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Neoplastic "Black Ops": Cancer's Subversive Tactics in Overcoming Host Defenses

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Abstract

Metastatic cancer is usually an incurable disease. Cancers have a broad repertoire of subversive tactics to defeat the immune system. They mimic self, they down-regulate MHC molecules so that T cells are blind to their presence, they interfere with antigen presentation, and they produce factors that can kill T cells or paralyze their response to antigens. Furthermore, the same powerful machinery designed to prevent harmful autoimmune responses is also acting to protect cancers. In particular, cancer is protected with the help of so-called regulatory immune cells. These unique subsets of cells, represented by almost every immune cell type, function to control responses of effector immune cells. In this review, we will discuss the evidence that cancer actively promotes cross-talk of regulatory immune cells to evade immunosurveillance. We will also discuss the role of a newly described cell type, regulatory B cells, by emphasizing their importance in suppression of antitumor immune responses. Thus, cancer not only directly suppresses immune function, but also recruits components of the immune system to become traitors and protect the tumor from immune attack.

Keywords

Cancer escape; regulatory T cells; Tregs; tumor-evoked regulatory B cells; tBregs; cancer immunotherapy

Effector immune cells can control cancer progression

The immunosurveillance theory of Thomas and Burnet proposes that the host immune effector cells recognize and eliminate aberrant and malignant cells (1). The fact that human trials of cancer screening appear to detect cancers that subsequently regress and do not threaten a patient's life (so called overdiagnosis) lends further support to this notion (2). This theory is now widely accepted and has significant implications in the development of cancer-combating strategies. The presence of $CD8^+$ cells in a tumor is a prognostic marker of a favorable clinical outcome in some cancer patients (3, 4), and adoptive transfer of tumor-reactive $CD8^+$ CTLs can yield a significant response rate in patients with melanoma (5, 6). It should be noted that the immunosurveillance theory also correctly predicts that immune cells can distinguish cancer cells despite their poor immunogenicity and similarity to self. Indeed, cancer patients have low but detectable levels of humoral and cellular responses to self and weakly immunogenic tumor-associated antigens (TAAs, the products

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of normal or mutated genes preferentially expressed in malignant cells). A practical implication of this fact is that a number of important TAAs, such as tyrosinase, gp100, MAGE, NY-ESO-1, OFA-iLRP (7–11), and human T cell lines specific for these antigens were cloned and used to successfully induce antitumor cellular immune responses (7, 12–14). Overall, a wealth of knowledge obtained from both preclinical studies and trials in cancer patients indicates that cytolytic $CD8^+$ T cells can effectively combat cancer. However, the process also requires the engagement of other "accessory" immune cells, such as tumor-infiltrating dendritic cells (DCs), B cells, macrophages, $CD4^+$ T cells, natural killer (NK) cells, granulocytes, mast cells, and others. For example, the generation and maintenance of $CD8^+$ T cells requires $CD4^+$ T cells to provide Th1-type help (15, 16). Some data indicate that cancers can also be directly controlled by tumor-reactive $CD4^+$ T cells and Th17-type $CD4^+$ T cells (17–19). Similarly, although NK cells and macrophages can kill tumor cells (20, 21), they provide important inflammatory help to counteract a cancer-promoting milieu.

Cancer-induced immune suppression and cancer escape

Despite an active participation of antitumor effector immune cells, cancer eventually progresses often revealing a biphasic progression pattern of growth, such as in BALB/C mice with orthotopic murine 4T1 breast cancer (22). This tumor grows slowly for a period of time and then grows very rapidly metastasizing to various organs. The biphasic pattern of tumor growth is abolished in the absence T and B cells, suggesting that cancer manipulates T and B cells to successfully progress. Interestingly, in a mouse model of spontaneous pancreatic cancer, this control may also occur very early in cancer progression, as preinvasive lesions did not contain signs of infiltrating immune effector T cells (23). Although cancer can escape from effector immune cells utilizing numerous mechanisms, the wealth of recent findings indicate that it also gets help from the immune system itself, particularly the component of the system designed to prevent or control the induction of harmful autoimmune responses. This immune augmentation of cancer progression involves the recruitment and activation of various immune cells, which can occur as early as at the stage of preinvasive lesions (23). For example, cancer recruits tumor-infiltrating leukocytes with suppressive and tumor-promoting functions (24–26), such as myeloid-derived suppressive cells (MDSCs) and regulatory T cells (Tregs). Thus, despite initial regression of murine 4T1 breast cancer in mice, cancer eventually regains its growth capacity and metastasizes into various organs (22), raising an interesting possibility that cancer progression is also a result of a successful immunosurveillance. In fact, antitumor acute responses (and tissue injury associated with, for example, expression of angiostatic chemokines CXCL9 and CXCL10) can also trigger "protective" responses designed to prevent the induction of harmful autoimmune responses. This condition combined with the genetic instability of cancer cells can provide benefits for the outgrowth of cancer subsets that lose antigenic epitopes or/and express immunomodulatory and tumor-promoting factors (27). Although the presence of tumor-infiltrating leukocytes is a key hallmark of cancer (28), the outgrowth of cancer variants and the changing tumor environment can induce sequential recruitment of leukocytes promoting chronic and suppressive inflammation. As a result, Th1 inflammatory conditions, often associated with cell-mediated clearance of cancer cells (29), are usually changed to Th2 responses that suppress anti-tumor Th1 responses and $CD8^+$ CTLs (30–32). In fact, at least in vitro, the same DCs that primed Th1 responses at early stages can preferentially prime Th2 and nonpolarized T cells at later time of activation (33). Similar changes are also observed during infection of some pathogens. For example, to survive, mycobacteria induce a sequential production of T helper type 1 (IFNγ and TNFα) and type 2 (IL-4, IL-5 and IL-13) cytokines (34). On the other hand, cancer can also directly promote Th2-skewed responses by producing the thymic stromal lymphopoietin (TSLP) (35–37), an IL-7-like type 1 inflammatory cytokine that is often associated with the

induction of Th2-type allergic responses (38, 39). Cancer-produced TSLP induces expression of IL-10 and IL-13 (40), cytokines that promote cancer escape by activating NKT cells (41), MDSCs (42, 43), and suppressive regulatory T cells, such as iTregs and Tr1 cells (44, 45). Interestingly, the inactivation of the TSLP/TSLPR axis alone is sufficient to inhibit breast cancer progression and metastasis in mice (40).

Cancers appear to utilize regulatory immune cells at early stages of their development. Once a cancer reaches a certain size, it may no longer need regulatory immune cell assistance, as its inflammatory milieu is cytotoxic for effector immune cells (46) resulting in reduced numbers of T, B and NK cells and an immunocompromised state in patients with advanced disease (Fig.1). Cancer and cancer stroma express numerous immunomodulatory and cytotoxic factors (47), including the β-galactoside binding protein (also known Galectin-1/Gal-1 in its dimeric lectin form after a non-covalent homodimerization at $>10^{-5}$ M (48–50). Among the plethora of responses depending on the activation state of target cells and the binding with cell surface glycoproteins (49), βGBP induces cell-cycle arrest and cell death of activated T cells (51, 52). In its monomeric form, βGBP shuts down Ras-GTP loading, ERK activation and cell proliferation through the inhibition of the p110 catalytic subunit of PI3K (53). Thus, the strategies to combat large tumor burden cancers will probably differ from immunotherapeutic approaches designed to combat cancer relapse and metastasis. Since cancer vaccines utilize the host immune system, they only seem to work well at the minimal residual disease stage, a time when the patient's cancer-induced immunosuppressive state can be controlled. The T cells in a patient with advanced cancer typically have defects in signal transduction that make them poorly responsive to activation signals (54). One strategy to overcome this particular form of tumor-induced immunosuppression is to introduce a novel signaling molecule that recognizes a tumor-associated antigen and is itself capable of activating the T cell that expresses it. This approach has been used in refractory chronic B-cell leukemia. Remissions were induced in three patients by adoptive transfer of ex vivo activated T cells expressing chimeric antigen receptors (CARs expressing anti-CD19 single chain antibody fused with intracellular signaling molecule) (55). It remains to be seen whether this exciting result will lead to a long-term disease-free state or the emergence of CD19-negative tumor variants. As we recently reported, the successful elimination of established T-cell tumors in immunocompromised mice by targeting their chemokine receptor CCR4 with TARC-toxin promotes the outgrowth of tumor escapees that did not express CCR4 (56).

Cancer utilizes regulatory immune cells designed to prevent harmful autoimmunity

Since Sakaguchi's seminal finding of regulatory T cells (Tregs) (57, 58), a new field has emerged demonstrating the existence and the importance of other regulatory immune cells in the control of autoimmune diseases (59, 60). Moreover, these unique immune cells are also cleverly hijacked by cancer, and as such their presence is associated with a poor disease outcome in patients with a variety of malignancies (61–63). The nature of these cells, including Tregs, and the mechanisms of their functions are being elucidated. The majority of Tregs represent a small and unique population of $CD4^+$ T cells that express high levels of CD25 (IL-2Rα), CTL-associated antigen 4 (CTLA-4) and scurfin, a fork-head box P3 gene product (FoxP3) (64), accounting for less than 5% of $CD4^+$ T cells and 20% of $CCR4^+CD4^+$ T cells in human peripheral blood (65). Although Tregs regulate responses to self- and alloantigens (58), their suppressive activity is difficult to segregate from activated $CD4^+$ T cells (66), specifically Th2-polarized cells that produce immunosuppressive cytokines (67, 68) and suppress Th1 polarization without affecting T cell proliferation (65). In contrast, Tregs mostly mediate a contact-dependent suppression of proliferation of T cells (57, 69, 70) acting either directly or indirectly through the inhibition of APCs (64, 71, 72) utilizing FasL/

Fas- and PD1/B7-H1-dependent processes (65, 73–76), or secreted factors such as IL-10, TGF-β, IL-27 and IL-35 (77–80). Yet, the choice of a particular regulatory pathway may depend on the nature of cells or the strength/type of stimulation. While memory-type $CD25^+CD4^+$ Tregs (T_{REM}) and Tr1 primarily utilize soluble factors, such as IL-10 and TGFβ (66, 81, 82), other ex vivo activated/generated Tregs use cell contact-dependent perforin/granzyme-mediated regulatory processes (44, 45). In contrast, human natural Tregs prevent activation of $CD8^+$ T cells by self-antigens utilizing FasL and βGBP (65, 83).

Tregs play an important role in cancer progression by inhibiting antitumor activity of tumor-infiltrating effector T cells (61–63), DCs (64, 71, 72) and by adversely affecting NK cell-mediated cancer killing (84). In fact, Tregs can directly suppress NK cell activity (85) or hamper their accumulation by blocking the generation of mature NK cells through short-range interactions with NK precursors (86). They can also indirectly affect activity of NK cells by abrogating their cross-talk with DCs (87). Tregs also regulate NK cell activity utilizing surface-expressed TGFβ (88). We recently reported that $CCR4^+$Tregs utilized βGBP to directly inactivate protective NK cells and thereby to promote breast cancer lung metastasis (73). Thus, Treg and NK counts are often inversely correlated in peripheral blood of both tumor-bearing mice and humans with advanced stage cancers (73, 89). The decrease in numbers of tumor-infiltrating NK cells ($CD56^+$) cells is an unfavorable clinical prognostic factor in patients with Hodgkin's disease and colorectal cancer (90, 91). Conversely, the restoration of NK activity is often a sign of better cumulative survival outcome in cancer patients (92, 93).

Besides Tregs, cancer also uses help from other immune cells, such as myeloid/myeloid-derived suppressive cells (MSC/MDSCs), M2-type macrophages and tumor-associated macrophages (TAM), tumor-infiltrating DCs and plasmacytoid DCs, NKT and B cells (Bregs) (73, 94, 95). The impairment in any parts of this machinery can not only exacerbate autoimmune diseases, but also activate immunosurveillance. For example, dysfunction in Tregs leads to the spontaneous onset of autoimmune disorders (59) and inversely correlates with tumor immunosurveillance and positive disease outcome in cancer (60, 61, 63). Tregs protect against CNS immune disease in murine models (96), but their infiltration is associated with human metastatic brain tumors (97). The $B220^+$ plasmacytoid DCs prevent clonal expansion of tumor-specific T cells in tumor-draining LNs (98) utilizing the same mechanism used for the protection of immune privileged sites from T cells (99), i.e. indoleamine 2,3-dioxygenase (IDO, an immunoregulatory enzyme)-mediated tryptophan depletion. NKT ($CD4^+$ CD1d-restricted T cells) cells, as important controllers of Th polarization (100), actively suppress antitumor $CD8^+$ CTLs (101).

Cancer recruits and activates a variety of myeloid cell types, such as M2 macrophages (102, 103), TAMs (25) and MDSCs (26), to utilize their prosurvival, angiogenic and immunomodulatory functions. Interestingly, unlike Tregs that only account for a fraction of immune cells, the majority of immune cells in tumor-bearing mice and cancer patients are often comprised by MDSCs (24, 71). This heterogeneous group of CD11b-expressing (in mice) immature myeloid cells consists of two major subsets, immature and mature granulocytic and monocytic cell lineages. Phenotypically, they are divided into granulocytic and monocytic cells, as defined by the level of Gr-1 expression, $Gr\text{-}1^{High}$ in granulocytic and $Gr\text{-}1^{Int}$ in monocytic (71). Although still debated, both subsets of MDSCs participate in tumor escape by suppressing T-cell activity through the use of reactive oxygen species (ROS) and nitric oxide (NO) (94, 104). For example, MDSCs differentially express two enzymes of L-arginine metabolism, iNOS and arginase I, to regulate the availability of L-arginine (24, 105) and interfere with CD3ζ and IL-2 receptor signaling of T cells (106, 107). While granulocytic arginase I-producing MDSCs control T-cell activity by reducing plasma levels of L-arginine in human renal cell carcinoma (108), monocytic $CD14^+HLA\text{-}DR^{-/lo}$

myeloid cells suppress activity of human metastatic melanoma-associated T cells by expressing TGFβ (109). The expression of iNOS and arginase I in MDSCs can be further increased by cytokines and immunomodulatory factors, such as IFNγ and IL-13 present in cancer milieu or produced by other cells, including MDSCs themselves and NKT cells (43). Moreover, IL-13 produced by NKT cells also promotes the generation of tumor-promoting M2 macrophages (102) and activates IL-13 receptor-expressing Gr-1^{+}CD11b^{+} myeloid cells to express TGFβ, thereby suppressing antitumor CD8^{+} CTLs (101). Similarly with Th2 CD4^{+} T cells, NKT cells also regulate T-cell polarization by producing IFNγ, IL-4, IL-13 and TNFα (100). Thus, by promoting recruitment and cross-talk of tumor-infiltrating cells, cancer further enhances its suppressive milieu to permit cancer progression and metastasis. Interestingly, while at least in vitro, the T-cell activity can be inhibited by a relatively low numbers of MDSCs and Tregs, it remains puzzling why cancer mobilizes and drastically expands MDSCs causing splenomegaly (24, 71). Is it to promote a systemic immunocompromised state and/or to provide cancer survival factors? MDSCs and M2 macrophages produce various immunomodulatory cytokines, such as GM-CSF, IL-1β, IL-6 and TGFβ (103, 110, 111), that can both directly and indirectly support survival and metastasis of murine breast cancer 4T1 cells (71, 72). Despite this and the presence of expanded MDSCs, the inactivation of Tregs alone is sufficient to completely abrogate lung metastasis (40, 73). Moreover, therapeutic benefits of the Treg depletion are often seen early in tumor progression and are lost in mice with established tumors (112). Thus, Tregs and MDSCs may have different roles in cancer escape: Tregs are to protect cancers at early stages of their progression and metastasis when the cells are most vulnerable to attacks from effector cells; while MDSCs are to provide beneficial conditions for cancer progression and survival and to induce systemic immunosuppression. Moreover, both cells mutually support each other, as the activated MDSCs induce the generation of tumor-specific FoxP3^{+}Tregs (72, 113), while the depletion of Tregs alone significantly reduces the number of MDSCs in tumor-bearing mice (40, 73).

Chemokines as key controllers of immune responses

Cancer utilizes almost every imaginable way to escape immune effector cells. Chemokines play an essential role in this evasion as regulators of immune cell migration and promoters of pro-survival and anti-apoptotic signals (114, 115) through the activation of PI3K/Akt and the ERK1/2 signalling cascades (116, 117). For example, some cancers produce CXCL8 to enhance proliferation and survival of CXCR1- and CXCR2-expressing endothelial cells (118, 119), while MIP-1γ activates NF-κB ligand-induced osteoclast differentiation and survival (120). Functionally, chemokines are distinguished as inflammatory (inducible) or homeostatic (constitutive) based on their pathophysiological activities, and angiostatic or angiogenic based on their ability to influence neovascularization (121). While inflammatory chemokines are expressed during infection or tissue damage by resident and infiltrated leukocytes, homeostatic chemokines are usually produced constitutively in discrete microenvironments to maintain the physiological trafficking of immune cells (121). Cancer retains infiltrating myeloid cells by down-regulating their chemokine receptors, such as CCR2 in TAMs of human ovarian carcinoma (122). Cancer reduces infiltration of DCs in prostate and head and neck squamous cell carcinoma by inhibiting expression of CXCL14 (123, 124). On the other hand, by excessive production of CXCL9, CXCL10, CXCL11 and CXCL12, cancer can repel DCs, monocytes, neutrophils and T cells (125–127). Overall, cancer produces chemokines to differentially affect recruitment of immune cell subsets to benefit escape from immunosurveillance. For example, by expressing CCL17 and CCL22, cancer recruits CCR4-expressing Tregs to promote tumor growth (15, 63) and, as we recently found, to facilitate breast cancer lung metastasis by inactivating protective NK cells (73). Although CCL2 can directly induce IL-4-mediated Th2 polarization (128), it recruits TAMs and Gr-1-expressing macrophages (122, 129) promoting cancer escape and

pulmonary metastases. Its receptor CCR2 is expressed at high levels on monocytic lineage MDSCs that mediate NO-dependent suppression of $CD8^+$ T cell proliferation (104), indicating their possible recruitment to CCL2-expressing sites. In general, expression of CCL2, CCL17, CCL22, CXCL1-3 and CXCL5, CXCL6, CXCL8 and CXCL12 can be associated with a poor disease prognosis in cancer patients (130), while production of CXCL9 and CXCL10 is considered a sign of a positive antitumor immune activity, such as infiltration of CXCR3-expressing effector $CD8^+$ T cells, Th1 $CD4^+$ T cells and NK cells (131).

Regulatory B cells and cancer

Among a multitude of functions, B cells produce autoreactive antibodies, act as a potent antigen-presenting cells and express both stimulatory and regulatory cytokines. B cells are involved in pathogenesis of various autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes mellitus (T1D) and multiple sclerosis (MS); and their depletion using the anti-CD20 antibody, rituximab provides some clinical benefits in patients (132)). However, the role of B cells in cancer progression and escape remains poorly understood. B cells can function as potent antigen-presenting cells promoting antitumor adaptive immune responses, as activated human B cells can present antigens and induce robust T-cell responses in vitro (133). While the depletion of CD20-expressing B cells increases tumor burden in the lungs of mice intravenously injected with B16-F10 melanoma, the injection of CpG-activated B cells inhibits tumor growth (134). Moreover, the preferential loss of $CD27^+$ (135) and $CD19^+$ (136) B cells is detected in advanced stage melanoma and other solid tumors. Thus, although these results clearly indicate the importance of B cells in cancer control, they also raise a possibility of cancer-supporting B cells. In fact, for a long time B cells were also associated with carcinogenesis of methylcholanthrene-induced (137, 138) or transplanted tumors (139). The mechanism of their involvement appears to be as complex as the multitude of B cell functions, such as production of immunoglobulins, immunomodulatory factors and cytokines, and antigen presentation and T-cell activation (132). As in autoimmune diseases, such as RA (140) and in the induction of insulin resistance (141), the immunoglobulin depositions induce FcR and complement-mediated chronic inflammation that creates a hospitable environment for cancers (142). For example, immunoglobulins produced by infiltrating B cells induce inflammation in premalignant tissues and cause tumor growth in an HPV16-induced spontaneous model of carcinogenesis (143). Moreover, immunoglobulins can also serve as a carrier for TGFβ and thereby mediate suppression of cellular immune responses (144, 145). Recently, tumor-infiltrating B cells, recruited by death of androgen-deprived cells, were shown to produce lymphotoxin-α/β and promote androgen-independent growth of prostate cancer cells by inducing the nuclear translocation of IKKα and activation of STAT3 (146). On the other hand, the loss or inactivation of B cells can also lead to the reduction of MDSCs and Tregs (40, 73, 134), suggesting that B cells may mediate suppression of immune responses by regulating other immune cells. In fact, B1 cells promote M2 polarization of macrophages and TAMs though the production of IL-10 (147). B cells isolated from tumor-bearing mice inhibit $CD4^+$ T cell-mediated help for CTLs (148); and $B220^+$ B cells from nasal antigen tolerized mice not only induce T-cell anergy, but also render these cells suppressive for other T cells (149). B cells from patients with advanced stage solid tumors have reduced ability to activate T cells to express IFNγ and IL-2 (135).

Until recently, the existence of regulatory B cells (Bregs) has mostly been shown in the autoimmunity. For example, T-cell-mediated autoimmune responses can be prevented in mice by a small subset (1–2% of $B220^+$ cells) of IL-10-producing $CD1d^{High}$ $CD5^+$ B cells (so called B10 regulatory cells) (150). Similarly, B1b ($CD5^-CD1d^{High}$ $B220^{Low}$ $CD11b^+$ IgM^+) regulatory cells in mice are linked with protection from murine chronic colitis (151,

152) and $CD19^{+}CD24^{High}CD38^{High}$ B cells in humans are associated with protection from systemic lupus erythematosus (153). Interestingly, in the majority of these cases Bregs appear to utilize IL-10 as a chief effector molecule (150, 153–155). In fact, LPS activation of naïve B cells alone can induce IL-10 production and suppress T-cell responses inducing H-Y antigen tolerance (156). Although the participation of these Bregs in cancer is not known, it is possible that cancer may also utilize them, at least, to promote IL-10-mediated Th2 polarization. Recently, we demonstrated that Bregs are actively involved in breast cancer lung metastasis (40). In fact, we found a unique and poorly proliferative B cell subset that phenotypically resembled immature B2 cells but expressed constitutively active Stat3 and high levels of IL2Rα (CD25), B7-H1 and CD81. These cells, designated tumor-evoked Bregs (tBregs), are required for lung metastasis, as breast cancer cells cannot metastasize in their absence (Fig.2). However, tBregs differ functionally from other Bregs (150, 152, 155) and LPS- or BCR-activated B cells (156, 157), as the suppressive activity of tBregs does not require IL-10 or other known suppressive pathways, such as B7-H1-PD1, Fas-FasL, and IL27/IL35. The main function of tBregs in lung metastasis is to induce the generation of $FoxP3^{+}$Tregs utilizing TGFβ (40), the cells that are required for the inactivation of NK cells (73). In addition, tBregs and Tregs may also act through the control or activation of MDSCs, as their absence in tumor-bearing mice results in drastic loss of MDSCs. Interestingly, in this orthotopic 4T1 breast cancer model, the metastatic and non-metastatic subsets of cancer cells played separate but complementary functions. While non-metastatic cancer cell subsets mostly induce the generation of tBregs from normal B cells, the metastatic cancer cells produce TSPL and CCL17 to promote Th2 polarization (35) and recruit $CCR4^{+}$ Tregs (73). The non-metastatic cancer subsets also express B cell survival factor BAFF to actively maintain tBregs. In fact, BAFF and APRIL (a proliferation-inducing ligand) can be found expressed in human solid tumors, including breast carcinomas (158, 159), suggesting an interesting possibility that these factors also support survival of tBregs in humans. So far, tBreg-like cells have been in vitro generated from normal human donor B cells treated with conditioned media of human cancer lines, such as breast, ovarian and colon carcinomas (40). The clinical implication of our findings is that, as long as cancer persists, it will induce the generation of tBregs and thereby initiate the chain of suppressive events. Hence, tBregs may be a useful target of therapy. They must be controlled to efficiently combat cancers, for example, by using B-cell-depleting or targeting IL2Rα antibodies. However, the depletion of B cells with the anti-CD20 antibody, rituximab, did not provide clinical benefit in cancer patients with renal cell carcinoma (160). Thus, it remains to be elucidated whether the lack of efficacy is due to a preferential depletion of “good” and activated B cells as in RA and SLE (132), while suppressive B cells and tBregs are left unaffected by rituximab.

Concluding thoughts

Cancer-host interactions are complex and result in the loss of intrinsic and extrinsic controls that eventually lead the immune system to become an active cancer accomplice. To do this, cancer uses inflammation, a dual edged condition also considered as the key factor for the eradication of cancers since an anecdotal cure of Saint Peregrine in the thirteenth century and William Coley’s patients with sarcoma more than 100 years ago. However, cancer actively regulates this process to create inflammation that directly or indirectly promotes its own survival through the regulation of anticancer immune responses. It appears that the choice between cancer progression and regression depends on whether the response is acute or chronic, strong or relatively weak and persistent. The lessons of the Coley’s vaccine and numerous modeling studies suggest that by inducing robust acute inflammation, for example, with the use of so-called “danger signal” activators that imitate bacterial infection (161), the immune responses can be shifted towards cancer eradication. Since the expression of CXCL9 and CXCL10 usually inversely correlate with human cancer progression (165), their induction seems to inhibit tumor progression by directly reducing angiogenesis,

inhibiting production of tumorigenic factors and chemokines CXCL8, CXCL1-3, and CXCL5, and recruiting IFNγ-producing Th-1 type $CD4^+$ T cells and NK cells (118, 131, 165). For example, the progression of human non-small cell lung cancer, melanoma and esophageal, ovarian, lung cancers was controlled by neutralizing expression of CXCL5 (162), CXCL1-3 (163) and CXCL8 (164). Alternatively, immunosuppressive environment and cancer metastasis can also be controlled if their chemokine receptors are blocked (166). For example, antagonist-peptides T22 and T140 and bisphosphonates that that block CXCR4 were able to suppress tumor invasion metastasis in mice (167, 168). Recently, by delivering toxic moieties fused with CCL17, a chemotoxin formulation that specifically kills $CCR4^+$ cells (56), we were able to control breast cancer lung metastasis in mice (73), further confirming the importance of targeting the CCL17-CCL22/CCR4 axis to control immunosuppressive $CCR4^+$ cells (63, 73). Although these manipulations may finally allow us to harness the power of the immune system, none of them alone is sufficient without an active engagement of the adaptive immune system. In fact, as we recently demonstrated, the chemokine-based vaccine strategies that induce local inflammation (by recruiting immune cells) together with the targeted delivery of tumor self-antigens can elicit potent therapeutic anticancer immunity in tumor-bearing mice (Fig.2) (169–171). We hope these effects can be effectively brought to bear against cancer in man.

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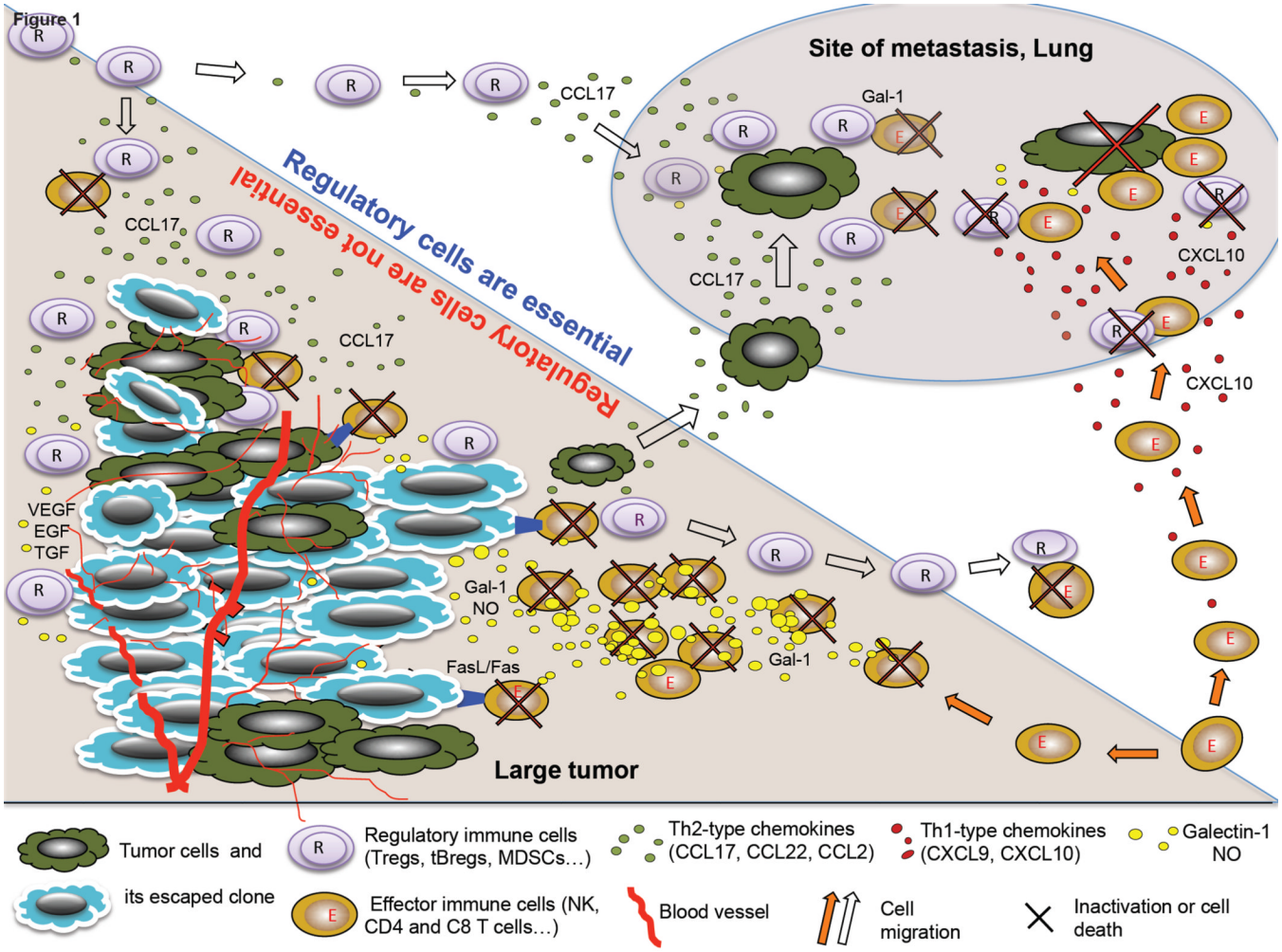
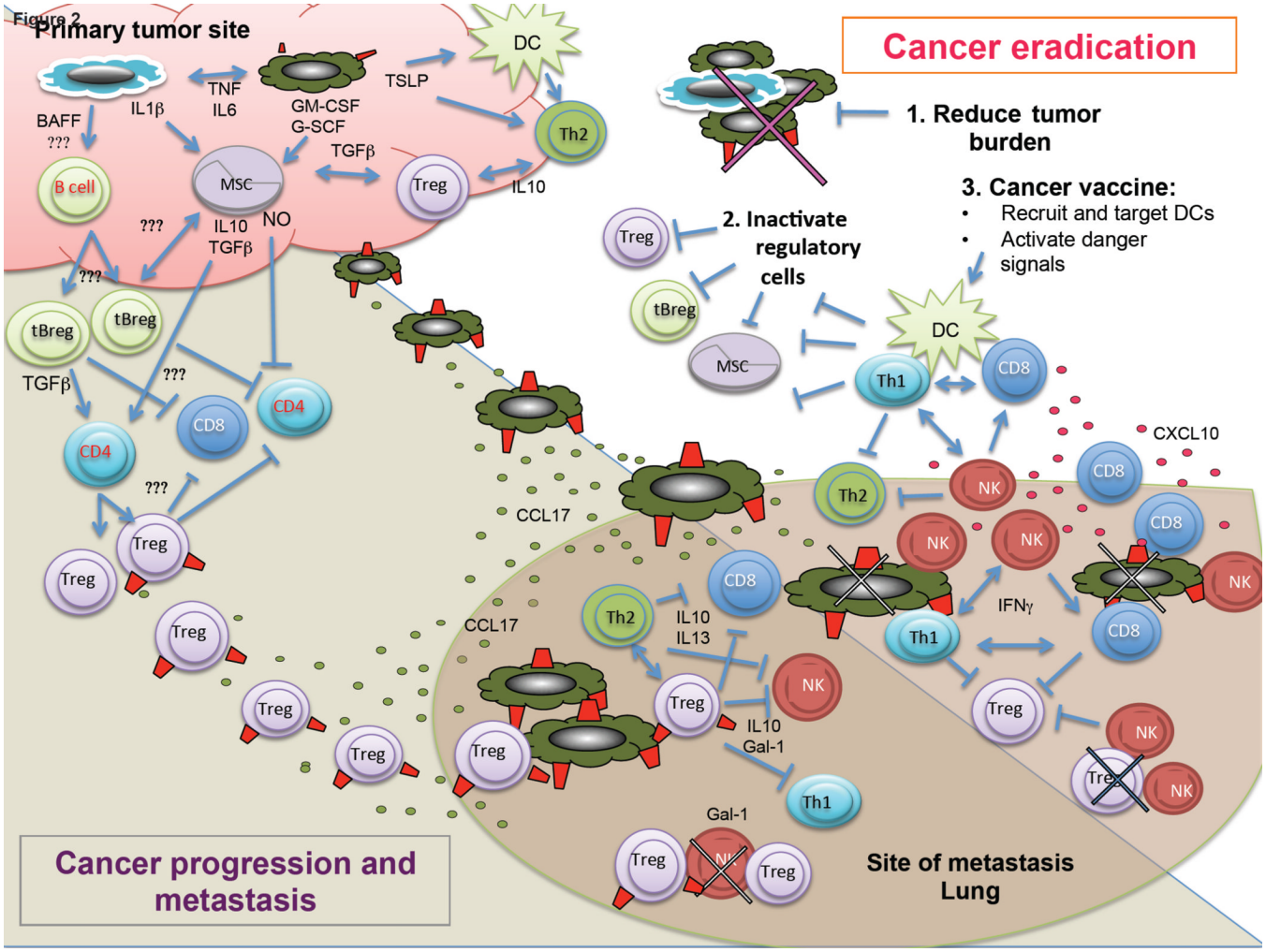


Figure 1. Corrupt immune cells are mostly required for small tumors and for metastasis

In contrast, the large tumors can efficiently kill or inactivate effector immune cells utilizing FasL/Fas axis and NO and producing various cytotoxic factors, such as galectin-1 (Gal-1). Yet, they still use regulatory immune cells probably to further promote systemic immune suppression and to produce tumor survival factors (VEGF, EGF, and TGF). They may also be utilized to activate production of chemokines, such as CCL2, CCL17, and CCL22, to prepare distant sites of metastasis thereby facilitating infiltration of metastatic cancer cells. The expression of CCL17 and CCL22 also enables recruitment of Tregs to directly neutralize anticancer NK cells using Gal-1. In the absence of regulator cells, the effector immune cells can efficiently eliminate the metastasizing cancer cells. The induction of Th1-type cytokine and chemokines (CXCL10) recruits more effector immune cells to counter act and neutralize regulatory immune cells.



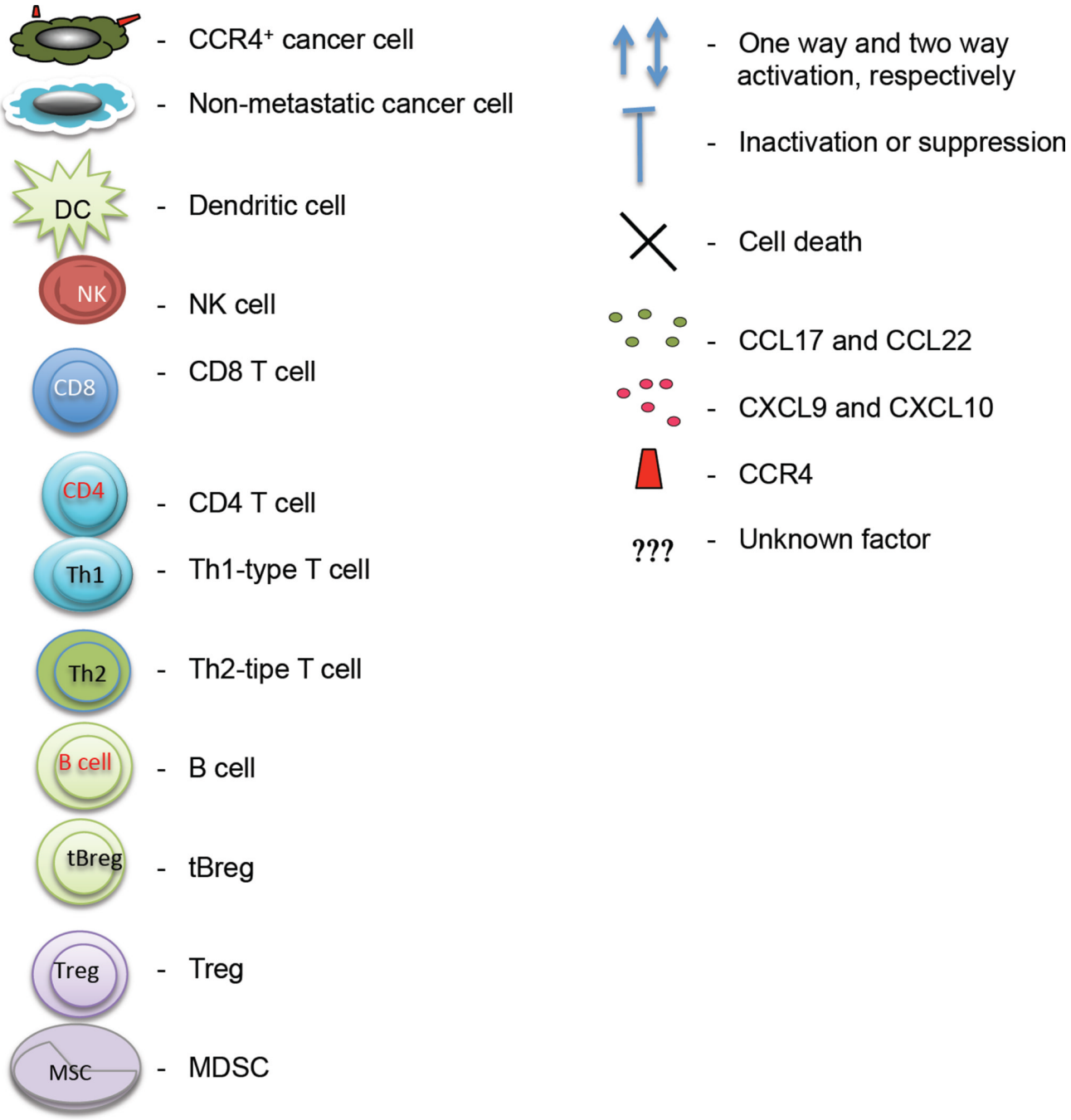


Figure 2. Proposal for a three-step cancer immunotherapy

Immunotherapy is most effective against residual diseases, i.e. when tumor burden is already reduced and at least one regulatory cell subset is inactivated. Both metastatic and non-metastatic cancer cells need to be targeted. Metastatic cells produce TSLP that directly, or through the education of DCs, induces Th2-type skewed T cell responses. As a result of a cross-talk between metastatic and non-metastatic cancer cells at the primary tumor site, expression of CCL17 and CCL22 is remotely induced in the lungs to recruit $CCR4^+$ cancer cells and Tregs. The role of Tregs is to neutralize or kill antitumor NK cells using galectin-1 (Gal-1) and, thereby, to facilitate survival of metastasizing cancer cells in the lungs. Using unknown soluble factors, non-metastatic cells convert normal B cells into tBregs. Although

tBregs can directly suppress T cells, they also induce conversion of $FoxP3^+$ Tregs using TGFβ. Cancer can be controlled utilizing chemokine-based cancer vaccines, such as proinflammatory chemokine fused with a tumor-associated antigen (Chemokine-TAA). Chemokine-TAA not only targets DCs, but also induces danger signal responses, resulting in a robust antitumor Th1-type $CD4^+$ T cell and $CD8^+$ CTL responses. To enhance efficacy of chemokine-TAA, Tregs or tBregs need to be inactivated, for example, by utilizing specific antibody or toxic moieties. As a result, suppressive milieu at the residual cancer sites is reversed enabling recruitment and activation of antitumor Th1-type $CD4^+$ T cells, $CD8^+$ CTLs and NK cells.