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STAT3 activation
A key factor in tumor immunoescape

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Abbreviations: APC, antigen presenting cell; C/EBP, CCAAT-enhancer-binding protein; CD, cluster differentiation; CDK, cyclin dependent kinase; CSF-1, colony stimulating factor-1; CTL, cytotoxic T lymphocyte; DC, dendritic cell; DC-SIGN, dendritic cell-specific ICAM-3 grabbing nonintegrin; DNA, deoxyribonucleic acid; EGF, epidermal growth factor; ERK, extracellular signaling regulated kinase; FGF, fibroblast growth factor; FOXP3, forkhead box P3; G-CSF, granulocyte colony stimulating factor; GFI-1, growth factor independent-1; GM-CSF, granulocyte macrophage colony stimulating factor; HSP, heat shock protein; ICAM, intracellular adhesion molecule; IFN, interferon; IL, interleukin; JAK, Janus activated kinase; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MDSC, myeloid derived suppressor cell; MHC, major histocompatibility complex; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NFκB, nuclear factor-κB; NK, natural killer; NO, nitric oxide; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; PIAS, protein inhibitor of activated STAT; RANTES, regulated on activation normal T cell expressed and secreted; RAR, retinoic acid receptor; RNA, ribonucleic acid; ROR, RAR related orphan receptor; ROS, radical oxygen species; shRNA, short hairpin RNA; siRNA, small interfering RNA; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TAM, tumor associated macrophages; TCR, T cell receptor; TDE, tumor-derived exosome; TGF, transforming growth factor; Th, T helper cell; TLR, Toll-like receptor; TNF, tumor necrosis factor; Treg, regulatory T cell; VEGF, vascular endothelial growth factor

Cancer growth is controlled by cancer cells (cell intrinsic phenomenon), but also by the immune cells in the tumor microenvironment (cell extrinsic phenomenon). Thus cancer progression is mediated by the activation of transcription programs responsible for cancer cell proliferation, but also induced proliferation/activation of immunosuppressive cells such as Th17, Treg or myeloid derived suppressor cells (MDSCs). One of the key transcription factors involved in these pathways is the signal transducer and activator of transcription 3 (STAT3). In this review we will focus on STAT3 activation in immune cells, and how it impacts on tumor progression.

Introduction

STAT3 belongs to the signal transducer and activator of transcription (STAT) family of signal responsive transcription factors which consists of seven members encoded by distinct genes. In non-stimulated cells, STAT3, like other STATs proteins, is kept in an inactive cytoplasmic form. Then, once activated, STAT3 translocates into the nucleus where it behaves as a transcription activator for a broad array of targeted genes. Typically, STAT3 activation is induced by phosphorylation on a critical tyrosine residue (Tyr 705) that triggers STAT3 dimerization thanks to reciprocal phosphotyrosine-SH2 domain interactions.[1] Even if multiple tyrosine kinases have been described as intracellular activators of STAT3 activity (such as EGFR, Src, ERK), the phosphorylation of STAT3 on tyrosine 705 is mainly regulated by members of Janus-activated kinases with JAK1 as key modulator.[2] In addition to tyrosine 705 phosphorylation, STAT3 is also activated through serine (Ser 727) phosphorylation. This phosphorylation is commonly regulated by protein kinase C, mitogen-activated protein kinases, and CDK5. Finally, the reversible acetylation of STAT3 by histone acetyltransferase on a single lysine residue (Lys 685) represents a third mechanism of STAT3 activation.[3] Acetylated STAT3 has been depicted as a way to enhance stability of STAT3 dimers which are required for DNA-binding and transcriptional activity.

Regulation of STATs protein activation is dictated by negative regulators such as PIAS (protein inhibitor of activated STAT) and SOCS (suppressors of cytokine signaling) proteins as well as protein tyrosine phosphatases. SOCS proteins prevent the JAK from activating STAT3 by direct and indirect interactions with tyrosine kinase SH2 domains.[4] They also drive the blockade of STAT3 to bind to receptor subunits. To date, SOCS protein family comprises eight members and SOCS3 seems to be the most specific inhibitor of STAT3 signaling pathway. Contrary to SOCS molecules, PIAS proteins are nuclear factors. They negatively regulate STAT transcriptional activity through several mechanisms, particularly by interacting and thus blocking DNA binding activity.[5] Protein tyrosine phosphatases remove phosphates from activated STATs which represent a third level of

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Table 1. Main STAT3 modulators and their effects in immune cells

Cell type	pSTAT3 modulators	Effects
CD4⁺ T cell	IL-27	↑ proliferation
Th17	IL-6, IL-21, IL-23	↑ RORγt and RORα expression and ↑ cell differentiation ↑ IL-21, IL-23R and IL-17
	IL-6 (in combination with TGF-β)	↑ CD39 and CD73 expression and ↑ adenosine production
Treg	IL-2	↑ Foxp3 expression (associated with pSTAT5) and ↑ inhibitory functions
	IL-6, IL-27	↓ Foxp3 expression (with an inhibition of pSTAT5 binding) and inhibition of differentiation
		↑ IL-6, TGF-β (with Foxp3 cooperation)
Macrophage	IL-6	Inhibition of differentiation
		Inhibition of Ag presentation to CTL, ↑ IL-23 and ↓ IL-12 production
		Inhibition of LPS-mediated cytokine production (e.g., RANTES and IL-12)
	Tumor cell supernatants	↑ IL-10, βFGF and VEGF-A production and ↑ angiogenesis
DC		Inhibition of Ag presentation to CTL and ↓ TLR induced IL-12 and TNF-α production
	Tumor cell supernatants, IL-6 and exosomes	↓ differentiation (with an IL-6 amplification loop)
MDSC	exosomes	↑ immunosuppressive functions (with an IL-6 amplification loop)
	Tumor cell supernatant	↑ production of VEGF and βFGF and ↑ angiogenesis
		↑ expression of NOX2, ↑ production of ROS and ↑ immunosuppressive functions

STAT modulation. Numerous phosphatases have been described to regulate STAT3, such as PTEN,[6] CD45,[7] SHP-1 and SHP-2.[8,9] Moreover, kinases such as phosphatidylinositol 3-kinase (PI3K) could regulate STAT3 phosphorylation in a different way, depending on the model used. STAT3 pathway is inhibited by PI3K in melanoma cells, whereas it is activated in laryngeal papillomas or in breast cancer stem-like cells with an emphasis in PTEN deficient cells.[6,10,11] Lastly, STAT3 has also been shown to go through ubiquitination-dependent proteosomal degradation.[12]

It is now well established that STAT3 signaling is a major intrinsic pathway driving apoptosis, inflammation, cellular transformation, survival, proliferation, invasion, angiogenesis and metastasis of cancer.[13,14] However, compelling evidence has now showed that STAT3 is constitutively activated in many human cancers.[15] Indeed, many receptor signaling pathways are excessively stimulated in tumoral context and lead to persistent activation of STAT3. Many regulated genes induced by STAT3 in turn activate the same STAT3 pathways and keep a stable feedforward loop going between tumor cells and tumor-interacting immune cells. In addition, the own tumorigenic properties of STAT3 highlight its oncoprotein status by driving malignant properties related to chronic inflammation.[16,17] Thereby, this explains that STAT3 has been characterized as a central actor for inflammation-induced cancer. STAT3 activation occurs in both cancer and stromal cells thereby allowing a crosstalk between these two cellular types. This activation is rapid and transient under normal biological conditions and mediated by a large number of extracellular stimuli including cytokines (IL-6, IL-10, IFNs, TNF-α…) and growth factors (i.e., EGF, G-CSF, GM-CSF, VEGF and Her2/Neu). Active oncogenic proteins, such as Src and Ras, as well as chemical carcinogens and other molecules also have the ability to activate STAT3 (see ref. 18 for extensive review).

It is now well established that carcinogenesis induces the appearance of danger signals which could drive tumor rejection by the immune system (a phenomenon called immunosurveillance). Some cancer cells could escape this rejection by limiting tumor antigen expression (a phenomenon called immunoediting) or by inducing active immune tolerance mechanisms.[19] These mechanisms include the proliferation and local accumulation of immunosuppressive cells, including regulatory T cells (Tregs), Th17 cells and myeloid-derived immunosuppressive cells (MDSCs). This tolerance (a phenomenon called immunoescape) prevents cancer rejection by the immune system and blunts the efficacy of immunotherapy as demonstrated in several mouse models.[20]

This review is dedicated to the current knowledge of the impact of STAT3 activation in immune cells (summarized in **Table 1**) on the balance between immunosurveillance and immunoescape.

STAT3 and T Cells

T lymphocytes or T cells play a central role in the adaptative immune response of the host to cancer.[21] Tumor-infiltrating CD4 and CD8 T cells are associated with clinical outcome and survival in colorectal cancer,[22] breast[23] and lung cancers.[24] CD4 T cells called helper T cells are capital in the generation of T cell immune response by their capacity to drive and coordinate all components of the immune response. Current cancer immunotherapies are therefore designed to induce or enhance T cell reactivity against tumor antigens.[25] Among T cells, different subsets have been described (regulatory T cells, cytotoxic T cells and T helper) with distinct functions that could be regulated by STAT3.

Th1/Th2. T helper (Th) cells assist other hematopoietic cells in immunologic processes, including activation of CTL, natural killer (NK) cells and macrophages. Th cells become activated when they are stimulated with antigens presented by MHC class II molecules that are expressed on the surface of antigen

presenting cells (APCs) such as dendritic cells. Once activated, they proliferate rapidly and secrete small proteins called cytokines that regulate or assist the active immune response. These cells can differentiate into one of several subtypes (e.g., Th1, Th2 and Th17), which secrete cytokines to regulate immune response.[26]

The expression levels of Th1 cell genes coding for Interferon-γ (IFNG), TAP1 and Granzyme B (GZMB) are significantly higher in colorectal tumors than in normal tissue. This high expression of Th1 cytotoxic genes was associated with significantly improved disease-free survival rates and patients with low expression of those genes had earlier recurrence.[27] Tbet, the master transcriptional regulator for Th1 differentiation is induced by T cell receptors (TCRs) and IL-12 stimulation.[28] In contrast, GATA3 is the master control for Th2 differentiation after stimulation with IL-4.[29] IL-27, a member of the IL-6/IL-12 family produced by macrophages and dendritic cells, favors the Th1/Th2 differentiation balance toward Th1 by upregulating Tbet (independently of STAT1), downregulating GATA3 (through a STAT1-dependent mechanism) and suppressing proinflammatory cytokine production such as IL-2, IL-4, and IL-13.[30] Even if IL-27 activates STAT3, all those events were STAT3-independent. In this context, only the IL-27 dependent $CD4^+$ T cell proliferation was mediated by STAT3 (with an upregulation of c-myc and pim-1 transcription) and not by STAT1.[30,31]

Th17. Th17 cells are $CD4^+$ T cells induced by TCR triggering with IL-6 and transforming growth factor (TGF)-β stimulation. After induction, IL-23, an IL-12 family member, maintains Th17 cell polarization.[32] Th17 cells have emerged as key driver of a wide range of autoimmune disorders, including inflammatory bowel disease, psoriasis and ankylosing spondylitis.[33] Th17 cell expansion was observed in human cancers such as ovarian, melanoma, breast or colon cancers.[34] In colorectal cancer, patients with low expression of Th17 genes seemed to have a prolonged disease-free survival.[27] Despite these early observations, the role of Th17 cells in cancer immunity remains controversial.

Th17 are characterized by the expression of the transcription factors RORγt and RORα.[35] In addition, STAT3 is also indispensable for Th17 cell differentiation, because STAT3 ablation in CD4 cells in mice results in an absence of Th17 differentiation.[36] Moreover, many in vitro studies have shown that STAT3 can be activated downstream of receptors for several pro-inflammatory cytokines including IL-6, IL-21 and IL-23 leading to the regulation of RORγt, RORα, IL-21, IL-23R and IL-17 expression along with the development and the stabilization of Th17 cells.[37-41] In the absence of STAT3, other signaling pathways are engaged, such as STAT1 pathway, leading to the induction of the Th1 cytokine IFNγ.[38]

Our team found that in vitro Th17 cells generated with IL-6 and TGF-β and in vivo tumor-infiltrating Th17 cells express CD39 and CD73 ectonucleotidases. This ectonucleotidase catalytic machinery leads to the cleavage of extracellular ATP into the immunosuppressive molecule adenosine which could suppress effector T cells. The expression of ectonucleotidases is dependent on STAT3 and the zinc finger protein growth factor independent-1 (Gfi-1), which is a transcription repressor. IL-6-driven STAT3 binds to the promoter regions of CD39 and CD73 and TGF-β-mediated downregulation of Gfi-1 removes its transcriptional inhibition during Th17 cell differentiation, both leading to the transcription of ectonucleotidases. CD39 expression on Th17 cells promotes tumor growth, suggesting that expression of ectonucleotidases dictates the immunosuppressive fate of Th17 cells in cancer.[42] The generation of Th17 cells from naive T cells activated with IL-1β, IL-6 and IL-23 in the absence of TGF-β has been reported. Unlike Th17 cells generated with TGF-β and IL-6, these Th17 cells generated without TGF-β were highly pathogenic in vivo[43] and failed to express ectonucleotidases.[42] These observations suggest that STAT3 and Gfi-1 determine the effector immunoregulatory fate of Th17 cells through regulation of ectonucleotidase expression.[42]

Treg. Regulatory T cells (Treg) are suppressor T cells that maintain peripheral immune tolerance.[44,45] Conversion of naive CD4 T cells in $CD4^+/CD25^+$ Treg cells can be achieved through co-stimulation with TCRs and TGF-β. Such stimulation induce the expression of FOXP3 the master transcription factor for Tregs.[46] These T cells accumulate in tumors and in the peripheral blood of patients with cancer[47] and the increased Treg frequency has been generally considered as a marker of poor prognosis in cancer presumably because of Treg-mediated suppression of antitumor immunity, which benefits the tumor.[48-50]

The role of STAT3 in regulating *Foxp3* expression by Tregs appears to be context-dependent. In vitro, IL-2 induces the binding of STAT3 and STAT5 to a highly conserved STAT-binding site located within the first intron of the *Foxp3* gene leading to the upregulation of FOXP3 expression in purified $CD4^+CD25^+$ T cells but not in $CD4^+CD25^-$ cells.[51]

In tumor-infiltrating Tregs both STAT3 and STAT5 can bind to a STAT consensus site in the *Foxp3* promoter and enhances FOXP3 expression which seems to be important in maintaining Tregs' inhibitory functions.[51-53] Low-dose IL-2 treatment of patients with metastatic cancer or chronic myelogenous leukemia after allogeneic hematopoietic stem cell transplantation resulted in an increase in the frequency of $CD4^+CD25^+$ cells in peripheral blood as well as FOXP3 expression in $CD3^+$ T cells.[51] In Treg cells, activated STAT3 and FOXP3 interact together and co-operatively regulate IL6 and TGFβ1 genes, which likely endow Treg cells with the ability to suppress Th17 cell-mediated inflammation and fatal colitis.[54] In addition, IL-21, which activates STAT3, does not activate STAT5 and has no effect on Treg viability, activation or function.[55] On the contrary, in naive T cells induced to differentiate into Tregs in vitro, IL-6 or IL-27 inhibit the differentiation to the Treg lineage in a STAT3-dependent and STAT1-independent manner.[40,56] In another context, STAT3 binds to a silencer element within the *Foxp3* locus[57] and could also inhibits STAT5 binding to its binding element in *Foxp3* promoter,[58] both inhibiting FOXP3 expression. Moreover, the modulation of STAT3 activity in Tregs by molecular compounds could lead to an inhibition of their activity. Thus, WP1066 (an inhibitor of STAT3 signaling) enhances T cell cytotoxicity against melanoma through inhibition of $FOXP3^+$ Tregs, suggesting that STAT3 is required for immunosuppressive functions of Tregs.[59]

STAT3 and Myeloid Cells

APCs lie at the center of this critical decision of the immune system, since these cells have been shown to capture antigens in the periphery, migrate to the lymphoid organs and present processed peptides to T cells in a way that may lead to either priming tolerance induction.[60]

Macrophages. Macrophages can differentiate into two subsets, M1 and M2 macrophages, based on their capacity to produce IL-12 or IL-10, respectively. M1 has potent microbicidal properties and promotes Th1 responses, whereas M2 supports Th2-associated effector functions.[61,62] M2 macrophages are further subdivided into several subtypes including tumor-associated macrophages (TAMs). TAMs accumulate at the tumor site by tumor-derived signals, such as macrophage colony-stimulating factor (M-CSF/CSF-1) and monocyte chemoattractant protein-1 (MCP-1) or chemokine (C-C motif) ligand-2 (CCL2). They actively contribute to tumor growth by releasing proangiogenic cytokines and growth factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), colony stimulating factor-1 (CSF-1), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (βFGF). They also produce arginase-1, IL-10 and TGF-β, which inhibit the antitumor function of T cells and natural killer cells leading to tumor tolerance and the impairment of antitumor immunotherapies efficacy.[63-65]

IL-6 inhibition of macrophage colony-stimulating factor (M-CSF)-induced colony formation observed in mice was abolished in mice mutated for the gp130-STAT1/3 signaling, suggesting that the IL-6/STAT3 pathway could regulate macrophage homeostasis.[66]

Cheng et al. were the first to show that disruption of STAT3 signaling in either macrophages or bone marrow-derived dendritic cells (DCs) renders these APCs capable of restoring the responsiveness of tolerant T cells from tumor-bearing mice suggesting an important implication for cancer immunotherapy. The finding that peritoneal elicited macrophages with a targeted disruption of STAT3 have a constitutively activated phenotype and are more prone to produce inflammatory mediators in response to LPS (such as RANTES, MIP-1α, MIP-1β, MIP-2, IP-10, IL-6 or IL-12) points to STAT3 signaling as a negative regulatory pathway in these cells. This could be due to an increased STAT1 activity (leading to high production of inflammatory factors e.g., RANTES or IL-12) or a lack of IL-10 production.[67] The same team showed that macrophages derived from conditional STAT3 knockout mice are superior to wild-type macrophages in terms of their ability to prime cognate CTL responses, and to cross-present tumor-derived antigen to CTLs in vitro leading to a stronger proliferation of CTLs and an increased production of IFN-γ and tumor necrosis factor (TNF)-α. In the same way, ablating STAT3 in hematopoietic cells results in rapid activation of innate immunity by CpG (a TLR9 ligand), with enhanced production of IFN-γ, TNF-α and IL-12, and activation of macrophages, neutrophils and natural killer cells (which present strong STAT1 activation) and with an eradication of B16 melanoma tumors.[68] Targeting STAT3 signaling represents therefore an enticing strategy to augment CTL responses in the tumor-bearing host.[69] Immunosuppressive activities of TAMs correlate with over-activated STAT3 signaling of the cells and disruption of STAT3 activity of TAMs can enhance rat immune response to breast cancer.[70] In glioblastoma, tumor-infiltrating macrophages were shown to be predominantly STAT3-positive M2 macrophages which is associated with a poor prognostic for those patients.[71] The same team proposed corosolic acid and oleanic acid as new compounds for tumor prevention and therapy through their capacity to suppress M2 polarization of macrophages and tumor cell proliferation by inhibiting STAT3 activation and IL-10 production.[72,73] In intrahepatic cholangiocarcinoma (ICC), patients with high counts of $CD163^+$ M2 macrophages showed poor disease-free survival. Tumor cell supernatant from ICC cell lines (such as HuCCT1) induced the production of IL-10 and VEGF-A by macrophages through activation of STAT3 and polarization toward the M2 phenotype.[74] This was confirmed by the fact that macrophages isolated from mouse tumors displayed activated STAT3 and induced angiogenesis in an in vitro tube formation assay via STAT3 induction of angiogenic factors, including VEGF and βFGF.[75] STAT3 signaling within the tumor microenvironment induces a procarcinogenic cytokine, IL-23, via direct transcriptional activation of the IL-23/p19 gene in tumor-associated macrophages, while inhibiting a central anticarcinogenic cytokine, IL-12, thereby shifting the balance of tumor immunity toward carcinogenesis.[53] The M-CSF-inducible DC-SIGN (dendritic cell-specific ICAM-3-grabbing nonintegrin or CD209) expression along monocyte-to-macrophage differentiation is dependent on JNK and STAT3 activation. This effect is potentiated by STAT3-activating cytokines IL-6 and IL-10 produced by STAT3-activated tumor cells. DC-SIGN contributes to the release of factors (IL-10) that would maintain STAT3 activation in tumor cells, thus implying that DC-SIGN favors the maintenance of an activated STAT3 context in the tumor stroma, which would compromise the ability of tumor-associated macrophages (and DCs) to generate effective antitumor responses and maintain an immunosuppressive environment.[76]

However an anti-tumoral role of STAT3 in macrophages has been proposed. This came from studies that investigated the importance of STAT3 in macrophages through an indirect manner, using SOCS3 conditional knockout mice in macrophage. These macrophages presented a prolonged activation of STAT3 (but also a reduced activation of STAT1) and simultaneously exerted anti-inflammatory (e.g., through IL-6 and TNFα down-regulation) as well as anti-tumor effects (through MCP2/CCL8-mediated anti-metastatic effect).[77] In that respect Lipoxin A4, by inducing the phosphorylation of STAT3, mediates monocyte differentiation into M2 subtypes that present anti-tumorigenic activities.[78] The discrepancies between these studies could be explained by the fact that SOCS3 and lipoxin signaling should regulate other pathways such as NFκB.

Tumor-STAT3 could also modulate macrophage fate. For example, in murine melanomas, natural STAT3 activity is associated with tumor growth and reduction of lymphocytes, NK cells, neutrophils and macrophages infiltration. In addition, blocking STAT3 triggers tumor cells to produce TNF-α and

IFN-β capable of activating macrophage production of NO and RANTES in vitro and in vivo, leading to macrophage-mediated, nitrite-dependent cytostatic activity against tumor cells.[79,80] Finally the inhibition of phagocytosis and the secretion of IL-10 induced by glioma cancer stem cells conditioned medium were reversed when the STAT3 pathway was blocked in the glioma cancer stem cells.[81]

Dendritic cells. Dendritic cells represent a terminally differentiated stage of monocytes and are the key antigen-presenting cells of the immune system. DCs play a main role as immune sentinels in the initiation of T-cell responses to microbial pathogens, tumors and inflammation.[82,83]

The first evidence that STAT3 is important for DC fate was observed in mice lacking expression of STAT3 in hematopoietic progenitors. These animals present profound deficiency in the DC compartment and abrogated Flt3L effects on DC development.[84] However, the same mice bearing a tumor present enhanced function of dendritic cells, T cells, NK cells and neutrophils, and a decreased tumor progression.[85] DCs derived from LysMcre/STAT3(flox/flox) mice displayed higher cytokine production (such as IL-12 and TNFα) in response to TLR stimulation, activated more efficiently T cells and intratumoral administration of these DCs significantly inhibited MC38 tumor growth.[86] The more important production of IL-12 in STAT3 deficient cells has been attributed to the capacity of STAT3 to suppress NFκB/c-Rel mediated IL-12 transcription.[53] One could speculate that it is also true for TNFα, a known NFκB target gene. Moreover, ablating STAT3 in myeloid cells increases CpG-induced dendritic cell maturation (which present high levels of activated STAT1), T-cell activation, generation of tumor antigen-specific T cells and long-lasting antitumor immunity in B16 melanoma tumor model.[68] In the same way, CpG-bound STAT3 siRNA were used to downregulate STAT3 expression specifically in myeloid and B cells. Then silencing STAT3 in these cells significantly augments dendritic cell engagement and effector functions of adoptively transferred $CD8^+$ T cells in vivo, with an upregulation of effector molecules such as perforin, granzyme B and IFN-γ.[87] The mechanism showing that STAT3-deficient DCs could activate more efficiently T cells than wild-type DCs was completed by Kortylewski et al.[85] Indeed mice lacking STAT3 in myeloid compartment of tumor stroma, including DCs and macrophages, showed reduced numbers of tumor-infiltrating $CD4^+CD25^+$/$Foxp3^+$/$Lag\text{-}3^+$ Tregs, which was accompanied by increased $CD8^+$ effector T cells. Reduced expression of MHC class II and costimulatory molecules due to constitutive STAT3 activation in tumor-residing DCs contribute to the expansion of tumor-infiltrating $FOXP3^+$ T cells.[85] Finally IL-6 has been shown to be a potent suppressor of bone marrow-derived DC activation/maturation and a regulator of DC differentiation in vivo, through STAT3 phosphorylation. Then an IL-6-gp130-STAT3 amplification loop controls DC differentiation/maturation and may represent a critical target for controlling T cell-mediated immune responses.[88] In this way, mammary tumor-derived exosomes block the differentiation of murine myeloid precursor cells into DCs in vitro. This was associated with an increased level of IL-6 and phosphorylated STAT3 and was blocked when bone marrow cells come from IL-6 knockout mice, suggesting that tumor cells could dampen DC differentiation through an autocrine STAT3 activation by IL-6.[89] IL-6 could also be produced by cancer cells and impact on STAT3 activation in DCs. Thus tumor cell conditioned medium from murine colon carcinoma or fibrosarcoma cells induced activation of JAK2 and STAT3 (but not STAT1 or STAT5), which was associated with an accumulation of immature myeloid cells and an inhibition of their differentiation into mature dendritic cells.[90-92] More precisely, this observation was confirmed by the fact that soluble factors released by pancreatic cancer cells, IL-6 and G-CSF, are responsible for inhibiting DC differentiation and activation respectively in a STAT3-dependent manner.[93]

In humans, STAT3-depleted DCs with adenoviral STAT3 short hairpin RNA (shRNA) were also capable of producing more cytokines under TLR stimulation (such as IL-12 and TNF-α), and they induced tumor Ag-specific T cells more efficiently than control DCs.[86]

The production by tumor cells of molecules regulating DC activation could be induced by STAT3. Blocking STAT3 in tumor cells (by anti-sense oligonucleotides or STAT3β to displace STAT3α DNA binding) increases expression of proinflammatory cytokines (such as IFN-β, TNF-α and IL-6) and chemokines (RANTES and IP-10) that activate innate immunity and dendritic cells, leading to tumor-specific T-cell responses.[80] On the contrary, constitutive STAT3 activity (by transformation with v-src or transfection with a constitutively active STAT3 i.e., STAT3C) reduces production of pleiotropic factors (e.g., IL-6 and RANTES) and inhibits dendritic cell functional maturation. It is surprising that STAT3 negatively regulates IL-6 production by tumor cells in this context, because IL-6 has been largely described to be a target of STAT3 (for reviews, see refs. 14 and 94). Nevertheless, tumor-derived factors (that were not defined in this study) inhibit dendritic cell maturation through STAT3 activation in progenitor cells.[80]

MDSCs. MDSCs have been identified in humans and mice as a population of immature myeloid cells with the ability to suppress T cell activation.[95] In tumor-bearing mice, these cells have been shown to markedly expand in lymphoid organ and blood when mice are inoculated with transplantable tumor cells or when tumors spontaneously develop in transgenic mice with tissue-restricted oncogene expression.[96] In addition, an increased MDSC frequency was detected in the blood of patients with different types of cancers.[97,98] In mice and humans, MDSCs from tumor bearers induce antigen-specific MHC class I restricted tolerance of $CD8^+$ T cells and are one of the major suppressors of antitumor immunity.[99]

STAT3 is probably one of the main transcription factors that regulate MDSC function. MDSCs from tumor-bearing mice have markedly increased levels of phosphorylated STAT3 compared with immature myeloid cells from naive mice.[91] Moreover, ablation of STAT3 expression through the use of conditional knockout mice or selective STAT3 inhibitor (JSI-124 that did not affect pSTAT1, pSTAT5 or pSTAT6) markedly reduced the expansion of MDSCs, promoted accumulation of dendritic cells and increased T-cell responses in tumor-bearing mice.[85,90,91] Then, activated STAT3 has been proposed to be the main

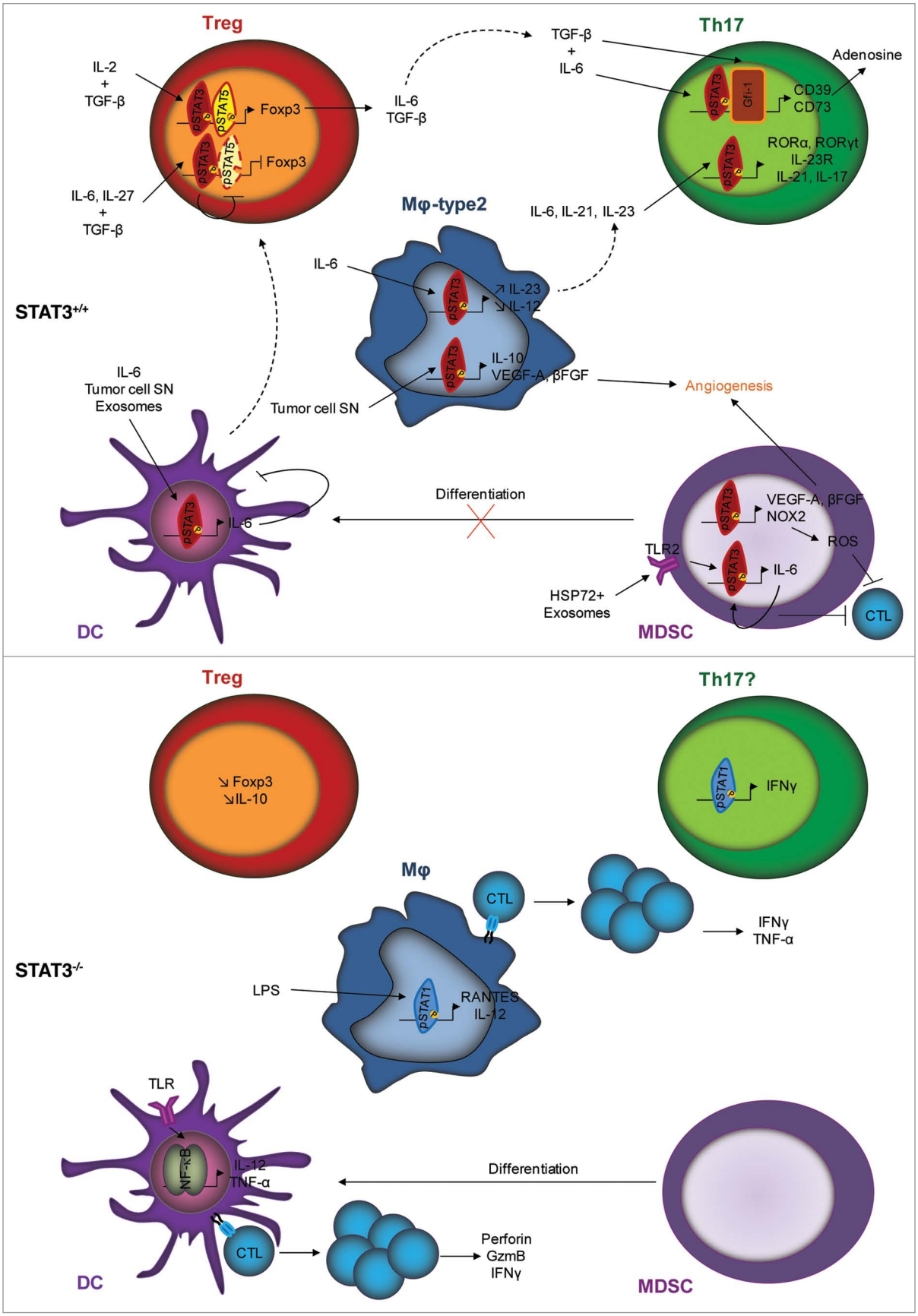

Figure 1. For figure legend, see page 7.

Figure 1 (See opposite page). Effects of STAT3 presence ($STAT3^{+/+}$) or absence ($STAT3^{-/-}$) within immune cells. STAT3 activation leads to the activation of Treg and Th17 cells, the differentiation of M2 macrophages, the accumulation of MDSCs and the absence of functional DCs. STAT3 deletion or inhibition leads to the inhibition of Treg and Th17 cells, the differentiation of MDSCs into DCs, a good antigen presentation to CTLs by macrophages and DCs leading to an anti-tumor immune response.

regulator of MDSC expansion. We have shown in contrast that tumor-derived exosome (TDE)-associated HSP72 triggered STAT3 activation in MDSCs in a TLR2/MyD88-dependent manner through autocrine production of IL-6. This mechanism is also relevant in cancer patients, as TDEs from a human tumor cell line activated human MDSCs and triggered their suppressive function but not MDSC expansion in an HSP72/TLR2-dependent manner.[100] How to explain these discrepancies? It is well known that in myeloid cells, STAT3 signaling drives the expression of Bcl-xL, c-myc, cyclin D1 or survivin, which prevents cell apoptosis, promotes cell proliferation, and prevents differentiation to mature cell types.[14,101] Moreover, in vivo STAT3 ablation or inhibition mediates the differentiation of MDSCs into DCs, leading to the observation of an absence of MDSCs and to the conclusion that STAT3 mediates MDSC expansion. In our hands, tumor cell supernatant triggers two distinct molecular pathways in MDSCs: tumor-derived soluble factors trigger the activation of ERK, which results in the expansion of MDSCs, while TDEs trigger the activation of STAT3 without promoting MDSC expansion.[100] Nevertheless, STAT3 controlled the G-CSF-responsive induction of C/EPBβ (CCAAT-enhancer-binding protein β) expression in myeloid progenitor cells.[102] The transcription factor C/EBPβ was reported to play a crucial role in controlling the differentiation of myeloid precursors to functional MDSCs, with a correlation between C/EBPβ expression and $CD11b^{+}$ $Gr\text{-}1^{+}$ MDSC accumulation in response to G-CSF.[103,104] Moreover, when C/EBPβ is deleted in bone-marrow cells they lose the ability to differentiate in vitro into functional MDSCs. It is conceivable that STAT3 can at least partially induce MDSC generation via upregulation of C/EBPβ.[102]

Finally, recent works have highlighted the importance of signaling pathways downstream of STAT3 that are responsible for MDSC differentiation. As mentioned above for macrophages, MDSCs isolated from mouse tumors displayed activated STAT3 and induced angiogenesis in an in vitro tube formation assay via STAT3-dependent induction of angiogenic factors, including VEGF and βFGF.[75] In MDSCs, STAT3 controls the NADPH oxidase NOX2 expression leading to the production of ROS responsible for MDSC-induced immune suppression in murine mammary, colon, lung carcinoma, thymoma, sarcoma models and in patients with head and neck cancer.[105] In mice, the myeloid-related protein S100A9, a direct target of STAT3 is responsible for an enhanced MDSC production in EL4 or CT26 tumor-bearing mice. Mice lacking this protein mounted potent antitumor immune responses and rejected implanted tumors an effect reversed by administration of wild-type MDSCs from tumor-bearing mice to S100A9-null mice.[106]

Conclusion

STAT3 activation within immune cells is responsible for the accumulation and the activation of immunosuppressive cells, such as Treg, Th17 and MDSCs, the differentiation of macrophages toward the M2 phenotype and the absence of functional DCs. On the contrary the deletion or the inhibition of STAT3 in these cells leads to the inhibition of Treg and Th17 cells, the differentiation of MDSC, a good antigen presentation by macrophages and DCs leading to an anti-tumor immune response mediated by CTL activation (**Fig. 1**). Thus, because STAT3 also regulates genes involved in biological functions of cancer cells, it makes this pathway a promising therapeutic target. Then STAT3 could be inhibited directly with peptides or natural compounds or indirectly by blocking upstream signaling pathways such as IL-6 and JAK2 pathways (for review, see ref. 107).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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