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Lymphocytes in cancer development: polarization towards pro-tumor immunity

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Abstract

The classic view that the role of immune cells in cancer is primarily one of tumor rejection has been supplanted by a more complex view of leukocytes having both pro-and anti-tumor properties. This shift is due to the now well recognized capabilities of several myeloid cell types that foster pro-tumor programming of premalignant tissue, as well as the discovery that subsets of leukocytes also suppress development and effector functions of lymphocytes important for mediating anti-tumor immunity. In this review, we focus on the underappreciated role that T lymphocytes play in promoting tumor development. This includes, in addition to the role of T regulatory cells, a role for natural killer T cells and CD4$^+$ T helper cells in suppressing anti-tumor immunity and promoting cancer growth and metastasis.

Keywords

Lymphocytes; Inflammation; Cancer; Tumors

INTRODUCTION

Leukocyte infiltration into developing *tumors* is now considered one of the hallmarks of cancer development (1). It is thought that the initial immune response to an early neoplasm mirrors the response to acute tissue injury, with sequential infiltration by various myeloid populations leading to eventual infiltration by lymphocytes (2). However, as the kinetics of tumor development and the neoplastic cells themselves alter the local immune microenvironment, making inferences between an immune response to injury/infection and tumor development is difficult. Regardless, if clearance of the would-be cancer cells is not achieved and the initial acute inflammatory response fails to resolve, there inevitably results a state of chronic inflammation within the local tissue. It is now well established that chronic inflammation fosters early cancer development through a number of mechanisms mediated primarily by myeloid-lineage cells, including tumor-associated macrophages, immature myeloid cells that can possess suppressive activity, and Tie2-expressing monocytes (3,4). The immune microenvironment of a neoplastic tissue encompasses not only the composition of infiltrating

leukocytes, but also the bioeffector function of these cells within the tissue. Thus, both the presence of a cell within a tumor and expression of tissue-specific cytokines, chemokines and other immune mediators profoundly influence whether an anti-tumor or pro-tumor immune response is elicited (4,5).

Although merely responding to tissue damage in the form of inflammatory cues, tumor-infiltrating myeloid cells rapidly respond to soluble and insoluble signals emanating from the neoplastic microenvironment. Responses take the form of dramatically altered gene expression programs that alter bioeffector functions of the immune cells. These often result in increased expression of factors/mediators that enhance growth and survival of neoplastic cells, as well as activating and sustaining angiogenic responses, furthering tissue remodeling, and squelching anti-tumor immune programs (4). Chronic inflammation in tissue resulting from infection or autoimmune disease can also alter the risk of cancer development by providing an environment permissive for initiated preneoplastic cell survival and subsequent proliferation, as well as through production of DNA damaging compounds such as reactive oxygen and nitrogen species that increase mutation frequency (6). While all of these aspects of solid tumor development are susceptible to regulation by infiltrating immune cells, in the context of this review, we will focus on aspects of carcinogenesis regulated by infiltrating lymphocytes, as mechanisms regulated by myeloid cells have been reviewed elsewhere (5–9).

T lymphocytes

T cells develop in the thymus from a common lymphoid progenitor and are defined by expression of a T cell receptor (TCR) that is responsible for recognizing antigens presented by the major histocompatibility complex (MHC) family of genes (also called human leukocyte antigen or HLA). T cells are classically divided into either $CD8^+$ cytotoxic lymphocytes (CTL) or $CD4^+$ T helper (T_H) cells that recognize peptides presented by MHCI or MHCII, respectively (Fig. 1). T_H cells are further divided into interferon (IFN–γ and tumor necrosis factor (TNF)-α expressing T_H1 cells and interleukin (IL)-4, IL-5 and IL-13 expressing T_H2 cells. This simplified view of the T cell compartment has been expanded upon by the identification of a range of additional subtypes, including T follicular helper cells (T_{FH}), IL-17 expressing T_H cells (T_H17), and regulatory T cells (T_{reg}) (10). Paralleling these subtypes in the $CD4^+$ T cell compartment, type 1, type 2, and type 17 $CD8^+$ T cells (T_C1, T_C2, T_C17), as well as regulatory $CD8^+$ cells, have all been described (11–13). There also exist two 'innate-like' T cell subsets that can be activated either by cytokines or TCR stimulation. Natural killer T (NKT) cells recognize glycolipids presented by the non-classical MHC molecule CD1d (14), while γδ T cells are not MHC restricted and recognize a diverse range of molecules, including soluble non-protein antigens (15). All of these T lymphocyte subsets have been examined for their role in tumor development and anti-tumor immunity, each with unique roles in directing the immune response.

Cytotoxic T lymphocytes

Mice harboring specific immune-based genetic deficiencies are more susceptible to formation of carcinogen-induced sarcomas, and depending on the specific defect, are also more prone to develop certain spontaneous tumors and lymphomas (16,17). The ability of immune-deficient mice to reject and/or inhibit the growth of many, but not all cell lines is also impaired. Numerous studies have shown that, due to their ability to produce IFNγ and directly kill target cells, both $CD8^+$ CTLs and natural killer (NK) cells are the critical mediators of the anti-tumor response (16). γδ T cells, which share characteristics with both CTLs and NK cells, are also involved in the anti-tumor response in epithelial tissues such as the skin (18), where they can be the dominant T cell population (15). The relative importance of CTLs, NK cells, and γδ T cells is highly dependent upon the cancer model being used. Even in the skin, genetic-deficiency of αβ T cells increases sarcoma formation following administration of methylcholantrene (MCA),

but not 7,12-dimethybenz[a]anthracene (DMBA) or 12-O-tetradecanoylphorbol-13-acetate (TPA) (18), while the absence of CD8^{+} T cells does not influence the development of neoplasms in a mouse model of de novo squamous carcinogenesis, e.g., K14-HPV16 mice (19).

Epidemiological studies of cancer incidence in acquired immune deficiency syndrome (AIDS) and organ transplant patients reveal that the relative risk (RR) for cancer development varies considerably depending upon organ site and cancer etiology (17,20) where viral-associated cancers, in particular Human Herpes Virus 8-associated Kaposi's sarcoma, Epstein-Barr virus-associated Non-Hodgkin's lymphoma and HPV-associated squamous carcinoma, are elevated in immune suppressed individuals due largely to lack of ability to provide protection against viral infections or viral reactivation in the absence of T cells (21). That said, some cancer types occur with increased frequency in selected groups of immune compromised patients for reasons unrelated to immune suppression for example, chronic exposure to carcinogens (tobacco) for thoracic malignancies, whereas head and neck, esophageal, gastrointestinal and pancreatic cancers are increased in liver transplant patients associated with prior history of alcohol (and tobacco) use (22,23). On the other hand, the RR for the most common non-viral associated solid tumors of epithelial origin, including breast and prostate, are decreased in immune suppressed patients, with some of these having a RR less than 1.0 (17,20).

Examination of T cell infiltrates in tumors reveals cells that can display activation markers and are able to recognize tumor antigens (16), indicating that some tumors are indeed immunogenic and can induce an anti-tumor immune response. Tumor antigens encompass both neo and overexpressed antigens, e.g., c-myc, HER-2/neu and p53 (24), as well as host/stromal cell-derived antigens unique to individual tumors. Clinical studies have even reported accumulation of autoantibodies against extracellular matrix (ECM) components, including anti-collagen type I, III and V, as well as anti-fibronectin antibodies that accumulate in lung cancer and nasopharyngeal patients (25). How then do neoplastic cells, expressing mutant proteins in an *inflammatory* microenvironment that seemingly engenders a robust T cell response, avoid killing by cytotoxic cells? Importantly, lymphocytes do not act in isolation, and their effector functions are largely dependent upon the release of cytokines and binding of inhibitory and activating receptors to ligands expressed by other leukocytes, stromal cells, and even neoplastic cells. For example, NK cells are potent regulators of CD8^{+} T cell responses through their release of IFNγ, which provides a maturation signal for tissue resident DCs and assists in CD8^{+} T cell effector function; meanwhile, cytokines released by mature DCs and activated T cells are important for promoting NK cell effector function (26). These interweaving regulatory pathways are necessary to initiate, direct, maintain and eventually shutdown an appropriate immune response. Such pathways are also necessary to prevent an inappropriate immune response: despite central tolerance through negative selection of self-reactive lymphocytes, peripheral tolerance mediated by cytokines, inhibitory receptors and immune regulatory cell types is necessary to prevent autoimmune disorders. As cancer cells are largely recognized as 'self', it is not surprising they utilize similar mechanisms that effectively dampen anti-tumor immunity; thus, T lymphocytes are intimately involved in regulating both pro- and anti-tumor immunity and chronic inflammation within the tumor microenvironment.

Regulatory T cells

Since the rediscovery of suppressor T cells as CD4^{+}CD25^{+} regulatory T (T_{Reg}) cells, and their further characterization as cells expressing glucocorticoid-induced tumor necrosis factor receptor (GITR), cytotoxic T-lymphocyte antigen (CTLA)-4, and uniquely, the transcription factor forkhead box P3 (FoxP3), this T cell subset has become an intense focus of cancer research. T_{Reg} cells can develop in the thymus or can be converted in the periphery by exposure to transforming growth factor (TGF)-β (27). These 'natural' and 'inducible' T_{Reg} cells,

respectively, utilize the same mechanisms to mediate immune suppression and may perform overlapping functions (28). A number of regulatory T cells that do not conform to the CD4$^+$CD25$^+$FoxP3$^+$ phenotype have also been described, but these remain poorly characterized (29) and evidence of a role for these cells in cancer has so far been limited to the isolation of IL-10 producing CD8$^+$ T cells from human ovarian tumors (30). Immune suppression of CD4$^+$ and CD8$^+$ T cell responses by T$_{Reg}$ cells is mediated both in the lymphoid organs where T cells activation occurs, and in the tissues (31). Interestingly, in a pancreatic islet allograft model, T$_{Reg}$ cells were shown to enter the inflamed tissue and then migrate to the lymph nodes, where these sequential series of steps was found to be associated with increasing graft survival time (32).

While a host of molecules have been described as important for mediating T$_{Reg}$ cell suppression, these molecules have been broadly classified as acting in one of four ways (33): *i.* cytokine inhibition, such as with TGF-β, IL-35 and IL-10; *ii.* direct cytolysis of effector T cells through perforin and granzyme; *iii.* metabolic disruption, such as IL-2 deprivation and cyclic adenosine monophosphate (cAMP) transfer; and *iv.* inhibition of DC function, such as through binding of CTLA-4 to CD80/86 and the induction of indoleamine 2,3-dioxygenase (IDO). These mechanisms appear to have overlapping but non-redundant roles, with the degree of importance being tissue and model dependent (34). This may be significant in cancer, as CD4$^+$CD25^{high} tumor infiltrating T lymphocytes from patients with head and neck squamous cell carcinoma (HNSCC) were found to mediate suppression through IL-10 and TGF-β, while the same population from peripheral blood did not express these cytokines and were less able to suppress proliferation (35). A role for IL-10 and TGF-β has also been revealed in mice following injection of a fibrosarcoma cell line (36), while a role for perforin- and granzyme B-dependent killing of NK and CD8$^+$ T cells was found in mice after injection of a lymphoma cell line (37). Other than these studies, the mechanisms by which T$_{Reg}$ cells mediate suppression in cancer has been largely ignored. Instead, research has been focused on correlating T$_{Reg}$ cell infiltration with prognosis and attempts to deplete T$_{Reg}$ cells with anti-CD25 antibody based therapies (29).

Suppression by T cells in solid tumors was first suggested by Fujimoto et al. (38), cumulating in work by North and colleagues, who showed that the ability to reject a 2nd subcutaneous injection of a fibrosarcoma cell line was inversely correlated to increased suppressor activity of CD3$^+$CD4$^+$ cells over time (39,40). These findings have since been expanded to show that depletion of CD25$^+$ cells, which are largely CD4$^+$FoxP3$^+$, reduces tumor growth of some tumor cell lines (29), as well as MCA-induced fibrosarcomas (41,42). Infiltration of T$_{Reg}$ cells into the tumor is observed in all of these models, while an increased percentage of T$_{Reg}$ cells in the periphery is observed only in some instances. In one study using a fibrosarcoma cell line, depletion of CD4$^+$ cells resulted in CD8$^+$ T cell-dependent tumor regression in 50% of the mice (36), that was increased to 100% following either CD4$^+$ or CD25$^+$ cell depletion when another fibrosarcoma-derived cell line expressing a strong antigen was used (36). Together with other studies, experimental systems such as these indicate that initiation of a CD8$^+$ T cell response is possible during tumor development, depending on the immunogenicity of the tumor antigens involved, but that local and/or systemic immune suppression by T$_{Reg}$ cells can limit their effectiveness. Unfortunately, these results have been limited in scope to transplantable tumor models representing few tumor types, and thus further investigation is required to determine whether T$_{Reg}$-dependent immune suppression is applicable to a wide range of spontaneous tumors, as might be expected.

Increased number of tumor-infiltrating FoxP3$^+$ cells is associated with poor prognosis in several cancers, including hepatocellular carcinoma (43), ovarian carcinoma (41), pancreatic ductal carcinoma (44), cervical cancer (45), non-small cell lung carcinoma (46), HNSCC (35), and breast cancer (47,48); with varying degrees of prognostic value regarding patient

outcome. As with some mouse models, increased frequency of T_{Reg} cells in peripheral blood of some cancer patients has been reported (49). Peripheral blood T_{Reg} cells from patients with ovarian cancer displayed equal suppressive capacity compared to tumor-derived T_{Reg} cells (41). This contrasts with the study of patients with HNSCC (35), highlighting the potential for differences in the immune response based on cancer type and/or etiology.

Attempts to translate T_{Reg} research into the clinic have focused around $CD25^+$ cell depletion using denileukin diftitox. Known commercially as Ontak, this compound composed of IL-2 fused to a portion of diphtheria toxin has been approved for treating $CD25^+$ cutaneous T-cell leukemia and lymphoma (29). Ontak administration has been demonstrated to reduce the numbers of peripheral T_{Reg} cells and improve T cell activation in a small number of patients with either lung, ovarian, breast, or renal cancer; either alone or in combination with DC-based vaccination (50,51). Other small studies have confirmed these findings, but as before, objective clinical responses were rare. Furthermore, a larger study with NSCLC patients observed no objective clinical responses, and almost half of the patients suffered side effects usually associated with IL-2 immunotherapy (52). Dosage optimization thus remains an issue, as does route of administration, as intratumoral injection of anti-CD25 antibodies demonstrating efficacy in mouse models (36). Determination of which cancer types are most suitable for T_{Reg} depletion is also required, as the ability of $CD25^+$ cell depletion to improve anti-tumor immunity is known to depend on the tumor type (53). Finally, combinatorial therapies, such as $CD25^+$ cell depletion with blockage of immunosuppressive molecules such as CTLA-4 and programmed death (PD)-1 (54), has great potential to overcome immune suppression within the tumor, although the induction of autoimmune diseases will likely remain an issue for patients.

T helper cells

Lineage commitment between $CD4^+$ and $CD8^+$ T lymphocytes occurs during development within the thymus. Further differentiation of the $CD4^+$ lineage, with the exception of natural T_{Reg} cell development, requires T cell activation through MHCII and co-stimulatory molecules, as well as cytokine dependent signaling that is responsible for directing the cell towards a particular lineage (Fig. 1). Classical differentiation of $CD4^+$ T cells into T_H1 and T_H2 cells, mediated by IL-12 and IL-4 respectively, was recently updated to a include a new T_H17 lineage (10,55). T_H17 cells are induced by a combination of IL-6 and TGF-β and mediate their effects through secretion of IL-17(A), IL-17F, IL-21 and IL-22 (56). Many of these cytokines increase the severity of autoimmune diseases in mouse models by promoting inflammation, but also appear to be functionally important in protecting against some extracellular pathogens, possibly by mediating leukocyte recruitment through the induction of chemokine expression (57,58).

T_H17 cells have been observed in patients with ovarian (59), prostate (60) and gastric cancer (61), and high numbers of IL-17 producing cells in hepatocellular carcinoma patients is an indicator of poor prognosis (62). In mice, transgenic expression of IL-17 by cell lines increases tumor growth by promoting angiogenesis (63,64), knockdown of the IL-17 receptor in 4T1 mammary carinoma cells reduced survival and tumor growth (65), and IL-17 depletion delays development of chemically-induced papillomas (66). This effect appears to be mediated by a pathway of IL-17 inducing IL-6 production by both neoplastic and stromal cells, that in turn leads to activation of Stat3 (67). Not only is Stat3 involved in upregulating genes that promote tumor growth and immune suppression (68,69), but it also mediates expression of IL-17 (70), potentially leading to a dangerous feedback loop (Fig. 2).

However, as with inflammation in general, IL-17-dependent inflammation may have both positive and negative effects on tumor growth, depending on the tumor model. MC38 colon cancer cell growth is enhanced in IL-17-deficient mice (71), while adoptive transfer of *in*

vitro polarized T_H17 cells specific for a B16 melanoma antigen can induce tumor regression (72). This effect appears to depend upon IFNγ, as blocking antibodies preventing T_H17 cell transfer from causing tumor regression (72), while reduced frequency of IFNγ producing cells were observed in IL-17-deficient tumors (71). Adding to the confusion is that both IL-21 and IL-22 activate Stat3, and IL-21 can promote Th17 differentiation (56). Meanwhile, CD8$^+$ T cells primed in *vitro* in the presence of IL-21 provide a more robust anti-tumor response upon adoptive transfer, and IL-21 therapy is currently in clinical trials for cancer treatment (73).

Not surprisingly, CD4$^+$ T cell-deficiency (and elimination of T_H1, T_H2, T_H17 and most T_{Reg} cell populations) has differential effects in different mouse tumor models (19,74–77). In general, T_H1 polarization is related to anti-tumor effects, while T_H2 polarization is thought to promote tumor formation (20,78,79). Direct targeting of T_H1 development and effector functions, through IL-12 and IFNγ respectively, clearly indicate a role for T_H1 in tumor rejection (80). Genetic deficiency in IFNγ or IFNγ receptor 1, or anti-IFNγ antibody treatment, increases MCA-induced sarcomas (81,82), with loss of IFNγ also shown to increase the rate of spontaneous lymphomas and lung adenocarcinomas (83). Similarly, IL-12 genetic deficiency increases the frequency of chemically-induced sarcomas (84) and papillomas (85), while exogenous IL-12 treatment has the opposite effect (86). Notably, IL-12 dependent rejection of a sarcoma cell was blocked by administration of neutralizing anti-IFNγ antibodies (87). IFNγ production is not limited to T_H1 cells however, and production by CD8$^+$ T cells, γδ T cells, NK cells, and NKT cells is also dependent upon IL-12 (88). In one study, loss of IFNγ expression by γδ T cells was found to account for the increase in MCA-induced carcinogenesis in IFNγ-deficient mice (89). Thus, at least in the skin where a higher percentage of γδ T cells are found, the importance of T_H1 cells may be minimal.

Perhaps the best evidence for a specific role for T_H1 polarization is in mice deficient for signal transducers and activators of transcription protein 6 (STAT6), which display increased T_H1 polarization due to the block in IL-4 signaling (16). These mice were able to reject tumors formed through injection of a mastocytoma cell line that grew permissively in normal mice (90) and were more resistant to growth of the 4T1 mammary carcinoma cell line (91). Cells from the lymph node of tumor bearing STAT6-deficient mice produced more IFNγ following secondary stimulation (90), while splenocytes from these mice displayed increased killing against the cell lines (90,91). STAT1-deficient mice meanwhile display increased T_H2 polarization due to the block in IFNγ signaling, and are more susceptible to tumor development (92). These results are consistent with the notion that T_H1 polarization increases IFNγ production, leading to more robust anti-tumor immunity through improved CTL responses. Importantly, direct effects of IFNγ on inhibiting proliferation, promoting apoptosis, and inhibiting angiogenesis have been observed, and loss of sensitivity to IFNγ reduces the immunogenicity of tumors (88). Intriguingly, IFNγ has been found to increase expression of MHCI on MCA-induced sarcomas, thereby improving CTL killing (93), suggesting an addition mechanism by which IFNγ production by T_H1 and other lymphocytes may assist anti-tumor immunity.

By virtue of reduced IFNγ production, T_H2 polarization is likely to be detrimental to the anti-tumor response. T_H2 polarization is dependent upon, and leads to, production of IL-4. This differs from T_H1 and T_H17 polarization, which are not induced by their respective cytokines, although these are involved in lineage stabilization (10,55). In addition to reducing T_H1 polarization, IL-4 may have direct immunosuppressive effects on CD8$^+$ T cells, as *in vitro* activation of naïve CD8$^+$ T cells in the presence of IL-4 reduces effectiveness of adoptive transfer of tumor-specific transgenic T cells (94,95). It should be noted however, that these cells, termed T_C2 (as opposed to T_C1 CD8$^+$ T cells activated in the presence of IL-12), were still able to improve survival from B16 melanoma cells in the lung when transferred in greater quantities (95). Subsequent work by the same group showed that IL-4 and IL-5 expression by

the adoptively transferred T_C2 cells was important in mediating this effect (96). Recombinant expression of IL-4 by several tumor lines also greatly improves clearance (97), and one study found that ovalbumin (OVA)-specific T_H2 polarized cells helped clear B16 melanoma lung colonies expressing OVA through recruitment of eosinophils (98). Is IL-4 production, and by extension T_H2 polarization, therefore protective? Probably not, since the increased anti-tumor response does not depend upon endogenous IL-4, but instead upon an effective T_H1 and CTL response by infiltrating leukocytes (96,99). By promoting inflammation and leukocyte recruitment, IL-4 production does appear to improve anti-tumor immunity. However, this protective effect is time-dependent, with later stage tumors more resistant to the adoptive transfer of IL-4 producing cells $CD8^+$ cells (95). Adoptive transfer of IL-4 producing T_H2 cells, or administration of IL-4 intravenously, also increases lung colonization of B16 melanoma cells injected intravenously (100). Thus, while IL-4 has the potential to improve anti-tumor immunity, its use may be limited therapeutically to inducing acute inflammation prior to the development of a chronically inflamed tumor microenvironment, with a T_H2 type response itself being detrimental for anti-tumor immunity.

Also produced by T_H2 cells, IL-13 affects the immune response in many of the same ways as IL-4 through activation of Stat6 (101). As with IL-4, IL-13 expression by tumors can improve the anti-tumor response (102), while tumor inhibiting endogenous IL-13 can prevent recurrence (103). T cells do not express the type II IL-4 receptor necessary for binding to IL-13 however, and IL-13 instead appears to inhibit the CTL response indirectly by increasing TGF-β production by myeloid cells in the tumor (104). Notably however, the source of this IL-13 did not appear to be T_H2 cells in this model, but instead NKT cells (103). Both IL-4 and IL-13 are considered to be the inducers of the M2 phenotype in monocytes/macrophages, reducing inflammatory cytokine expression and increasing expression of immune suppressive cytokines, resulting in indirect immunosuppression (7,105). We have recently reported that IL-4 also affects late-stage mammary cancer development by mediating the activity of tumor-associated macrophages (TAMs) (77). In the MMTV-PyMT mouse model of mammary carcinogenesis, lung metastasis was dramatically reduced by genetic deficiency of either CD4 or IL-4Rα (which would prevent IL-4 and IL-13 signaling). *In vitro*, IL-4 expression by T_H2 cells was found to increase expression of epidermal growth factor (EGF) by TAMs, enhancing the ability of neoplastic cells to extravasate into the circulation. IL-4 and IL-13 may also affect tumor growth independent of their effects on the immune system, as many cell types express the type II IL-4 receptor that binds both IL-4 and IL-13 (106). This has not been addressed by many studies, but IL-4 has been shown to reduce angiogenesis by inhibiting endothelial cell migration (107,108). IL-4 and IL-13 also inhibit proliferation of several epithelial cancers, although IL-13 can also promote proliferation and/or inhibit apoptosis of some hematological malignancies (101) as well as breast carcinomas in some models (109).

Natural Killer T cells

NKT cells play a key regulatory role in directing a T_H1 or T_H2 polarized immune response through the rapid production of IFNγ, TNFα, IL-4, and IL-13 following stimulation. This is observed in mice deficient for NKT cells, as infections in these mice, particularly bacterial or parasitic, are often more severe (110). As with conventional CTL and T_H cells, NKT cells develop in the thymus and express the α and β chains of the TCR. Instead of recognizing a peptide presented by MHC class I or II molecules however, the TCR expressed by NKT cells recognizes glycolipid antigens presented by CD1d, a non-classical member of the MHC family (14). Type I, or invariant, NKT cells (*i*NKT) express a specific alpha chain variable (V) and joining (J) region (Vα14-Jα18 in mice, Vα24-Jα18 in humans) in combination with a limited number of β chains, and were identified for their ability to recognize α-galactosylceramide (α-GalCer). Type II NKT cells, while also recognizing CD1d, express a variety of αβ TCR chains and are activated by glycolipids that remain poorly defined.

Interest in targeting NKT cells for anti-cancer therapy began with the discovery that treatment with α-GalCer increased the survival time of mice injected with B16 melanoma cells (111). The anti-tumor effects of α-GalCer (112) and IL-12 treatment (113) were subsequently found to depend upon the presence of *i*NKT cells. Jα18-deficient mice, that lack *i*NKT cells, were also found to be more susceptible to MCA-induced fibrosarcomas (84). Although capable of directly lysing tumor cells in a perforin-dependent manner, a series of studies by Godfrey and colleagues utilizing MCA-induced fibrosarcomas demonstrated that both NKT-dependent immune surveillance and protection provided by IL-12/α-GalCer therapy was dependent upon IFNγ production by NKT cells, leading to CTL-dependent and NK-dependent anti-tumor responses, respectively (84,114,115). It has yet to be determined if these same mechanisms are at play in solid tumors of epithelial origin or in models of de novo cancer development.

In addition to possible direct effects of IFNγ production by NKT cells on CTL and NK cells, α-GalCer treatment has been shown to act as an adjuvant through NKT-dependent DC activation and IL-12 production (116,117). Pulsing DCs *in vitro* with α-GalCer prior to adoptive transfer also more effectively prevents liver metastasis of B16 melanoma cells than injection of α-GalCer (118), possibly by improving long-term IFNγ production and limiting T_H2 cytokine expression (119,120). Unfortunately, despite these successes in murine tumor transplantation models, injection of α-GalCer, α-GalCer pulsed DCs, or transfer of α-GalCer activated NKT cells all proved ineffectual in early clinical trials in patients with a range of cancer types, even though NKT activation was evident (121).

Using transplantable sarcoma models in which immune surveillance was evident, work by Berzofsky and colleagues found increased resistance of CD1d-deficient mice to tumor development (122). This was due to the absence of IL-13 producing NKT cells in these mice (103), which were responsible for promoting TGFβ production by splenic $CD11b^+Gr-1^+$ immature myeloid cells in a model using NIH/3T3-derived cell lines (104). By comparing Jα18-deficient (lacking type I *i*NKT cells) to CD1d-deficient mice (lacking both type I and type II NKT cells), they concluded that type II NKT cells were responsible for inhibiting immune surveillance in several models where CD25-depletion had no effect (123). Importantly, in agreement with other studies, α-GalCer treatment increased protection in these models (124). Meanwhile, stimulation of at least a portion of type II NKT cells with a sulfatide compound reduced protection, and could even counteract the protection offered by α-GalCer treatment (124). The most parsimonious explanation for these observations is that IFNγ production by type I NKT cells improves the CTL and NK cell responses, while IL-13 production by type II NKT cells inhibits the immune response (Fig. 2). As T, NK, and NKT cells are unresponsive to IL-13, the results also suggest that the effects of NKT cells depend on an intermediate cell, such as DCs (125). It should be noted however that studies using other tumor injection models found that improved protection of CD1d-deficient mice was not related to IL-13 (126,127). Protection through adoptive transfer of NKT cells is also dependent on whether the NKT cells are $CD4^+$ or $CD4^-$, and upon the tissue used to isolate NKT cells, which relates at least partially to production of IL-4 (128). In humans, peripheral blood $CD4^-$ NKT cells expressed only IFNγ and TNFα, whereas $CD4^+$ NKT cells produced both T_H1 and T_H2 type cytokines following stimulation (129,130). A possible role for cytotoxic effector functions by NKT cells can also not be ruled out. Song and colleagues found that human NKT cells can directly kill monocytes pulsed with lysate from human neuroblastoma cell lines (131). Using immunocompromised mice, the authors found that human NKT cells reduced the number of monocytes in the tumors and inhibited growth of the xenograft, indicating that NKT cells also inhibit pro-tumor immunity by killing tumor-associated macrophages. Tissue localization and tumor type may therefore greatly affect cytokine expression by NKT cells and determine whether they promote or inhibit the anti-tumor response.

CONCLUSIONS

Although first described over 20 years ago (132), the complexity by which the immune system directs a T_H1 or T_H2 response is only now being appreciated. The concept of polarized populations of immune cells has now been expanded to include CD8$^+$ CTLs, macrophages, and NKT cells. Whether these populations can be defined by genetic programs remains to be determined, but the effects of this polarization on the anti-tumor response demonstrates the importance for further research. Inhibiting immune suppression by blocking the activity of regulatory immune cells, or blocking self-suppression of the CTL response by inhibitory molecules such as PD-1 and CTLA-4, holds great potential for improving anti-tumor responses. These approaches will likely be enhanced by therapies that also dampen the effect of pro-tumor immune molecules released by other leukocytes that enhance angiogenesis, tissue remodeling and cell survival pathways, and in combination, may increase clinical efficacy of adoptive transfer therapies to engender durable anti-tumor immunity. Early clinical results have shown success in some of these areas, although the ability to inhibit peripheral tolerance and improve the anti-tumor response appears directly related to the severity of autoimmunity that is also induced. Being able to release the full potential of the immune response, while also being able to appropriate direct and control that response, is key to the future of immunotherapy.

Acknowledgments

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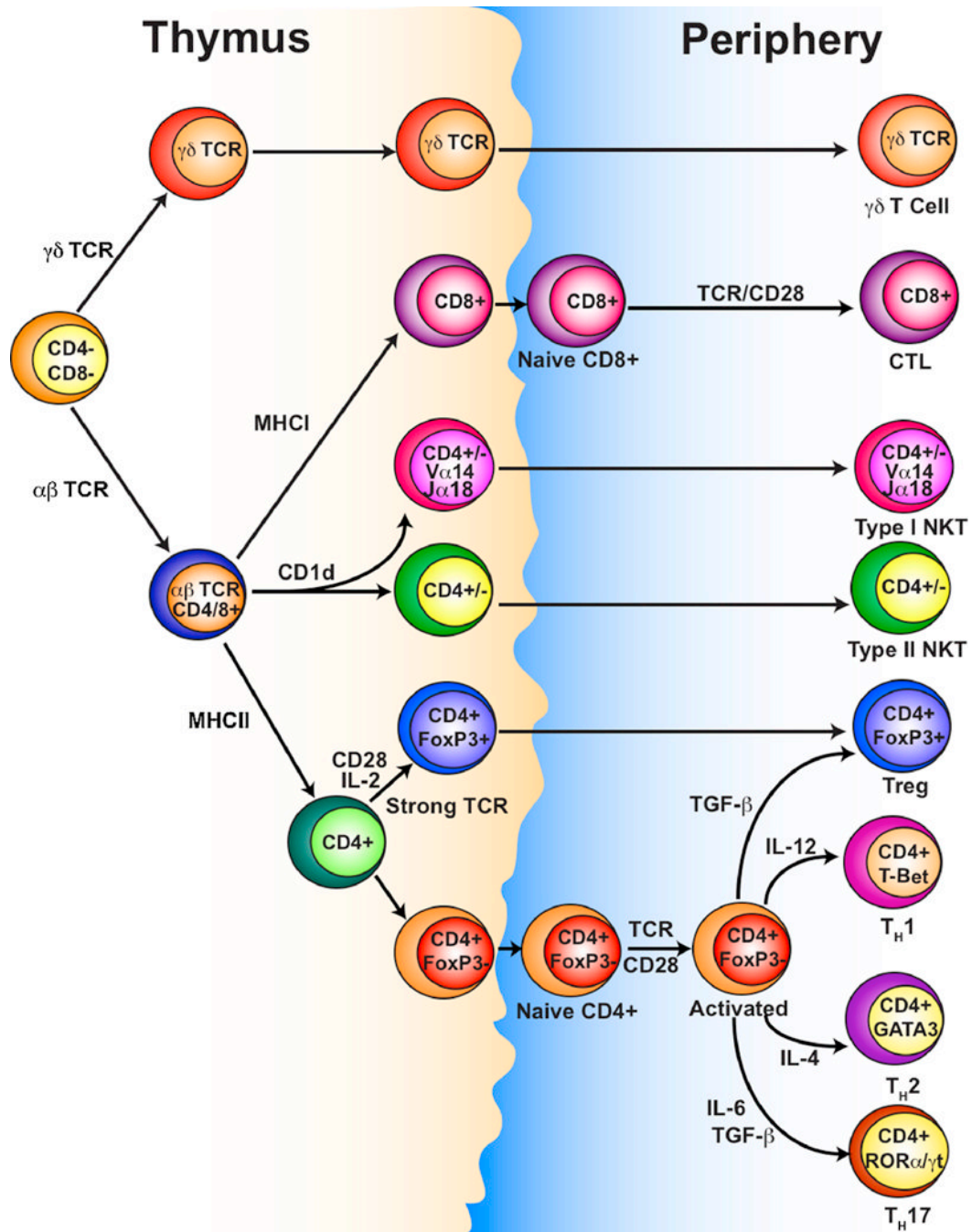
Figure 1. T cell lineages and subsets
Successful rearrangement and expression of a TCR determines lineage commitment between
γδ and αβ T cells. Recognition by αβ T cells of MHCI, MHCII, or CD1d drives CD8^{+}, CD4^{+}
or NKT cell development, respectively. Strong recognition of peptide:MHCII complexes by
CD4^{+} cells drives natural T_{Reg} cell development in the thymus, otherwise CD4^{+} T cells
differentiate into T_H1, T_H2, T_H17 or inducible T_{Reg} cells following activation in the periphery,
with polarization directed by IL-4, IL-6, IL-12 and TGF-β. Type I NKT cells are defined by
expression of specific α-chain regions (Vα14-Jα18 in mice, Vα24-Jα18 in humans), but the
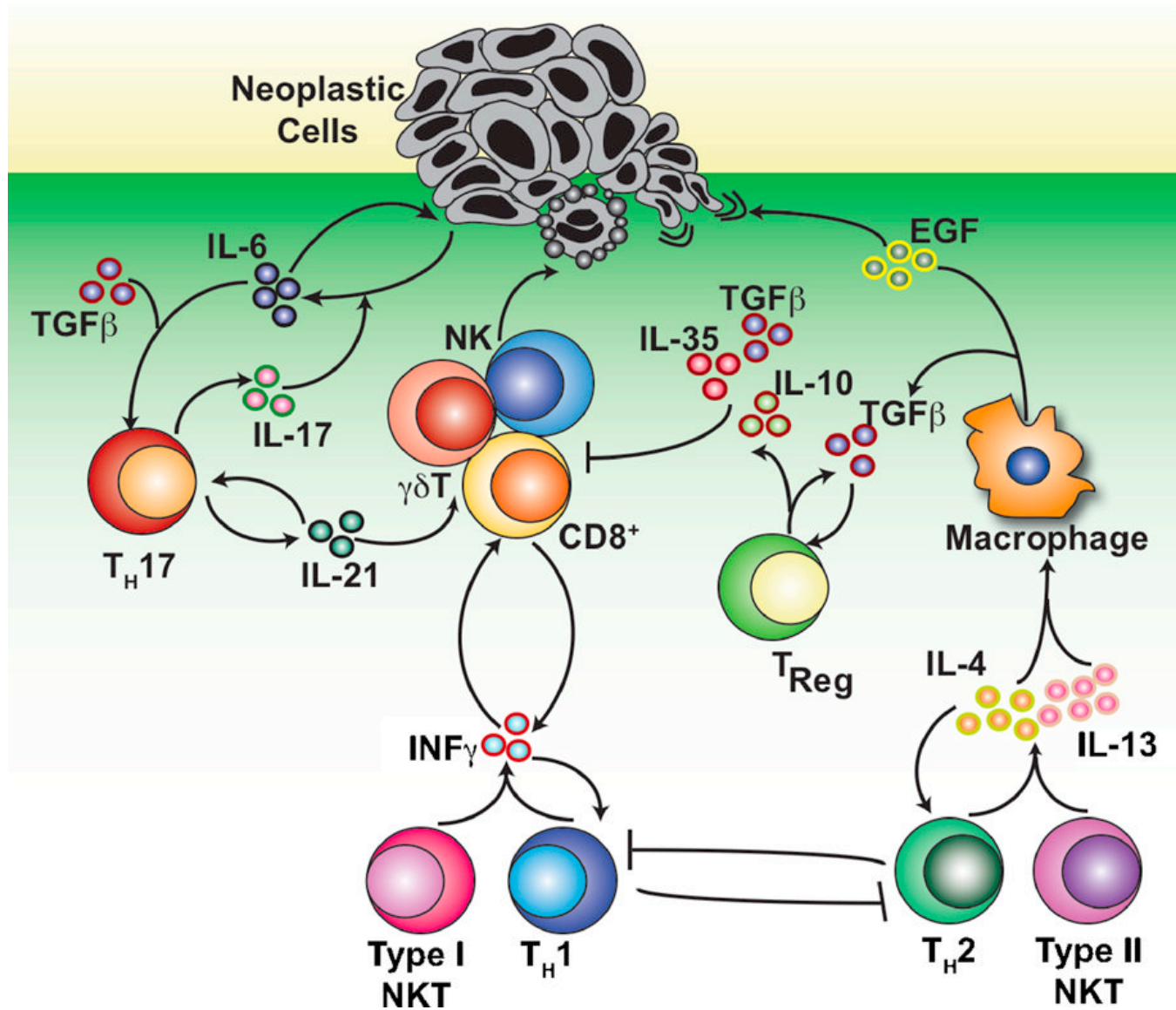reason for functional differences between type I and type II NKT cells is unclear.

Figure 2. T cell-derived cytokines regulate pro- and anti-tumor immunity
NK cells, γδ T cells, and CD8$^+$ CTLs mediate anti-tumor immunity by inducing cell death in neoplastic cells. The cytotoxic effector functions of these cells are supported by IFNγ released from T$_H$1 and type I NKT cells, as well as by self-production of IFNγ that further drives T$_H$1 polarization. T$_H$2 polarization opposes T$_H$1 polarization, and the release of IL-4 and IL-13 by both T$_H$2 and type II NKT cells can direct macrophages towards an M2 phenotype. Macrophages polarized by IL-4 promote metastasis through the release of EGF, while production of TGFβ suppresses the immune response directly, or indirectly through promotion of T$_{Reg}$ development. In the presence of IL-6, TGFβ can also promote T$_H$17 polarization. IL-17 induces the production of IL-6 by tumor cells, which both promotes tumor cell growth and further drives T$_H$17 polarization, while IL-21 has been shown to enhance CTL effector function. Multiple cell types and pathways have been omitted for clarity.