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Review Article

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Keywords

Cancer immunotherapy: a cancer treatment that relies on coaxing the immune system to selectively target cancerous cells

Cytotoxic T lymphocyte: a type of white blood cell that identifies infected or cancerous cells; releases enzymes to destroy the target cell

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Immune Response Regulation has Therapeutic Potential in the Treatment of Cancer

Abstract

Background: Depending on their path of differentiation, immune cells can have opposing roles in tumour progression. As a result, during growth, tumours undergo selective pressure to produce immunosuppressive factors that contribute to tumour growth, angiogenesis, and metastasis. This review discusses the contribution of different macrophages and T cells to tumour progression, as well as their role in current cancer immunotherapies.

Methods: We searched for articles online through McGill Library with search terms including the names of different immune cells along with "polarity", "tumour progression", or "cancer immunotherapy". Cancer therapies "CTLA-4 blockade", "Ipilimumab", "adoptive cell transfer", and "PD1 inhibition" were also used as search terms.

Summary: Depending on the cell types involved, crosstalk between different immune cells in the tumour stroma can contribute to either the development or the inhibition of tumour growth. Certain therapies such as adoptive cytotoxic T lymphocyte (CTLs) transfer and CTLA-4 & PD1 inhibition work by enhancing CTL tumoricidal responses, and have produced durable responses in a small but significant group of patients. Other therapies work by skewing the phenotype of tumour associated macrophages from pro-tumorigenic to anti-tumorigenic. However, disrupting the balance between immune cell functions risks triggering inflammatory disorders such as autoimmunity. Therefore, future directions in cancer immunotherapy include targeting potential responders and restricting therapeutic mechanisms to the tumour microenvironment.

Introduction

Inflammation at the tumour site is thought to enable several hallmarks of cancer such as sustained tumour cell proliferation and survival as well as angiogenesis and metastasis. (1) For instance, several bone marrow-derived cells such as macrophages, neutrophils, mast cells and myeloid progenitors contribute importantly to the onset and maintenance of angiogenesis, the formation of vasculature required to sustain tumour growth. Furthermore, these cells have been found to promote metastasis by producing matrix-degrading enzymes and by stimulating the epithelial-to-mesenchymal transition involved in tumour cell invasion. (1) Not only do tumour cells undergo selective pressure to populate their microenvironment with immune cells that foster their progression, they also undergo pressure to evade destruction by tumoricidal leukocytes, such as cytotoxic T lymphocytes (CTLs), natural killer cells, and specific subsets of macrophages and neutrophils. (1) Tumour immune evasion is thought to rely on reduced antigen presentation at the tumour cell surface, on the production of immunosuppressive molecules, and on the recruitment of immune cells that inhibit the activity of anti-tumour leukocytes. The interaction of tumours with immune cells in their microenvironment has warranted the development of therapies aimed at stimulating tumour-cytotoxic immune cells and at inhibiting the tumour-leukocyte interactions that promote cancer progression. This review focuses on the role of certain T cells and macrophages in tumour progression and therapy, as these cell types are among the most studied in the context of tumour immunology. However, several other immune cells and non-immune cells have important roles in tumour progression. To date, adoptive transfer of tumour-specific T cells as well as T cell checkpoint inhibition have resulted in durable responses to cancer, including some complete responses lasting several years, and have increased overall survival. Other therapeutic strategies involve inhibiting the recruitment, differentiation, or function of immune cells, such as regulatory T cells and M2-type macrophages that inhibit anti-tumour immune responses and foster tumour immune evasion and progression.

Cytotoxic Lymphocytes in Cancer Therapy

CTLs are a subset of CD8+ T cells in which every clone harbours a unique antigen specificity. CTLs specific to a tumour-associated antigen can kill tumour cells presenting the antigen with Major Histocompatibility Complex (MHC) class I at their surface. The cytotoxicity of a CTL requires the binding of its T cell receptor (TCR) to this peptide-MHC class I complex on the tumour cell surface. (2) CTLs can then trigger the apoptosis of a tumor cell via their expression of the ligand for the Fas receptor found on the tumor cell membrane. CTLs also express perforin, which forms holes in the tumour cell membrane through which the CTL injects granzymes. These granzymes activate the caspases that drive apoptosis. IFNγ produced by CTLs upregulates Fas expression and is therefore important for inducing tumor cell apoptosis. (2)

In order to mount an anti-tumour immune response, antigen-presenting cells such as macrophages or dendritic cells must take up tumour antigens at the tumour site and travel to tumour-draining lymph nodes. Once at the lymph nodes, they activate CD4+ and CD8+ T cells specific to the antigen by presenting the antigen-MHC complex to the T cell receptor and by presenting co-stimulatory ligands to the T cell. CD4+ T cells are called T

helper (Th) cells because they stimulate the activation or differentiation of other immune cells. Th1 cells are induced by the cytokine IL-12 produced by M1 macrophages and dendritic cells. (2) They stimulate the activation and proliferation of CD8+ CTLs. In the presence of IL-4, IL-6, or IL-10, CD4+ cells differentiate into Th2 cells that mediate opposing responses with respect to Th1 cells. (3, 4) Th2 cells inhibit anti-tumour responses mediated by CTLs and M1, and instead promote M2 pro-tumorigenic macrophages. (Fig. 1)

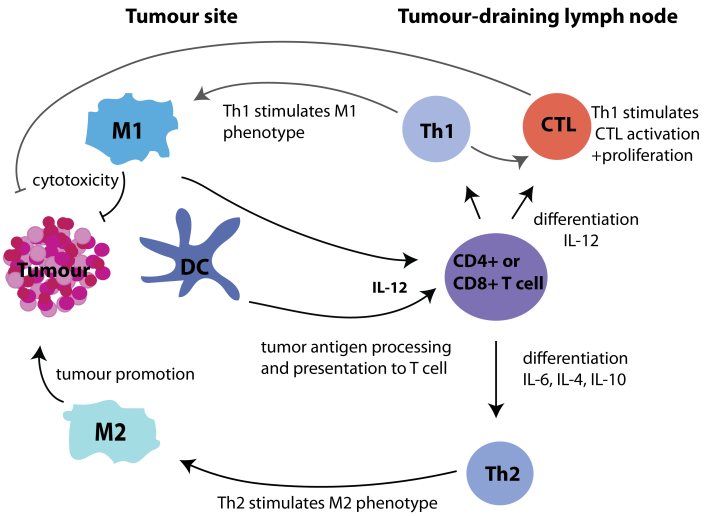

Figure 1. Opposing Immune Responses in Tumour Progression. Dendritic cells and M1 macrophages process tumour antigens and present them to naïve T cells in tumour-draining lymph nodes. These antigen-presenting cells can produce IL-12 that stimulates the differentiation of Th1 cells and CTLs. Th1 cells stimulate the production of CTLs and also promote M1 macrophages. On the other hand, IL-6, IL-4, and IL-10 induce the differentiation of Th2 cells that promote M2 macrophages and inhibit anti-tumour responses mediated by Th1 cells, CTLs, and M1.

All nucleated cells routinely present fragments of endogenous peptides in complex with MHC class I molecules at their surface. To prevent autoimmunity, T cells that are reactive to self antigens undergo negative selection in the thymus where epithelial cells present most, if not all genome-encoded self antigens in complex with MHC molecules. (2) Given that negative selection in the thymus depends on T cell receptor (TCR) signalling, T cells with high affinity to self antigens are eliminated in the thymus, while TCRs with low affinity for self antigens are weakly activated and may escape elimination. There is accumulating evidence suggesting that anti-tumour responses are mediated by low affinity effector T cells. Recent research report the ability of some of low affinity effector T cells to escape negative selection in the thymus and in the lymph nodes. (5) Low affinity T cells require interaction with a greater number of antigen-MHC complexes on the target cell surface compared to high affinity T cells, which can become cytotoxic upon binding a single complex. For this reason, low affinity T cells are more likely to target transformed cells that overexpress a certain peptide shared with normal cells – the transformed cell will present a greater density of the peptide-MHC complex at its surface. (5) This is thought to be the case for T cells targeting melanocyte differentiation antigens such as tyrosinase, gp100, and MART-1, as well as for those targeting HER2 overexpressed in certain breast tumours. However, this tumour specificity is far from perfect and the stimulation of autoreactive T cells in cancer immunotherapies can result in the destruction of healthy tissue. For instance, immune therapies that stimulate CTL-mediated responses against the melanocyte differentiation antigens overexpressed in melanoma destroy normal melanocytes in the eye and ear, leading to uveitis and hearing loss in some patients. (6, 7)

Other tumour antigens are restricted to tumour cells. One category of tumour-restricted antigens are the cancer-testis antigens. (2, 8) These peptides are encoded by genes normally expressed in male germ cells and are not usually presented to T cells, since male germ cells do not express MHC molecules. Some tumours activate the expression of antigens such as melanoma associated antigen (MAGE) or NY-ESO-1 in oesophageal cancer, melanoma, breast cancer, prostate cancer, bladder cancer, or non-small-cell lung carcinoma. (2, 9) However, these proteins are also expressed in the thymic cortex, so T cells specific to these antigens have low affinity for the peptides due to the pressures of negative selection. Other tumour-restricted antigens are derived from mutant proteins, most often from point mutations such as in Ras or p53. (2) For instance, T cells specific to a K-Ras point mutant epitope were isolated from pancreatic tumours. (10) T cells specific to point mutated Ras did not recognize wild type Ras. (11) Other tumour antigens are processed from fusion proteins, such as BCR-Abl, or proteins that undergo defective post-translational modifications such as underglycosylated mucin (MUC-1) in breast and pancreatic cancers. Finally, viral oncogenes can also be expressed as tumour antigens. Not all abnormal proteins can bind MHC molecules and be presented to T cells. However, it is now widely accepted that most tumours are immunogenic either via their surface overexpression of shared antigens or their presentation of modified self or viral peptides.

It is not clear whether mutant proteins generate more immunogenic epitopes than non-mutated self-peptides. Further evidence suggests that the context of antigen presentation is more important than the degree of self-ness or non-selfness of tumour antigens. (8) For instance, infection with a tumour-associated virus triggers acute inflammatory responses that activate T cells to more effectively destroy infected and transformed cells. This is supported by the higher incidence of viral-associated cancers in immunodeficient individuals. On the other hand, chronic antigen presentation and inflammation seem to promote immune tolerance and T cell anergy. Adenoviral DNA vectors encoding tumour antigens are currently being used to stimulate the Th1/CTL response against self-antigens. For instance, a study by Naveh et al. (2013) investigated the transduction of dendritic cells with a replication-deficient adenoviral vector encoding three melanoma antigens: MART-1, tyrosinase melanocyte antigens, and MAGE-A6 cancer-testis antigen. (12) Viral DNA stimulates dendritic cells to present antigen to T cells and secrete cytokines such as IL-12 that go on to promote the differentiation of Th1 cells and CTLs. This method is particularly effective at activating tumour specific CTLs since the viral vector promotes antigen presentation by dendritic cells in complex with MHC class I molecules. Acute inflammatory responses triggered by dendritic cells in response to viral DNA may be a method of preventing tolerance and promoting stronger T cell responses against tumour antigens.

A barrier to effective anti-tumour CTL responses involves the ability of tumour cells to escape detection by downregulating components of the complex cellular machinery responsible for the processing and presentation of endogenous peptides at their surface. These components include the catalytic proteasome subunits LMP2, LMP7, and LMP10 that generate peptide fragments; the transporters TAP1 and TAP2 that carry these peptides into the endoplasmic reticulum; ERAP1 that trims the peptides for loading onto the MHC class I complex; and ER chaperones such as tapasin that enable the assembly of the MHC-peptide complex. Human bladder cancer, melanoma, and colourectal carcinoma specimens having reduced MHC class I surface expression were found to have downregulated one or more of these components. (13) Loss of LMP7, TAP1, and ERAP1 were associated with reduced overall survival in human cervical cancer. (14) Therefore, targeting the mechanisms by which tumour cells downregulate peptide-MHC class I surface expression has strong potential to contribute to effective cancer immunotherapy. Other mechanisms by which tumour cells avoid destruction by CTLs include the expression of inhibitory ligands such as PDL1, the production of factors such as IL-10 and TGF-β that inhibit CTL function, and the recruitment of regulatory T cells that also block CTL activity.

Regulatory T cells inhibit CTL-mediated responses

Tumours may escape destruction by CTLs by promoting and recruiting another CD4+ T cell subset called regulatory T cells (Treg). Treg cells

inhibit the activation of lymph node T cells that bear the same antigen specificity as that of the Treg cell itself. They can therefore prevent the development of T cells that will target tumours. This inhibition results from a receptor called CTLA-4 on the membrane of Treg cells that competes with greater affinity compared to the T cell activating receptor CD28 for its ligand B7 on antigen-presenting cells. (15) The activation of CD28 via its engagement with B7 is essential for T cell proliferation upon TCR-antigen interaction. CD28-mediated signalling induces T cell production of the cytokine IL-2 as well as the assembly of the IL-2 receptor. This process enables the necessary IL-2 autocrine signalling for T cell survival and proliferation. (2) Furthermore, Treg cells have a greater capacity to bind IL-2 and may therefore sequester it from other T cells. (2) Treg cells can also kill dendritic cells bearing the same antigen specificity. (16) IL-10 produced by Treg cells in the tumour stroma inhibits the maturation and antigen presenting activity of dendritic cells and macrophages. (17) Therefore, Treg cells inhibit the activation and expansion of tumour-specific Th1 cells and CTLs in lymph nodes both by blocking the presentation of tumour-antigens to T cells, and by blocking the CD28 and IL-2 signalling necessary for T cell survival and proliferation. At the site of the tumour, Treg cells can inhibit CTL activity via membrane-bound or secreted transforming growth factor beta (TGF-β). (18) Treg cells have been shown to downregulate INFγ and perforin production by CTLs via direct cell-cell interactions. (19) Treg cells are induced by TGF-β; the presence of elevated levels of TGF-β upon the presentation of a tumour antigen to naïve CD4+ T cells in tumour-draining lymph nodes may promote their differentiation into Treg cells. Production of IL-10 and TGF-β by human cervical cancer cells is associated with decreased CTL function, inhibition of type 1 T cell polarity, and tumour invasiveness. (4) Studies relating to breast and ovarian cancer report increased Treg infiltration with disease stage in association with reduced relapse-free survival, and reduced overall survival. (20, 21)

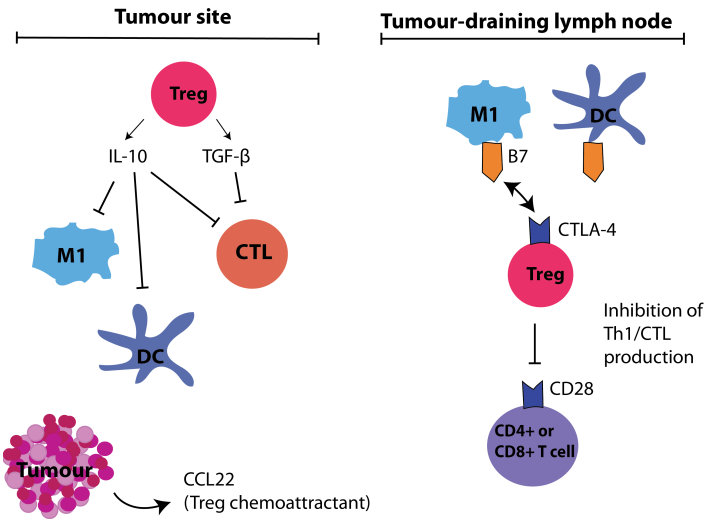

Figure 2. Regulator T Cells Inhibit Anti-Tumour Immunity. At the tumour site, Treg cells secrete IL-10 that inhibits M1 macrophages, dendritic cells, and CTLs. Membrane-bound TGF-β on Treg cells also inhibits CTL activity. Tumours produce the Treg chemoattractant CCL22. In tumour-draining lymph nodes, CTLA-4 on Treg cells sequesters B7 ligand from its receptor CD28 on naïve T cells, inhibiting their activation and proliferation into Th1 cells and CTLs.

Tumour cells may also evolve the ability to recruit Treg cells. In a study by Benevides et al. (2013), Treg infiltration was observed in breast invasive ductal carcinomas (IDC) as opposed to healthy controls, and was correlated with IDC elevated expression of CCL22, a chemokine that attracts Treg cells. Blocking the interaction between CCL22 and its receptor CCR4 on Treg cells using a CCR4 agonist has therapeutic potential by inhibiting the recruitment of Treg cells to lymphoid organs and to the tumour site. (22) Studies in mice have shown that the administration of tumour antigens as vaccines along with the administration of a CCR4 small molecule antagonist enhances the production of tumour-specific CTLs. (23) Tumour cells and their associated macrophages were shown to secrete CCL22 in ovarian carcinoma. (20) Therefore, tumours may have the ability to promote tolerance to any tumour antigen by producing factors that inhibit CTL-mediated responses and by promoting the production and recruitment of Treg cells.

Adoptive T Cell Transfer

Some of the most striking advances in cancer immunotherapy conducted in melanoma treatment were inspired by cases of spontaneous regression that encouraged the trial of T cell-targeted immunotherapies in metastatic melanoma patients. The most durable responses have been reported for immunotherapies such as adoptive T cell transfer and the inhibition of the T cell receptors CTLA-4 and PD1 that promote CTL anergy.

Adoptive T cell transfer therapy involves culturing a patient's tumour-infiltrating T cells ex-vivo in several cultures established from single cell suspensions and then selecting for Th1 cells and CTLs that are specific to the patient's tumour antigens. This is done by co-culturing the T cells with tumour cells and selecting for IFNγ production. The T cells are cultured in the presence of IL-2 to stimulate their proliferation. The selected T cells are then injected into the patient's circulation.

Adoptive cell transfer (ACT) has shown promising results from three clinical trials on metastatic melanoma patients. (6) Tumour-infiltrating T cells from metastatic lesions were cultured in the presence of IL-2 and tested for tumour specificity via the methods described above. They were injected back into patients along with IL-2 after lymphodepleting non-myeloablative (NMA) chemotherapy. Twenty-two percent of patients experienced complete responses, of which 93% had disease free survival at five years. The five-year survival rate of the entire cohort was 29% compared to about 5% following the standard of care or IL-2 therapy. Patients that received lymphodepleting chemotherapy followed by ACT had an objective response (OR) rate of 48%, whereas patients who also received low dose or high dose full-body irradiation prior to ACT had an OR rate of 52% and 72%, respectively. The complete response rates for these three cohorts were 12%, 20%, and 40%. The proportion of complete responses and their durability is much higher than in BRAF inhibitor therapies, which have a 6% complete response rate, and decarbazine, which has a 1% complete response rate. (6) Interestingly, patients who had previously received CTLA-4 blockade therapy showed an increased overall survival rate.

Non-lytic doses of irradiation (up to 20 Gy) enhance the susceptibility of cancer cells to cytotoxic T cell-mediated killing. They promote the upregulation of Fas expression at the tumour cell surface, as well as the expression of MHC class I and intracellular adhesion molecule 1 (ICAM-1), which binds receptors on T cells. (24) The increased susceptibility to CTL attack as a result of irradiation may explain in part the higher OR rates seen in patients having undergone irradiation.

Other studies have reported the synergy and potential clinical benefit of combining adoptive T cell transfer with the BRAFV600E inhibitor Vemurafenib in melanoma patients. BRAFV600E inhibition upregulates the surface expression of melanocyte-differentiation antigens MART, gp100, and tyrosinase with MHC class I complexes and therefore enhances the recognition of melanoma cells by T cells having low affinity for these antigens. (25) MAPK inhibition could also enhance CTL recognition of other cancer cell types, but it is important that the inhibitor be specific to a mutant upstream kinase only found in transformed cells to avoid destruction of normal cells by CTLs. The kinase inhibition also sensitizes melanoma cells to induced apoptosis by CTLs. (25) Furthermore, it has been shown that mutant BRAF signalling upregulates the production of IL-1 by melanoma cells, which increases tumour-associated fibroblast expression of ligands for the inhibitory receptor PD1 on T cells. Activation of the PD1 death receptor on CTLs inhibits their activity and can lead to their apoptosis. Melanoma cells from patients undergoing Vemurafenib treatment express less IL-1 than before treatment, and their tumour-associated fibroblasts are less able to inhibit T cell cytotoxicity in vitro. (26) Clinical trials are currently evaluating the safety and efficacy of combining Vemurafenib and

ACT therapy in melanoma patients.

Sipuleucel-T (Provenge) is a form of adoptive cell transfer therapy approved for metastatic castration-resistant prostate cancer. The therapy target expanding T cells specific to the prostate antigen, prostatic acid phosphatase, that is overexpressed in 95% of prostate cancers. (27) The method involves isolating a patient's peripheral mononucleated blood cells, which include T cells, macrophages, and dendritic cells. The cells are cultured in vitro with a fusion protein of prostatic acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor. The fusion protein promotes the uptake of the tumour antigen by macrophages and dendritic cells and its presentation to T cells. A double blind randomized phase III trial was conducted by Kantoff et al. (2010) in which 512 patients were given either three injections of leukocytes cultured with the fusion protein or leukocytes cultured with no antigen over two weeks. (28) The study reports that 46 out of 63 patients from Sipuleucel-T group (73%) had proliferative T cell responses to the fusion antigen at six weeks post-infusion. Median survival was increased by 4.1 months, and the probability of survival at 36 months was 32% for the group receiving Sipuleucel-T, compared to 23% for the placebo group. However, this survival increase is modest compared to the ACT trials conducted in melanoma patients.

CTLA-4 blockade: Ipilimumab

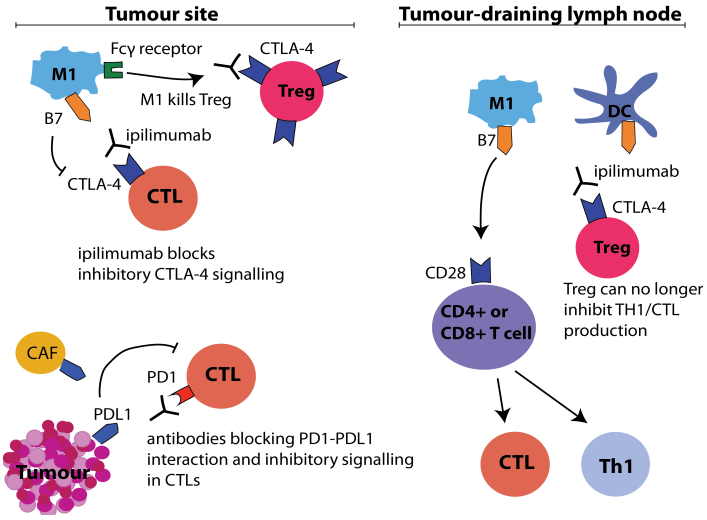

Figure 3. T Cell Checkpoint Inhibition. At the tumour site, Ipilimumab inhibits the interaction of the inhibitory receptor CTLA-4 on CTLs with its ligand B7 on other immune cells. Ipilimumab may also enable the depletion of Treg cells at the tumour site by binding Treg CTLA-4 and mediating the destruction of Treg cells by macrophages bearing Fcγ receptors that interact with the constant region of Ipilimumab. Other antibodies inhibit the interaction between the inhibitory receptor PD1 on CTLs and its ligand on tumour cells and on cancer-associated fibroblasts (CAF).

Other cancer immunotherapies enhance CTL killing of tumour cells by blocking their inhibitory receptors, CTLA-4 and PD1. (Fig. 3) Ipilimumab is a fully human, monoclonal IgG antibody against CTLA-4, an inhibitory receptor expressed on activated T cells. This then induces T cell anergy upon interaction with its ligand, B7, on macrophages or dendritic cells at the tumour site. Ipilimumab can also block Treg inhibition of CTL production in lymph nodes by binding CTLA-4 on Treg cells and inhibiting its sequestration of activating ligands away from T cells. Furthermore, recent evidence suggests that Ipilimumab depletes Treg cells at the tumor site by promoting the killing of Treg cells. Treg cells are killed by macrophages located in the tumor stroma that bind the constant region of the antibody via their Fcγ receptor. (29) It is thought that effector T cells are not targeted by macrophage cytotoxic activity because they express much less CTLA-4 than Treg cells. Therefore, Ipilimumab enhances the production and function of CTLs by blocking inhibitory interactions at the tumour site and in lymph nodes. Ipilimumab was approved in 2011 for the treatment of metastatic melanoma. Clinical data indicates that patients receiving Ipilimumab alone had an OR rate of 13%, whereas patients who received IL-2 had an OR rate of 25% with a 17% rate of complete responses, and higher overall survival. (30) IL-2 is known to promote CTL survival, proliferation, and secretion of IFNγ. Toxicity of CTLA-4 inhibition involves immune-related adverse effects (IRAEs) due to the role of CLTA-4 in regulating inflammation and undesirable autoimmune responses. Grade 3 or 4 IRAEs were observed in 10% to 15% of patients receiving Ipilimumab. Treatment with Ipilimumab improved overall survival with a 34% reduction in probability of death compared to tumour vaccines, and 26% compared to IL-2 therapy alone. (31) Responses to Ipilimumab treatment are strikingly durable and associated with significant increases in overall survival. Results from three phase II trials indicate that previously treated patients receiving Ipilimumab have 4 year survival rates of 20% to 28%, and treatment naïve patients have 4 year survival rates of 38% to 50%. (32) In a phase III clinical trial, 1 and 2 year survival rates for patients receiving Ipilimumab were 46% and 24% compared to 25% and 14% in the gp100 vaccine control group. (33) The safety and efficacy of Ipilimumab therapy is currently being evaluated in trials for other cancers such as non-small-cell lung cancer and prostate cancer.

PD1 blockade

Another T cell checkpoint inhibition therapy that has revealed striking clinical benefits in some patients is PD1 blockade. Tumour cells can inhibit CTL cytotoxic activity by expressing ligands for the PD1 programmed death receptor on CTLs. (34) Tumour PD1-ligand (PDL1) interaction with PD1 on T cells inhibits CD8+ T cell proliferation and production of cytokines such as IFNγ, and can also lead to T cell apoptosis. A phase I clinical trial conducted by Topalian et al. (2012) evaluated the safety and response to treatment with an anti-PD1 antibody. OR rates were 18% for non-small-cell lung cancer patients, 28% for melanoma patients, and 27% for renal cell carcinoma patients. Grade 3 or 4 drug-related adverse effects were reported in 14% of patients, along with 3 deaths from pneumonitis out of 296 patients, of which two were patients with non–small-cell lung cancer and one was a patient with colourectal cancer. Ninety percent of patients had received three or more infusions of Ipilimumab 12 weeks before enrolling in the trial. (35) In a follow up study on 39 patients treated with anti-PD1, three patients experienced durable objective responses. (36) One metastatic colourectal adenoma patient had been refractory to multiple chemotherapy regimens before anti-PD1 therapy and developed a complete and durable response with no recurrence at the time of data collection three years later. A patient with renal metastatic carcinoma also experienced a complete response to anti-PD1 ongoing at the latest follow up four years off therapy. A third patient had melanoma, and prolonged administration of anti-PD1 stabilized disease resulted in a partial response lasting several years. Thus, there exists a correlation between tumour cell expression of PDL1 and response to anti-PD1 therapy.

Another anti-PD1 monoclonal antibody, Lambrolizumab, was evaluated in a phase I trial on 135 patients with metastatic melanoma. (37) The highest dose cohort (10mg/kg) showed a 52% OR rate, and overall response in the whole dose escalation cohort was 38%. There were grade 3 or 4 drug-related adverse effects in 13% of patients, and most responses were durable at follow up after 11 months.

Adoptive T cell therapy and checkpoint inhibitors against PD1 and CTLA-4 have resulted in durable complete responses in a small but significant proportion of patients, and have shown improvements in overall survival. There is a great need to determine the characteristics of patients that will respond to these therapies in order to better target these treatments. The limitations of adoptive T cell therapy relative to small molecule inhibitors are its labour-intensive requirements and cost. Given the success of Ipilimumab and anti-PD1, small molecule inhibitors which prevent restrictions to the production and activity of tumour specific T cells appear as a more feasible and effective cancer immunotherapy.

However, stimulating CTL tumour-specific responses by expanding these cells or by inhibiting their checkpoints may not be sufficient to mount an effective anti-tumour response. For instance, this therapeutic approach may be ineffective against tumours that have downregulated antigen presentation at their cell surface and that can escape recognition by CTLs. Another approach is to skew the tumour microenvironment from pro-tumorigenic to anti-tumorigenic. This is achieved by countering the tumour's ability to promote the differentiation and recruitment of immune cells which otherwise contribute to tumour progression. As previously discussed, different subsets of infiltrating T cells have opposing effects on tumour progression. Th1 cells play a significant role in promoting CTL and M1 mediated tumour cytotoxic responses, and their activity is opposed by Th2 cells and Treg cells. Th17 cells represent another subset of CD4+ T helper cells found in the tumour microenvironment; however, their role in tumour progression remains controversial. Their production of the cytokine IL-17 has been associated with reduced disease-free survival of breast cancer patients after chemotherapy. (38) IL-17 promotes the production of IL-6 by tumor cells and results in IL-6 autocrine signaling that promotes tumour cell proliferation, survival, angiogenesis, and invasion. (39-44) Some studies report the production of INFγ by Th17 cells and suggest plasticity in the Th17 cytokine profile. (45, 46) However, Th17 production of INFγ is driven by the transcription factor T-bet that is responsible for Th1 differentiation. Therefore, this transition from IL-17 to INFγ production may result from a transition from the Th17 to the Th1 phenotype. Indeed, this transition occurs naturally in inflammatory responses to reduce the strong inflammatory effects of IL-17. (46) Furthermore, the different subsets of T helper cells have important effects on tumour progression through their crosstalk with other immune cells. The crosstalk between T helper cells and macrophages has an important role in determining whether a tumour-cytotoxic response will be initiated or shut down. These interactions can be generally divided into type 1 and type 2 responses. The type 1 response consists of a positive feedback loop between Th1 cells and M1 macrophages. Th1 cells stimulate the differentiation of M1 macrophages that are tumour cytotoxic and that present antigen to T cells and stimulate their differentiation into Th1 cells and CTLs. (2) The type 2 response involves the interaction between Th2 cells and pro-tumourigenic M2 macrophages, whereby Th2 cells promote M2 differentiation. (47) M2 macrophages foster tumour growth, angiogenesis and invasion. (48) They are also immunosuppressive and interact with Treg cells. Importantly, the phenotype of T helper cells and macrophages is plastic and depends on the signals they receive. (2) Therefore, these response axes can be skewed by the tumour toward a type 2 response, or skewed by therapeutic intervention toward a type 1 response. The following section will discuss these responses in more detail, as well as the therapies aimed at their manipulation.

Macrophages in cancer progression and therapy

Similarly to T cells, macrophages can have pro-tumorigenic or anti-tumorigenic properties depending on their path of differentiation. (Fig. 4) Macrophages are generally classified into two effector types: M1 and M2. Often, macrophages in the tumour microenvironment are polarized toward M2 functions and contribute to tumour growth, angiogenesis, and metastasis. (49) These macrophages are often called tumour-associated macrophages (TAMs). A study following surgically treated renal cell carcinoma patients observed that high proportions of M2 and low proportions of M1 macrophages in tumours were associated with reduced survival, whereas higher M1 presence was associated with increased survival. (50) IFNγ produced by Th1 cells, NK cells, or CTLs promotes the differentiation of M1 macrophages that can kill tumour cells via nitric oxide and tumour necrosis factor (TNF) production. (51) Th1 cells are crucial for the production of M1 macrophages and CTLs. M1 macrophages express elevated levels of MHC class II molecules and produce IL-12, enabling them to present antigen to CD4+ T cells and induce their differentiation into activated Th1 cells that will go on to stimulate a CTL response. Th1 cells, M1 macrophages, and CTLs can, taken together, positively regulate each other and mount an anti-tumour response. IL-4 produced by Th2 cells promotes immunosuppressive M2 macrophages that have reduced antigen presentation ability. M2 macrophages secrete IL-10 that stimulates PDL1 expression and suppresses CTL activity, in addition to inhibiting dendritic

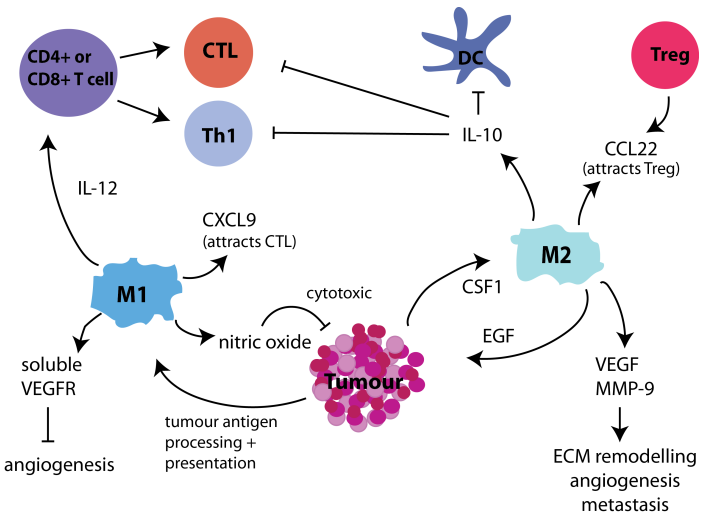

Figure 4. Opposing Roles of Macrophages in Tumour Progression. M1 macrophages inhibit tumour progression by promoting Th1 and CTL responses, inhibiting angiogenesis, and producing tumour-cytotoxic nitric oxide. M2 macrophages inhibit Th1 and CTL mediated anti-tumour responses and promote angiogenesis and metastasis. Tumour cells produce CSF1 and M2 cells produce EGF in a paracrine loop. CSF1 attracts macrophages and promotes an M2 phenotype. EGF promotes tumour progression and further production of CSF1.

cell antigen presentation and IL-12 production. M1 macrophages produce the chemokine CXCL9 that attracts CTLs whereas M2 macrophages produce CCL22 that attracts Treg cells. (20)

The polarity of macrophages is associated with the opposing activities of the transcription factors NF-kB and STAT3. NF-kB activates the production of IL-12, and the switch from active to inactive NF-kB in macrophages appears to be central to tumour malignancy. (52, 53) IL-10, Vascular endothelial growth factor (VEGF), and IL-6 are activators of STAT3 in macrophages, and STAT3 induces the production of IL-10 and downregulates IL-12. (54) In this mechanism, STAT3 activity is important for immune tolerance to tumours; inhibition of STAT3 was shown to enable the activation of T cells. (55) Several studies suggests that the dichotomy of M1/M2 macrophages is not clear cut and that MI and M2 are the classified phenotypes at both ends of a spectrum of gene expression in macrophages. (56) The properties of macrophages in tumour progression may depend on their relative pro-tumorigenic and anti-tumorigenic activity, and there is substantial evidence that tumours regulate these properties to their advantage.

Macrophages are recruited to hypoxic areas of tumours via their binding to VEGF, CSF-1, and MCP-1 chemoattractants produced by tumour cells in response to the activation of HIF-1 transcription factor. Several tumour cells and their associated stroma also acquire the ability to produce other macrophage chemoattractants such as CXCL12, CXCL-8, and CCL9. (48, 57) In these hypoxic areas of the tumour, upregulation of HIF-1 in macrophages results in the production of VEGF and FGF2 angiogenic factors involved in the angiogenic switch that is important for tumour growth and metastasis. (35, 41, 43-44) Macrophages at the tumour site also secrete proteases such as matrix metalloproteinases that degrade the extracellular matrix (ECM) surrounding tumours and enable vessel remodelling as well as tumour cell migration. Furthermore, ECM degradation releases growth factors and pro-angiogenic factors into the tumour microenvironment. M2 macrophages are associated with these angiogenic properties, and M1 macrophages can actually secrete soluble VEGF receptor that neutralizes VEGF. (58) Finally, when tumour cells extravasate, they induce tissue resident macrophages to produce Matrix metallopeptidase 9 (MMP-9), leading to the release of VEGF. The resulting tissue and vessel remodelling enables the establishment and growth of metastases.

CSF1 is an important macrophage chemoattractant and differentiation fac-

tor for tumour invasiveness. CSF1 secreted by tumours can skew the phenotype of surrounding macrophages from M1 to M2. (59, 60) Consistent with these findings, the over-expression of CSF1 in breast tumours and the high density of tumour-associated macrophages correlate with poor prognosis. (46, 48) Importantly, an EGF-CSF1 paracrine loop contributes to tumour growth and invasiveness. Macrophages recruited to the tumour site via CFS1 secrete EGF that, amongst other EGFR-signalling effects on tumour growth, induces their epithelial to mesenchymal transition and promotes further secretion of CSF1 by tumour cells. (48) Macrophages and tumour cells migrate together toward blood vessels, and macrophages facilitate the intravasation of tumour cells and can associate with metastatic cells in circulation. (49) Transgenic mice lacking CSF1 have delayed progression to invasive mammary carcinoma and a large reduction in the incidence of metastasis, whereas expression of CSF1 in mammary epithelium and increased macrophage tumour infiltration accelerates tumour progression and increases metastasis to the lung. (61)

These data underline the therapeutic potential of inhibiting CSF1 or its receptor. Clinical trials are currently evaluating CSF1/CSF1R blockade. A study by Strachan et al. (2013) evaluated the effect of blocking CSF1 signalling in murine cervical and mammary cancer models. They found that the turnover of macrophages in the tumour microenvironment is dependent on CSF1 therefore the inhibition of CSF1R signalling may be therapeutically effective in depleting them. Treatment with CSFR1 or CSF1 inhibitors significantly decreased the number of tumour infiltrating macrophages and neoplasm size, and also resulted in increased tumour infiltration of CD8+ T cells. (60) Another study reported that TAMs had the ability to promote cancer stem cell traits via STAT3 signalling; and that efficacy of chemotherapy was increased when mice were treated with a CSF1R inhibitor, due to a decrease in STAT3-dependent chemoresistance. (62)

Another macrophage-targeted therapy involves skewing the phenotype of TAMs rather than depleting them. A study by Rolny et al. (2011) investigated the ability of an anti-angiogenic histidine-rich glycoprotein (HRG) to induce an M2 to M1 switch in tumour-associated macrophages in mice via its downregulation of PIGF growth factor. Expression of factors produced by M2, such as IL-10 and CCL22, was reduced and expression of M1 factors, such as IL-12 and CXCL9, was upregulated. This change in TAM phenotype was associated with increased antigen presentation, CTL infiltration and function, and tumour cell lysis. (63) Other agents have also revealed therapeutic potential by affecting macrophage polarity. For instance, biphosphonates are administered to advanced breast and prostate cancer patients to reduce cancer-induced bone disease. They have been shown to act on macrophages, derived from the same progenitors as osteoclasts, by downregulating their production of angiogenic factors and MMP-9 and upregulating Inducible nitric oxide synthase (iNOS), which is important for macrophage cytotoxic activity. (64)

Another study in humans and mice evaluated the capacity of a CD40 agonist to activate macrophage tumoricidal activity. Treatment of pancreatic ductal adenocarcinoma patients with demcitabine chemotherapy and agonist CD40 antibody increased the response rate from 5.4% with demcitabine alone to 19% with the combination therapy. A 30% response rate was observed for the same treatment in mice, and the response was dependent on macrophages that became tumoricidal in response to CD40 activation. TAMs also upregulated their expression of IL-12, MHC class II, and costimulatory molecules necessary to present antigen to and activate tumour specific T cells. However, no significant increase in patient survival was observed. (65, 66)

One of the most striking anti-tumour responses observed in a mouse model triggered by macrophage repolarization was seen by Guiducci et al. (2005) in mice bearing mammary carcinomas or colon carcinomas. The mice were treated with a combination that showed a synergistic effect in promoting anti-tumour immunity: CpG ligand for TLR9-activating receptor on macrophages, plus anti-IL-10R and CCL16 macrophage chemokine. TAMs that had previously secreted IL-10 switched phenotype to TNFa and IL-12 producing macrophages, and increased their production of nitric oxide. 60% and 90% of the mice rejected colon and mammary tumours, respectively. About 30% of the response depended on macrophage tumoricidal activity, and the rest of the response depended on T cell cytotoxicity. Tumour specific CTL activity was observed as early as seven days after treatment. (67)

However, there are potential caveats to skewing macrophage phenotype, or inhibiting their recruitment. M2 macrophages play an important role in resolving inflammation via their immunosuppressive characteristics and their mediation of tissue repair at sites of injury or infection. (2) Promoting the cytotoxic activity of M1 macrophages at the expense of M2 macrophage functions may lead to persistent tissue damage without resolution or repair at non-tumour sites. M2 macrophages are essential for wound healing and restored tissue homeostasis. Without functional M2 macrophages, persistent tissue damage may lead to chronic inflammation, altered tissue homeostatic set points, and chronic disease. (68) For instance, suppression of M2 macrophage function has been shown to produce massive inflammation of the gut due to the role of these cells in controlling such inflammation. (68) Also, immunosuppressive macrophages are required to reduce reactivity against apoptotic cells in the spleen. Therefore, inhibition of M2 macrophage function may promote responses against self molecules such as DNA, a response found in systemic lupus erythrematosus and related autoimmune syndromes. (68) At the site of the tumour, persistent M1 macrophage activity and the resulting tissue damage may further drive chronic inflammation and the immunosuppressive activity of cell types other than macrophages. Indeed, other immune cells follow the same general polarity seen in macrophages with respect to their role in tumour progression. For instance, neutrophils have been shown to mirror M1-M2 macrophage polarity and can be classified into N1 and N2 neutrophils. N1 neutrophils are tumour cytotoxic and stimulate antigen presentation by dendritic cells as well as the tumour cytotoxic activity of natural killer cells. N2 neutrophils are induced by TGF-β and are immunosuppressive in addition to contributing to tumour cell proliferation, angiogenesis, and invasion. (69) Therefore, it is uncertain whether therapies stimulating M1 function at the expense of M2 are effective and whether the potential benefits outweigh the risks of inflammatory disorders. Additionally, it is not clear how long stimulated M1 activity can last without triggering a wave of immunosuppression and pro-tumorigenic activity from other immune cells recruited to the tumour site.

Conclusion

Immune cells have opposing properties in tumour progression, and the manipulation of their effector functions holds therapeutic potential. Adoptive T cell transfer and CTLA-4 or PD1/PDL1 inhibition have so far resulted in strikingly durable responses in a small but significant proportion of patients. There is evidence for the synergistic effect of coupling immune-based therapies with other cancer therapies, from chemotherapy and irradiation to oncogene inhibitors. Finally, skewing the phenotype of macrophages to suppress their tumour-promoting M2 functions and enhance their M1 tumoricidal activity and antigen presentation may be a significant component of cancer immunotherapy, since macrophages are the most abundant immune cell in the tumour stroma and have important contributions to tumour growth and invasiveness. The extensive crosstalk between macrophages and T helper cells further supports the promise of skewing macrophage polarity in order to mount effective tumour cytotoxic responses and inhibit pro-tumorigenic and immunosuppressive macrophage-T cell interactions. Therefore, future developments in cancer immunotherapy should strive to regulatie these opposing networks of immune cells and their interactions in order to enhance the cooperative activity of Th1 cells, M1 macrophages, and CTLs while suppressing M2 macrophages, Treg cells, and other inhibitory interactions that reduce the effectiveness of tumoricidal responses. However, systemically tipping the balance between regulatory immune cells and pro-inflammatory immune cells may come at the cost of inflammatory disorders, resulting in autoimmunity and chronic disease. Developing cancer immunotherapies will benefit from efforts to target these therapies specifically to the tumour microenvironment, as well as from targeting potential responders.

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