated with the exacerbation and severity of several chronic inflammatory diseases, such as in multiple sclerosis [40], Sjögren syndrome [41] and renal allografts during rejection [42], they correlate with the development of protective primary and secondary immune responses during viral infection [32, 43]. Intratumoral TLS contain a few mature dendritic cells (DC) in a T cell

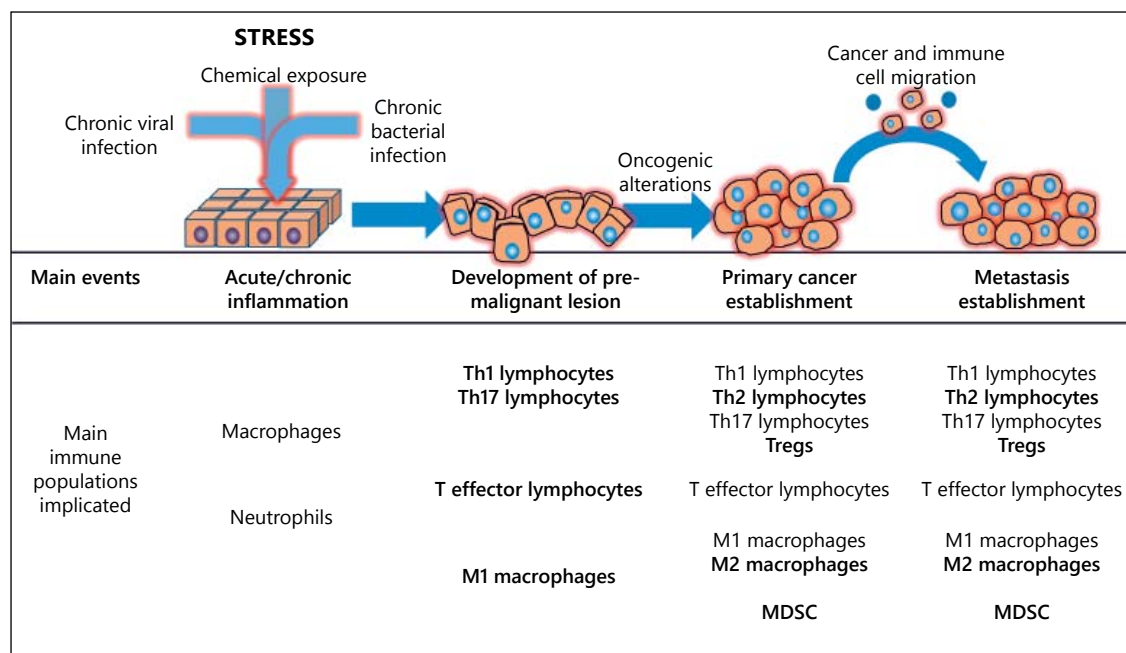


Fig. 1. The immune contexture in cancer development. Cancer development is a very complex and dynamic process that usually begins with the establishment of a chronic stressful event that leads to the infiltration of inflammatory cells, including macrophages and neutrophils. The subsequent development of histological atypia reflects several abnormalities in the cellular physiological processes. Subsequently, oncogenic mutations in these same cells can lead to the development of a cancerous lesion. Interestingly,

the transition from a premalignant lesion to cancer is characterized by a shift of an immune contexture dominated by Th1, Th17, T effector cells and M1 macrophages to a microenvironment where Th2 cells, Tregs, M2 macrophages and MDSC orchestrate the response. The metastatic spread of the malignant cells can occur in the early or late stages of tumor development. It is unknown if the immune infiltrating cells arrive either from cells educated in the primary lesion or 'de novo' in the metastatic tumor.

zone adjacent to the B cell follicles. Data on lung cancer have demonstrated that the density of mature DC, which home exclusively in TLS, is associated with a coordinated Th1/CD8 cytotoxic response and a favorable clinical outcome for lung cancer patients [28–30]. In addition, a high density of follicular B cells correlated with long-term survival both in untreated, early-stage patients and in advanced-stage patients treated with neoadjuvant chemotherapy [44]. Thus, TLS may orchestrate the local intratumoral T and B cell responses and play a protective role in cancer, as they do during infection.

The fine analysis of the immune and inflammatory tumor microenvironments brings, therefore, a highly dynamic image of the events that occur during cancer development. This extensive set of complex and intricate interactions between the components of the tumor microenvironment has profound consequences for tumor evolution, defined by the TNM classification. Indeed, recent breakthrough advances in this field have shown that a well-defined local immune score is a major and independent prognostic factor at all stages of disease progres-

sion, in primary as well as in metastatic tumors [26, 45]. This review will describe the immune contexture of primary and metastatic tumors, its impact on patient outcome and the importance of the local 'immunoscore' and of the immune contexture in the clinical setting.

The Immune Contexture of Primary and Metastatic Tumors

Following the cancer-initiating genetic modifications of normal cells, a tumor starts growing firstly as a benign 'in situ' tumor. This precancerous stage is characterized by a tumor with well-defined borders and is contained in a milieu of immune and inflammatory components (fig. 1). Thus, the 'in situ' microenvironment shows a strong Th1 and macrophage M1 orientation with a high production of IL-12, interferon gamma (IFN γ) and inducible nitric oxide synthase, which activate Th1 and natural killer (NK) cells and directly kill tumor cells or inhibit tumor cell proliferation [46]. The transition from a

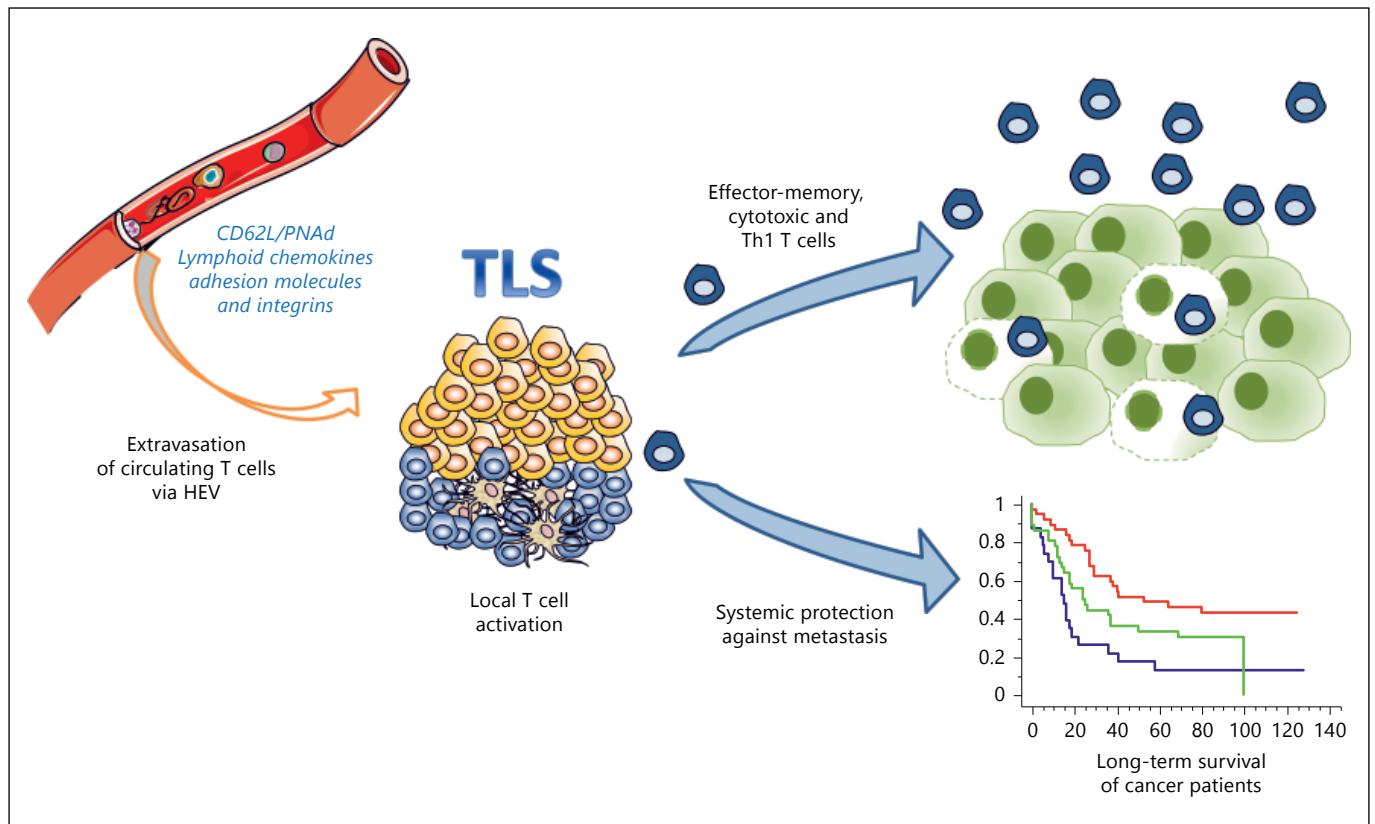


Fig. 2. Imprinting of the local immune contexture by cancer-associated TLS. In some tumors, the presence of a favorable or at least a permissive milieu allows the neogenesis of TLS. Under this con-

dition, TLS may modulate the microarchitecture of the tumor microenvironment, i.e. its vasculature, the immune infiltrate and ultimately the clinical outcome of the cancer patient.

precancerous stage to an invasive tumor is marked by a shift from a Th1 to a non-Th1 (mainly Th2) phenotype and from an M1 to an M2 phenotype. The local level of $\text{IFN}\gamma$ decreases, that of IL-6 increases as well as that of indoleamine 2,3-dioxygenase and VEGF. The tumor loses its neat barriers and locally invades the surrounding tissue by modulating the expression of integrins or cadherins with the help of urokinases, metalloproteinases and growth factors secreted by tumor cells and other components of the tumor microenvironment (fibroblast, pericytes or immune cells such as invasive macrophages) [47]. This process is also supported by the mechanisms of angiogenesis and increased vascular permeability induced by the tumor, particularly through growth factors such as VEGF [48–51], bFGF (basic fibroblast growth factor), angiopoietin or ephrin [52–54]. During this invasive local tumor stage, the kinetics of the evolution of the primary tumor towards a more aggressive metastatic tumor are not totally fixed by a predefined program. In fact, the transition to a metastatic tumor depends on the genetic

characteristics of the tumor cells and on the host response; here immune elements are essential. The genome of tumor cells is highly plastic and undergoes multiple alterations as the tumor develops. Gene mutations, but also chromosome amplifications and deletions and epigenetic modifications resulting in gene silencing or synthesis of abnormal proteins, accumulate, rendering tumor cells more aggressive. Not only do tumor cells become autonomous in order to proliferate, they also acquire characteristics that favor their migration and metastasis. Thus, the tumors produce VEGF, which increases the neovascularization that they will need to counteract local hypoxia. During this process, hypoxia-inducible factor (HIF)-1 α acts as a master transcription switch for the regulation of oxygen homeostasis [55]. Indeed, under normoxic conditions, the von Hippel-Lindau tumor suppressor protein binds to HIF-1 α and promotes the ubiquitinylation of the complex for its rapid proteasomal degradation [56–58]. Under hypoxic conditions, HIF-1 α binds to the promoters of genes that mediate angiogene-

sis such as the gene encoding VEGF [59, 60]. Tumors also secrete chemokines that attract myeloid cells, resulting in the local suppression of antitumoral immune reactions [61]. Finally, tumors undergo immunoediting that allows them to escape immune attack [62]. Under a selection pressure, it is possible that tumor variants, less immunogenic and/or more resistant to lysis, emerge. In an environment more conducive to their survival (immunosuppression), these tumor cells, that have undergone a Darwinian selection, are able to survive and enter into an equilibrium state in which the innate and adaptive immune system control the tumor [62, 63]. This phase of tumor development can take several years, as is suggested by the clinical data of transplantation patients [64, 65]. Ultimately, some tumor cells will acquire mutations that allow them to escape the control of the immune system, proliferate and give rise to a clinically detectable tumor. Thus, the immunoediting theory suggests that the hosts 'sculpt' their tumor cells to become less immunogenic and that this process requires immunocompetence, and does not in fact occur in individuals who are immunodeficient due to primary genetic or acquired deficiencies or because they are on immunosuppressive treatment [66, 67].

So the microenvironment of primary tumors contains variable densities of immune cells, including T cells, B cells, NK cells and DC, in addition to various populations of macrophages, myeloid-derived suppressor cells (MDSC), neutrophils and mast cells [9] (fig. 1). A closer look into the organization of the immune microenvironment reveals that the various cells are not randomly distributed within a tumor. Thus, T cells are found not only at the invasive margin of the tumor, but also in the core (or center) where some CD8⁺ T cells are found in close interaction with tumor cells. The majority of NK cells are also found in the invasive margin, but dispersed in the tumor stroma and often in an anergic state accompanied by the downregulation of activating receptors such as DNAM1, CD16, Nkp30 [68–71] and a specific local gene expression signature [72]. The inhibition of NK cell cytotoxicity can be reproduced in vitro, upon culture of NK cells with tumor cells [68, 73].

A striking characteristic of the tumor microenvironment is the presence of TLS localized at the invasive margin of the tumor and composed of a T-cell-rich area adjacent to a B cell follicle [28, 29, 44, 45, 74] (fig. 2). Mature DC infiltrate the T cell zone where they are in close contact with CD4⁺ and CD8⁺ T cells. Follicular DC, tingible body macrophages and follicular helper T cells (T_{FH}) are in contact with B lymphocytes within the B cell zone. The

B cells proliferate actively and can form a typical germinal center where the somatic hypermutation and class switch recombination machineries are activated [29, 44]. These lymphoid islets are reminiscent of the TLS, which are considered to be a privileged site for the generation of local adaptive immune reactions. For example, in a model of infection using influenza virus in mice lacking secondary lymphoid organs, it was shown that IFN γ and TNF α -secreting CD8⁺ T cells could be generated in situ and were able to control viral infections and that a T cell memory response could be induced within these structures [32, 43]. A specific molecular signature consisting of a set of adhesion molecules, integrins and chemokines for the recruitment of T cells has been identified within the TLS of human lung cancers [28]. It comprises the selectin CD62L, which is most likely involved in the crossing of the PNA⁺ high endothelial venules (HEV) by T cells [28]. In accordance with these observations, an increase of both CD62L⁺ cells and PNA⁺ HEV has been observed in gastric carcinoma when compared to normal gastric mucosa [75]. The presence of CD62L⁺ cells is usually found in SLO, where its interaction with PNA expressed by HEV plays a key role in the recruitment of both naïve and central-memory T cells [76]. Overexpression of 3 selective integrins α L, α 4 and α D is associated with the presence of T cells infiltrating the TLS of lung cancers, and their ligands (ICAM-2 and ICAM-3, VCAM-1 and MAdCAM-1) expressed on T cells have been detected on the HEV of TLS [28]. Interestingly, stronger expression of VCAM-1 and MAdCAM-1 has been linked to better survival in colorectal carcinoma [77].

A specific pattern of chemokine expression is selectively associated with the T lymphocytes that are present in lung cancer TLS (CCL5, CCL17, CCL19, CCL21, CCL22, CXCL13 and IL-16) in accordance with the expression of their receptors by the TLS-infiltrating T cells [28]. CCL19 and CCL21 are involved in the recruitment of CCR7-positive activated/mature DC as well as naïve and central-memory T cells to SLO. Intratumoral injection of exogenous CCL19 and CCL21 induces a strong influx of immune cells, mainly comprising effector T cells and activated DC in solid tumors; this is associated with tumor regression [78–81]. Thus, the CCR7 ligands CCL19 and CCL21 seem to be involved in the migration of T cell subsets and mature DC in cancer-associated TLS. CCL19 is secreted by mature human DC and may play a crucial role for TLS maintenance in lung cancer. The chemokine CXCL13 is known to recruit some B cell subpopulations and T_{FH} through its unique receptor CXCR5. It could be hypothesized that CXCL13 plays a major role in the re-

cruitment of T_{FH} , in accordance with the presence of T cells within the germinal center of the lung cancer TLS. The chemokine CCL5, which is commonly expressed in an inflammatory environment and plays a role in Th1 lymphocyte recruitment [82, 83], has been associated with a good prognosis in early-stage lung adenocarcinoma [84]. Thus, CCL5 could be responsible for the recruitment of a subset of memory Th1 $CD4^+$ or $CD8^+$ T cells that, once recruited into the TLS, could be reactivated and then leave the lymphoid structure in response to other CCR5 ligands in the tumor microenvironment. CCL17 and, to a lesser extent, CCL22 gene expression are also associated with TLS. These chemokines share the CCR4 receptor, which is expressed on central memory Th2 T cells, cutaneous T cells and regulatory T cells [85, 86]. CCL17 and CCL22 are able to recruit effector T cells [87–90] and Tregs in cancer mouse models [90–93]. Human CCL22 expression is associated with tumor regression [88–90, 94] and good prognosis in lung cancer [95] whereas in breast cancer, it correlates with Treg infiltration and poor prognosis [91]. Of note, the same set of chemokines involved in the T cell migration in the TLS of lung cancers has been identified as directing the migration of T cells subsets in SLO, with the exception of IL-16. The expression of this selective set of chemoattractants in TLS strongly suggests that TLS may represent a gateway for the recruitment of peripheral T cells into lung tumors. Bona fide SLO germinal centers contain T_{FH} cooperating with B cells. Indeed, T_{FH} were recently detected in peritumoral TLS attracted by CXCL13 and IL-21 [96] and were found to predict an improved disease outcome in breast cancer [97] and colorectal cancer [96].

Strikingly, intratumoral naïve T cells can only be found in the TLS. We thus proposed that tumor-induced TLS are important in shaping efficient antitumor immune responses. In this model, naïve T cells enter into the T cell zone via HEV, become educated by mature DC and migrate toward the tumor nests or recirculate through lymphatic vessels to secondary lymphoid organs. Here, the T cells can be subsequently reactivated to control metastatic cells [30, 98] (fig. 2).

These observations have been made in primary tumors and until recently, the immune status of the metastatic microenvironment was poorly known. An evolutionary perspective from the tumor side would predict that metastatic tumors have undergone immunoediting [62, 63, 67] and may have become immunologically silent when they eventually metastasize to a distant organ. This view has recently changed after investigators began to analyze the immune microenvironment of metastatic sites thoroughly. Al-

though the metastatic tumor cells may have escaped the control of the immune system in the primary tumor, the immune infiltrate in metastatic sites remains present and active [74, 99]. Our recent assessment of a large series of primary tumors documented that metastatic sites may have an immune microenvironment similar to that of primary sites, with T and B lymphocytes, NK cells, macrophages, granulocytes and mast cells located in the center and at the invasive margin of the tumor nests and organized in TLS [45]. Unexpectedly, it was found that although some quantitative differences in lymphoid cell densities between different tumor sites are observed, a positive correlation was observed between the immune cell densities in the primary tumor and in the subsequent metastatic sites in the same patient. These data suggest an ‘imprinting’ of the immune contexture by the malignant cells/host interactions at the early stages of tumor development. The natural history of the disease is therefore that of a dynamic evolution of the cancer cells to escape and/or subset the initial imprinting in order to render the immune reaction blind, anergic or suppressed. Moreover, comparative histological analysis has revealed profound similarities in the morphology, organization and vascularization of primary and metastatic tumors. For example, in colorectal cancer, tumors form glands that are surrounded by an abundant and collagenous stroma and by a high density of TLS. In contrast, in renal cell carcinoma, cohesive tumor cell nests are separated by a thin and highly vascularized stroma with rare TLS. These results further emphasize the predominant influence of the origin of the tumor in the composition and organization of the immune infiltrate that is reproduced throughout the course of the disease [45].

The Immune Microenvironment: A Strong Prognostic Factor for Clinical Outcome

The immune landscape described above may vary from tumor to tumor but also, more importantly, from patient to patient even at a given stage. At the time of diagnosis, or of surgery, the immune landscape is a hallmark of how the patient and the tumor have interacted. Since different elements of the microenvironment may impact the clinical evolution of cancer differently and since the tumor immune microenvironment at the clinical stage results from an ‘imprinting’ during the preclinical phase, it is reasonable to postulate that its analysis may provide prognostic and, potentially, theranostic markers. During the last decade, comprehensive analyses have been conducted in many different cancers to characterize

and assess the clinical impact of the various components of the tumor microenvironment. Jass [100] and Clark et al. [101] reported in the late 1980s that a strong lymphocytic infiltrate correlated with a favorable prognosis in epithelial tumors. In 2003, Zhang et al. [102] reported the analysis of a large cohort of ovarian cancers in which a high density of intratumoral CD3⁺ T cells were associated with longer survival. The most comprehensive study was performed on annotated tumor libraries collected from large cohorts of colorectal cancer patients. It was first reported that the presence of high densities of memory T cells correlated with the lack of early signs of metastasis, i.e. venous emboli, lymphatic invasion and perineural infiltration [103]. The quantification of the T cell infiltration both in the center of the tumors and at the invasive margin revealed that the two regions were important to consider, with a high density of memory T cells in both being associated with the best prognosis in terms of disease-free and overall survival [26]. The analysis of the functional orientation of the immune microenvironment showed that a high and coordinated expression of genes governing adaptive immunity with a Th1/cytotoxic orientation correlated with a good prognosis in terms of tumor recurrence and overall survival. A fine analysis of the immune microenvironment in patients with different tumor sizes and at different TNM stages revealed that although the percentage of patients with high densities of memory T cells decreased in large primary tumors, it was always low in patients with recurrence, suggesting that the local memory T cell response was particularly efficient in controlling the emigration and nidation of potentially metastatic tumor cells [104]. In colorectal cancer, the impact of other T cell subsets was less striking than that of memory Th1/cytotoxic T cells. High densities of Treg cells and B lymphocytes correlate with good prognosis [105, 106]. In contrast, high densities of Th17 lymphocytes correlate with an unfavorable prognosis [105]. Th2 lymphocytes have no significant clinical impact [105], and the role of tumor-associated macrophages in colon cancer remains more controversial [107, 108]. An increased infiltration of NK cells is generally associated with a good prognosis [109]. These data led Pages et al. [27] to propose an immunoscore based on the determination of two immune markers (CD3/CD8 or CD3/CD45RO or CD8/CD45RO) in two regions (center of the tumor and at the invasive margin) of the tumor. In this model, the maximum score of 4 was associated with the best survival and the minimum score of 0 was associated with poor survival; the prognostic value for the patients' clinical outcome was confirmed in a limited series of random-

ly selected patients. This immunoscore is being tested in a worldwide effort on samples from 10,000 colorectal cancer patients to help establish the immunoscore as an independent prognostic marker for colorectal cancer [110, 111].

The immunoscore approach has been utilized in many laboratories in a large array of different types of tumors. Similar findings concerning the favorable clinical impact of a memory Th1/cytotoxic immune microenvironment have been reported in melanoma [112, 113] and head and neck [114], breast [115, 116], ovarian [102, 117, 118], esophageal [119, 120], prostate [121, 122], lung [123, 124], pancreatic [125], anal [126] and urothelial [127] cancer. The clinical impact of other T cell populations was less concordant. Th2, Th17 and Treg lymphocytes were associated with poor prognosis in half of the evaluated cases and with no impact or good prognosis in the other half [9]. It is, however, striking that in a large number of tumor types, a worldwide consensus emerges that a strong Th1/cytotoxic orientation is associated with favorable prognosis, suggesting that the immunoscore could be applied to cancers other than colorectal cancer and could become a reference test in oncology.

There are, however, some discrepancies in the overall consensus. Thus, a high infiltration of CD8⁺ or CD45RO⁺ T cells has been reported by several authors to correlate with poor survival of patients diagnosed with renal cell cancer [128–131], ocular melanoma [132, 133], hepatocellular carcinoma and lymphoma [134–136]. These paradoxical findings reflect the complexity of the tumor microenvironment, and suggest that some of its components can buffer or even reverse the positive impact of a Th1-oriented adaptive reaction. Previous reports by our group suggest that the presence of a strong inflammatory microenvironment, the presence of macrophages, Th2 cells and the expression of VEGF can abrogate, at least to some extent, the good impact on prognosis associated with a Th1-oriented response [45, 137, 138]. Further research is needed in this field to better understand this trend.

From the Immunoscore to the Immune Contexture

The immunoscore, based on the density, location and structural orientation of memory CD8⁺ T cells, is a powerful prognostic marker in many cancers. However, a high density of these cells does not always correlate with favorable prognosis. This observation raised a question about the functionality as well as the interplay of these T cells with other components of the tumor microenviron-

ment that may affect clinical outcome. For instance, high VEGF expression counteracts the favorable prognosis associated with high expression of granulysin – a molecule produced by cytotoxic lymphocytes – in primary colorectal cancer, i.e. patients with high granulysin and high VEGF expression in their tumor have a prognosis similar to patients with low granulysin expression [137]. In addition, high VEGF expression and intense vascularization in the primary tumors of renal cell carcinoma and ovarian, colorectal and lung cancer are associated with a poor clinical outcome [139–141].

We recently investigated the impact of key elements of the immune, inflammatory and angiogenic tumor microenvironment on the prognostic value of CD8⁺ T cells. We paid great attention to the role of the TLS in primary lung cancer, and found that a high density of mature DC, a marker of lung cancer-associated TLS, correlates with good, disease-free, disease-specific and overall survival [29]. In addition, we found that high TLS density was associated with a strong coordinated expression of genes governing Th1/cytotoxic functional orientation, but that such coordinated gene expression did not occur in tumors with scarce TLS [30, 142]. This finding suggests that intratumoral T cells may be different when they are generated in tumor-adjacent TLS than when they are directly recruited from the peripheral blood to the tumor. Indeed, patients with a high density of mature DC and CD8⁺ T cells in their tumor have a favorable outcome whereas patients with a low density of mature DC have a poor prognosis even when they have a high density of CD8⁺ T cells [30, 142].

We further investigated the role of TLS and other microenvironmental factors in lung metastases from colorectal and renal cell cancers. These two cancer types differ in that the former has numerous TLS and poor vascularization while the latter has rare TLS and high tumor-associated neoangiogenesis [45, 143]. We found that a high density of CD8⁺ T cells in the center and at the invasive margin of lung metastases from colorectal cancer correlated with long-term survival [45] as in the primary tumors [27, 143]. In contrast, a high density of CD8⁺ T cells in metastases from renal cell cancer [45] correlated with poor survival as in the primary tumors [131]. Analyzing the other components of the tumor microenvironment in depth, we found that strong CD8⁺ T cell infiltration in colorectal metastases correlated with a low expression of genes governing inflammation (IL-6 and STAT3) and angiogenesis (VEGF) whereas in renal cell metastases, the correlation between the strong infiltration and the expression of these genes was positive. It has been shown that VEGF and IL-6 could induce the inhibition of DC

differentiation and promote the differentiation of monocytes into macrophages [144–148]. Many studies have also suggested that VEGF plays an important role in tumor development by inducing a noncoordinated immune response, affecting Th1 and cytotoxic responses and contributing to the progression of the tumor [149, 150]. Our data suggest that the inflammatory and angiogenic components of the tumor microenvironment modulate the impact of the immunoscore, even when there is a Th1/cytotoxic orientation [45]. Numerous studies have reported that in renal cancers, intratumoral lymphocytes were anergic [151, 152], but once extracted from their environment, they were able to lyse tumor cells *ex vivo* [153]. Thus, in addition to disrupting the innate and adaptive immune responses, the unique characteristics of the renal cell carcinoma microenvironment are able to modulate the impact of the T cell infiltration. In the case of lung metastases from renal cell cancer, we reported that a high density of NK cells in the tumor stroma correlated with favorable prognosis in terms of overall survival [45], similar to the findings of Donskov and von der Maase [154]. There may therefore be two situations: (1) a strong Th1/CD8⁺ T cell infiltration accompanied by (2) a weak inflammatory and angiogenic environment that combine to be a major positive prognostic factor. The fact that, in primary colorectal cancer, Th17 cells which promote inflammation and Treg cells which may inhibit inflammation are of bad and good prognostic value, respectively, reinforces this hypothesis [105]. When a high CD8⁺ T cell infiltration is associated with a weak intratumoral Th1/cytotoxic functional orientation due to a lack of TLS and/or a strong inflammatory and angiogenic environment, it is paradoxically associated with poor prognosis. However, it is important to note that the immune pattern is the strongest independent factor for overall survival in colorectal and renal cell carcinoma metastases [45]. These observations led us to propose that the immunoscore should be completed by the characterization of the immune contexture of human tumors [9], taking into account not only the location, density and functional orientation of memory Th1/cytotoxic T cells but also the quality and density of TLS and the overall inflammatory and angiogenic components of the tumor microenvironment.

The Immune Contexture as a Therapeutic Factor

The last few years have witnessed the emergence of novel efficient immunotherapies, such as a sipuleucel-T for castration-resistant prostate cancer [155], cell therapy

with engineered T cells for B cell malignancies [156], anti-tumor monoclonal antibodies [157–159], T-cell-engaging bispecific antibodies in non-Hodgkin lymphoma [160] and anti-checkpoint antibodies in melanoma [161, 162] lung cancer or pancreatic cancer [163]. These therapies have the potential to induce long-term survival in responding patients that is usually not due to the classic rapid tumor shrinkage seen after chemotherapy [163–166].

Interestingly, a strong antitumoral immune response before and after treatment has proven to be an important predictor of the response to immunotherapies. Indeed, the expression of immune-related genes in the tumor either before or after treatment with anti-CTLA-4 antibodies in melanoma seems to accurately predict the therapeutic response in the clinical setting [167]. Furthermore, patients with a broader antibody response against tumor antigens have a higher probability of tumor regression [168]. Posttreatment activation of antigen-presenting cells and T cells has been also associated with a good response to cell therapies (i.e. sipuleucel-T) in patients with advanced prostate cancer [169] and to MAGE-peptide vaccination in patients with advanced melanoma [170]. A higher density of immune cells before and after treatment is also a common hallmark in the tumors of patients who respond to treatment [171]. Taken together, these observations support the use of the immunoscore as a biomarker to predict the ability of a patient to respond to immunotherapies [110, 111].

Indeed, a common indication of how a patient will respond to an immunotherapy is the presence or induction of a high density of intratumoral T cells, prompting the use of the immunoscore to follow patients receiving immunotherapies. Moreover, treatment with ‘classical’ chemotherapies, antiangiogenic molecules or even antitumor cytotoxic monoclonal antibodies can induce long-term survival in patients when the therapies induce a memory-adaptive T cell response [172, 173] and/

or decrease myeloid-derived suppressor cells and regulatory T cells [174–177] or normalize tumor-associated vasculature [178, 179]. It appears, therefore, that while classic cancer treatments with surgery and chemotherapy result in a short-term tumor reduction, it is only treatments which modulate the immune microenvironment by increasing Th1/cytotoxic memory T cells and/or decreasing inflammation and angiogenesis that will induce a durable response and increase survival. Combinations of several therapeutic approaches aimed to reach this goal are being actively developed.

Concluding Remarks

The recent decade has witnessed a change in the view of cancer as an autonomous cellular disease [3] to that of a regulated disease involving the immune, inflammatory and angiogenic components of the microenvironment [7]. A greater understanding of this tumor/host microenvironment interaction will likely stimulate the development of prognostic and theranostic biomarkers, the identification of novel target antigens for therapeutic intervention and the improvement in tools for the long-term management of cancer patients.

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