An overview of the tumor microenvironment, from cells to complex networks (Review)

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Abstract. For a long period, cancer has been believed to be a gene disease, in which oncogenic and suppressor mutations accumulate gradually, finally leading to the malignant transformation of cells. This vision has changed in the last few years, the involvement of the tumor microenvironment, the non-malignant part of the tumors, as an important contributor to the malignant growth being now largely recognized. There is a consensus according to which the understanding of the tumor microenvironment is important as a means to develop new approaches in the therapy of cancer. In this context, the present study is a review of the different types of non-malignant cells that can be found in tumors, with their pro or antitumoral actions, presence in tumors and therapeutic targeting. These cells establish complex relations between them, through cytokines, exosomes, cell adhesion, co-stimulation and co-inhibition; these relations will also be examined in the present work.

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Abbreviations: IL, interleukin; CCL, CXCL, chemokines; EGF, epidermal growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TAM, tumor associated macrophage; TGFβ, transforming growth factor-β; TLR, Toll-like receptors; PDGF, platelet-derived growth factor; CAF, cancer-associated fibroblast; SDF, stromal cell-derived factor; ROS, reactive oxygen species; ECM, extracellular matrix; TME, tumor microenvironment; GF, growth factors; MDSC, myeloid-derived supressor cell; TNFα, tumor necrosis factor-α; PGI2, prostaglandin I2; NO, nitric oxide; LPS, lipopolysaccharide; Ang, angiopoietin; Hif-1, hypoxia-inducible factor-1; NET, neutrophil extracellular trap; CTL, cytotoxic T lymphocyte; DAMPs, damage-associated molecular patterns; LT, LB, T, B lymphocytes; NK LTs, natural-killer lymphocytes; APC, antigen-presenting cell; DC, dendritic cell; ADCC, antibody-dependent cellular cytotoxicity; Th, T-helper lymphocyte; T, Breg, T, B-regulatory lymphocyte; PDL-1, Programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TIM-3, T cell immunoglobulin and mucin-domain containing 3 IDO-Indoleamine 2, 3-dioxygenase; CAR, chimeric antigen receptor; MHC, major histocompatibility complex

Key words: microenvironment, biogenesis, immunity, network

Contents

1. Introduction

According to the general logic of tissue architecture and dynamics, a tissue that expands needs to build a vascular network, an interstitium and it needs the help of other supporting cells in order to survive and to grow. This is the case of the epithelia that regenerate, of the embryo that develops, of wounds that heal and, finally, of benign and malignant tumors (1).

In the meantime, even if it has its independent growth, the tumoral tissue is connected with the rest of the body; different cells infiltrate it, either as homeostatic elements, or as an attempt to fight against it, or even recruited by the tumor to help it, contributing in these different ways to the shaping of the tumoral growth (2).

These interactions proved to be important in the development of tumors and are the object of the present review.

2. Cellular participants to the tumor microenvironment

Cells of the innate immune system and other stromal cells

Macrophages. They have important functions as the first line of defense against pathogens and tissue damage: phagocytic, antigen-presenting, inflammatory cytokines and chemokine secretion (3).

According to these roles, macrophages can be found in different activation states, M1 state for inflammation and immune defense, and M2 for tissue homeostasis and regeneration. In fact, the spectrum of macrophage activation is much more complex and intermediate forms between these states can be found (4). The macrophage is a versatile cell and transitions between states can occur, under the influence of external conditions and cytokine milieu.

In cancer, macrophages are thought to be recruited by local conditions-hypoxia and necrosis- and by cytokines and chemokines from the tumor cells, such as colony stimulating factor-1 (CSF-1), interleukin 34 (IL34), as well as IL6,

C-C motif chemokine ligand 2 (CCL2) or CXCL10. They are mainly in the M2 state, produce IL10, transforming growth factor β (TGFβ), growth and angiogenic factors like epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), chemokines (CCL2, 5, 3, 8, 22), they do not secrete IL12 and do not present tumor antigens. They are called tumor associated macrophages (TAMs) and they contribute to the immune suppression and angiogenesis, migration and invasion and recruit other immune cells (reviewed in 5).

There is also a M1-Th1 component in tumors, triggered by tumor antigens, mainly in the initial stages, which contributes to the antitumor defense (6).

What seems to determine a protumoral profile in macrophages is the exposure to factors from the tumor (ILs 4, 6, 10, TGFβ, exosomes), while Toll-like receptors (TLR) ligation through damage-associated molecular patterns (DAMPs) or agonists or exposure to interferon-γ (IFNγ) will turn them into antitumoral macrophages (5).

The presence of tumoral macrophages is generally linked to an unfavorable prognosis (7). By consequence, tumoral macrophages are considered for inhibition (Table I). Given the versatility of these cells, 'educating' them is also taken into account (8).

Fibroblasts. They are the main component of the connective tissue and also of the tumor stroma; they secrete the intercellular matrix and fibrils, sustain tissues, contribute to the tissue and wound healing, to fibrosis and, when activated, to inflammation. Specific markers are vimentin, smooth muscle actin-α (SMAα), fibroblast activation protein (FAP) (9).

According to these roles, there are sub-populations of fibroblasts: tissue resident fibroblasts, which realize tissue turnover and sustaining, fibroblasts with regeneration function that migrate to injured tissues and contribute to healing, inflammatory fibroblasts, activated by immune cells; there are also sub-populations of fibroblasts specific for each body region, with specific HOX gene codes (10).

In cancer, fibroblasts are induced by tumor cells, together with blood vessels, through factors such as FGF or PDGF (platelet-derived growth factor), as well as by hypoxia in tumors. They have a predominantly activated state, due to the action of IL1, TGFβ, PDGF, stromal cell-derived factor (SDF) and reactive oxygen species (ROS) (11) and are known as cancer-associated fibroblasts (CAFs). They are the majority of the tumor stromal cells.

CAFs secrete the extracellular matrix (ECM) and also active substances such as cytokines, growth factors (GFs) like TGFβ, HGF, SDF and MMPs, through which they shape the microenvironment of tumors, they are angiogenic through VEGF and PDGF and support the tumor growth and invasion. They usually negatively modulate the antitumor immunity through chemokines (CCL2 and 5) and cytokines (TGFβ), attracting T-regulatory lymphocytes (T regs), myeloid-derived suppressor cells (MDSCs) and also helping tumor cells to migrate (through CXCL12) (11,12).

Fibroblasts are also subjects of immunotherapy (Table I) (reviewed in 12).

Endothelial cells. Together with pericytes, they form the boundaries of capillaries and regulate the flow of substances and cells from within the vessel out and backwards. They are dynamic structures, responding to cytokines, growth factors and other active substances like IL1, tumor necrosis factor-α (TNFα), IFNγ, IL4, PGI2-prostaglandin I2, NO-nitric oxide, VEGF, FGF and secreting active substances (IL1, TNFα, IL6, IL8, IL20, IL33, LPS-lipopolysaccharide), through which they participate in the inflammatory processes and augments them when needed (13).

The endothelial cell also participates in the regeneration and healing processes, responding to angiogenic factors (VEGF, FGF, Ang-angiopoietin) and secreting them (13).

In tumors, due to the multiplication of cancer cells, there is hypoxia, which leads to the hypoxia-inducible factor-1 (Hif-1)-dependent augmentation of VEGF and other angiogenic factors (14); this causes angiogenesis. The vessels that form are different from normal vessels, being tortuous, disorganized, with few or no pericytes and to some of their length without walls (15).

The result is a modified metabolism in tumors, changes in the physical qualities of the ECM, metastasization, abnormal distribution of drugs and abnormal trafficking of immune cells to and from the tumor.

Neutrophils in tumors. Their normal function is to respond to pathogens at the beginning of the immune response, through phagocytosis and extracellular traps (NETs). Through the cytokines they produce, they amplify the response of other cells in inflammation. In addition, they present antigens and contribute to the end of the inflammatory process, by phagocytizing dead cells (16).

There are fewer neutrophils than macrophages in tumors, attracted by chemokines such as IL8 from the tumor cells or by inflammation and necrosis. They are short-lived, but they have a turnover and contribute to the process in two opposite ways: they can be antitumoral (N1 neutrophils), especially in a milieu with IL12 and TNF, and this effect requires the presence of $CD8^+$ cells (17).

They can be protumoral through the secretion of MMP9, HGF and VEGF, where the neutrophil depletion leads to the cancelling of the angiogenic switch (18). This is the natural state of neutrophils in tumors and this is one of the reasons for which blocking IL8 reduces tumor growth (17). TGFβ is believed to be the main cause for this protumoral profile, called N2, while IFNγ turns the neutrophils into N1 tumoricidal cells (17).

As a rule, neutrophilic infiltration in solid tumors is associated with worse prognosis (19). A greater number of circulating neutrophils, reflected in the neutrophil/lymphocyte ratio, is associated with worse prognosis in many tumors (20).

Eosinophils in tumors. The eosinophils have a role in clearing parasitic and some bacterial infection. They participate in the immune response, especially in the Th2 type. They are activated by IL5 (3) and secrete more than 30 cytokines, such as IL1, 2, 3, 4, 5, 9, 10, 12, 13, 17, 25, IFN, TNF, chemokines such as CCL11, MIP, MCP, CCL5 and growth factors NGF, PDGF, EGF, TGFα and β (21).

Eosinophils were shown to be tumoricidal in some tumors, where the presence of $CD8^+$ cells was needed (22). However, they are an important source of IL4, which is Th2 polarizing and protumoral (23). Nonetheless, eosinophils are effective

Table I. Cells that compose the tumor microenvironment.

Type of cell	Specific markers	Recruitment in tumors	Protumoral actions	Determinants of the pro/antitumoral role	Antitumoral actions	Presence in tumors/prognostic associations	Targeting	(Refs.)
Macrophage (TAM)	CD68, CD11b^{+} HLADR^{+}, -M1-CD86^{+} CD80^{+} INOS^{+} -M2-CD 163^{+} or CD206^{+}	CCL2, 5 CXCL12 CSF1, VEGF (from the tumor cells)	M2-like:-angiogenesis (Vegf, IL8, Ang-2, FGF, MMP9) -EMC remodeling (MMPs 9, 12) -Growth factors (EGF, FGF, PDGF) immunosuppression (Arg, PDL1, Fasl, IL10, TGF) adhesion to the tumor cells, co-migration (ICAM1, VCAM, PECAM) -Recruitment of other cells (CXCL17, 22, 24). -M1-contribution to oncogenesis through inflammation	-For the protumoral role-GCSF, L10, TGFβ, IL4, 13, tumor exosomes -For the antitumoral role: IFNγ IL12, GM-CSF, DAMP, TLR, NLR agonists, apoptotic cells	M1-Supports Th1, NK LTs -Recruitment of defensive cells (CXCL9, 10) -Directly tumoricidal (ROS, phagocytosis) -ADCC -Th1 effector	-Frequent (10-50% of the tumor mass) -Mainly M2 Associated with poor prognosis in: breast, gastric, pancreatic, oral cancer, lymphoma -Good prognosis in melanoma, cervical, esophageal, colorectal cancer	Anti CCL2/CCR2, -Anti CSF1, CSF1R -Anti IL6R, PIK3CA, STAT3 -Anti CXCL12, CXCR4 -IFNγ, IL12 (aiming at educating or eliminating TAMs)	(3,4,6)
Fibroblast (CAF)	SMA-α FAP Vimentin	FGF, PDGF SDF (from the tumor cells)	Secretion of growth factors (PDGF, TGF) angiogenesis (VEGF, CXCL12) myeloid cells recruitment (CCL7) adhesion to the tumor cells (ICAM, Itg a11, IGF KGF, HGF) invasion ECM secretion, remodeling (MMPs) -Immunosuppression (TGFβ) -Inflammation (IL6) -Resistance to therapies	-For the protumoral role: -Mainly TGFβ -IL6 -Exosomes from tumor cells	Normal fibroblasts restricts tumor initiation, while CAFs promote tumor growth	Main component of the tumor stroma, in some situations (pancreas) being up to 80% of the tumor mass Associated with poor prognosis	Normalization or elimination of CAFs -Anti FAP, TGFβ, FGF, FGFR PDGFR, CXCL12, CXCR4, IL6, PGE2	(7,9,10)

Table I. Continued.

Type of cell	Specific markers	Recruitment in tumors	Protumoral actions	Determinants of the pro/antitumoral role	Antitumoral actions	Presence in tumors/prognostic associations	Targeting	(Refs.)
Endotheliocyte	CD31	-Angiogens: -VEGF, PDGF, FGF, Ang2, IL8, CXCL1, 2, 3, 5, 12 -ILs 1, 6, 23 (from tumor and associated cells	-Nutritive support of tumors -Dysregulated network, maximally dilated→hypoxia -Increase in NCAM→cell influx, angiogenesis -Increases resistance to therapy -Adhesion to the tumor cells, interendothelial adhesion→ invasion -VEGF→decreased ICAM-1, VCAM→decreased influx of immunocytes	-For the protumoral role, angiogens, decreased ICAM-1 -For the antitumoral role: Proinflammatory ILs (IL1, 6, 36, TNFα) →increased cells influx	Release of proinflammatory ILs, chemokines→increased ICAM-1, VCAM- → influx of defensive cells (LTs, monocytes)	In all tumors; presence is necessary for tumor survival	Anti-VEGF (bevacizumab) -Anti-PDGFR, Ang2, Tie2, αvδ3 Itg. -Depletion of M2.N2 -IL1 should precede adoptive therapy (to increase cell adhesion to the endothelium)	(12,13,97)
Neutrophil (TAN)	$CD16^+$ $CD66^+$ $CD15^+$	CXCL1, 2, 3, 5, IL8, GMCSF, G-CSF (from the tumor cells, macrophages)	Angiogenesis (through VEGF), -EMC remodeling (MMPs 8, 9) -Tregs recruitment-(CCL17) -Myeloid cell recruitment (IL8) -Adhesion to the tumor cells, migration -Arginase and i-NOS are immunosuppressive -Inflammation in tumors -NETs are thrombogenic	N2 polarization (protumoral)-mainly TGFβ N1 polarization-IFNγ, TNFα	Tumoricidal-directly through degranulation, phagocytosis -Through ADCC -Th17 effector Chemokines secretion- -CCL19, 20-for DCs -CXCL9, 10-for LTs Stimulation of $CD8^+$, NK (through TNFα) Antigen presentation, stimulation of LB in lymph nodes	TANs accumulate in correlation with tumor stage -poor prognosis in: melanoma, renal, hepatocellular, neck, lung, pancreatic cancer -NLR associated with poor prognostic in many tumors	-Anti-G-CSF -IFNγ, anti-TGF -Anti-IL8, anti-CXCR1, 2; -Anti-IL17 -MABs targeting markers -NET targeting	(15,17,18)
Eosinophil	$CD193^+$ $Siglec\text{-}8^+$ $CD15^+$	CCL11, 24, 26, IL8, CXCL1 -DAMP -IL5;-PGE2 ICAM-1, V-CAM	Angiogenesis secretion of Th2 cytokines	For the antitumoral role: DAMP, necrosis -A context of Th1 response favors the antitumoral actions -Contributes to immune surveillance	Tumoricidal -Directly through degranulation independent of Th2 -Th2 effector -Through ADCC -Stimulation of $CD8^+$ LTs -Antigen presentation	Associated with favorable prognosis in gastric, ovarian, nasopharyngeal, colorectal, lung, esophageal, lung, prostate cancer -Unfavorable-Hodgkin lymphoma, cervical cancer -TABE (Tumor-associated blood eosinophilia) unfavorable prognostic in renal, gallbladder, breast, pancreatic tumors	-Adoptive therapy -Combined therapy eosinophil-lymphocyte increases the inhibition of the tumor growth on models	(20,22,24)

Table I. Continued.

Type of cell	Specific markers	Recruitment in tumors	Protumoral actions	Determinants of the pro/antitumoral role	Antitumoral actions	Presence in tumors/prognostic associations	Targeting	(Refs.)
Mast cell	FCεRα1^{+} CD117^{+} Triptase^{+} -Activated- CD203c^{+}	SCF VEGF IL8 CCL2 CXCL1, 10, 15 (from the tumor cells)	Angiogenesis (they accumulate near CD31): -Through histamin, PDGF, IL8 -EMC remodeling -Proteases, MMPs, heparin -Immunosuppresion-IL10, TGFβ, adenosine -Secretion of Th2 ILs→ accumulation of M2 macrophages; these ILs have protumoral roles -Genotoxic through ROS and inflammation	For the antitumoral role-TLR2 activation -For the protumoral role-exposure to the TME	-Activation of CD8^{+} LTs -Recruitment of defensive cells -Antigen presentation -It is not directly tumoricidal -IL9-stimulates CD8, CD4^{+} LTs -Through IFNγ, TNFα they stimulate the defense	-Negative role in: Hodgkin lymphoma, CLL bladder, thyroid, esophageal, breast, prostatic, pancreatic colorectal cancer -Positive role in: breast, lung, ovarian cancer -No association: Renal, lung cancer	Targeting where it has a negative role -c-Kit inhibitors -Sylimarin inhibits Mc recruitment, MMPs 2, 9 -Cromolyn	(26,27)
Myeloid-derived suppressor cell (MDSC)	-Monocytic- CD11b^{+}CD14^{+} CD15^{-} HLA-DR- -Polinuclear CD66b^{+}CD15^{+} CD14-HLA-DR-	CCL2, 5, CXCL5, 8, 12, GMCSF, VEGF, from tumor cells-soluble or exosomal	-Immune suppression through Arg, ROS, galectin (TCR nitration) -ADAM17-↓ E-selectin -Angiogenesis -At the ingestion of tumoral exosomes, MDSC express IL6, VEGF -MDSCs (in ovarian tumors) have decreased miR101→stemness	STAT3, NFκB; tumoral exosomes + TGFβ, PGE2α		-Present in most tumors, correlated with stage -Associated with poor prognosis in: breast, lung, pancreatic, uterine, prostatic tumors, HCC, glioma	-Inhibition of CSFR -Inhibition of CCL2 -Sildenafil (↓NO) -Inhibition of exosomes release (dimethyl amiloride) -Education through IFNγ, IL1β, IL4, TNFα, TLR-ligands	(71,98)
Dendritic cell (DC)	Conventional- CD11c^{+} HLADR^{+} CD1c$^{+/-}$, CD141$^{+/-}$ -Plasmacytoid- CD123, 303^{+} -Activated- CD83^{+}	CXCL9, 10, 12, 14, CCL19, 20, 21	-Dysfunctional DCs in antigen presentation, maturation, infiltration -Tolerogenic DCs-expressing PDL-1, IDO, Arg→ stimulation of LTreg, inhibition of CD8^{+}, CD4^{+} LTs, macrophages, neutrophils -Immature DCs (MDSC) -DC deficiency→Th1 deficiency, bias towards Th2 in tumors; also CD8^{+} deficiency	Protumoral role: -DCreg profile due to TGFβ, IL10, tumoral exosomes -Inhibition through PDL, Gal. IDO, Arg -Antitumoral-action on TLRs (DAMP, apoptotic cells), IFNγ, TNFα	Presents antigen to CD8, CD4^{+} LTs -Through IL12, IL15 -Stimulation of Th1, CD8^{+}, NK LTs -Role in the Th1, 2.9.17, Tfh, Treg differentiation -Direct cytolitic action -Stimulation of memory LTs	-Mature DCs-good prognostic in melanoma, head-neck, colorectal. bladder, oral, gastric, uterine cancer -Plasmacytoid DCs-associated with poor prognostic in melanoma, glioma, breast, ovarian, oral, gastric, renal and lung cancer	-DC-based vaccines -Anti-PDL.TIM-3, -Anti-IL10, TGFβ, miR155 -CD-40 agonists -SiRNA anti STAT3	(39,40)

Table I. Continued.

Type of cell	Specific markers	Recruitment in tumors	Protumoral actions	Determinants of the pro/antitumoral role	Antitumoral actions	Presence in tumors/prognostic associations	Targeting	(Refs.)
$CD8^+$ lymphocyte	$CD3^+$ $CD8^+$ Gzm, Perf. -activated: $CD69^+$, $CD25^+$ -exhausted $TIM\text{-}3^+$, $LAG3^+$	CXCL9, 10, CCL5 (from DCs, M1, some tumor cells)	-In oncogenesis (for example in HCC) -Self-inhibition through the upregulation of PDL-1 by IFNγ	-Inhibited through signals from the tumor, from CAFs, TAMs, Treg (PDL-, TIM3-L Fasl, B7-H3) -It depends on antigen presentation, co-stimulation and ILs from DCs -Support from eosinophils, Th1, Th9, NK cells	-Cytotoxic through direct contact (Granzyme, perforins) -Recruitment, stimulation of other cells	-Infiltrating $CD8^+$LTs associated with good prognosis in many cancers like breast, colorectal, renal, prostatic, bladder, ovarian cancers	Adoptive therapies bispecific antibodies Anti PDL-1, anti-CTLA4 -ILs 2, 9, 12, 15, 18 21, 27 -CXCL9, 10	(43-45)
$CD8^+$ memory lymphocyte	$CD3^+$ $CD45RO^+$ CCR7 $(CD197)^+$ (central) $CD127^+$	CXCL9,10 CCL3.4,5, 8,14	In some tumors (lung, ovarian)-exhausted	-Differentiation through IL15, IL33, TGFβ -CD1c	-Cytotoxicity (through granzyme, perforins) -Secretion of IFNγ, TNFα -Chemokines for other cells (CCL3, 4, 5) -Through CD103 they inhibit tumors expressing E-cadherin -Response to vaccines requires $CD8^+$ mem	-Associated with good prognosis in: ovarian, lung, urothelial, uterine, breast, bladder tumors, glioma ($cd103^+$) -Exception: colorectal cancer -Marker of immunogenic tumors	-Vaccines -Adoptive therapies -Anti-PDL-1	
NK lymphocyte	$CD56^+$ $CD3^-$ $CD16^+$	CX3CL1	-Inhibited in many tumors due to the immunosuppressive milieu	Stimulation through ILs 2, 12, 15, 21-antitumoral	-Immune surveillance -Cytotoxic on cells with low MHC, $MICA^+$ cells -Through ADCC, CDC, -Stimulation of $CD8^+$LT, DCs -IL12, 2, 15, IFNγ secretion	Associated with good prognosis in gastric, colorectal, liver, lung, renal cancer and others	-Adoptive therapy, CAR-NK -IL-IL2, 12, 15 -Anti-PDL, anti-KIR anti-NGG2A -MAB, BsAB for CD16 or NCR-tumor antigens	(34,35)
γδ T lymphocyte	$CD3^+$ $\gamma\delta TCR^+$	CCL5 CXCL9, 10, LFA, VLA1-4 L-selectin, Integrin αvβ7	γδ-17 subset-production of IL17 in colorectal tumors→chronic inflammation, angiogenesis, recruitment of myeloid cells -Suppressive subset	-Tumoral phosphoantigens or injection of phosphoantigens stimulates the cytolitic activity -The immune suppression from tumors-they become suppressive	-Presents antigens to $CD4, 8^+$ -Non-peptidic antigens -Through NKG2D, TRAIL perforins, granzyme -ADCC -Secretion of IFNγ, TNFα, IL12, 15, 36-stimulation of DCs -Rapid response -Cooperation with NK, LB (γδTfh subset)	The strongest association with favorable prognostic in solid cancers, leukemias, lymphomas -Negative association through the γδT17 subset in ovarian, bladder, colorectal cancer	-Phosphoantigens -Bisphosphonates -Adoptive therapy -Multi-immunocyte-γδT+αβT or CIK-with IL17 measurement -Modifying the IL balance-giving ILs 21, 15, 12, 36	(28-30)

Table I. Continued.

Type of cell	Specific markers	Recruitment in tumors	Protumoral actions	Determinants of the pro/antitumoral role	Antitumoral actions	Presence in tumors/prognostic associations	Targeting	(Refs.)
NKT lymphocyte	$CD56^+$ $CD3^+$		NKT II subset-secretion of ILs 4, 13→↑ M2, MDSC Inhibits LT $CD8^+$; stimulates LB, LTh2	-Sulfatides from the tumor→activation of NKTII-protumoral -ILs polarize NKT LTs pro or anti-tumoral -α-Gal-cer promotes antitumor immunity	Glycolipidic antigens presented on CD1d -in CMH-deficient tumors -Secretion of IFN, activation of NK -Rapid response to IL, DAMP-TLR -Adaptive response regulation -IL12, CD40 → DCs, NK, CD8	-Associated with good prognosis in myeloma, lung cancer -They protect from some tumors, being necessary for this effect	-Adoptive therapy-CIK cells -The agonist α-Gal-cer. -Anti IL4, 13, TGFβ -α-Gal-cer.-adjuvant for vaccines	(31-33)
Th1 lymphocyte	$CD3^+$ $CD4^+$ $T\text{-}bet^+$	CXCL9,10. CCL5 from APCs, certain tumor cells	Inhibited in many tumors through Th2, M2, Treg, TGF, IL10, IL4 -Exhausted by chronic exposure to tumoral antigens -Self-inhibition through the upregulation of PDL-1 by IFNγ	For the antitumoral role-IL12, 18, 27, TNFα	-Tumoricidal through M1 Mϕ -stimulates $CD8^+$, NK LTs -DC licensing -Necessary for an efficient antitumoral response or response to vaccines especially with exhausted $CD8^+$ LTs or tumors $CD8^+$ resistant or expressing Fasl -Recruitment of $CD3^+$ cells -$CD4^+$ CTLs	Associated with favorable prognosis in many tumors, from which melanoma, colorectal, ovarian, breast, cancer, multiple myeloma	-Stimulation with IL2, IL12, IL18, IL27 -In adoptive therapy -CXCL 9, 10	(46,47)
Th2 lymphocyte	$CD3^+$, $CD4^+$ $GATA3^+$	-CCL 17, 22 (tumor cells, M2 macrophages) or locally polarized	-Inhibition of Th1 -Through the Th2 cytokines which are protumoral -Non-stimulation of $CD8^+$ LTs -Stimulation of M2 macrophages -Stimulation of B lymphocytes-sometimes protumoral	-DC dysfunction→↓IL12 -TSLP (from CAFs) →bias towards Th2 in tumors	-Together with Th1 they contribute to a complete response including to vaccines with/without the intervention of $CD8^+$ LTs -Through the tumor cell necrosis -Through eosinophils -In adoptive therapy they eradicate Th1 or $CD8^+$-resistant tumors -IL4 necessary for the development of $CD8^+$ LTs	Associated with good prognostic in some tumors like breast cancer and lymphoma -Poor prognostic in gastric, pancreatic, ovarian cancer -No association in colorectal cancer	-IL12, IFNγ -Anti IL4 (models) -For the positive role: -Adoptive therapies -Vaccines (when both Th1 and Th2 ILs increase)	(22,49,50)

Table I. Continued.

Type of cell	Specific markers	Recruitment in tumors	Protumoral actions	Determinants of the pro/antitumoral role	Antitumoral actions	Presence in tumors/prognostic associations	Targeting	(Refs.)
Th9 lymphocyte	$CD3^+$ $CD4^+$ $IL9^+IFN\gamma^-$		IL9 stimulates mast cells that can be protumoral	Th9 polarization: $IL4^+$ TGFβ	-IL9-stimulates $CD8^+$LTs, mast cells (CCL20) -IL21-stimulation of NK cytolysis, IFNγ secretion of $CD8^+$LTs -Direct cytolysis through Granzyme	-Presence in tumors has been reported	-Adoptive therapy-sometimes more effective than Th1 -The effect is mainly through $CD8^+$	(55)
Th 17 lymphocyte	$CD3^+$ $CD4^+$ $IL17^+$ $RORYt^+$	CCL18, 20, 22, CCL 4, 5	-Th17 cytokines (IL17, 22, 26) are protumoral, proangiogenic -Through the LTh17-reg subset -Through the inflammation which is protumoral	-For the anti-tumoral role IL12 through stimulation of Th17-1 subset -For the protumoral role-TGFβ-stimulation of Th17-reg subset	-Cytotoxic through neutrophils -In a Th1 milieu they contribute to the defense -Stimulation of B lymphocytes through IL21 -Through Mϕ and DC -Through the Th17-1 subset-secretion of TNFα, IFNγ -Recruitment of $CD8^+$LTs, neutrophils, LB, DCs in tumors (CCL20, 2, 7, CXCL9, 10)	-Adoptive transfer-good effect -Natural Th17LTs-associated with poor prognosis -Better prognosis in ovarian, prostatic, lung cancer, seminoma -Worse prognosis in pancreatic, colorectal tumors, HCC	-Adoptive therapies with LTh17 -Inhibition of IL17 IL22, 23, 26	(42,51-54)
T regulatory LT	$CD3^+$ $CD4^+$ $Foxp3^+$ CD25 $(IL2R)^+$	-CCL22, 28 from tumor cells -Activation of RAS→ infiltration with Tregs	-Immunosuppression -Angiogenesis -Inhibition of immunotherapy -Inhibitory potential of Tregs increases much in cancer	-TGFβ, IL10, IL35, IL2 -Other cells stimulate Tregs (MDSC, M2, Breg, CAF) -Adenosine from tumor cells	-Positive role where the protumoral inflammation is dominant -Versatility under the influence of LTh2; (TGF^+IL4) LTreg become LTh9, antitumoral	-Present in cancer, associated with worse prognostic in many cancers like breast, ovarian, gastric cancer -Associated with good prognosis in colorectal, breast cancer and lymphoma	-Anti TCR, Foxp3, -Anti CD25 (IL2R) -Anti PDL1, CTLA4 -IFNγ -GITR, TNFR inhibition -LTreg education	(57,58,60)
T-follicular lymphocyte (Tfh)	-CXCR5 (homing in follicles) -Bcl6		Tfh2, 17 subsets-protumoral action	-Antigen presentation by DCs, LBs, IL6, ICOS, TGF→Tfh differentiation -Stimulation by DCs, Mϕ through IL6, 21, 27	-Secretion of ILs 21, 4, 12 -CXCL13→influx of LBs, generation of tertiary lymphoid organs (TLO) -Affinity maturation of ABs -Differentiation of LB mem -Rescuing LTs from anergy in the TLO -Associated with increase of $CD8^+$, Th1 LTs (IL21) -Inhibition of LTreg	-Associated with favorable prognosis in breast, colonic cancer (presence of TLOs) -Unfavorable prognosis in gastric cancer	-Adoptive therapy -CD19-directed CAR-T-cells	(28,57)

Table I. Continued.

Type of cell	Specific markers	Recruitment in tumors	Protumoral actions	Determinants of the pro/antitumoral role	Antitumoral actions	Presence in tumors/prognostic associations	Targeting	(Refs.)
B lymphocyte (LB)	-$CD19^+$ -Plasma cells $CD138^+$	CXCL 13	-They inhibit the antitumor response -Immune complexes occupy FcR of neutrophils, mast cells, macrophages →angiogenesis -The Breg subset inhibits $CD8^+$, NK, Th1, NKT, LTs and stimulates LTregs through IL10, TGFβ -Lymphotoxin-lyphangiogenesis	-The activation state seems to determine the pro-or antitumoral profile -Activated-antitumoral, resting-protumoral -Breg-protumoral -Plasma cells-antitumoral	-Presents antigen to $CD4^+$LTs -ADCC -Complement activation -Some LBs are cytotoxic -IL2 secretion -In some models LBs were necessary for the Th1, $CD8^+$ response -Resistant to immuno-suppression	-Associated with good prognosis in breast, hepatocellular, biliary, gastric cancer -Negative role in melanoma pancreatic, lung, oral cancer	-Adoptive therapies-successful -Antibody usage according to the B lymphocyte model (MABs) -Inhibition where they have a proven negative role (rituximab) -Association of adoptive B and T LTs superior to single cell therapy	(61,62)
B-memory lymphocyte	$CD19^+$ IGD- $CD27^+$				-Tumor antigens-weakly immunogenic, sometimes tolerogenic- →Bmem LTs are important for vaccines -Bmem LTs present antigens to $CD8^+$LTs, costimulation (CD40-CD27)		-Searching epitopes with effect on Bmem is important	

IL, interleukin; CCL, CXCL, chemokines; EGF, epidermal growth factor-, FGF, fibroblast growth factor, VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TGFβ, transforming growth factor-β; TLR, Toll-like receptors; NLR, nod-like receptor; ROS, reactive oxygen species; ECM, extracellular matrix; TME, tumor microenvironment; GF, growth factors; MDSC, myeloid-derived suppressor cell; TNFα, tumor necrosis factor-α; PGI2, prostaglandin I2; NO, nitric oxide; CAM, cell adhesion molecule; Itg, integrin; LPS, lipopolysaccharide; Ang, angiopoietin; Hif-1, hypoxia-inducible factor-1; NET, neutrophilic extracellular trap; DAMPs, damage-associated molecular patterns; LT, LB-T, B-lymphocyte; TAN, Tumor associated neutrophil; NKLTs, natural-killer lymphocytes; Mϕ, macrophage; DC, dendritic cell; LT-T, lymphocyte; LB-B, lymphocyte; ADCC, antibody-dependent cellular cytotoxicity; CTL, cytotoxic lymphocyte; Th-T, helper lymphocyte; T, Breg-T, B-regulatory lymphocyte; PDL-1, programmed death-ligand; IDO, Indoleamine 2, 3-dioxygenase; CAR, chimeric antigen receptor; MHC, major histocompatibility complex; CD, Cluster of differentiation; Inos, inducible nitric oxide synthase; HCC, hepatocellular carcinoma; HLA, human leukocyte antigen; KIR, Killer-Ig-like-receptors; NCR, natural cytotoxicity receptors, TRAIL, TNF-related apoptosis-inducing ligand; FasL, Fas-ligand; CIK, citokine-induced killer cells; MAB, monoclonal antibody; ICOS, inducible T-cell costimulator; GITR, glucocorticoid-induced TNFR-related protein; CLL, chronic lymphocytic leukemia; →, results in; ↑, increasing of ; ↓, decreasing of.

in the CTL (cytotoxic T lymphocyte)-resistant tumors on models (24) and in tumors with cells engineered to express IL4 there was a rich infiltration with eosinophils and macrophages, which had tumoricidal effect (25).

The eosinophilic infiltration in tumors (except Hodgkin lymphoma) is associated with good prognosis (26).

Mast cells in tumors. Mast cells are cells with an important role in the innate and adaptive immunity. They are involved in the immune defense of the mucosal barriers and express TLRs 1-7, 9 and Fcε receptors (FcεR). They recognize DAMPs and release inflammatory mediators contained in their granules or cytokines (IL1, 6, TNF) and recruit other cells such as neutrophils, eosinophils, $CD8^+$ and natural-killer lymphocytes (NK LTs). The mast cells present antigens via MHCI or II (major histocompatibility complex), they stimulate DCs (dendritic cells) and contribute to angiogenesis (27).

In tumors, mast cells can play either pro or antitumoral role (Table I) (reviewed in 28). Mast cells exposed to the tumor microenvironment are mostly protumoral, while the action on their TLR-2 receptors has been shown to stimulate an antitumoral profile in them (29).

Lymphocytes of the innate immune system

γδ T-cells. They form 0.5-5% of the lymphocyte population, being present mostly in the gut and in the skin. γδ T-cells act on phosphoantigens, but they also present antigens to $CD8^+$ and $CD4^+$ LTs and cooperate with NKs (3). Of all immune cells in tumors, it is the subset with the strongest association with good prognosis in cancer (30).

However, there are also protumoral subsets: γδ-17 LTs, which secrete IL17, and a suppressive subset (31). The suppressive TME can turn γδ LTs into suppressive LTs. They are extensively studied for adoptive and other therapies (Table I) (reviewed in 32).

NKT cells. They respond to hydrophobic antigens, presented by CD1d-type MHC. There are two main subsets of NKT, type I with an invariant T-cell receptor (TCR), which is antitumoral by stimulating DCs, $CD8^+$ and NK LTs, and type II, which is mostly protumoral (33).

There are also NKT-17, Tfh (T follicular)-like and Treg-like subsets, with dominant protumoral activity (34).

NKT cells are also intensively studied for adoptive therapies (CIK cells), for selective stimulation of NKT1 with α-galactosyl-ceramide (α-Gal-Cer), for interleukin therapy and others (reviewed in 35) (Table I).

NK lymphocytes. They are main antitumor defenders acting on cells with low level of self-proteins (MHC-I) like some tumor cells. They also possess NKG2D receptors for atypical MHC, such as MICA and Fcγ receptors, through which they perform antibody-mediated cell destruction (ADCC). They stimulate $CD8^+$ LTs and DCs through the IFNγ they secrete, receiving in turn support from Th1 (T-helper 1 lymphocyte), Th9, $CD8^+$ LTs and M1 macrophages through ILs 9, 12, 15, 21 and type I and II IFNs (3,36). NK cells often become exhausted and suppressed in the inhibitory TME.

They are also investigated for adoptive cell therapy, CAR-NK therapy and others (reviewed in 37) (Table I).

Innate lymphoid cells (ILCs). ILCs possess CD127 (IL7R) and have three main subsets: ILC1 that secrete IFNγ, having some antitumoral activity (38), ILC2, with pattern of secretion such as Th2 LTs and ILC3, with secretion pattern like Th17 LTs. Intervention of these subsets in cancer resembles that of their adaptive counterparts (36). An antitumoral role has been found for ILC3 in some models (39).

Dendritic cells (DCs). DCs are a heterogeneous cell population present in every tissue and they are professional antigen-presenting cells to LTs. They also secrete cytokines (ILs 1, 2, 6, 12, 15, 18, 37, 23, 27, 7, 37, 31, 10, IFNs) and chemokines (IL8, IL16, CCL9). They express MHC II type proteins. DCs have myeloid or plasmacytoid origin. Depending on the type of antigen exposure and cytokines they secrete or are exposed to, DCs contribute to the Th1, Th2, Th17 polarization or to immune tolerance (40).

In cancer, dendritic cells present tumor antigens to LTs, their number being linked to good prognosis (41). Their depletion leads to the depletion of LTh1 cells, which is detrimental. Tumor cells influence infiltrating DCs, lowering their number and antigen presenting capabilities. They become tolerogenic DCs, which contribute to the immune suppression in the TME and accumulation of Treg cells. Cytokines from the tumor (IL10, TGFβ, VEGF, low IL12) and activation of PD receptors contribute to this (reviewed in 42). Certain miRNAs (micro-RNAs) also influence the behavior of DCs (43).

Cells of the adaptive immune system

$CD8^+$ T lymphocytes (CTLs). CTLs represent the main antitumor defenders in the human organism; they act after tumor antigen presentation by DCs (1), also receiving support from Th1, Th9 and M1 macrophages (reviewed in 44). $CD8^+$ LTs are active especially in the earlier stages of the tumor development. Later, they develop exhaustion and apoptosis due to the activation of programmed death (PD) receptors and to the immunosuppression of the TME (45).

The presence of $CD8^+$ lymphocytes is associated with good prognosis in many cancers (46). Given its position as key player in the antitumor immune defense, this subset is the most extensively used in different immunotherapeutic approaches, immune checkpoint blockade, adoptive therapies, bispecific antibodies, interleukin therapy, chimeric antigen receptors (CAR)-engineered cells and others (reviewed in 47) (Table I).

$CD4^+$ T lymphocytes. They are in different states of polarization, depending on which cytokine combinations act on them (1):

The Th1 (T-helper-1) subset polarizes in the presence of IL12 from M1 macrophages and IFN, to which also IL18, IL27, IL1α contribute; they are also contributors to the antitumor defense (48) (Table I). It has been shown that an intact $CD4^+$ component is necessary for an efficient antitumoral response (49).

The Th2 subset is polarized by ILs 4, 13, 19, 25 and 33 from mast cells, NK and $CD4^+$ mem ($CD4^+$-memory) cells (1,22) and secrete Th2 cytokines such as ILs 4, 5, 10, 13, 25, 31, 33. In tumors, they are not favorable to the defense process, because of the secretion of IL4 and 13 that inhibit the

Th1 response, and of the cytokines mentioned above, which have mainly a pro-tumoral effect (50). Until recently, the Th2 subset was considered almost entirely protumoral, but in the light of recent data, it was shown that Th2 lymphocytes can also contribute to the antitumor defense, being necessary for some of its observed effects (25,51) (Table I). This is the reason why Th2 LTs were studied for adoptive therapies, with good effect (52).

The Th17 subset is polarized in the presence of IL1β, IL6, IL23 and TGFβ and they secrete ILs 17, 22 and 26, through which they sustain the tumor growth (53). There is a plasticity of the Th17 cells, which can be reprogrammed in Th1 or Treg lymphocytes (54). As in the case of the Th2 subset, the initial view that Th17 is protumoral was reconsidered in the light of some data that showed a beneficial role, especially in adoptive therapy, with success even in cases that were $CD8^+$ and Th1-resistant (44,55,56) (Table I).

A particularity of the antitumoral immune response is just this combination of subsets that are usually mutually exclusive in the immune response, such as Th1 and Th2; in tumors they seem to coexist and even to cooperate, building an immune defense that is unique to tumors (44,51).

The Th9 subset, through the ILs 9 and 21, which have a stimulatory action on the $CD8^+$ cells, are contributors to the antitumor defense. Th9 lymphocytes also have a direct tumoricidal action (57).

The Th22 subset secretes IL22, whose action is protumoral (58).

T follicular LTs (Tfh) are associated with good prognosis in many tumors (30). They support the antitumor defense by building the tertiary lymphoid organs in tumors, which are associated with good prognosis (59).

The Treg (T-regulatory) subset polarizes in the presence of TGFβ from the tumor and associated cells and has an immunosuppressive and by consequence protumoral action through TGFβ and ILs 10 and 35 that it secretes, and through other inhibitory mechanisms (reviewed in 60) (Table I).

Their presence is associated with poor prognosis (61). An exception is represented by tumors in which there is a strong inflammatory component, where the presence of the Tregs is beneficial (62). They are considered for inhibition through different strategies (60,62) (Table I).

B lymphocytes. They are antitumoral in some tumors such as breast, hepatocellular, gastric and biliary tumors, while being protumoral in others (melanoma, pancreatic, lung, oral cancers) (reviewed in 63) through different mechanisms (64) (Table I).

A more detailed review of the pro and antitumoral role of the mentioned cell types of in tumors, along with the factors that drive their pro or antitumoral state, can be found in Table I and in some other recent works (30).

3. The tumor microenvironment, from biogenesis to complex relations

Complicated networks are generated through the interaction of these cells, having as result either the promotion or the inhibition of the tumoral growth. In a previous work, we have reviewed these pro and antitumoral networks, the way in which they interact and the therapeutic opportunities that the understanding of these complex relations may open to immunotherapy (65). In the present study, these networks are analyzed in their dynamics, starting from the biogenesis of the TME, with the same objective of exploring the ways in which the knowledge of this network structure may help to improve therapy.

The biogenesis of the tumor microenvironment

Angiogenesis and stromagenesis. In their continuous expansion and proliferation, tumor cells arrive at a certain point where they become hypoxic and they need to build a vascular network.

Some studies show that hypoxia does not have any role, the activation of the oncogene being enough to cause the overexpression of angiogens (66); other studies also reveal a hypoxia- and Hif-1-dependent mechanism (67). In fact, to the embryo, a main regulator of angiogenesis is the partial pressure of O_2 (PpO_2).

It has been shown that the angiogenic switch takes place early in the evolution of tumors, even in the premalignant stages (18).

Stromagenesis. The tumor cells secrete FGF, through which they recruit fibroblasts from the surrounding tissues; other sources of fibroblasts are thought to be the endothelial and the tumor cells, through metaplasia. The fibroblasts secrete the ECM, which is dysregulated in tumors, with a different collagen content and structurally altered, compared to normal ECM (11).

Recruitment of cells in tumors. The tumors secrete chemokines: CCL2, IL8, CXCL12, CXCL1, 2, 3, GM-CSF, CCL 5, CCL17, sometimes also anti-tumoral chemokines CXCL9 and 10. This leads to the accumulation of monocytes, neutrophils, regulatory lymphocytes (68).

The cells arrive in tumors for several reasons: they enter, as in any tissue, due to a basal secretion of chemokines (but dysregulated in cancer); they also enter, in an increased number, in the case of the existence of distress signals from within the tumor. This leads to the overexpression of inflammatory chemokines (IL8, CCL2, 5, CXCL9, 10) and infiltration of the tumor with defensive cells.

It has been shown, under experimental conditions, that the activation of an oncogene leads to the overexpression of chemokines such as IL8, CCL2, CCL17, leading to the infiltration with myeloid or lymphoid cells (69).

Some studies report on auto-inflammation in the tumor because the activation of the EGFR or other oncogenic pathways would also lead to the activation of the NFκB pathway with consecutive secretion of IL1, 6, TNF and inflammation of the tumor environment. The NFκB pathway can carry oncogenic mutations itself (reviewed in 70).

This cell infiltration in tumors occurs from early stages of the tumor development, even from preneoplastic stages (71).

Under the influence of tumor-generated factors (IL4, 10, TGFβ, exosomes), these cells acquire a protumoral profile, of tissue reconstruction and immune suppression (M2, N2, Treg) (72).

Immature myeloid cell recruitment. Tumor-secreted factors (IL1, IL6, GM-CSF, TGFβ, CXCL12) act at the level of the bone marrow and trigger an accelerated myelopoiesis, having as result immature myeloid cells (MDSCs), which accumulate in the tumor, leading to immunosuppression and favoring tumor growth (73). This phenomenon is more advanced as the tumor progresses.

Immune suppression in tumors. The tumor microenvironment is immunosuppressive. This immunosuppression takes place through a few mechanisms, from which: the overexpression of inhibitory molecules by the tumor and by the tumor-associated cells (PDL-1, B7-H3, TIM-3 ligands, CD47) and of death factors (FasL); cytokines such as TGFβ, ILs 4, 10, 35; secretion of exosomes with immunosuppressive content; recruitment of suppressive cells (Treg, M2, MDSCs); metabolites (lactate, adenosine), hypoxia, increased hydrostatic pressure in tumors (Fig. 1) (reviewed in 74). The nature of this immunosuppression becomes more clear from some experiments with conditionally activated oncogenes; it has been shown that following the oncogene inactivation, the TME was infiltrated by immune cells ($CD8^+$, NK LTs), that destroyed tumors (75). Insight into the mechanisms of this relation between oncogenes and immunity showed that activation of Myc led to the upregulation of both CD47 and PDL-1 on the tumor cells (76).

The immunosuppression was an effect of the oncogene activation, along with the others mentioned above (angiogenesis, cell recruitment).

The level of immunosuppression is directly correlated with the tumor progression (74). Other characteristics complete the picture of the TME:

Physico-chemical qualities of the ECM: high hydrostatic pressure, hypoxia, high lactate and adenosine content, IDO-Indoleamine 2, 3-dioxygenase-from MDSCs (77).

Inflammation: In tumors, inflammation can be both pro or antitumoral. It has been shown that inflammation exerts a tumor-promoting role in cancer, through multiple mechanisms such as angiogenesis, release of genotoxic product like ROS, enhanced survival, stimulation of proliferation, stemness or invasion of tumor cells (reviewed in 70); this is true especially with regard to chronic inflammation. On the contrary, experimental data also support a positive role of inflammation in cancer (78). This happens especially in the presence of a strong Th1 component and in the acute phase of inflammation (79).

Proliferation, angiogenesis, cell recruitment and immune suppression - a coordinated program? The events that occur in the TME (proliferation, angiogenesis, cell recruitment and immune suppression) are recognized hallmarks of cancer (80). Considering them as a whole made some researchers compare tumors to a tissue regeneration process, like the one that occurs after a tissue destruction or after the resolution of an infection (81).

This program is triggered by stimuli such as growth factors or signals of termination of the immune response, and has the same components: cell multiplication, angiogenesis, recruitment of cells with regeneration potential (macrophages, neutrophils, fibroblasts, Tregs, epitheliocytes) and immunosuppression.

The program is stopped when signals of tissue integrity and completion (integrin or cadherin signaling, hippo pathway signals) are received.

By contrast, in cancer the program is started aberrantly by the activation of oncogenes, is dysregulated and does not respond to stop signals.

There is some experimental evidence that supports this analogy (82). The comparison is not 100% accurate, but it can serve as starting point for some therapeutic considerations (83).

Immune response in tumors. The presence of the danger signals from the hypoxic or necrotic tumor cells triggers an innate response from macrophages, neutrophils, mast cells and eosinophils, directed against the tumor.

On the other hand, dysregulation of MHC provokes a tumoricidal response from the NK LTs, while particular antigens, phosphoantigens or lipids, will awake the response of cells like γδ or NKT-1 cells.

The antigen presentation from antigen-presenting cells (APCs) such as DCs, but also B-lymphocytes (LBs), macrophages and even neutrophils, mast cells or eosinophils opens the way for the intervention of $CD8^+$CTLs, which is completed by the activation of CD4 T-helper lymphocytes, with their effector mechanisms (effector cells of Th subsets, M1 macrophages, neutrophils and eosinophils) (Fig. 2) (3,84).

There is an adequacy of the immune response to a large array of stimuli. The diversity of signals and antigens in tumors requires the deployment of such a great diversity of cells, as mentioned above. The immune response is multimodal and adequate to all types of antigens and stimuli.

There is also a cooperativity in the immune response, as indicated in the first section and in Table I. This cooperation occurs between the cells of the innate immunity, between the innate and adaptive immunity and, finally, within the adaptive response (Fig. 2).

The immune system, with a complexity far beyond what could be described in this review, is still working as a unit, but a unit with adaptable modules (85). This unity is achieved through a network of signals between cells, both soluble (interleukins, chemokines), exosomal and through direct contact through cell adhesion, co-stimulation and co-inhibition.

The immune system is extremely efficient. A great body of experimental evidence shows that innate cells such as macrophages, neutrophils, eosinophils, NK, NKT or γδ cells can be strong tumoricidal elements, sometimes completely eradicating tumors (86). Clinical evidence from the above-mentioned prognostic association (30) and from new treatments such as bispecific antibodies also shows that there is an extreme efficiency of the lymphocyte when it faces the tumor cells (87).

Unfortunately, this is not always the situation in tumors. They are not eradicated, but continue to grow in spite of such a powerful system that is directed against them.

What are the reasons for this situation? The answer resides in the way in which the immune system and the tumors interact.

Interaction between the immune system and tumors. The first problem that the immune system encounters is the nature of the tumor antigens: they are not true non-self elements,

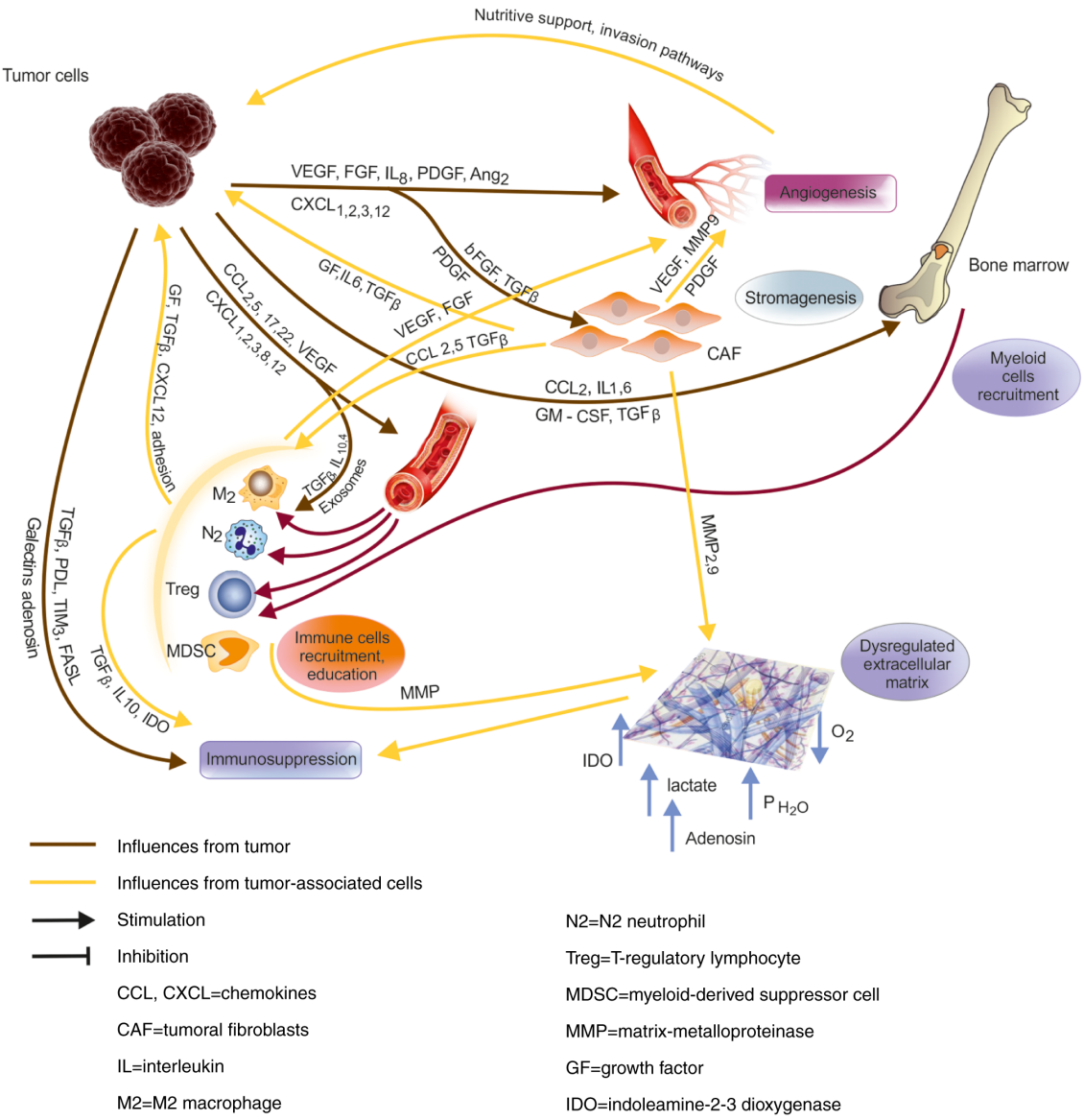

Figure 1. Biogenesis of the tumor microenvironment. The development of the tumor leads to angiogenesis and to recruitment of different cells in the tumor; immunosuppression appears also as a consequence of the tumoral growth. The tumoral fibroblasts secrete a dysregulated extracellular matrix which contributes to the immune suppression. Complex relations are established between the resulting microenvironment components.

but rather an altered self. There are also many stop signals for immunocytes in tumors, such as CD-47 or PD-L1. The tumor antigens are changing through mutations of an unstable genome, another hallmark of cancer (80).

Another problem is represented by the physico-chemical qualities of the environment in which these cells work; in tumors there is acidosis, lactate, hypoxia, IDO and an increased hydrostatic pressure; the blood vessels are modified and do not offer enough cell adhesion molecules (CAMs) for extravasation of leukocytes. By consequence, the immune response is weakened and less efficient (77).

Furthermore, there is immune suppression in tumors; the lymphocytes have to overcome this barrier as well, which they do, but at the expense of losing much of the efficiency of their response. They finally become exhausted and ineffective against tumors (74).

A major problem is that innate immune cells, that prove so tumoricidal in experiments, are subjects of tumor-secreted factors that transform them into cells supporting the tumor. The lymphocytes of the adaptive response lose the important support of these cells, weakening once more in their capacity of response (88).

A seemingly paradoxical situation is that some of the components of the immune response in tumors have protumoral effects themselves; this is the case of the chronic, smoldering inflammation that accompanies tumors, and also of some types of adaptive response, the Th2, Th17 response, some γδ or NKT-cell subsets, as mentioned earlier (Table I and Fig. 3). This situation is caused by the fact that interleukins can act as growth factors, can promote angiogenesis especially in a situation of chronic inflammation and, acting on epithelia, including tumor cells, they can promote proliferation and cell survival (reviewed in 65).

Finally, the dysfunction of the dendritic cell in tumors leads to a misbalance between lymphocyte subsets, with a

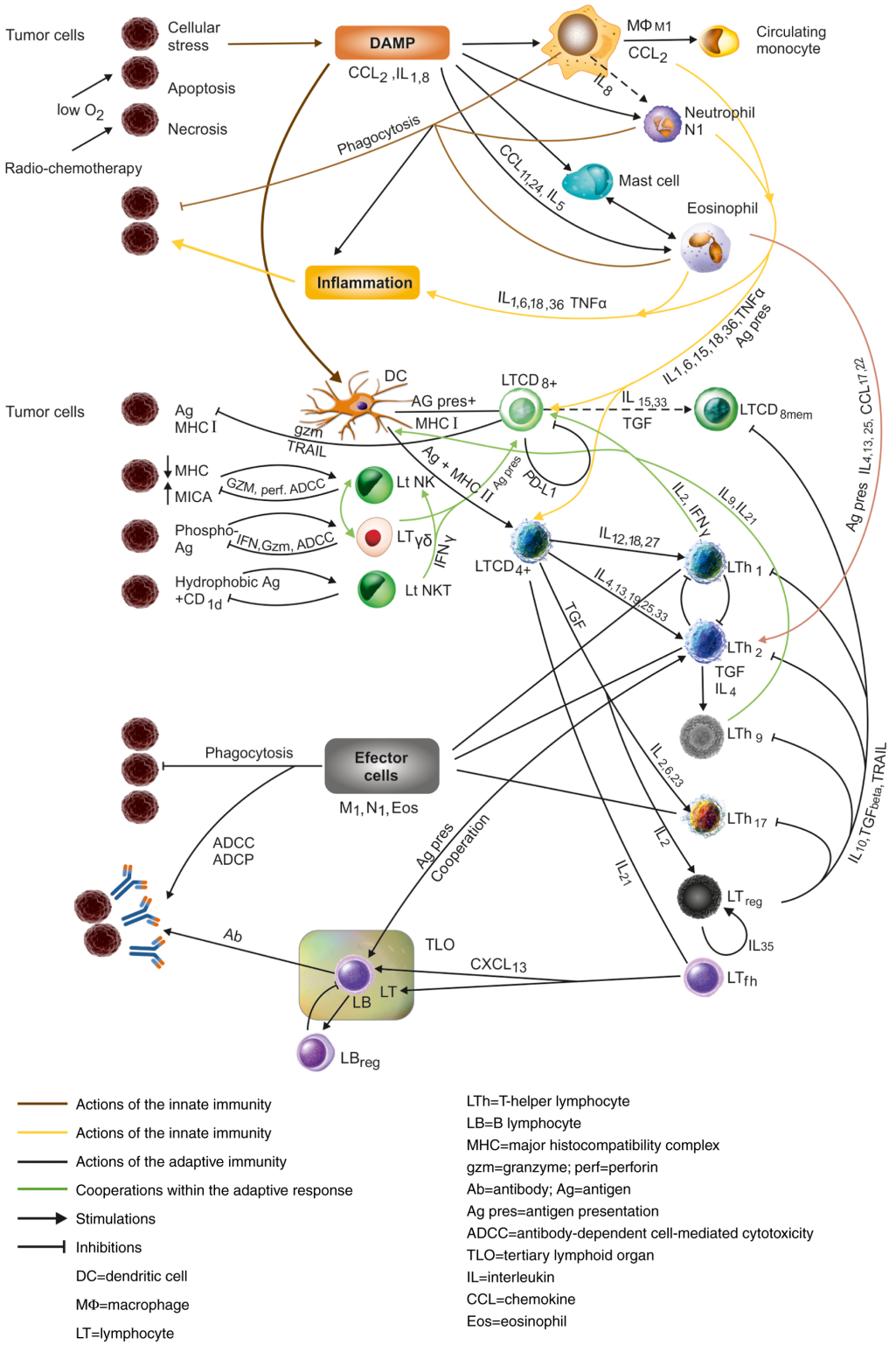

Figure 2. Immune response in malignant tumors. The presence of the danger signals within the tumor triggers an innate immune response; the antigen presentation by the APCs (antigen-presenting cells) leads to the activation of the $CD8^+$ and $CD4^+$ lymphocytes; low level of MHC proteins or increased atypical MHC such as MICA activate NK lymphocytes, while particular subsets such as γδ or NKT-cells respond to antigens like phospho-antigens or CD1d-presented lipidic antigens. A multimodal immune response develops, adequate to the signals that the tumor presents. The different segments of the immune system cooperate for an optimal response.

bias towards Th2 and regulatory subsets and a decrease of the Th1-M1 subsets. As shown earlier, it is this misbalance that is harmful to the antitumor defense, and in this situation, Th2 and Th17 lose most of their antitumoral activity and become mainly protumoral (42).

As a result, the immune system becomes gradually inefficient and the tumors continue to grow.

Varieties of tumor microenvironment. Tumors are heterogeneous structures and their TME differs from one tumor to

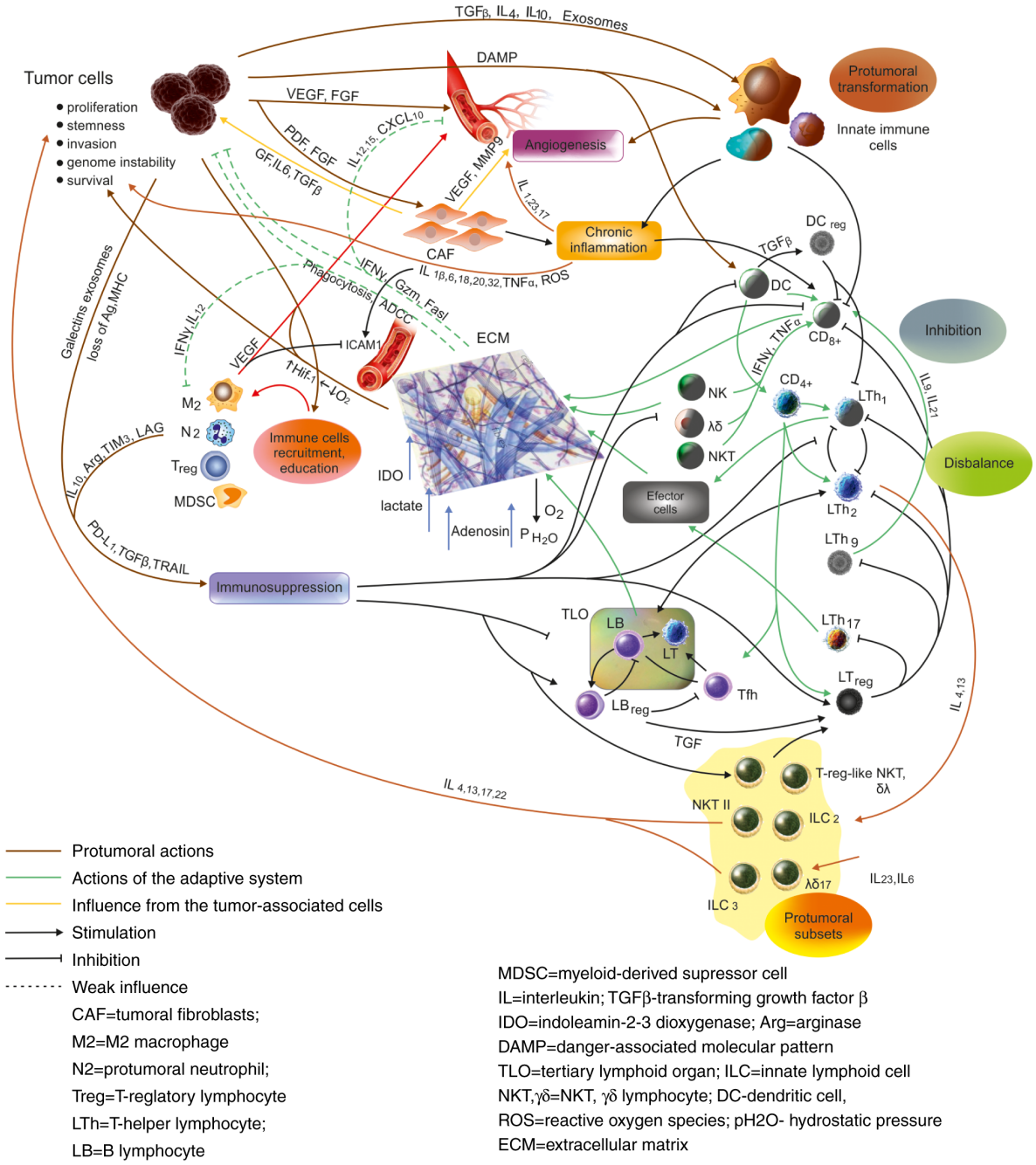

Figure 3. Relations between the tumor and the immune response. The immune response and tumors interact in multiple ways. The immunosuppression from the tumor microenvironment inhibits the antigen-specific lymphocytes, while innate immune cells are influenced by tumor-derived factors, cytokines and exosomes to aquire a protumoral profile. The dysregulated extracellular matrix contributes to the suppression, increasing also the survival and the invasiveness of the tumor cells; chronic inflammation that develops supports the tumoral growth, while certain profiles of adaptive response such as Th2 or Th17 are also mainly protumoral.

another. A recent study showed that there are at least six types of TME in tumors, based on which of these networks predominate, the subtype with angiogenesis, with inflammation, interferon-dominant, TGFβ-dominant, lymphocyte-depleted and immunologically quiet tumors. The authors suggest that these data should be incorporated in the future strategies of cancer therapy (89).

There are also differences between the different locations of tumors (90), between stages and even between patients. The type of carcinogen may also cause differences concerning not only the genomic alterations, but also the profile of the immune response that follows (91,92). These differences between tumors should prompt the development of more personalized approaches in immunotherapy (93). Personalized approaches are a developing field, and they involve the use of biomarkers such as tissue expression of checkpoint molecules, serum cytokines profile or proteomic approaches to direct precision targeted therapy of tumors (93,94).

The role of neuroendocrine factors. To complete the picture of networks in tumors, the role of the neuro-endocrine factors has to be mentioned; indeed, there is experimental data that demonstrate an influence of the nervous and endocrine system on the

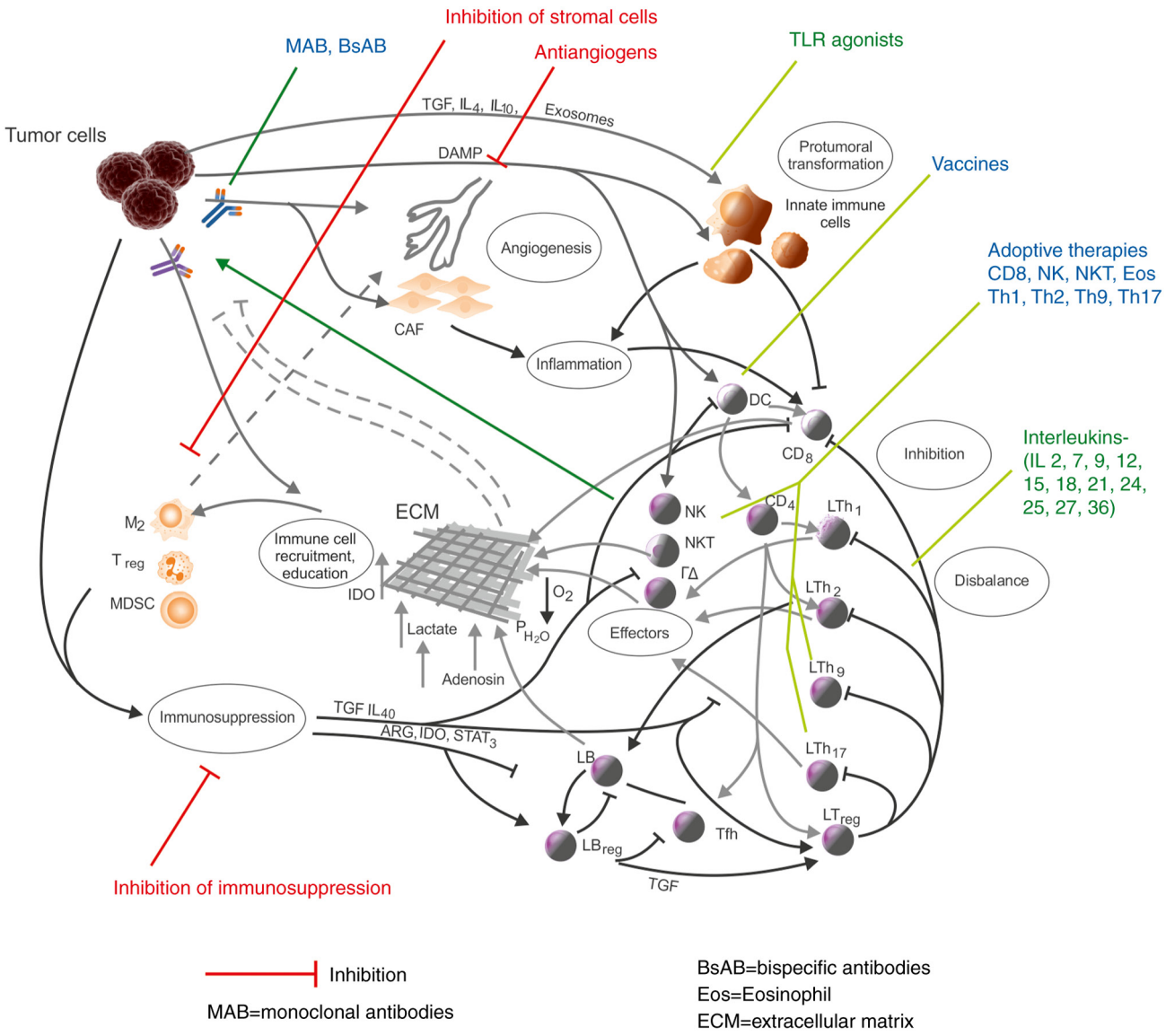

Figure 4. Immunotherapy adresses tumors by inhibiting angiogenesis, tumor-associated cells and immunosuppression, and also by stimulating different cells with tumoricidal potential, through antibodies (monoclonal or bispecific), adoptive therapies, vaccines, TLR stimulation or interleukins. A network view of the different immunotherapies is shown.

immune response (95). This is also true for the tumor immunology, since both immunocytes (96) and cancer cells (97) possess receptors for catecholamines, cortisol or different neuropeptides. This fact can be therapeutically exploited, where there are receptors in the tumor. It has been shown that through these receptors, the nervous and the endocrine systems can modulate the tumoral growth and invasion (98). The neuroendocrine factor, and through it the psychological factor, proved to be not neglectable factors in influencing the prognosis of patients with tumors.

Networks and immunotherapy. A new factor, immunotherapy, has recently entered this dynamic relation between tumors and the immune system.

One side of the immunotherapy is an inhibitory one, which addresses the tumoral side of the environment; its targets are angiogenesis (99), tumor associated cells (100), immuno-suppression (101) and inflammation (70).

Another side is the positive immunotherapy, which uses parts of the immune response to attack tumors. Antibodies (monoclonal or bispecific) are used to direct immune cells against the tumor cells, immune cells themselves are used in adoptive therapy, vaccines are used to strengthen the antigen-specific response, TLR agonists are used to stimulate innate cells, and interleukins are used to stimulate the defense (101,102) (Fig. 4).

Immunotherapies must be considered in the larger frame of immune networks in tumors; such a perspective opens the way of new strategies, which result from the network structure of the TME (103) and completes intelligent approaches like bispecific antibodies, CAR-engineered lymphocytes or the attempt to modify the antigenic interface of tumors (104). Undoubtedly these are efficient therapeutic means and have proven results, but they also have limitations, which may, at least partially, be due to the existence of these inhibitory loops that work in the TME.

Subsequently, the analysis of the immune networks in tumors is an area of increasing interest, because it is expected to offer solutions based on the understanding of the structure of these networks in the TME.

4. Conclusions and perspectives

The present study is an attempt to decipher the complex pro and antitumoral networks that form and interact in the tumor microenvironment.

The study underlines the great potential of immunotherapies; however, based on the existence of this network structure of the TME, it suggests that therapeutic approaches should be network-based and should take into account all these complex interactions within the microenvironment of tumors.

At present, performant imaging and computational approaches going as far as the single-cell level begin to enter clinic (105), computer-based learning is used to project anticancer molecules (106), but also network medicine begins to enter all fields of pathology (107), including tumor immunology and immunotherapy.

Immunotherapy is at its beginnings, but much progress has been done in recent years, based on the growing knowledge of immunobiology and genomics of malignant tumors.

Apart from the progress in the molecular and cellular biology, the knowledge of the tumor microenvironment as a whole, with its complex network of cells and cytokines, will contribute to the development of the immune therapy in the years to come.

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Authors' contributions

OF contributed in the conception of the study and wrote the manuscript. VC contributed in the conception of the study and revised it critically for important intellectual content. Both authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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