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REVIEW ARTICLE

Th2-mediated anti-tumour immunity: friend or foe?

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Abstract

The concept that the immune system can recognise tumour cells and either eliminate them (tumour immune surveillance) or select for immunologically resistant variants (immunoediting) is gaining general acceptance by immunologists. In terms of an adaptive immune response to cancer, however, much of the research has focused on the response of cytotoxic $CD8^+$ T lymphocytes to tumour-specific antigens and the production of Th1 cytokines by $CD4^+$ and $CD8^+$ T cells. In contrast, Th2-mediated immunity has traditionally been viewed as favouring tumour growth, both by promoting angiogenesis and by inhibiting cell-mediated immunity and subsequent tumour cell killing. While there is evidence that components of type 2 inflammation, such as B cells and interleukin-10, do promote tumour growth, there are also many studies demonstrating the anti-tumour activity of $CD4^+$ Th2 cells, particularly in collaboration with tumour-infiltrating granulocytes, such as eosinophils. In this review, we examine all the components of type 2 immunity and their effects on tumour growth. Collectively, from this analysis, we conclude that there is a great potential for the development of Th2-mediated immunotherapies that harness the cytotoxic activity of eosinophils.

Introduction

Tumour immune surveillance and immunoediting describe the processes by which the immune system has the inherent capacity to recognise and modify tumour development without external therapeutic intervention (1). By understanding and harnessing the anti-tumour immune response, cancer immunotherapy aims to stimulate the immune system to destroy tumours. Current evidence suggests that tumour immune surveillance/immunoediting involves a complex mixture of components from both the innate and the adaptive immune systems. However, to date, the majority of the research into anti-tumour immunity has focused on the dendritic cell (DC)-driven response of cytotoxic $CD8^+$ T lymphocytes (CTLs) to tumour-associated antigens (TAAs) presented by major histocompatibility complex (MHC) class I molecules on tumour cells. Many types of vaccines incorporating peptide antigens, proteins, DNA and whole tumour cells, as well as adoptive transfer of tumour-reactive CTLs, have been tried, although generally these approaches have had limited clinical success [reviewed in (2, 3)]. There are many problems associated with generating CTL-mediated anti-tumour immunity [reviewed in (2, 4)], a major one being that 'successful' tumours that emerge in patients have often been immunoselected to evade recognition by CTLs. Tumour cells may lose the ability to process and present antigen and therefore evade immune recognition, with loss of MHC class I expression (5), as well as antigen-processing molecules [reviewed in (6)] reported in both human tumour specimens and human and murine tumour cell lines. Tumour cells may also evade immune recognition through the loss of the target antigens (2). This has led to the search for alternative approaches to CTL-mediated cancer immunotherapy.

One approach has been to examine the potential of tumour-specific $CD4^+$ T cells to eliminate tumours. Unlike $CD8^+$ T cells, $CD4^+$ T cells can respond to MHC class I-negative tumours, which are resistant to CTL lysis. TAAs can be presented on MHC class II molecules to $CD4^+$ T cells by bystander antigen-presenting cells in and around the tumour, reducing the likelihood of immunoselection (4, 7, 8). In the past decade, it has become apparent that there is an important role for $CD4^+$ T cells in anti-tumour immunity.

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Injection of either a $CD4^+$ T-cell-activating cancer vaccine (8) or *in vitro* generated tumour-antigen-specific $CD4^+$ T cells (9, 10) has been shown to clear established tumours from mice. For example, Hung et al. (8) showed that a vaccine against B16 melanoma induced anti-tumour immunity, which was absent in mice lacking $CD4^+$ T cells. The $CD4^+$ T cells within the tumours produced both type 1 and type 2 cytokines, and tumour clearance was reduced in mice deficient in interferon (IFN)-γ, interleukin (IL)-4 and IL-5 (8). Similarly, Nishimura et al. (9) demonstrated that injection of ovalbumin (OVA)-specific $CD4^+$ T cells expressing either a Th1 or a Th2 cytokine secretion profile was able to completely clear established tumours of the B-cell lymphoma A20-OVA. Studies from our own laboratory have also demonstrated a critical role for antigen-specific $CD4^+$ T cells exhibiting a cytokine profile characteristic of Th2 cells in the clearance of established B16 melanoma metastasis (7). However, in contrast to the study by Nishimura et al., $CD4^+$ Th1 cells were ineffective in inducing tumour clearance (7). More recent studies have reiterated a role for $CD4^+$ T cells that induce either type 1 (11) or type 2 (12) inflammation or a mixture of both (13) in tumour clearance. Thus, tumour-antigen-specific $CD4^+$ T cells have the ability to effectively eradicate established tumours. Whether a type 1 or type 2 immune response is required appears to differ depending on the tumour type and the model system used.

Anti-tumour immunity initiated by Th1 cells appears to be dependent on the local production of IFN-γ, which induces the infiltration of macrophages (8, 9, 11) and/or $CD8^+$ CTL (9, 10) into the tumour. IFN-γ-activated macrophages have been demonstrated to inhibit the growth of MHC class II-negative myeloma (11) and B16 melanoma cells (8), most likely through the production of nitric oxide (8). Although this process was found to be CD8 independent, many Th1 anti-tumour responses appear to be dependent on IFN-γ-mediated activation of the CTL response, with tumour clearance ablated in mice depleted of $CD8^+$ T cells (9, 10). This process was also found to be dependent on expression of the adhesion molecule leukocyte function-associated antigen-1 (LFA-1) (9). LFA-1 is required for the cell–cell interactions involved in CTL-mediated cytotoxicity and migration of lymphocytes into inflammatory sites (14) and presumably tumour tissue (9). A major problem associated with CTL-based immunotherapy is that the infiltration of CTLs into large tumour masses and direct CTL–tumour cell interactions are difficult to achieve *in vivo* (4). This, together with the fact that Th1-activated CTL-mediated anti-tumour responses are still sensitive to loss of target TAAs or MHC class I expression, means that this type of therapy may not be a suitable alternative to approaches directly harnessing tumour-specific CTLs.

In contrast, an advantage of $CD4^+$ Th2 cells is that they can amplify the innate anti-tumour immune response by directly recruiting tumoricidal myeloid cells into the tumour to induce tumour clearance. Th2-mediated anti-tumour immunity appears to be dependent on the production of IL-4 (15–17) and recruitment of tumoricidal myeloid cells such as eosinophils (7–9, 15–19), often in collaboration with macrophages (15, 17–19). A more detailed discussion of the mechanisms of Th2-mediated anti-tumour immunity will be the focus of this review.

Key factors involved in Th2-mediated tumour immunity

There have been many reports suggesting that while a Th1-type anti-tumour immune response correlates with tumour regression, Th2-type cytokines downregulate anti-tumour immunity. This has largely been attributed to interference with anti-tumour CTL activity, particularly through IL-10-mediated inhibition of DC antigen processing and presentation and activation of immunosuppressive regulatory T cells. However, there is increasing evidence that particular components of the Th2-mediated immune response, such as IL-4 and eosinophils, can actually decrease tumour growth and initiate anti-tumour activity (Table 1, Figure 1). The key factors involved in Th2-mediated tumour immunity will be discussed below.

Th2 cytokines

IL-4 is the prototypical Th2 cytokine (20). Injection of IL-4 around the tumour-draining lymph node (15), directly into the tumour (17) or use of IL-4-secreting tumours (16, 17) has demonstrated a role for IL-4 in tumour clearance. IL-4 was demonstrated to induce an infiltration of eosinophils, macrophages and in some cases neutrophils and lymphocytes into tumours, which correlated with tumour clearance (15, 17, 18). Neutralisation of IL-4 using an anti-IL-4 monoclonal antibody resulted in an absence of infiltrating eosinophils and macrophages and concomitant loss of anti-tumour immunity (17). Tumour growth was also partially restored through neutralising IL-5 with an anti-IL-5 monoclonal antibody, which was accompanied by reduced eosinophil influx. In a similar study, depletion of granulocytes resulted in complete loss of anti-tumour immunity (18). Together, these studies suggest an important role for eosinophils in IL-4-induced tumour clearance.

There have been reports, however, that neutrophils, not eosinophils, induce IL-4-mediated tumour elimination because eosinophils were present in IL-5-secreting tumours, which were not rejected (21). However, studies within our laboratory investigating Th2-mediated clearance of B16 melanoma have shown that IL-5 was not required for eosinophil recruitment into the tumour or to induce tumour clearance (7), while the mere presence of eosinophils does not confer tumour immunity if the appropriate eosinophil-activating signals are not received (22). In contrast, B cells,

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Table 1 The key factors involved in Th2-mediated immunity and their effect on tumour growth

Factor	Effect on tumour growth	Key observations	References
IL-4	Negative	IL-4 induces tumour clearance by infiltrating granulocytes and is anti-angiogenic	(15–18)
IL-13	Negative	IL-13 induces granulocyte-mediated tumour clearance	(35, 36)
	Positive	NKT-cell-derived IL-13 inhibits CTL activation by macrophage secretion of TGF-β	(38)
IL-10	Negative	IL-10 promotes NK-cell-mediated tumour clearance and can inhibit angiogenesis	(53–55)
	Positive	IL-10 inhibits tumour cell lysis by CTLs	(44, 48–51)
Eosinophils	Negative	Eosinophils play an important role in IL-4-mediated tumour clearance in mice and can directly kill tumour cells *in vitro*	(7–9, 15, 17, 18, 63)
		High eosinophil counts in tumours are associated with a good prognosis	(56–61)
	Positive?	Eosinophils may promote tumour angiogenesis	(68–70)
aaMac	Negative	aaMac-derived arginase-1-mediated arginine depletion inhibits tumour growth	(77–80)
	Positive	Arginase-1 can inhibit CTL function and anti-tumour activity	(76)
Tc2 cells	Negative	IL-4-secreting Tc2 cells can induce tumour clearance	(84, 85, 87)
	Positive	Tc2 cells secreting high levels of IL-10 can inhibit tumour clearance	(88, 89)
B cells	Negative?	Tumour-specific antibodies may induce ADCC and tumour clearance	(99)
	Positive	B-cell-deficient mice have decreased tumour growth	(91–93)
		B cells inhibit CTL-mediated tumour clearance through IL-10 and formation of IC	(90, 97, 98)

aaMac, alternatively activated macrophages; ADCC, antibody-dependent cellular cytotoxicity; CTL, cytotoxic $CD8^+$ T lymphocytes; IC, immune complexes; IL, interleukin; NK, natural killer; NKT, natural killer T; TGF, transforming growth factor.

natural killer (NK) cells and mast cells do not appear to be required for IL-4-mediated tumour clearance (17). The presence of $CD8^+$ T cells has been reported as both unnecessary (17) and crucial (18) for IL-4-mediated tumour clearance and may depend on the individual tumour model being examined. However, $CD8^+$ T-cell participation is not related to CTL activity but may instead induce direct or indirect interactions with eosinophils (18). In this regard, the anti-tumour activity of type 2 $CD8^+$ T cells will be discussed in a subsequent section.

In contrast to type-1-mediated anti-tumour immunity that induces tumour cell apoptosis, type-2-mediated immunity appears to result in tumour necrosis (17). For example, IL-4 has been reported to possess anti-angiogenic properties (23), which could induce necrosis in solid tumours above a certain size. Similarly, IL-4 expression in glioma cells resulted in reduced vascularisation and tumour growth (24), possibly through the action of tumour-associated fibroblasts that have been demonstrated to inhibit angiogenesis when stimulated with IL-4 (25). Conversely, IL-4 has been reported to inhibit cell apoptosis, which could help promote tumour growth, with IL-4 treatment of prostate, breast and bladder cancers *in vitro* increasing expression of the anti-apoptotic genes cellular FLIP/FLAME-1 and Bcl-x_L, and protecting cells from CD95- and chemotherapy-induced apoptosis (26). Expression of these genes was also observed in tissue sections of IL-4 receptor (IL-4R) expressing tumours (26). However, this point remains controversial as *in vitro* treatment of renal cell carcinoma (RCC) with IL-4 and tumour necrosis factor (TNF)-α has been shown to increase RCC apoptosis (27).

To date, there have been several clinical trials of IL-4 therapy for the treatment of advanced RCC and metastatic melanoma (28–30), although in contrast to mouse and *in vitro* studies, the results have been disappointing with little anti-tumour activity reported. However, this may be because of the dose schedule or, more likely, the route of administration employed. In contrast to mouse studies in which IL-4 was delivered directly to the tumour site or draining lymph nodes, in clinical trials IL-4 administration has been systemic or localised to a site distant from the actual tumour. Thus, the lack of anti-tumour immunity observed may suggest that IL-4 localised to the tumour microenvironment is important and necessary to induce effective anti-tumour immunity. Similar to mouse studies, IL-4 therapy in human cancer patients has been shown to induce blood eosinophilia and increase eosinophil degranulation, following analysis of eosinophilic major basic protein (MBP) in serum and urine (31). However, the effect of eosinophils at the tumour site was not assessed.

While IL-4 has been demonstrated to be the most important cytokine involved in inducing type 2 inflammation (32), IL-13 is another cytokine upregulated during Th2-mediated immune responses. IL-4 and IL-13 can both bind

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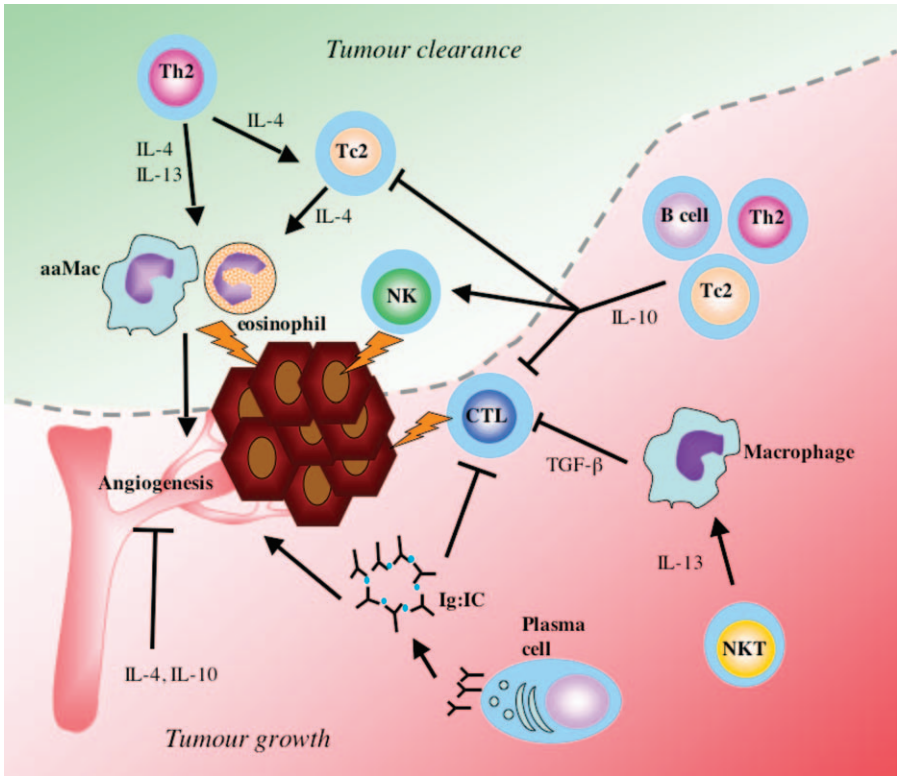


Figure 1 Balancing the good and the bad in Th2-mediated inflammation within tumours. The model shows how different components of Th2-mediated immunity can either enhance tumour clearance (green zone) or promote tumour growth (red zone). Secretion of interleukin (IL)-4 or IL-13 by $CD4^+$ Th2 cells can induce an influx of eosinophils and alternatively activated macrophages (aaMac) into the tumour and promote anti-tumour cytotoxic activity. Similarly, IL-4-secreting $CD8^+$ Tc2 cells may collaborate with eosinophils and macrophages to induce tumour clearance. Conversely, Th2 and Tc2 cells, along with B cells, may produce IL-10, which inhibits $CD8^+$ T lymphocyte (CTL)-mediated anti-tumour activity by interfering with antigen processing and presentation. CTL activity is also inhibited by transforming growth factor (TGF)-β secretion by $CD11b^+$ macrophages following activation with the Th2 cytokine, IL-13, derived from natural killer T (NKT) cells. However, IL-10 can also activate natural killer (NK) cell anti-tumour activity against CTL-resistant tumours, promoting tumour clearance. Plasma cells promote tumour growth by the secretion of anti-tumour antibodies that form immune complexes (Ig:IC) that interfere with CTL-mediated tumour cell lysis and together with tissue remodelling leucocytes, such as eosinophils and macrophages, promote tumour angiogenesis.

to the same receptor, the type II IL-4R, which is composed of the IL-4Rα chain and the IL-13Rα1 chain and consequently have very similar biological activity (33, 34). Similar to IL-4, overexpression of IL-13 has been shown to induce an anti-tumour immune response mediated by infiltrating myeloid cells. While IL-4 anti-tumour activity is predominately mediated through eosinophils and macrophages, IL-13 anti-tumour activity was reported to be mediated by neutrophils and macrophages (35, 36). Thus, regression of an IL-13-secreting tumour was observed to correlate with a significantly increased influx of $Gr\text{-}1^+$ and $MAC\text{-}3^+$ leucocytes, deemed neutrophils and macrophages respectively (36). However, as eosinophils also express Gr-1 and are associated with IL-13-mediated inflammation (37), they cannot be excluded as the effector cells in IL-13-mediated tumour clearance, similar to that observed for IL-4.

Conversely, IL-13 has also been reported to inhibit anti-tumour immunity through the suppression of IFN-γ secretion and CTL activity (38). In this model using a murine fibrosarcoma, IL-4 did not suppress anti-tumour activity, suggesting that the immune suppression is unique to IL-13. Although closely related, it has recently become evident that IL-4 and IL-13 can also have independent effects on Th2-mediated inflammation in the context of asthma, ulcerative colitis and responses to nematode parasites (34). In addition to the type II IL-4R, IL-13 can also signal through a receptor called IL-13Rα2 (39, 40), which exclusively binds IL-13, enabling IL-13 to have distinct functions. Thus, the fact that IL-13, but not IL-4, was able to inhibit anti-tumour immunity suggested that this may be mediated by IL-13Rα2. Indeed, inhibition of IL-13 activity with a soluble IL-13Rα2-Fc restored anti-tumour immunity (38). IL-13 was produced by tumour-infiltrating natural killer T (NKT) cells that activated $CD11b^+Gr1^+$ myeloid cells to produce transforming growth factor (TGF)-β that downregulated tumour-specific CTL activity. The anti-tumour activity of NKT-cell-derived IL-13 is supported by the observation that in a lung metastasis model of colon cancer, tumour

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growth was partially inhibited in NKT-cell-deficient (CD1d knockout) or IL-13-depleted mice, but not in IL-4-deficient mice, compared with wild-type controls (41).

Interestingly, increased expression of IL-13Rα2 has been observed on many types of tumours including lymphoma, RCC, glioblastoma, head and neck cancer, hepatoma, pancreatic cancer, breast cancer, prostate cancer and colon cancer (34). However, with the exception of lymphoma, breast cancer and pancreatic cancer, the impact of IL-13 on tumour growth remains to be elucidated. Furthermore, it appears that the effect of IL-13 may be different for different tumour types. While in Hodgkin's lymphoma, IL-13 appears to be used as an autocrine growth factor by Reed–Sternberg cells (34, 42), overexpression of IL-13Rα2 in breast and pancreatic cancer appears to promote tumour regression (43). Thus, when human breast (MDA-MB-231) and pancreatic (PANC-1) cancer cell lines were stably transfected with IL-13Rα2, tumour cell growth was inhibited *in vivo*, but not *in vitro* (43). The transfected cancer cells appeared to produce higher levels of IL-8, which induced an influx of neutrophils and macrophages and was associated with tumour regression. While the infiltrating neutrophils were observed to secrete IL-13, it was not clear whether this was the mechanism of anti-tumour cytotoxicity.

While IL-10 was originally classified as a type 2 cytokine because of its inhibitory effects on IFN-γ and IL-12 production by Th1 $CD4^+$ T cells and its ability to stimulate B cells (44), it is now clear that it can regulate both type 1 and type 2 cytokine responses (44, 45) with $CD4^+$ Th1 cells recently shown to be an important source of IL-10 in immune responses to protozoan and *Leishmania major* infections (46, 47). However, as many of the reports suggesting that Th2-type cytokines downregulate anti-tumour immunity have attributed this effect to IL-10 immunosuppression, we have included it in this review. In the context of tumour immune surveillance, IL-10 has been considered to promote tumour evasion. Treatment of cancer cell lines with recombinant IL-10 *in vitro* decreases MHC class I expression and sensitivity to tumour-specific CTLs (48). Furthermore, in mice over-expressing IL-10, it was found that the IL-10 inhibited DC antigen processing and presentation, resulting in increased tumour growth (49, 50). IL-10 may also activate immuno-suppressive regulatory T cells that may suppress anti-tumour CTL activity *in vivo* (51). Inhibition of IL-10 through anti-IL-10 or anti-IL-10 receptor antibodies, as well as anti-sense oligonucleotides, has been demonstrated to improve or restore anti-tumour immunity in several animal studies (44). In clinical studies, tumour biopsies from patients with cancers such as melanoma, RCC, ovarian cancer and several types of adenocarcinoma have demonstrated an increase in IL-10 compared with healthy tissue controls (51), suggesting a role for IL-10 in the evasion of the anti-tumour immune response. Furthermore, the demonstration of a higher expression of IL-10 in metastatic than in primary tumours has led to the proposal that IL-10 increases tumour metastasis (52).

In contrast, there is mounting evidence that IL-10 is not always immunosuppressive and does not always promote tumour immune escape (44). Tumours that secrete IL-10, while showing decreased CTL sensitivity as a result of loss of MHC class I expression, have increased susceptibility to NK-cell-mediated lysis *in vitro* and *in vivo* (53, 54). Additionally, IL-10 has been reported to possess anti-angiogenic properties (55), which may help inhibit tumour growth. Thus, IL-10 appears to have complex pleiotropic effects on anti-tumour immunity being able to either enhance or decrease tumour immunogenicity.

Eosinophils

Eosinophils have been observed in tumour infiltrates in many clinical and animal studies. Whether these cells actively contribute to anti-tumour immunity or are mere bystanders has been a source of debate. However, their role as a bystander is now being questioned following studies showing a direct correlation with eosinophil infiltration and positive prognosis, suggesting that eosinophils may play an active role in tumour clearance. For example, in patients with oesophageal squamous cell carcinoma, gastric cancer, head and neck cancer and colorectal carcinoma, a high number of infiltrating eosinophils has been associated with increased survival (56–60) and in the case of colorectal cancer, decreased metastasis (61).

A role for eosinophils in anti-tumour immunity is supported by several mouse studies examining the mechanisms of Th2-mediated tumour clearance. The anti-tumour activity of IL-4 originally reported by Tepper et al. (62) was found to be dependent on infiltrating eosinophils and macrophages (17, 62). This observation was confirmed by several other studies that investigated IL-4-dependent anti-tumour activity in other types of mouse tumours (15, 16, 18). Studies examining the anti-tumour effect of antigen-specific $CD4^+$ T cells also identified a role for eosinophils in tumour regression. Nishimura et al. (9) demonstrated that Th2-mediated induction of tumour necrosis was accompanied by a prominent infiltration of eosinophils, while Hung et al. (8) suggested that Th2 cells within a tumour produced cytokines that recruited and activated eosinophils, which were important in tumour destruction. Furthermore, in IL-$5^{-/-}$ mice that display decreased numbers of circulating eosinophils, a lack of eosinophils at the tumour site was shown to correlate with a loss of anti-tumour immunity (8).

Studies from our laboratory have demonstrated that tumour-specific $CD4^+$ T cells, exhibiting a cytokine secretion profile characteristic of Th2 cells, are capable of clearing established lung and visceral metastases of the B16-F1 CTL-resistant melanoma. Clearance of B16 metastases by the Th2 cells was found to be completely dependent on

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the eosinophil chemokine, eotaxin, with degranulating eosinophils within the tumours being associated with tumour regression (7). Tumour clearance was also partially dependent on the transcription activator signal transducer and activator of transcription 6 that is important in IL-4 and IL-13 signalling through the type II IL-4R (7), suggesting that these Th2 cytokines play a supportive role in eosinophil-mediated tumour clearance. Additionally, recently published studies using the methylcholanthrene (MCA)-induced fibrosarcoma model confirm an important role for eosinophils in tumour clearance (63). MCA-induced tumour incidence was examined in genetically modified mouse strains that had either an enhanced or a reduced level of circulating eosinophils. Tumour incidence and growth was significantly attenuated in IL-5 transgenic mice that had a high level of eosinophilia within and surrounding tumours compared with wild-type controls. Conversely, tumour incidence was greatly increased in mouse strains that had either a reduced or a complete absence of tumour-infiltrating eosinophils (63). Subsequent *in vitro* studies demonstrated that eosinophils can directly kill MCA-induced fibrosarcoma cells (63). Human eosinophils have also been demonstrated to bind and destroy the breast cancer cell line MCF-7 (64) and inhibit the growth of DU-145 and PC-3 prostate cancer cells (65) *in vitro*. However, the mechanisms by which eosinophils mediate tumour destruction remain to be elucidated.

Although Th2-mediated clearance of B16 lung tumours was found to be totally dependent on eotaxin, studies using a B16-eotaxin-expressing melanoma, while inducing eosinophil recruitment into the tumour, were insufficient to induce eosinophil degranulation and tumour clearance [unpublished data, (22)]. Similar results have been obtained for several IL-5-secreting tumours (21). This suggests that additional signals are required to induce eosinophil cytotoxic activity. It is well established that eosinophils contain cytotoxic granule proteins, such as MBP, eosinophil peroxidase, eosinophil cationic protein and eosinophil-derived neurotoxin, that are capable of inducing tissue damage and dysfunction (66), and these mediators could be harnessed to directly kill tumour cells. Given the high toxicity of these granule products, and the potential damage induced, it is not surprising that very specific activating factors may be needed to induce degranulation and killing of tumour cells. Alternatively, tumour-specific antibodies could earmark tumour cells for killing by eosinophils by antibody-dependent cellular cytotoxicity as has been demonstrated for eosinophil-mediated elimination of helminth parasites (67). It is also possible that eosinophils act in concert with other myeloid cells to induce tumour eradication. For example, in many of the studies highlighting a role for eosinophils in tumour clearance, an influx of macrophages was also noted (7, 8, 15, 17, 18, 62). A potential role for macrophages in Th2-mediated tumour clearance will be discussed in more detail in the following section.

In contrast, it has been proposed that eosinophils may promote tumour growth by inducing angiogenesis. In this regard, eosinophils have been implicated in tissue remodelling in several Th2-mediated pathologies, such as asthma, as well as in chronic inflammation associated with parasitic infections (68). Several factors that are known to promote angiogenesis, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), TNF-α, granulocyte–macrophage colony-stimulating factor and IL-8, are synthesised by eosinophils and stored in their granules (68). Eosinophils within the airways of human asthmatic patients have also been shown to express VEGF and FGF-2 (69). Furthermore, eosinophils can induce endothelial cell proliferation *in vitro* and induce the formation of new blood vessels (70). Nevertheless, a direct role for eosinophils in promoting tumour angiogenesis remains to be elucidated. In fact, in one study, no correlation was found between macrophage, eosinophil and mast cell content in non-small-cell lung carcinoma biopsies and tumour angiogenesis, as measured by vascular density and microvessel number (71).

Alternatively activated macrophages

Macrophages are heterogeneous and differentiate into at least three to four subtypes in response to specific tissue- and immune-related stimuli (72). In response to the type 2 cytokines IL-4 and IL-13, macrophages differentiate into the alternatively activated phenotype (72). Alternatively activated macrophages (aaMacs) are characterised by the production of arginase-1 and reduced production and release of nitric oxide through arginase-1 suppression of nitric oxide synthase (NOS)-2 (72). aaMacs and arginase-1 have recently been shown to play important roles in Th2-mediated immunity. Infection of mice with the gastrointestinal parasite *Heligmosomoides polygyrus* is known to trigger a highly polarised Th2 response, which was recently demonstrated to involve activation of aaMacs by IL-4-producing $CD4^+$ T cells, with subsequent clearance of *H. polygyrus* by the aaMacs dependent on production of arginase-1 (73). Production of arginase-1 by aaMacs has also been linked to Th2-mediated pathologies such as asthma (74).

Currently, macrophages infiltrating tumours, known as tumour-associated macrophages (TAMs), are considered as having a negative effect on anti-tumour immunity and are equated with poor prognosis. The ability of TAMs to increase tumour growth and promote angiogenesis as well as to decrease anti-tumour T-cell responses is well established and reviewed extensively elsewhere (75). However, macrophages have also been demonstrated to directly kill tumour cells *in vitro* and *in vivo* (8, 11). Thus, the role of macrophages remains controversial and may differ

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depending on the tumour microenvironment. Interestingly, although production of arginase-1 by TAMs has been reported to inhibit anti-tumour CTL activity, by inhibiting cell proliferation and re-expression of CD3 (76), there are also many reports detailing the sensitivity of tumour cells to arginine depletion *in vitro* and *in vivo* (77–79). Furthermore, the arginine depletion enzyme, pegylated arginine deaminase, is now being developed as a potential anticancer agent and has entered phase II and phase III clinical trials (80). Recently, our laboratory has observed that IL-4-activated macrophages possess cytotoxic activity against B16 melanoma cells *in vitro* through a mechanism that appears to be dependent on production of arginase-1 (unpublished data). However, whether macrophages, through the production of arginase-1, also play a role in Th2-mediated tumour clearance *in vivo* remains to be fully elucidated. Interestingly, IL-4 or IL-13 stimulation of DCs, fibroblasts and epithelial cells induces the expression of genes associated with the differentiation of macrophages into the alternatively activated phenotype (81). Thus, it is possible that these cells are also providing important signals and factors in the effector phase of Th2-mediated tumour clearance and should be further investigated.

Type 2 CD8+ T cells

Similar to $CD4^+$ T cells, $CD8^+$ T cells can be classified into two distinct types of effector cells based on their cytokine secretion profile following antigen encounter (82, 83). Type 1 CD8+ T cells (Tc1) secrete IFN-γ, IL-2 and TNF-α, and type 2 CD8+ T cells (Tc2) predominately produce IL-4, IL-5 and IL-10. The role of $CD8^+$ CTL in tumour clearance is well established and considered to be largely dependent on production of IFN-γ and perforin-mediated or Fas-mediated cytotoxic activity (84). While the anti-tumour activity of Tc1 cells is outside the scope of this review, of interest are a number of recent studies that examine the role of Tc2 cells in anti-tumour immunity. In mice, the adoptive transfer of antigen-specific Tc2 cells resulted in the eradication of established B16 melanoma and EG7 thymoma tumours and increased mouse survival compared with untreated controls (84, 85). However, in comparison with Tc1 cells, Tc2 cells were reported to be less effective in controlling tumour growth, requiring 5- to 25-fold more Tc2 cells to establish the same degree of tumour elimination as seen with Tc1 cells. While Tc1-mediated tumour clearance was found to be significantly impaired in IFN-γ-deficient mice, this was not the case for mice given Tc2 therapy, leading the authors to propose that the Tc1 and Tc2 cells operate by different mechanisms. It was suggested that Tc2 cells may facilitate tumour rejection through their interaction with other tumour-infiltrating leucocytes such as macrophages, NK cells and eosinophils (84), although neither the presence of nor the requirement for these leucocytes was examined (84). However, this proposition is consistent with several studies discussed earlier in this review that report a role for $CD8^+$ T cells in Th2-mediated or IL-4-mediated tumour clearance where infiltrating eosinophils and macrophages were also deemed important (9, 10, 16, 18, 86). Furthermore, in a supporting study, adoptive transfer of IL-4-producing $CD8^+$ T cells cleared established lung metastasis by a process dependent on $CD8^+$ T-cell-derived IL-4 and host NK cells and partially dependent on granulocytes (87).

In contrast, two other recent studies have reported a complete lack of anti-tumour activity by Tc2 cells. First, Kemp and Ronchese (88) observed that while adoptive transfer of antigen-specific Tc1 cells completely inhibited tumour establishment in mice subsequently challenged with a Lewis lung carcinoma cell line, protection was not afforded by transfer of Tc2 cells. Second, incorporation of IL-4 into the immunogenic HLA-CW3-expressing P815 (P.CW3) mastocytoma was reported to inhibit anti-tumour immunity (89) by changing the responding $CD8^+$ T cells from the Tc1 to Tc2 phenotype. A possible explanation for the discrepancy between these studies and earlier reports could be the level of IL-10 secretion by the Tc2 cells in the different model systems. In both cases, a lack of Tc2 anti-tumour activity was accompanied by high levels of IL-10 production that was not reported for Tc2 cells able to induce tumour clearance. The immunosuppressive effects of IL-10 on anti-tumour immunity are well documented (see previous section) and may counteract the anti-tumour potential of Tc2 cells.

B cells

An important function of $CD4^+$ T cells with a Th2 cytokine phenotype is to provide 'help' for antigen-specific B cells and facilitate immunoglobulin (Ig)G1 and IgE antibody responses. Some studies have suggested that such responses favour tumour growth. In fact, increased humoral immunity has been reported to be a factor in several chronic inflammatory diseases, such as ulcerative colitis, asbestosis, hepatitis C virus (HPC)-related cirrhosis and chronic obstructive airway disease, which are also associated with increased risk of developing cancer (90). Furthermore, several autoimmune diseases resulting from B-cell hyperactivity are associated with cancer (90). Together, these studies suggest a negative role for B cells in anti-tumour immunity. Indeed, mice lacking B cells have been reported to have a higher resistance to the EL4 thymoma, a colon carcinoma and the B16 melanoma compared with wild-type controls (91, 92). B-cell-deficient mice also displayed a better response to immunisation with melanoma vaccines and subsequent tumour challenge than wild-type mice, suggesting that B cells may inhibit anti-tumour immunity (93). Furthermore, in patients with advanced colon carcinoma,

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partial depletion of B cells using rituximab, a chimeric monoclonal antibody specific for human CD20, resulted in a reduction in tumour burden in 50% of patients (91).

The mechanism(s) of B-cell inhibition of anti-tumour immunity are most likely to be multifaceted (90). First, B cells may produce cytokines such as IL-10, which may inhibit the anti-tumour immune responses as discussed previously, and IL-6, which has been reported to be a growth and differentiation factor for several types of tumour cells (94, 95). However, secretion of these cytokines is not restricted to a type-2-mediated immune response (44, 95). Second, experiments in B-cell-deficient mice have suggested that B cells may promote tumour growth by inhibiting IFN-γ secretion and CTL and NK cell activity (92, 93). Last, the secretion of anti-tumour antibodies and the formation of immune complexes (IC) is associated with an increased tumour burden and a poor prognosis. Tumour-specific antibodies may bind to tumour cells and mask T-cell epitopes, inhibiting a T-cell response, while IgG has also been reported to form a complex with TGF-β that can inhibit anti-tumour responses by CTLs (96). Furthermore, deposition of IC is known to recruit myeloid cells that can initiate pro-angiogenic and tissue remodelling pathways (90, 97). Consistent with this, formation of IC consisting of anti-tumour antibodies and tumour antigens has been shown to increase the infiltration of tissue remodelling leucocytes into tumours and enhance tumour formation, growth and metastasis in mice (97, 98). Consequently, the elimination of B cells and tumour-specific IC deposition resulted in diminished tissue remodelling activity and angiogenesis, as well as a reduction in tumour size and incidence (98).

Conclusions

Th2-mediated immunity has traditionally been considered to promote tumour growth, both by enhancing angiogenesis and by inhibiting cell-mediated immunity and subsequent killing of tumour cells. However, from the information presented in this review, it is clear that while elements of the type 2 immune response, such as humoral immunity and possibly IL-10, have been demonstrated to have a negative impact on anti-tumour immunity, other components of the Th2-mediated immune response have a demonstrated ability to clear established tumours (Table 1, Figure 1). Of particular interest are myeloid cells, such as eosinophils, which if appropriately activated appear to be able to efficiently destroy tumour cells *in vitro* and *in vivo*. Elucidation of the mechanisms of eosinophil-mediated anti-tumour activity holds promise for the development of anticancer immunotherapies in the future. Similarly, a better understanding of the role of IL-10 and it ability to both suppress and stimulate anti-tumour immunity may help improve the efficacy of future immunotherapies.

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