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The split nature of tumor-infiltrating leukocytes
Implications for cancer surveillance and immunotherapy

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Abbreviations: TIL, tumor-infiltrating lymphocyte; Th, helper T cell; Treg, regulatory T cell; NK, natural killer cell; MDSC, myeloid-derived suppressor cell; IFN, interferon; IL, interleukin; VEGF, vascular endothelial growth factor; ACT, adoptive cell therapy

An important development in tumor immunology was the identification of highly diverse tumor-infiltrating leukocyte subsets that can play strikingly antagonistic functions. Namely, "anti-tumor" vs. "pro-tumor" roles have been suggested for Th1 and Th17 subsets of $CD4^+$ T cells, Type I or Type II NKT cells, M1 and M2 macrophages, or N1 and N2 neutrophils, respectively. While these findings are being validated in cancer patients, it is also clear that the balance between infiltrating $CD8^+$ cytotoxic and $Foxp3^+$ regulatory T cells has prognostic value. Here we review the pre-clinical and clinical data that have shaped our current understanding of tumor-infiltrating leukocytes.

Introduction

A fundamental principle of cancer immune surveillance is that tumors are infiltrated by leukocytes, particularly lymphocytes, capable of recognizing and targeting transformed cells, thus leading to their elimination before the tumor becomes clinically apparent. Moreover, the efficacy of immunotherapy against established tumors presumably depends on lymphocyte recruitment and effector function within the tumor bed. However, a major obstacle to anti-cancer therapy is the local immune suppression commonly found within the tumor microenvironment.[1] While earlier work had focused on tumor cell-derived factors that inhibit the local immune response, the past few years have demonstrated a dramatic contribution of leukocytes themselves to this "pro-tumor" environment. Recent reports have further clarified this paradoxical leukocyte behavior by identifying a very heterogeneous set of subpopulations, both of lymphoid and myeloid origin, that can play strikingly antagonistic roles within the tumors they co-infiltrate.

The prototypic anti-tumor function, displayed by various lymphocyte subsets (**Fig. 1**), is cytotoxicity via the perforin/granzyme system or, alternatively, by engaging death receptors (such as Fas). These properties are further promoted by interferon γ (IFNγ), the signature Th1 cytokine that is, in fact, secreted by multiple cell types (see below), often together with tumor necrosis factor (TNF). By contrast, cytokines such as TGFβ or IL-10 are highly immunosuppressive, and other secreted factors, like VEGF, directly promote angiogenesis and thus tumor growth (**Fig. 2**). The detailed characterization of gene expression and cytokine profiles in leukocyte populations isolated from tumor biopsies (or draining lymph nodes) has been instrumental in revealing the heterogeneity of tumor-infiltrating leukocytes, both of lymphoid and myeloid nature, which we will discuss in this review.

Many of the key studies on tumor-infiltrating leukocytes have been performed in mouse tumor models. Although they present several important limitations, including the artificial homogeneity and laboratory selection of tumor cell lines used in transplantable models, the lack of relevant physiology (including interactions between autologous tumors and immune cells) in xenograft models, and the commonly short span (2–4 weeks) of all these tumor development experiments, animal models provide a unique possibility of tracking and manipulating cancerigenesis in vivo.

This notwithstanding, it is obviously essential to validate all the findings from mouse tumor models in human cancer samples. Therefore, in this review we will discuss and summarize the most recent advances, both in the laboratory and in the clinic, in our understanding of the biology of tumor-infiltrating leukocytes. We will highlight their anti- or pro-tumor functions in mouse models, and how these translate (or not) into prognostic value in cancer patients.

The Traditional Players: NK, $CD8^+$ T and Th1 Cells

It has been known for three decades that NK cells and $CD8^+$ T lymphocytes, including those extracted from tumor biopsies, can efficiently kill transformed cells. Collectively, these killer lymphocytes recognize two important types of tumor antigens (among others): processed peptides presented by MHC Class Ia proteins via TCRαβ; and non-classical (Class Ib) MHC proteins via NKG2D.[2] The latter, which is expressed on NK, $CD8^+$ and also γδ T cells, has been recently shown to be a key genetic determinant of cancer immune surveillance.[3]

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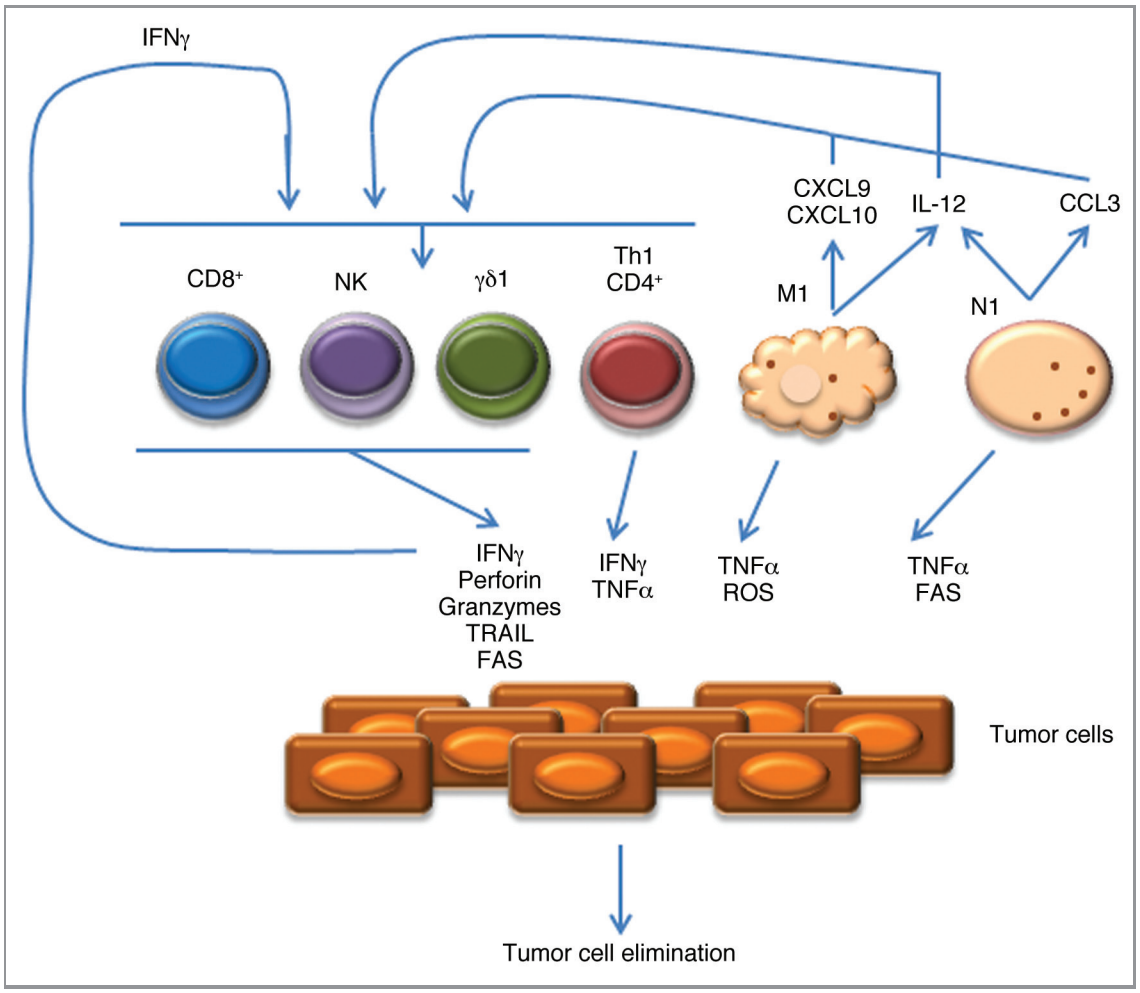

Figure 1. Anti-tumor infiltrating leukocytes and molecular mechanisms of action. Representation of the main anti-tumor lymphoid and myeloid cells. N1 and M1 refer to neutrophil and macrophage subsets, respectively. γδ1 and Th1 refer to IFNγ-producing γδ and CD4+ T cells, respectively. Depicted are also molecules produced by these leukocytes, including cytokines that impact on cell differentiation and expansion, and chemokines that control their recruitment/infiltration into tumors.

NK and CD8+ cells provide highly complementary anti-tumor strategies. Indeed, as demonstrated by the seminal work of Kärre and Kiessling, the downregulation of MHC class Ia, which is a common mechanism of evasion against CD8+ cells, renders tumors more susceptible to NK cell-mediated lysis. This "missing self" recognition by NK cells is based on a set of MHC class Ia-specific inhibitory receptors that include killer cell immunoglobulin-like receptors (KIRs) in humans, lectin-like Ly49 molecules in mice, and CD94/NKG2A heterodimers in both species; in fact, NK cells express a complex repertoire of inhibitory and activating receptors that calibrate this anti-tumor function, while ensuring self-tolerance.[4,5] In result, NK cells eliminate tumors that lack MHC class Ia expression; or that overexpress ligands for activating NK receptors like NKG2D or the natural cytotoxicity receptors NKp30, NKp44 and NKp46.[5] Furthermore, NK cells express high levels of low-affinity Fc receptor for IgG (CD16), which allows them to mediate antibody-dependent cell-mediated cytotoxicity (ADCC).[6]

NK cells have been described to infiltrate various types of tumors in the skin, lung, gut and kidney.[5] Recent data on human NK cells infiltrating highly aggressive non-small cell lung cancers (NSCLC) showed a profound alteration of their phenotype, with decreased ability to degranulate and to produce IFNγ, when compared with NK cells from distal lung tissues or blood from the same patients or from healthy donors.[7] This functional impairment of NK-TILs correlated with decreased expression of NKp30, NKp80, DNAM-1, CD16 and ILT2 receptors. Interestingly, among these, NKp30 has been shown to affect the prognosis of gastrointestinal stromal tumors through a specific pattern of alternative splicing.[8]

Various immunotherapeutic strategies have been proposed to tackle the common defects of NK cell activity in cancer patients:[5] activation of endogenous NK cells (with cytokines like IL-2, IL-15 and IL-18), NK-cell adoptive immunotherapy, NK-cell-based donor lymphocyte infusions and allogenic stem cell transplantation (SCT).[6] Although globally the objective responses

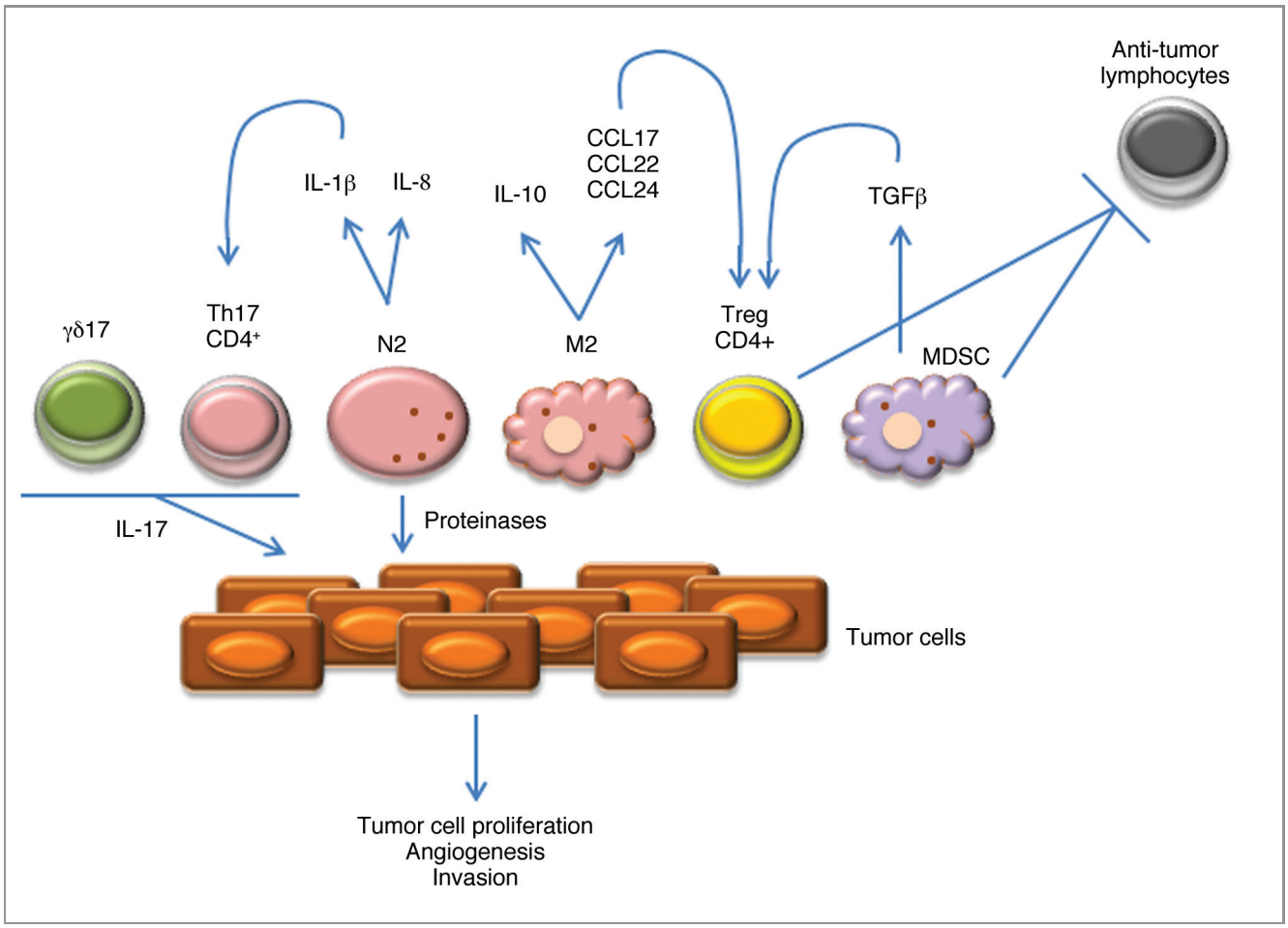

Figure 2. Pro-tumor infiltrating leukocytes and molecular mechanisms of action. Representation of the main pro-tumor lymphoid and myeloid cells. N2 and M2 refer to neutrophil and macrophage subsets, respectively. γδ17 and Th17 refer to IL-17-producing γδ and CD4+ T cells, respectively. Depicted are also molecules produced by these leukocytes, including cytokines that impact on cell differentiation and expansion, and chemokines that control their recruitment/infiltration into tumors.

have been disappointing, some data from allogenic and, more recently, haploidentical hematopoietic SCT have shown clinical (in the absence of adverse) effects mediated by NK cells.[5] This inspires further translational studies aimed at enhancing NK cell recruitment to tumors and their functional activity in situ.

With regard to CD8+ T cell-based immunotherapy, many recent efforts have focused in activating and expanding CD8+ tumor-infiltrating lymphocytes (TILs) ex vivo and then re-infusing them into the cancer patient—adoptive cell therapy (ACT). ACT of CD8+ TILs into lymphodepleted metastatic melanoma patients has shown very high objective response rates, ranging from 50% up to 81%.[9] In fact, TIL-ACT (combined with high doses of IL-2) has mediated cancer regression in 49–72% of melanoma patients, and durable complete responses, beyond 3–7 y, are currently ongoing in 40% of the patients.[10]

In pre-clinical models, adoptively transferred naïve CD8+ cells were shown to infiltrate melanoma lesions, be activated in situ and differentiate into functional cytotoxic T lymphocytes (CTLs).[11] The naïve status of the infused population appeared to be an important parameter, as the differentiation stage of CTLs inversely correlated with their anti-tumor efficacy in vivo.[12] The enhanced anti-tumor function of naïve T cells was related to sustained effector cell development, prolonged cytokine production, and increased expansion in vivo.

Transduction of tumor antigen-specific TCRs[13] or chimeric antigen receptors (CARs)[14,15] represent exciting prospects to increase the efficacy of cytotoxic ACT. These strategies have thus far enabled cancer regression in patients with metastatic melanoma, synovial sarcoma, neuroblastoma and refractory lymphoma or leukemia.[10]

In addition to cytotoxicity, IFNγ secretion is a key anti-tumor function of CD8+ and NK cells, who share this property with various other lymphocyte populations, most notably "helper type 1" (Th1) CD4+ cells. These were first described 25 y ago in the context of the "Th1/ Th2" paradigm of immunity to infection, and since then clearly implicated in promoting anti-tumor responses: Th1 cells enhance the cytotoxic functions of NK and CD8+ cells, upregulate MHC Class I expression in tumor cells (a direct effect of IFNγ), and support CD8+ cell proliferation through the secretion of IL-2.[16] Moreover, Th1 cells condition

the antigen-presenting capacity of DCs and macrophages, thus shaping the CTL response. In fact, the combination of Th1 cell therapy with local radiation therapy augmented the generation of tumor-specific CTL at the tumor site and induced a complete regression of subcutaneous tumors.[17]

"New" Effector TILs: γδ T, NKT and Th17 Cells

The "Th1/Th2" paradigm for CD4+ T cell differentiation has been recently revised with the addition of Th17 cells, characterized by the production of interleukin-17 (IL-17). IL-17-deficient mice were shown to be more susceptible (than wild type animals) to tumor growth and lung metastasis.[18,19] Adoptive transfer studies from the Restifo lab showed that in vitro generated Th17 cells were more efficient at eradicating tumors than Th1 cells,[20] and this was recently associated with stem cell-like properties of Th17 cells.[21] Importantly, adoptively transferred Th17 cells gave rise in vivo to Th1-like effector cell progeny,[21] and IFNγ was actually necessary for the protective effects of adoptively transferred Th17 cells.[20] These data suggest that acquisition of Th1-like properties are required for an anti-tumor function by Th17 cells.

In stark contrast to the previous studies, IL-17-deficient mice presented reduced tumor growth in other models such as B16 melanoma and MB49 bladder carcinoma,[22] DMBA/TPA-induced skin carcinoma,[23] or in a spontaneous intestinal tumor model (driven by a mutation in the tumor suppressor gene APC).[24]

The pro-tumor functions of IL-17 have been tightly linked to angiogenesis: IL-17 has been shown to act on endothelial, stromal and tumor cells to induce the expression of pro-angiogenic factors like VEGF, Angiotensins, PGE2 and IL-8, and thus promote tumor vascularization.[25] The precise conditions that determine pro- vs. anti-tumor functions of Th17 TILs remain unclear and require further investigation.

Although Th17 cells are important providers of IL-17, this cytokine can be abundantly produced by other tumor-infiltrating leukocyte populations. Namely, murine γδ T cells can be the major source of IL-17, not only in homeostatic conditions,[26] but also upon infection or tumor challenge.[27,28] Like for Th17 cells, the role of IL-17 produced by γδ cells within the tumor microenvironment is controversial: it has been associated both with angiogenesis and promotion of tumor growth[25,27]; and with CD8+ T cell recruitment and the therapeutic effects of chemotherapy against several subcutaneous tumor lines.[28,29]

While the recently discovered ability of γδ cells to make IL-17 has attracted much attention, these lymphocytes were previously characterized as strong cytotoxic and IFNγ-producing cells, and thus prototypic anti-tumor mediators. Consistent with this, seminal work by Girardi and Hayday showed a decade ago that mice lacking γδ T cells were significantly more susceptible to chemically induced tumors.[30] This phenotype was subsequently extended to transplantable,[31] spontaneous[32] and transgenic[33] tumors.

In the murine B16 melanoma model, γδ T cells were shown to infiltrate tumor lesions already at day 3 post-transplantation and to provide a critically early source of IFNγ.[31] This contrasts with the above-mentioned findings on IL-17+ γδ-TILs.[27,28] A more detailed characterization of γδ-TILs is therefore required in a wider set of pre-clinical tumor models. This should take into account the two functional γδ T cell subsets recently identified on the basis of CD27 (and CCR6) expression: CD27+ γδ cells make IFNγ but no IL-17, whereas IL-17 production is restricted to CD27- γδ cells.[34]

γδ T cell-based clinical trials have thus far concentrated on the highly IFNγ-polarized (and cytotoxic) Vγ9Vδ2 subset that constitutes most of γδ cells circulating in the human peripheral blood. As these cells are specifically reactive to non-peptidic phosphoantigens, they can be selectively activated and expanded both in vitro (for ACT) and in vivo. In cancer patients, γδ T cell-based immunotherapy has thus far produced objective responses in the range of 10 to 33%.[35] Future research should also take into account the important roles played by NK receptors, including NKG2D[36] and NKp30,[37] in tumor cell recognition by Vγ9Vδ2 cells and by Vδ1 cells (which predominate in tissues).

NKT cells also employ NK receptors, as well as CD1d-restricted TCRs to recognize tumor targets. The vast majority of these T cells are canonical or invariant NKT (type I NKT) cells that possess a specific TCRα rearrangement (Vα14Jα18 in mice; Vα24Jα18 in humans), associated with Vβ chains of limited diversity. All the other NKT cells that are CD1d-restricted and do not express this invariant TCR are called Type II NKT cells.[38,39] Although CD1d-deficient mice showed increased susceptibility to MCA-induced sarcomas,[40] there is evidence of functional heterogeneity also within NKT cells: while Type I NKT cells seem to be protective, Type II NKT cells mostly suppress tumor immunity.[39,41]

In terms of cytokine production, activated NKT cells are potent providers of IFNγ and IL-4 (and, to lesser extent, of IL-17). In the B16 metastatic melanoma model, a dual role of NKT cells was linked to immune suppressive IL-4 production by the thymus-derived subpopulation; and protective IFNγ production by liver-derived Type I NKT cells.[42]

Based on the pre-clinical evidence for an anti-tumor role of type I NKT cells, and the availability of a specific TCR agonist, α-Gal-Cer, several clinical trials have attempted to activate endogenous iNKT cells, or—more promising given by relative rarity of NKT cells in humans—perform ACT with (ex vivo expanded) Type I NKT cells. However, the clinical effects of αGal-Cer or NKT ACT have been very limited,[39] thus illustrating the difficulty in translating findings from animal models of cancer into improved immunotherapies.

The Inflammatory Phagocytes: TAMs and TANs

Macrophages and neutrophils are important myeloid cells of the innate immune system and major drivers of inflammatory responses. Given the long-established association between cancer and inflammation, it is not surprising that tumor-associated macrophages (TAMs) and neutrophils (TANs) can have great impact on the course of tumor progression. While most studies have associated TAM and TAN infiltration with promotion of tumor cell growth, some other reports have proposed some

anti-tumor roles. Once again, these opposing behaviors may be explained by heterogeneous TAM and TAN phenotypes, with distinct intra-tumor dynamics in various models.

Mirroring Th1/ Th2 polarization of $CD4^+$ T cells, two distinct subsets of macrophages have been recognized: the "classical" activated (M1) macrophage phenotype and the "alternatively" activated (M2) macrophage phenotype.[43] IFNγ drives the polarization toward M1 macrophages, which are characterized by abundant production of TNF, IL-12 and IL-23, CXCL9 and CXCL10, reactive nitrogen and oxygen species; and by high expression of MHC class II and costimulatory molecules (making them efficient antigen-presenting cells).[44] Conversely, IL-4 polarizes macrophages toward the M2 phenotype, which is associated with low levels of IL-12 but high levels of IL-10, IL-1RA and IL-1 decoy receptor. M2 cells also produce CCL17, CCL22 and CCL24, which results in the recruitment of Tregs and Th2 cells, eosinophils and basophils.[44]

The balance between M1 and M2 phenotypes seems to be controlled by NFκB signaling. Thus, NFκB targeting switched macrophages from an M2 to an M1 phenotype and led to ovarian tumor regression in vivo.[45] Nonetheless, the most frequent TAM phenotype seems to be M2.[43] Consistent with this, TAM depletion was associated with improved anti-tumor immunity in models of metastatic breast, colon and non-small lung cancers.[46] The pro-tumor roles of M2 macrophages derive from various molecular mechanisms, including the production of the pro-angiogenic mediator semaphoring 4D[47] and the invasive proteases cathepsins B and S.[48]

In the case of neutrophils, besides secreting cytokines and chemokines (such as IL-1β, IL-8, and IL-12), they produce large amounts of proteinases that remodel the extracellular matrix and promote the release of pro-angiogenic VEGF, thus supporting tumor cell growth and invasiveness.[49] Particularly important neutrophil proteinases are elastase[50] and matrix metalloproteinases MMP-8 and MMP-9.[51]

Despite being widely accepted as pro-tumor mediators based on multiple pre-clinical and clinical studies,[49] a dual nature of tumor-infiltrating neutrophils has also been suggested recently.[52,53] Thus, anti-tumor N1 and pro-tumor N2 subsets were described and modulated within tumors by TGFβ[52] or IFNβ.[54] Consistent with such a complex neutrophil activity within the tumor microenvironment, the concentration of reactive oxygen species also seems to determine either pro-tumor (genotoxicity at modest concentrations) or anti-tumor (cytotoxicity at high concentrations) effects.[49] Consequently, the depletion of total neutrophils can lead to either reduced[52] or increased[55] tumor burden, further illustrating the globally paradoxical roles of tumor-infiltrating leukocytes.

Immunosuppressive Leukocytes: Treg and MDSCs

Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of myeloid progenitors and precursors of macrophages, granulocytes and dendritic cells, which are better characterized by their strong capacity to inhibit both innate and acquired immunity[56] particularly T-cell responses.[57] Murine MDSCs can be identified by the expression of Gr1 (includes Ly6C and Ly6G, macrophage and neutrophil markers, respectively) and CD11b (characteristic of macrophages). In humans, MDSCs are characterized by a $CD11b^+$ $CD33^+$ $CD34^+$ $CD14^-$ $HLA\text{-}DR^-$ phenotype. Tumors produce various factors that promote MDSC expansion, such as IL-6, VEGF or GM-CSF, whereas they get further activated by local IFNγ, IL-1β or Toll-like receptor (TLR) signals.[57]

MDSCs use a diversity of mechanisms to suppress T-cell function, including the uptake of arginine and cysteine (essential amino acid for T cell activation) and the nitration of the TCR.[56] In addition, MDSCs have been recently shown to directly support tumor growth by promoting the epithelial-to-mesenchymal transition in melanocytes.[58]

The possibility of improving anti-tumor immune responses by targeting MDSCs has been explored in pre-clinical models. One of the chemical drugs that seem to be more effective for MDSC depletion was 5-fluorouracil (5-FU). In a model of thymoma EL4 cells transplanted subcutaneously, tumor-bearing mice treated with 5-FU showed reduced number of MDSC in tumor lesions. This associated with prolonged mouse survival and enhanced intratumoral $CD8^+$ T cell antigen-specific capacity to produce IFNγ.[59] Interestingly, combination therapy with an agent (cyclophosphamide, CTX) that reduces Tregs led to a synergistic protective effect. Consistent with this, another study showed that inhibition of MDSC and Treg function within B16 melanomas using blocking antibodies to CTLA-4 (already in clinical use—ipilimumab—in late-stage melanoma) and to PD-1 reduced tumor development and increased mouse survival.[60]

$Foxp3^+$ Tregs are well known to suppress the activation, proliferation and effector functions (such as cytokine production) of a wide range of immune cells, including αβ and γδ T cells, NK and NKT cells, B cells, macrophages and DCs. Suppressive functions displayed by Tregs include contact-dependent mechanisms, such as those that involve CTLA-4, PD-1 and GITR; and cytokine-mediated mechanisms such as TGFβ, IL-10 and IL-35.[61] TGFβ is particularly critical since, besides being strongly immunosuppressive, creates a potent positive feedback mechanism by instructing the differentiation of "inducible" Tregs.[1]

Experimental Treg depletion has been usually accomplished using anti-CD25 monoclonal antibodies, since there is a good correlation between CD25 and Foxp3 expression within $CD4^+$ T cells (although activated effector cells also upregulate CD25). Prophylactic Treg depletion in renal cell carcinoma and MCA carcinoma was shown to reduce tumor growth, with protection being dependent on $CD8^+$ and NK cells.[62,63]

While most studies have concentrated on the immunosuppressive function of Tregs, two recent reports have shown that they can also act by directly promoting tumor growth and dissemination. Thus, Treg TILs in ovarian cancer they secrete VEGF that promotes endothelial cell proliferation;[64] and in breast cancer they produce RANKL, which associates with lung metastasis.[65] Importantly, the latter study is one of many that demonstrates that Treg accumulation within tumors is a marker for poor clinical outcome.[66]

The Prognostic Value of Tumor-Infiltrating Leukocytes in the Clinic

Although a favorable association of high numbers of TILs in the primary tumors had been generally reported for decades in many human cancers, TILs had never reached the level of recognized prognostic marker (or proof for cancer immunosurveillance) probably due to their phenotypic and functional heterogeneity.[67] The recent observations that specific immune parameters have better prognostic value than standard staging systems, highlights the importance of the endogenous immune response in determining the clinical outcome. This may help to modify current classifications, and—most importantly—to identify the patients who would benefit the most from adjuvant immunotherapy.

Considering the data reviewed above, it is tempting to assume that good prognosis associates (for example) with $CD8^+$ and NK cells, whereas bad prognosis is linked to the accumulation of Tregs and MDSCs. Moreover, given the functional heterogeneity within many leukocyte populations, clearly distinct outcomes could be expected from Th1 vs. Th2 or Th17 $CD4^+$ subsets, M1 vs. M2 macrophages, N1 vs. N2 neutrophils. This level of refinement is obviously incompatible with traditional immunohistochemistry of cancer patient samples, thus requiring additional techniques like flow cytometry and molecular biology to provide an adequate characterization of tumor-infiltrating leukocytes. Furthermore, detailed imaging may also be important as to define the localization of TILs within the tumor mass. For example, in a pre-clinical model, $CD8^+$ T cells were recently shown to be trapped in the stroma and thus excluded from the core of tumor due to post-translational modifications (nitration) of the chemokine CCL2.[68] Of note, novel drugs that inhibited CCL2 nitration facilitated $CD8^+$ T cell infiltration and tumor regression.

The most comprehensive clinical studies correlating tumor-infiltrating leukocytes with disease outcome have been performed in colorectal cancer, where the general conclusion has been that disease free overall survival is positively associated with a coordinated Th1/ $CD8^+$ T cell infiltration[67] (**Table 1**). A similar result was reached for breast cancer;[69,70] and for hepatocellular carcinoma, where NK markers and the chemokines CCL2, CCL5 and CXCL10 were additional immune signatures predictive of patient survival (at early stages of the disease).[71]

By contrast, Treg infiltration has been generally associated with poor prognosis (**Table 2**). In ovarian carcinoma, melanoma, breast cancer, Hodgkin lymphoma and glioblastoma, the presence and frequency of Tregs correlated with tumor grade and with reduced patient survival.[66] These studies also highlighted the potential role for CCL17 and CCL22 (ligands for the chemokine receptor CCR4) in recruiting Tregs into tumors. The combined value of quantifying both $CD8^+$ and Treg TIL (antagonistic) subsets as prognostic of disease-free survival was demonstrated in hepatocellular carcinoma[72,73] and colorectal cancer.[74] However, in some cancer types, such as colorectal[75] and head and neck carcinomas,[76] Treg accumulation within tumors has been associated with favorable prognosis. This was suggested to be due to a dominant effect in suppressing infection-associated inflammation at mucosal interfaces.[77] Nonetheless, positive associations between survival and Treg numbers were also observed upon immunohistochemical analysis of biopsies from four types of lymphoma patients.[78,79]

Table 1. Tumor-infiltrating leukocytes associated with good prognosis for cancer patients

TIL	Cancer Type	References
$CD8^+$	colorectal cancer	74, 86, 87
	hepatocellular carcinoma	71, 72, 73
	esophageal carcinoma	84, 88, 89
	breast cancer	69
Th1 ($CD4^+$)	colorectal cancer	85
	hepatocellular carcinoma	71
	breast cancer	70
Th17 ($CD4^+$)	esophageal carcinoma	84
Tregs ($CD4^+$)	colorectal cancer	75
	head and neck carcinoma	76
	lymphoma	78, 79
γδ T cells	ovarian carcinoma	90
B cells	breast cancer	69
NK cells	esophageal carcinoma	84
	hepatocellular carcinoma	71

Whereas Th2 infiltration has been associated with poor prognosis in pancreatic cancer,[80] the role of Th17 TILs in human cancer is much more controversial. On one hand, Th17 cell infiltration has been correlated with poor prognosis in prostate cancer[81] and in hepatocellular carcinoma;[82] on the other, it has been associated with better overall survival in ovarian cancer[83] and in esophageal squamous cell carcinoma.[84] While the reasons for these discrepancies are unclear, it may be interesting to assess the co-production of IL-17 and IFNγ by Th17 cells, as well as their association with $CD8^+$ T cell recruitment.

Finally, a recent study attempted to integrate the prognostic values of Th1, Th2 and Th17 TILs by hierarchical clustering of signature gene transcripts in colorectal tumor specimens. The results showed that: the Th2 cluster did not correlate with prognosis; patients with high expression of the Th1 cluster had prolonged disease-free survival; and patients with high expression of Th17 cluster had poor prognosis.[85] In the future, we believe

Table 2. Tumor-infiltrating leucocytes associated with poor prognosis for cancer patients

TIL	Cancer Type	References
Th17 ($CD4^+$)	colorectal cancer	85
	hepatocellular carcinoma	82
	prostate cancer	81
Th2 ($CD4^+$)	pancreatic cancer	80
Tregs ($CD4^+$)	colorectal cancer	74
	hepatocellular carcinoma	72
	ovarian carcinoma	91
	breast cancer	92
MDSCs	esophageal, pancreatic and gastric	93
Macrophages	breast cancer	94
Neutrophils	renal cell carcinoma	95

that will be highly informative and important to collect similar data in many other cancer types.

Conclusions

The identification of highly diverse tumor-infiltrating leukocyte subsets and their distinct, sometimes antagonistic, functions in the tumor niche has been an important development in Oncoimmunology. This has allowed a better dissection and understanding of the interactions between immune components and tumor cells, not only in animal models but also in patients. We are now approaching an era where immune parameters will likely constitute some of the best prognostic markers for cancer progression/ regression. This will also allow a more insightful selection of patients to undergo immunotherapy as adjuvant treatment. Notwithstanding, many basic aspects of TIL biology still need to be clarified as to resolve key controversies in the field, such as the paradoxical behaviors of Th17 and γδ T cells (among others) in distinct cancer models/ types. Clinical studies must now routinely include in-depth population phenotyping and gene expression analysis in order to address the striking heterogeneity of the immune infiltrates. These future directions will be crucial to clinically promote an anti-tumor microenvironment and thus increase the success of cancer immunotherapy.

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