Anti-Metastatic Defense by CD8$^+$ T Cells

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Highlights

- CD8$^+$ T cells prevent metastasis in most steps of the metastatic cascade.
- The interaction between DTCs and CD8$^+$ T cells in the metastatic organ determines whether DTCs are eliminated, progress to make metastatic lesions or become dormant.
- CD8$^+$ T cell-derived cytokines can induce metastatic dormancy in DTCs.
- CD8$^+$ T cells play a major role in tumor cell clonal evolution by exerting selective pressure on tumor cells during metastatic progression.

Keywords: Metastasis, CD8$^+$ T cells, metastatic dormancy, immunoediting.

Abstract

Metastasis is an intricate process whereby tumor cells migrate from the primary tumor, survive in the circulation, seed distal organs and proliferate to create metastatic foci. $CD8^+$ T cells can detect and eliminate tumor cells. Research of $CD8^+$ T cell-dependent anti-tumor immunity has classically focused on their role in the primary tumor. There is increasing evidence, however, that $CD8^+$ T cells have unique anti-metastatic functions in various steps of the metastatic cascade. Here, we review the mechanisms whereby $CD8^+$ T cells control metastatic lesions. We discuss their role in each step of metastasis, metastatic dormancy and metastatic clonal evolution as well as the consequent clinical repercussions.

The metastatic cascade and anti-tumor $CD8^+$ T cell immunity

Most morbidity and mortality caused by solid tumors is due to metastasis. To metastasize, tumor cells must leave the primary tumor, **intravasate (see glossary)** into the circulation, survive as **circulating tumor cells (CTCs)**, **extravasate** into the target organ, adapt to a new environment as **disseminated tumor cells (DTCs)** and proliferate to create a macro-metastatic lesion[1]. The ability of DTCs to initiate new cancer lesions in distal organs resembles the capacity of **cancer stem cells** to initiate tumors[2, 3]. Indeed, multiple genes involved in metastatic spread, such as SOX-2 or SOX-9, are also associated with stemness[4, 5] and metastatic lesions often have similar histological features to the primary tumor in most human cancers. During the process of metastasis, tumor cells must evade the attack of different immune populations such as NK cells[6, 7] and T cells[8].

T cells can recognize tumor cells if the latter express **neoantigens** or **ectopic antigens**. The interaction between tumor cells and T cells is illustrated by the cancer immunity cycle[9]. Here, tumor cells release antigens that are taken up by antigen-presenting cells (APCs), such as dendritic cells (DCs). APCs process these antigens, mature, and migrate to the draining lymph nodes where they cross-present antigens to T cells. This process requires 3 signals: (i) Recognition of the antigen-derived peptide::major histocompatibility class I (MHC-I) complex by the T cell receptor (TCR), (ii) expression of co-stimulatory

molecules and receptors by the DC and $CD8^+$ T cell, respectively, and (iii) expression of activating cytokines by the DC[9]. Upon activation, $CD8^+$ T cells clonally expand, upregulate activation markers such as immune checkpoints and effector molecules, and migrate into the tumor. There, they recognize tumor cells that express the cognate antigen-derived peptide::MHC-I complex and kill them by effector molecules such as perforin, granzyme or by cytokines such as IFN-γ or TNF-α. Dying tumor cells will release antigens, thus fueling the cycle[9]. While research of $CD8^+$ T cell-dependent anti-tumor immunity has classically focused on the primary tumor, recent advances underscore a unique role for $CD8^+$ T cells in every step of the metastatic cascade. Because the primary tumor plays an essential role in priming of anti-tumor $CD8^+$ T cells, the role of $CD8^+$ T cells in metastasis should not be studied in models that omit T cell priming by the primary tumor, such as intravenous injection of tumor cells (Box 1).

$CD8^+$ T cells in the early steps of metastasis

The cancer immunity cycle is not equally effective in every tumor because a key step of this cycle, T cell priming, does not always spontaneously occur. Indeed, tumor-reactive $CD8^+$ T cells cannot be detected in a significant proportion of cancer patients[10]. In such situations, $CD8^+$ T cells are unlikely to play a role in restricting metastasis. To successfully metastasize, tumor cells must evade spontaneous or therapy-induced $CD8^+$ T cell attack during every step of the metastatic process (Figure 1).

Local invasion

Metastasis starts with invasion of adjacent tissues by the primary tumor. In this step, tumor cells face $CD8^+$ T cells that will try to eliminate them before they enter the circulation. To evade immune attack, tumor cells orchestrate the development of an immunosuppressive tumor microenvironment (TME)[11]. Tumor cells remodel the adjacent extracellular matrix aided by mesenchymal cells and myeloid cells. $CD8^+$ T cells can interfere with these populations, thus preventing tumor cell invasion. For example, extracellular vesicles secreted by $CD8^+$ T cells reduced local tumor invasion and metastasis by depletion of intratumoral mesenchymal cells[12]. However, it is still unclear whether $CD8^+$ T cells also directly interfere with the invasion capacity of tumor cells.

Epithelial-to-mesenchymal transition and intravasation

In some cases, carcinoma cells undergo an epithelial dedifferentiation, known as epithelial-to-mesenchymal transition (EMT), that can increase their metastatic potential[13]. EMT is not a binary process, and distinct intermediate states between epithelial and mesenchymal phenotypes exist[14]. EMT, however, seems not always required for metastatic colonization[15]. Indeed, in some cases, lack of EMT and presence of an epithelial phenotype favors metastasis formation[14]. Factors that inhibit $CD8^+$ T cell-mediated anti-tumor immunity, such as TGF-β, have been shown to promote EMT and increased metastasis *in vivo*[16]. Whether $CD8^+$ T cells directly regulate EMT, however, is unclear. A series of studies compared a breast cancer cell line derived from Her2/neu transgenic mice before and after undergoing *in vivo* immunoediting. In this model, *in vivo* $CD8^+$ T cell-mediated immunoediting was associated with increased EMT and stemness markers[17-19], however, this did not lead to increased primary tumor growth *in vivo*, and metastatic capacity was not evaluated[17]. T cell effector cytokines, such as IFN-γ and TNF-α, can induce EMT when incubated in vitro with breast[20], pancreatic[21] or prostate cancer[22] human cell lines. Nonetheless, whether *in vivo* T cell-derived IFN-γ and TNF-α can induce EMT and whether this leads to increased metastasis is unknown. The fact that immune surveillance promotes immune evasion may explain the apparent paradox that immune surveillance promotes EMT[14]. This hypothesis is supported by the fact that EMT results in upregulation of PD-L1 and downregulation of MHC-I, both of which render tumor cells resistant to $CD8^+$ T cells[23]. Therefore, it is also possible that, rather than directly inducing EMT, $CD8^+$ T cells preferentially eliminate cells that did not undergo EMT. After undergoing EMT and intravasation, tumor cells will circulate until dissemination into distal organs.

Survival as CTCs

CTCs must survive in the circulation until they arrive at the target organ. Because CTCs lack the immunosuppressive environment of the primary tumor, they may be more susceptible to immune elimination. However, CTCs can increase their metastatic potential by forming clusters with neutrophils[24], platelets[25] or other CTCs[26]. Such clusters may allow CTCs to evade immune surveillance. For instance, CTCs bind to platelets,

which protect them from NK cytotoxicity[25]. Platelets also secrete TGF-β[16], which is a known inhibitor of anti-tumor T cell immunity[27]. While the capacity of NK to eliminate CTCs has been widely described[6, 7], the role of $CD8^+$ T cells in this process is largely unknown. Although a correlation between the numbers of CTCs and the percentage of cytokine producing $CD8^+$ T cells in the circulation[28], as well as between PD-L1 expression by CTCs and worse outcome[29] suggest a role for $CD8^+$ T cells in controlling CTCs, direct experimental evidence is missing.

Extravasation into the target organ

Before CTCs can extravasate, they need to arrest their motion, potentially becoming an easier target for $CD8^+$ T cells. While the lead role of NK cells and patrolling monocytes in controlling CTCs in the capillaries is well established[6, 7, 30], the evidence for $CD8^+$ T cell involvement in this step is scarce. A study found that CTCs trapped in the capillaries of the lungs can activate conventional resident DCs. Depletion of these DCs promoted metastasis, presumably due to compromised $CD8^+$ T cell control[31]. Also, for this step in the metastatic cascade, further experiments are required to define a role for $CD8^+$ T cell-mediated killing of extravasating cells.

Control of disseminated tumor cells in the metastatic organ

Once CTCs have seeded the target organ, they will face $CD8^+$ T cell attack with three possible outcomes: **Metastatic dormancy**, complete elimination of DTCs by $CD8^+$ T cells, or immune evasion and consequently, metastatic lesions (Figure 2, Key Figure).

Metastatic dormancy

While in some cases metastasis is clinically apparent already at diagnosis, in others it appears years after resection of the primary tumor[32]. This phenomenon, known as metastatic dormancy, is particularly prevalent in certain cancers such as ER^+ breast cancer[33] and melanoma[34]. Indeed, patients who underwent breast cancer resection have increased mortality even 20 years after surgery[35]. Moreover, early-stage melanomas can have metastatic relapses decades after resection[36]. Such late metastatic relapses require awaking of dormant DTCs[37] and depend on tumor-cell-

intrinsic[38] and -extrinsic[37] mechanisms including the immune response. Observations in immunosuppressed organ transplant recipients, who developed tumors of donor origin after receiving organs from patients who were apparently cured of cancer decades ago[39], underscore the potency of the immune system to protect against metastasis. Metastatic dormancy differs from immune equilibrium[40]: Whereas metastatic dormancy is characterized by the presence of non-cycling DTCs, a balance between proliferation and apoptosis keeps the number of DTCs constant in immune equilibrium.

Different immune populations have been associated with metastatic dormancy including $CD8^+$[41] and $CD4^+$ T cells[42] as well as NK cells[43]. Recently, $CD39^+PD\text{-}1^+CD8^+$ T cells were identified as the essential immune population mediating metastatic dormancy in a preclinical breast cancer model[44]. Despite the expression of so-called **exhaustion markers**, $CD39^+PD\text{-}1^+CD8^+$ T cells were enriched in effector molecules and had a **tissue-resident** memory signature (Box 2). Using depletion and adoptive transfer experiments, $CD39^+PD\text{-}1^+CD8^+$ T cells were unequivocally shown to be essential for metastatic dormancy. In a cohort of breast cancer patients, the frequency of tumor-infiltrating $CD39^+PD\text{-}1^+CD8^+$ but not total $CD8^+$ T cells correlated with delayed metastatic relapse after resection (disease-free survival)[44]. In the same study, T cell-derived IFN-γ and TNF-α were identified as essential mediators of metastatic dormancy. This is consistent with the previously described capacity of these cytokines to induce senescence in tumor cells through activation of p16INK4a and downstream Rb protein hypophosphorylation leading to G1/G0 permanent growth arrest[45]. The involvement of T cell-derived effector cytokines in metastatic dormancy contrasts with their possible ability to drive EMT[20-22] as mentioned above. Because T cell-dependent promotion of EMT was linked to a cancer stem cell phenotype[19], it is possible that IFN-γ or TNF-α restrict the proliferation and activates stemness pathways in cancer cells, which support tumor cells in hostile environments such as the blood (EMT) or in distal organs (metastatic dormancy). While induction of cell cycle arrest may be the main mechanism underlying metastatic dormancy, involvement of cytotoxicity cannot be excluded. For example, $CD8^+$ T cells may mediate dormancy by killing most of DTCs and inducing cell-cycle arrest in those that escaped[46]. Furthermore, in a mouse model of spontaneous uveal melanoma,

DTCs migrated to the lungs early, where they showed limited proliferation and caused late metastasis. Depletion of $CD8^+$ T cells promoted proliferation of DTCs and the appearance of macro-metastasis[41]. Depletion of $CD8^+$ T cells also led to the awaking of dormant lung metastasis in a model of fibrosarcoma[47].

How dormant DTCs evade $CD8^+$ T cell-mediated killing is an aspect of special interest. DTCs seed the metastatic organs as small clusters or as single cells[26, 44, 48], making the orchestration of an immunosuppressive TME in which $CD8^+$ T cells lose function unlikely. Instead, DTCs preferentially seed perivascular niches[49], which are rich in immunosuppressive molecules[50, 51], and may benefit from systemic immunosuppression generated by the primary tumor[52]. Loss of MHC-I by DTCs in the liver was proposed as an evasion mechanism in a model of pancreatic cancer[48]. In this study, suppression of the epithelial phenotype and downregulation of MHC-I resulted from an endoplasmic reticulum stress response. This mechanism mimics the process of EMT described above, where tumor cells lose their epithelial phenotype and downregulate MHC-I[23]. Indeed, MHC-I downregulation is a common immune evasion mechanism in patients treated with **immune checkpoint inhibitors**[53]. Although downregulation of MHC-I could result in increased sensitivity to NK cells[23], the evidence that NK cells control solid tumors or DTCs is scarce[7].

Dormant DTCs can awake and progress to macro-metastases, but it is still unclear what drives this process. Although there is compelling evidence that adaptive immunity induces metastatic dormancy[41, 44, 48], it is unknown whether continued immunosurveillance is needed for maintenance of dormancy. Dependence on constant immunosurveillance was observed in some studies where $CD8^+$ T cell depletion resulted in awaking of dormant DTCs and macrometastasis[47, 48]. Consequently, awaking is only possible when dormant cells evade immune surveillance. On the one hand, DTCs can acquire resistance to $CD8^+$ T cell-mediated induction of senescence[46], however, it is unclear whether this happens during dormancy. Alternatively, the tumor-specific $CD8^+$ T cell response might wane over time, especially in the setting of minimal residual disease. Indeed, the memory response of tissue-resident T cells is unstable in the absence of antigen stimulation[54, 55]. On the other hand, DTC can awake due to activation of cellular proliferation programs. Particularly, inflammatory events such as cigarette smoke-derived LPS[56] or

surgery[57] seem to wake dormant tumor cells. Therefore, both inflammation and immune evasion may contribute to the awaking of dormant DTCs. However, whether constant immunosurveillance is always needed or whether strong proliferative signals can overcome immune control is still unknown.

The involvement of $CD8^+$ T cells in metastatic dormancy has direct clinical implications. PD-1 blockade reduced the number of dormant DTCs in the lungs in a preclinical model[44], presumably by increasing $CD8^+$ T cell activity. This finding may explain the observation that stage IV melanoma patients treated with PD-1 blockade had exceptionally durable responses[58]. Along these lines, the analysis of metastasis-specific readouts, such as metastasis free survival, in immunotherapy clinical trials could potentially improve our understanding on the influence of $CD8^+$ T cell stimulation in metastasis and metastatic dormancy. Furthermore, many cancer patients receive corticosteroids to treat cancer- and chemotherapy-related complications. Corticosteroids are known inhibitors of anti-tumor $CD8^+$ T cell responses[59, 60], and could contribute to metastatic relapses even by non-immune related mechanisms[61]. More studies are needed to understand the effect of corticosteroids and other cancer treatments in metastatic dormancy.

Immune-mediated elimination or immune evasion

The interaction between $CD8^+$ T cells and DTCs in the metastatic organ does not always lead to dormancy, and other scenarios exist (Figure 2).

There are a few studies showing that $CD8^+$ T cells eliminate all DTCs, thus preventing metastasis. This was seen in the EMT6 model of breast cancer, where the primary tumor primed $CD8^+$ T cells that killed all DTCs in the lungs by induction of apoptosis[62]. Another study in a spontaneous model of breast cancer showed that exposure of mice to an oral carcinogen increased the immunogenicity of the primary tumor by increasing its mutational burden and expression of CCL21 in tumor cells. This led to a stronger $CD8^+$ T cell response that eliminated DTCs in the lungs[63].

Alternatively, DTCs can evade immune surveillance and form metastatic lesions[27]. This can be due to resistance to $CD8^+$ T cell-mediated growth arrest induction or killing. Resistance to IFN-γ and TNF-α, which mediate growth arrest in metastatic dormancy[44],

is common in cancer[64, 65]. Additional mechanisms, such as secretion of TGF-β, can diminish T cell-mediated immune surveillance and thus promote development of macrometastasis[27]. Finally, any parameter that compromises efficient T cell priming by the primary tumor[10, 44] precludes $CD8^+$ T cell-mediated control of metastasis.

Modulation of systemic $CD8^+$ T cell immunity in metastatic disease

Despite systemic, protective immunity, some DTCs will eventually produce clinically apparent metastatic lesions. A locally compromised immune defense in the metastatic organ may be essential for metastatic outbreaks.

Modulation of immunity by environmental cues

Local conditions in the metastatic or dormant niche influence the anti-tumor T cell response. Organ-specific particularities can lead to the development of different immune contextures in different metastatic lesions. This was illustrated in a case of an ovarian cancer patient whose metastases progressed differently during off-treatment periods. Analysis of the individual lesions showed abundant T cell infiltration and clonal expansion in regressing lesions but immune exclusion in progressing lesions[66]. Similarly, co-occurrence of different immune landscapes within the same patient was recently reported in a case of a melanoma patient treated with immunotherapy[67] and in a cohort of colorectal carcinoma patients[68]. A recent study showed that lung metastases shared an immune signature that differed from the signature in other metastatic sites and, moreover, was independent of the origin of the primary tumor[69]. Furthermore, non-small cell lung cancer (NSCLC)-derived brain metastases contained less T cells and more macrophages compared with primary tumors[70]. This finding, however, could also reflect divergent evolution of metastatic cells towards a more immunosuppressive phenotype. The importance of the immune microenvironment in the target organ may explain why immunotherapy seems to be more effective in lymph node metastasis than in other organs[71, 72]. The abovementioned examples illustrate that the metastatic niche may have organ-specific features. In case of the liver, the influence of the metastatic niche seems to have systemic components. For example, patients with liver metastases – independently of the type of primary cancer – respond worse to immunotherapy than

patients with metastases in other organs[73]. The effect seems to involve dampening of systemic anti-tumor immunity by regulatory T cells[74] or loss of circulating activated $CD8^+$ T cells[73]. Metastatic infiltration of some organs, however, can support systemic anti-tumor immunity. This was recently described in a mouse model of melanoma with brain metastasis where the presence of extracranial disease increased the traffic of $CD8^+$ T cells to the brain which led to increased response to immune checkpoint blockade[75].

A wide array of local cues probably contributes to the heterogeneity of local and systemic anti-tumor immunity. For instance, individual organs contain different numbers and subtypes of tissue-resident T cells[54, 55] and myeloid cells[76], which influence protective anti-tumor immunity[77]. Also, metabolites[78, 79], microbiota and biophysical conditions[80] vary among different tissues and all influence T cell function. Consequently, mechanisms underlying immune evasion by DTCs presumably vary depending on organ-specific conditions.

Modulation of immunity by tumor-cell-intrinsic factors

Tumor-cell-intrinsic features, such as expression of neoantigens, immune checkpoint molecules, cytokines or MHC-I, can influence the TME and therefore, spontaneous as well as therapy-induced anti-tumor immunity[77, 81]. In addition, tumor-cell-derived factors often influence systemic immunity. Analysis of the immune infiltrate of various organs in multiple mouse cancer models showed that even localized tumors can alter the immune environment of distal organs leading to systemic immunosuppression[82]. The changes induced by the primary tumor included a re-organization of the T cell compartment and a recruitment of myeloid cells to the peripheric tissues. The latter can promote metastatic seeding of CTCs by creating a pre-metastatic niche[52].

The occurrence of cancer-related systemic immune suppression could be explained by the capacity of many tumors to secrete exosomes that are rich in PD-L1, inhibit $CD8^+$ T cells and are associated with reduced response to PD-1 blockade[83]. Systemic immunosuppression may restrict the defense to bacterial and viral infections[82], and indeed, sepsis is a common ultimate cause of death in cancer patients[84]. Furthermore, systemic immune suppression is particularly deleterious in the context of immunotherapy[85]. The observation that systemic immune suppression is reverted after

primary tumor resection[82] may have important consequences for the treatment of cancer patients, since it is unclear whether immunotherapy is more effective when given before (neo-adjuvant) or after (adjuvant) primary tumor resection. Nonetheless, while adjuvant therapy is not affected by primary tumor-mediated immunosuppression, neo-adjuvant therapy seems more effective in mouse models[86] and expands more tumor-resident T cell clones in cancer patients[87]. The possible superiority of neo-adjuvant immunotherapy is still unclear, but factors like the more abundant presence of antigen or the lack of post-surgical immunosuppression could potentially explain this.

Metastatic clonal evolution during systemic immune pressure

A tumor is composed of heterogenous tumor cells that continuously evolve due to accumulation of mutations and selective pressure. Intratumor heterogeneity explains why resistant tumor cells emerge upon treatment[88] and why metastasized cancers often differ from the primary lesion[68].

$CD8^+$ T cells are a major driving force for competition between different tumor cell clones[88], which differ in immunogenicity for various reasons[67, 89]. $CD8^+$ T cells preferentially eliminate the more immunogenic clones, thus selecting for immune-evasive clones[40]. This process is known as **immunoediting**[90] and is especially relevant during metastatic progression, as underlined by a study that analyzed the immune infiltrate and clonality of different metastatic lesions in colorectal cancer patients[68]. Another study in NSCLC showed that loss of MHC-I, which was identified as a sign of immunoediting in early-stage primary tumors, was further enriched in brain metastasis[89]. Although some tumor cell intrinsic features, such as EMT[14, 23] or activation of β-catenin signaling[91, 92], have been associated with both immune evasion and metastasis, it is unknown whether $CD8^+$ T cells preferentially control tumor cells that lack certain metastatic traits. The dynamic competition between the adaptive immune response and different tumor populations determines which tumor cell subclones will become prevalent in space and time over the course of metastatic disease[67].

Intratumor heterogeneity aids the tumor in coping with attacks by different classes of therapies including immunotherapy. For example, immune recognition and control of

mouse tumors composed of mixes of clones inversely correlated with the number of clones and genetic diversity of the tumor[93]. Along the same lines, a high degree of intratumor heterogeneity was associated with poor $CD8^+$ and $CD4^+$ T cell infiltration, increased infiltration of regulatory T cells and decreased survival in a cohort of breast cancer patients[94].

In addition, heterogeneity with respect to the immune infiltrate is observed between different lesions in the same patient but also even within the same lesion[68, 95]. Regional differences in tumor cell mutations seem an important driver of this immune heterogeneity. For example, a study of NSCLC patients described a correlation between the regional presence of $CD8^+$ T cell clones and mutations[95]. Thus, intratumor heterogeneity may result in a miscellaneous distribution of antigens and a heterogenous distribution of antigen-specific $CD8^+$ T cells. This can be especially relevant to the first steps of metastasis, since some studies suggest that metastatic tumor cell clones tend to originate from the center of the primary tumor[96], which means that the clonal anatomy of the primary tumor could potentially shield metastasizing populations from immune surveillance.

Concluding remarks and future perspectives

Recent advances in the fields of metastasis research and tumor immunology have emphasized the specific anti-metastatic functions of $CD8^+$ T cells that only now we are starting to understand. It seems plausible that priming of $CD8^+$ T cells by the primary tumor is important for preventing the early steps of metastasis (Figure 1). Whether and to which extent $CD8^+$ T cells can restrict CTCs, however, is largely unknown. After dissemination to the metastatic organ, DTCs presumably face immunosurveillance mediated by $CD8^+$ T cells primed by the primary tumor. Depending on many tumor-cell-intrinsic as well as -extrinsic variables – some of which are still unknown – DTCs are eliminated, driven into cell-cycle arrest (dormancy) or evade immune attack to form a metastatic lesion (Figure 2). Especially processes related to metastatic dormancy are enigmatic, thus, their understanding requires further investigation. The research

questions, which seem most relevant to our opinion, are discussed in the Outstanding Questions Box.

A better understanding of the anti-metastatic functions of $CD8^+$ T cells will potentially lead to the design of immunotherapies to better target or even prevent metastatic disease.

Figure 1. $CD8^+$ T cells control early steps of metastasis. Anti-metastatic $CD8^+$ T cell responses are most likely primed by the primary tumor. Ineffective priming promotes immune escape and metastasis. In contrast, appropriately primed $CD8^+$ T cells can prevent invasion and extravasation of tumor cells in the circulation by direct elimination and depletion of mesenchymal cells[12]. Invading tumor cells can undergo EMT which promotes immune evasion[23]. CTCs are mainly controlled by NK cells in the circulation, it is unclear whether $CD8^+$ T cells also control CTCs. CTCs bind to platelets[16, 25], other tumor cells[26] and neutrophils[24] to increase their metastatic potential and to escape immunosurveillance. NK cells and patrolling monocytes are the main controllers of extravasating CTCs. Extravasating tumor cells can also activate DCs, which prime $CD8^+$ T cells that could potentially eliminate extravasating cells[31]. Created with BioRender.com".

Figure 2. $CD8^+$ T cell-mediated control of DTCs. Once DTCs arrive to the target organ, they face $CD8^+$ T cell attack. The interaction between DTCs and $CD8^+$ T cells can lead to 3 different outcomes: (i) immune evasion by DTCs leading to proliferation, expression of the proliferation marker Ki67, and formation of macrometastasis[27], (ii) immune elimination of all DTCs by $CD8^+$ T cell effector molecules such as granzyme B (GzmB), perforin (PRF), IFN-γ and TNF-α[62, 63] and (iii) induction of metastatic dormancy by PD-1^+ $CD39^+$ $CD8^+$ T cell mediated IFN-γ and TNF-α[44]. Dormant DTCs can awake and cause macrometastasis. Alternatively, stimulation of $CD8^+$ T cells with PD-1 blockade can reduce the number of dormant DTCs[44]. Created with BioRender.com".

BOX 1: Models to study metastasis and anti-tumor immunity

Metastasis is a challenging process to reproduce in a model, and different approaches have been used to obtain mouse models that recapitulate human disease. Many mouse models are used to study (parts of) the metastatic cascade. Although most models are suitable to study some aspects of metastasis, most models are insufficient in one way or another.

Quantification and visualization of metastasis require tagging cell lines with fluorescent or bioluminescent proteins (e.g. luciferase, mCherry), because counting nodules is only possible for overt macro-metastasis. Dependent on the tag and genetic background of the mouse, such modified cell lines may be more immunogenic than the parental line and therefore, display different metastatic behavior[57].

Below we discuss three fundamentally different models for studying metastasis: (i) **Experimental metastasis**, which results from introducing tumor cells in the blood stream, and (ii) spontaneous metastasis, which is seeded from a primary tumor and (iii) genetically engineered mouse models (GEMM)

Experimental metastasis

Tumor cells can be introduced into the blood stream to seed metastasis in different organs. For instance, injection in the tail vein leads to lung metastasis[6, 16, 24, 25, 44] whereas intraportal or splenic injections cause liver metastasis[48]. The advantages of this approach are the high penetration (usually 100%) and the synchronized kinetics of metastasis development. A major disadvantage, which is too often ignored, is the absence of a primary tumor. First, there are no metastases without a primary tumor in clinical situation. Second, a primary tumor influences many variables including priming of adaptive immune defense, as well as systemic effects including immunosuppression and dysregulated hematopoiesis[82].

Spontaneous metastasis

In these models, tumor cells are injected **orthotopically** or subcutaneously to create a primary tumor that spontaneously metastasizes. This approach allows T cell priming by the primary tumor and permits a more holistic approach to study metastasis[82, 86]. It appears that only selected cell lines spontaneously metastasize in a useful proportion of

mice. The underlying reason is not understood in most cases and presumably is multifactorial. Because of this, most metastasis research uses only a few mouse tumor cell lines, which is concerning. Furthermore, the primary tumor results from a single injection of thousands to millions of tumor cells. Therefore, such models have some disadvantages: Besides the risk that injection of a large number of tumor cells may induce inflammation, cancer usually starts from a single transformed cell. Finally, using patient-derived xenografts or human cell lines only allows studying features unrelated to immune defense.

GEMM

Mice can be genetically engineered to develop different kinds of malignancies. GEMM avoid the inflammation created by tumor injection and allow the generation of spontaneous orthotopic cancers in any organ[15, 27, 41]. Tumors generated in GEMM can be preceded by pre-neoplastic lesions which better recapitulate human disease[27]. Moreover, GEMM are generally designed to express the same mutations as the human cancer they model[15, 27]. This is of special importance since cancer specific mutations have important consequences for tumor-specific immunity[27]. Despite similarities to human cancer, most GEMM develop multiple primary tumors[41], which differs from the human situation. A challenge, however, is that the timing and incidence of metastasis in GEMM oftentimes are heterogeneous, and genetic modification of tumor cells such as expression of fluorescent proteins, requires backcrossing.

Models of metastatic dormancy

Metastatic dormancy has been described in experimental metastasis[44, 48], orthotopic models with spontaneous metastasis[44, 47] and GEMM[41]. However, there are only a few models of metastatic dormancy in immunocompetent mice. While the aspects discussed for metastasis also apply to metastatic dormancy, we are only starting to understand the influence of anti-tumor immune response in this phenomenon, and further studies should clarify the influence of each model.

BOX 2: $CD8^+$ T cell memory in control of metastasis

Memory $CD8^+$ T cells respond promptly upon antigen re-encounter and play an important role in protection against infections and metastasis[97-99]. In murine models of pancreatic and breast cancer, transient exposure to primary tumors generated a T cell memory response that induced metastatic dormancy when mice were re-challenged with intravenous tumor cells[44, 48]. Memory T $CD8^+$ responses were also detected in patients with metastatic melanoma who responded to immunotherapy. In this cohort, the same $CD8^+$ T cell clonotypes were detected as effector memory $CD8^+$ T cells (T_{EM}) in the blood and tissue-resident memory $CD8^+$ T cells (T_{RM}) in the tumor and skin affected with vitiligo even 9 years after treatment. These memory $CD8^+$ T cells expressed an *IFNG*/*TNFA*-high signature that correlated with survival[99].

Among the different subsets of memory $CD8^+$ T cells, T_{RM} have emerged as one of the most relevant types of T cells involved in metastasis control[97, 100]. T_{RM} reside in non-lymphoid tissues and do not recirculate. They are characterized by the expression of CD69 and CD103, which contributes to its homing to peripheral tissues, and the lack of expression of lymph node homing receptors such as CD62L or CCR7[97, 98]. Their location predestines them for an immediate response in healthy or tumor tissues. Moreover, T_{RM} can propagate systemic anti-tumor immunity as described in a mouse model of melanoma where skin T_{RM} activated dendritic cells, which subsequently primed $CD8^+$ T cell systemically resulting in distal control of lung metastasis[100]. Indeed, abundance of T_{RM} correlates with improved survival in multiple human cancers such as triple negative breast cancer[77], melanoma[100] or NSCLC[101].

T cell memory is crucial not only for spontaneous or immune checkpoint-induced anti-tumor immunity but also for other cancer therapies such as cancer vaccines[102]. For example, in a cohort of $HER2^+$ breast cancer patients, vaccination against HER-2 induced expansion of memory $CD8^+$ T cells, which was associated with progression-free survival [103]. Expansion of memory $CD8^+$ T cells was also observed in melanoma patients that received a personalized neoepitope-derived mRNA vaccine[104]. These patients showed a decreased rate of metastasis after vaccination, which illustrates how memory T cell responses can lead to metastasis control[104].

Outstanding questions

- Can $CD8^+$ T cells control CTCs?
- How do dormant DTCs awake to create metastasis?
- How do CTCs and dormant DTCs evade immune surveillance?
- How can we stimulate $CD8^+$ T cells to eliminate dormant DTCs?
- Can local, immune-response-related cues in different organs explain the characteristic metastatic patterns that are observed in some cancer types?

Glossary

Cancer stem cells: Self-renewing, undifferentiated cancer cells which can give rise to differentiated cancer cells to create a heterogeneous tumor.

Circulating tumor cells (CTCs): Tumor cells that have left the primary tumor and entered the circulation.

Disseminated tumor cells (DTCs): Tumor cells that have settled in distant organs away from the primary tumor site after exiting the circulatory system.

Ectopic antigens: Proteins that are only expressed in some isolated anatomic locations, that become aberrantly expressed by tumor cells leading to immune recognition.

Extravasation: The process by which a tumor cell exits the circulation and reaches the metastatic organ.

Exhaustion markers: Markers that are upregulated during activation of T cells and that remain highly expressed on functionally impaired (exhausted) T cells. Such situation often occurs after persistent TCR stimulation.

Experimental metastasis: Injection of tumor cells in the circulation to generate metastatic lesions.

Immune checkpoint inhibitors: Antibodies that enhance anti-tumor immune response by blocking the interaction between inhibitory checkpoint receptors on immune cells and their ligands.

Immunoediting: Process by which the immune system eliminates immunogenic populations of tumor cells, leading to selection of less immunogenic populations.

Intravasation: The process by which a tumor cell enters the circulation.

Memory T cells: Antigen-specific T cells that persist after the antigen has been eliminated. Memory T cells rapidly proliferate and develop effector function upon re-exposure to the specific antigen, thus providing a fast protective response.

Metastatic dormancy: Process resulting from cell cycle arrest in DTCs for a prolonged period. Dormant DTCs can awake, start proliferating and produce metastasis.

Neoantigen: Mutated tumor-cell-specific protein that is absent in normal tissue

Orthotopic: Presence of a cell type or organ in a location in which it would be found under physiological circumstances.

Tissue-resident immune cells: Different immune populations that occupy certain tissues without recirculating.

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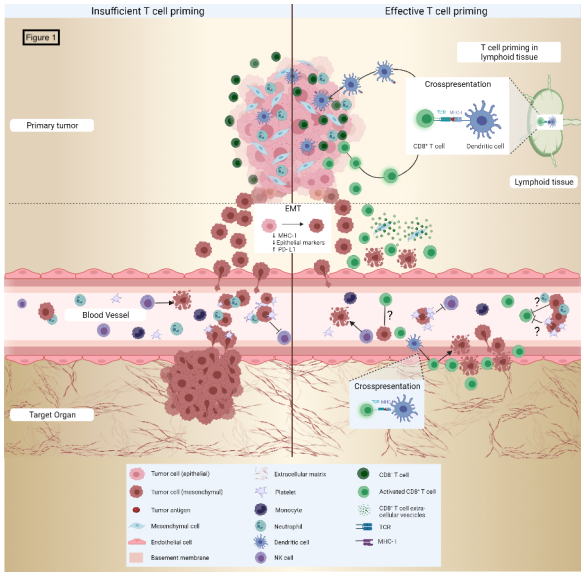
Insufficient T cell priming
Effective T cell priming
Figure 1
T cell priming in lymphoid tissue
Crosspresentation
Primary tumor
CD8+ T cell
Dendritic cell
Lymphoid tissue
EMT
↓ MHC-I
↑ Epithelial markers
↑ PD-L1
Blood Vessel
Crosspresentation
Target Organ
Tumor cell (epithelial)
Tumor cell (mesenchymal)
Tumor antigen
Mesenchymal cell
Endothelial cell
Basement membrane
Extracellular matrix
Platelet
Monocyte
Neutrophil
Dendritic cell
NK cell
CD8 T cell
Activated CD8 T cell
CD8 T cell exosomes
TCR
MHC-I