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The Immune Response in Paediatric Cancer

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Abstract: Recent years have seen something of a resurgence of interest in the role of the immune system in the initiation, development, control and treatment of human cancers. Growing evidence indicates a complex set of interactions between developing tumours and the host immune system. In this review, we examine the major types of tumour-infiltrating immune cells and discuss their significance in terms of both pro- and anti-tumour effects. In the second part of the review, we consider a number of the potential immune escape mechanisms which may be employed by tumours, including intrinsic signalling pathways such as STAT3 and NF-κB; cytokines such as TGF-β; metabolic pathways including indoleamine 2,3-dioxygenase (IDO) and loss of expression or shedding of immune mediators such as MHC class I and NKG2D. Although much of the literature in this field relates to adult malignancies, we focus in this review on paediatric cancers and discuss potential differences in the role of the immune system in these malignancies, compared to adult-type cancers.

Keywords: Cancer, angiogenesis, intrinsic signalling.

INTRODUCTION

Recent years have seen a renewed interest in the role of the immune system in the initiation, development, control and treatment of cancers. It is increasingly recognised that tumour development and progression relies not just on factors intrinsic to malignant cells, but also on their interactions with the local microenvironment to promote angiogenesis and facilitate invasion and metastatic spread [1]. There is growing evidence that interactions between cancer cells and components of the immune system are also of crucial importance, and that immune escape might reasonably be considered a seventh 'hallmark of cancer'[2]. The concept of immunoediting has developed from the previous theory of immunosurveillance, and explains the development of malignancy as a balance between cancer cells and the immune response [3]. In an initial *elimination* phase, the innate and adaptive immune responses are able to eradicate cancers before they become clinically overt. Subsequently, through selection pressure, cancer cells acquire immune resistance and in the *equilibrium* phase, the immune system, whilst still able to control the growth of overt tumours, is no longer able to eradicate them. With continued selection, cancer cells are ultimately able to *escape* the control of the immune system entirely and develop into overt tumours which then progress and metastasise beyond the control of the host immune system [4]. Experimental data have confirmed the existence of all three stages – elimination, equilibrium and escape – in a mouse model of chemical-induced carcinogenesis [5] and various lines of evidence support this model in human cancers [4]. A complex set of interactions between cancer cells and the immune system determine this balance between eradication and escape; these factors are explored further in this review.

Whilst, in the model outlined above, the immune system is cast primarily in a tumour-suppressor role, eradicating nascent tumours and limiting their growth and spread, there is also growing evidence that aspects of the immune response may themselves promote the development and subsequent growth of cancers. Chronic inflammation plays an important role in the development of many types of adult cancer, particularly carcinomas arising from inflamed epithelium [6-8]. The initiating cause may be infectious (e.g. *H. pylori* in stomach cancer or human papilloma virus (HPV) in cervical carcinoma), or non-infectious (e.g. asbestos exposure leading to mesothelioma or reflux oesophagitis leading to Barrett's oesophagus and ultimately to oesophageal adenocarcinoma), but in either case, proneoplastic inflammatory mediators (including cytokines, chemokines, prostaglandins and reactive oxygen species) are crucial in driving malignant progression. In addition to this 'extrinsic pathway' in which inflammation or infection is instrumental in driving the malignant process, the immune system and developing tumour may also be linked *via* an 'intrinsic pathway' in which oncogene activation within the nascent tumour leads not just to cancer cell proliferation, but also influences the local immune system [7].

The extent to which such processes are relevant to the development of paediatric cancers is at present less clear, since on the whole the aetiology of these diseases is less well understood. Paediatric cancers arise infrequently from epithelial tissues and their presence in young children, who have not had the same degree of cumulative tissue damage as adults, suggests different causative mechanisms. It is assumed that most paediatric cancers arise as a result of a series of inherited and/or acquired genetic events during rapid cellular proliferation during embryogenesis. Neverthe-

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less, with a few exceptions such as the Rb gene in retinoblastoma, specific molecular pathways, and indeed the relevant embryological cell of origin, have not in most cases been established. In terms of infectious causes, although there has been considerable interest in the possibility of a viral cause for the most common childhood malignancy, acute lymphoblastic leukaemia [9], studies searching for evidence of viral infection in Guthrie card blood samples of subsequently affected individuals have failed to prove a correlation [10-12]. Other than the well-established association of EBV infection with the development of Burkitt's lymphoma, Hodgkin's disease and post-transplantation lymphoproliferative disorder (PTLD), there is no convincing evidence for a role of infection or chronic inflammation in the aetiology of childhood cancers. However, whilst it appears likely that the 'extrinsic pathway' does not play a significant role in the development of paediatric cancers, there is evidence that 'intrinsic' signalling pathways within these tumours (such as PAX3-FKHR and STAT3 in alveolar RMS) can lead to changes in the immune environment.

Exploring the immune response to cancer is therefore a complex undertaking. For example, in interpreting the presence of tumour-infiltrating immune cells, whilst these cells may appear as part of the immune response to malignancy, their possible role in the original inflammatory origins of the tumour must also be considered. Similarly, the presence of immune effector cells does not necessarily imply an active anti-tumour immune response. The likely substantial differences in the aetiology of childhood cancers and 'adult-type' carcinomas must also be carefully considered. Differences in the roles of the immune system in the *initiation* of malignant transformation may impact on the interpretation of apparent immune *response* to a particular tumour entity.

TUMOUR-INFILTRATING LEUCOCYTES

The presence of leucocytes within solid tumours has long been recognised, but only in recent years have these infiltrates been systematically characterised, and correlations with clinical outcome determined. Components of the innate immune system which may participate in initial anti-tumour immune responses include NK cells, NKT and γδ T cells (both specialised types of T lymphocytes), macrophages and granulocytes. The subsequent adaptive immune response is characterised by the activation of antigen-specific T and B lymphocytes as a result of antigen uptake and presentation by dendritic cells. Tumour-specific cytotoxic $CD8^+$ T cells (CTLs), further activated by T_H1 $CD4^+$ T cells, may then migrate into the tumour to lyse tumour cells. However, the generation and activity of CTLs, particularly against any antigen-recognised as 'self', is tightly regulated and tumours employ multiple mechanisms to avoid detection and destruction by CTLs and other components of the immune system (Fig. **1**).

Tumour-Infiltrating Lymphocytes (TILs)

In considering the published literature on tumour-infiltrating lymphocytes, it is important to recognise that this cell population is by no means homogenous. Many studies have used CD3 as a marker of infiltrating T lymphocytes, although this does not allow a distinction between $CD8^+$ cytotoxic T cells, $CD4^+$ T helper cells, regulatory T cells (Tregs), NKT or γδ T cells. Although some studies have specifically assessed numbers of infiltrating $CD8^+$ cells, the presence of such cells within the tumour does not necessarily represent an anti-tumour immune response; based on $CD8^+$ staining alone, it is impossible to be certain of the antigen target of these CTLs. The potential role of $CD4^+$ T cells is similarly complex: whilst T_H1 cells may contribute to an anti-tumour immune response (through production of IFN-γ), T_H2 cells and Tregs produce, amongst other cytokines, IL-10 which may have an immuno-inhibitory effect on anti-tumour responses through inhibition of dendritic cell antigen processing and presentation to CTLs. However, whilst T_H2 cells may promote tumour growth through enhancing angiogenesis and inhibiting anti-tumour immune responses, other components of the T_H2 response have been shown to have anti-tumour effects [13]. A recently published report on adult Hodgkin's disease demonstrated that, whilst increased number of infiltrating Tregs was associated with a poorer outcome, large numbers of infiltrating T_H2 cells were, in fact, associated with significantly improved disease-free and event-free survival [14].

The above complexities notwithstanding, the presence within tumours of $CD3^+$ and/or $CD8^+$ T lymphocytes has been correlated with improved outcome in a large number of adult malignancies, including colorectal cancer [15] and related liver metastases [16]; endometrial cancer [17]; breast carcinoma [18]; melanoma [19]; epithelial ovarian cancer [20]; oesophageal carcinoma [21]; malignant mesothelioma [22] and squamous cell carcinoma of the lung [23]. Thus, it appears that evidence of host immune response to malignancy (as manifest through enhanced tumour lymphocytic infiltration) correlates with improved outcome. However, this association can be complicated by confounding factors. In renal cell carcinoma, for example, patients with substantial $CD8^+$ or $CD4^+$ lymphocyte infiltrates had increased recurrence and reduced survival, with high grade tumours more likely to have high numbers of TILs [24]. Interestingly, however, patients in whom there was evidence of proliferation of tumour associated $CD8^+$ lymphocytes (as evidenced by Ki-67 staining) had improved outcomes compared to those in whom TILs did not show proliferative activity. Thus, it is possible that proliferative activity of TILs may represent a surrogate marker for effective anti-tumour immunity [24].

Equivalent published data in relation to paediatric cancers are considerably more limited. Tumour-infiltrating lymphocytes have been isolated from neuroblastoma, Wilms' tumour, rhabdomyosarcoma, osteosarcoma and other soft-tissue sarcoma tumour samples, although in general these lymphocytes were difficult to expand in culture and showed little tumour-directed cytotoxicity [25-27]. Immuno-histochemical analysis of 12 paediatric tumours (including osteosarcoma, Wilms' tumour, rhabdomyosarcoma and a range of other tumour types) showed minimal lymphocytic infiltrates [27]. In neuroblastoma, Martin *et al.* [28] suggested a correlation between lymphocyte infiltration and improved survival, although these data are confounded by tumour grade since lymphocytic infiltrates were seen more frequently in low grade, differentiating tumours. In a

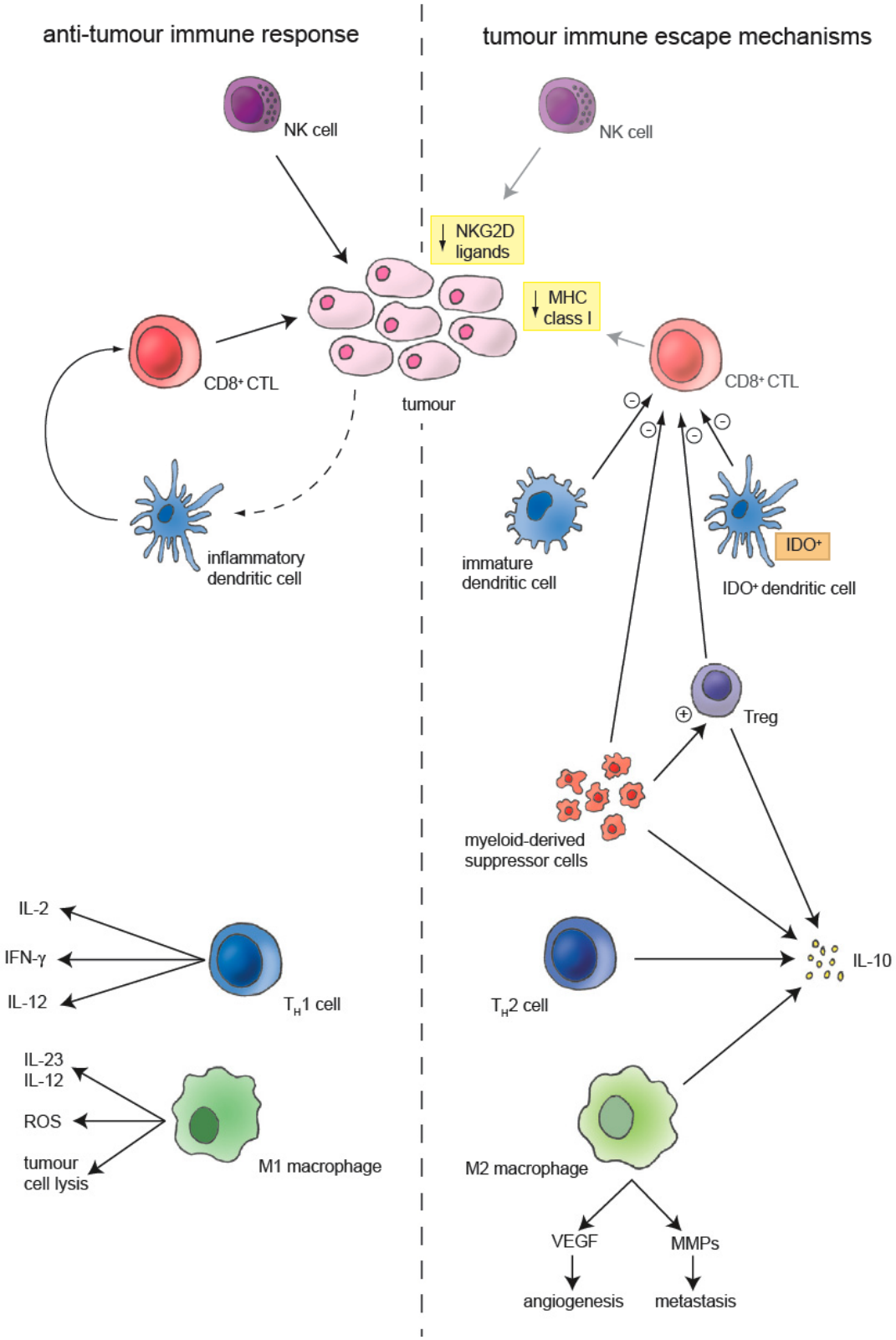

Fig. (1). Cellular pathways of tumour immune response and immune escape. The major cellular pathways of the immune response which may have anti- or pro-tumour effects are shown. NK cells and $CD8^+$ CTLs may directly target tumour cells for lysis; however this may be countered by decreased tumour expression of NKG2D ligands or MHC class I. Dendritic cells are important for priming an anti-tumour immune response, although immature DCs and IDO-expressing DCs may instead lead to the induction of tolerance. Myeloid-derived suppressor cells and regulatory T cells (Treg) may also suppress the anti-tumour CTL response. T_H1 cells and M1 macrophages produce pro-inflammatory cytokines which help to stimulate the anti-tumour immune response, whilst T_H2 cells (and other cell types) produce IL-10 which may have a predominantly inhibitory effect on the anti-tumour response. Tumour-associated M2 macrophages may promote tumour growth and metastases *via* a number of different mechanisms.

separate examination of 26 high-risk neuroblastoma tumour samples, there was minimal or undetectable infiltration of $CD8^+$ or $CD4^+$ T cells, $CD20^+$ B cells or $CD56^+$ NK cells within tumour nests [29], although in most patients $CD8^+$ or $CD4^+$ lymphocytes were present within the peritumoural stroma. Interestingly, the majority of patients had evidence of small numbers of circulating cytotoxic T cells against the tumour antigen survivin (expressed by all of the tumours in this study) and these CTLs were highly functional in *in vitro* assays. Thus, whilst some degree of anti-tumour immune response (at least to this particular antigen) appears to exist in neuroblastoma patients, these cells appear unable to infiltrate the tumour.

Although few studies have made a direct comparison, there is evidence to suggest that overall paediatric-type tumours contain substantially fewer infiltrating lymphocytes than adult epithelial malignancies. Vakkila *et al.* [30] examined 27 paediatric tumour samples (including neuroblastoma, Wilms' tumour, hepatoblastoma, rhabdomyosarcoma, osteosarcoma and Ewing's sarcoma) and 13 adult primary tumours (breast, oesophageal and colon carcinomas). They showed that whilst total numbers of tumour-associated leucocytes (TALs) were equivalent, the nature of these leucocytes varied markedly between typical adult-type epithelial carcinomas and paediatric sarcomas/blastomas. Whilst adult tumours contained approximately equal numbers of $CD3^+$ T lymphocytes, macrophages ($CD68^+$) and dendritic cells ($S100^+$), in paediatric cancers macrophages predominated (median 68% of all TALs), with substantially fewer infiltrating lymphocytes and dendritic cells.

One exception appears to be neuroblastoma patients with the paraneoplastic phenomenon opsoclonus-myoclonus-ataxia (OMA) syndrome. In a study of 54 neuroblastoma tumours, only limited focal lymphocytic infiltration was seen in control tumours, whilst 12 of 13 cases with OMA had extensive diffuse lymphocytic infiltration with lymphoid follicles [31]. Thus, in OMA cases, the immune response to neuroblastoma (comprising both infiltrating T and B lymphocytes) may contribute to the paraneoplastic syndrome seen, probably through the generation of auto-reactive antibodies [32].

Tumour-Associated Regulatory T-Cells

As discussed above, in many adult cancers the presence of significant numbers of tumour-infiltrating lymphocytes, which potentially represent the host immune response against the tumour, is associated with improved prognosis. Nevertheless, the total tumour-associated lymphocyte population can include not just cytotoxic lymphocytes (CTLs) with potential anti-tumour activity, but also immunosuppressive regulatory T cells [33]. Tregs are able to suppress the activity of CTLs by direct cell-cell contact and also secrete immunoregulatory cytokines such as transforming growth factor β (TGF-β) and interleukin-10 (IL-10). The presence of Tregs ($CD4^+$ $CD25^+$ cells expressing the nuclear transcription factor FOXP3) has been examined in many adult tumour types, typically by immunohistochemistry for FOXP3. The presence of high numbers of Tregs correlates with worse prognosis in many cancers, including renal cell [34], ovarian [35], pancreatic [36], breast [37] and hepatocellular carcinoma [38]. The correlation is not, however, always as clear. In astrocytic gliomas, the presence of Tregs correlates with tumour grade (being especially prevalent in high-grade tumours, i.e. glioblastoma multiforme), but does not independently correlate with outcome [39]. Somewhat counter-intuitively, increased Treg cellular density in colorectal carcinomas has recently been shown to correlate with *improved* outcome [40]. Of note, all these studies, despite their divergent conclusions, used a relatively uniform methodology of FOXP3 immunohistochemistry.

In adult cancers, Treg-mediated immunosuppression appears to play a crucial role in tumour immune evasion. Consequently there is growing interest in developing methods to modulate Treg function and overcome their immunosuppression [41, 42]. In paediatric cancers, however, the role of Tregs is much less clear and to our knowledge there are no published data on the presence (or otherwise) of Tregs in paediatric tumours. Preliminary data suggest that $FOXP3^+$ Tregs are very scarce or entirely absent in paediatric Wilms' tumour, neuroblastoma, osteosarcoma, Ewing's sarcoma and rhabdoid tumour samples (Hasan, Morgenstern, Sebire and Anderson, unpublished observations).

Natural Killer T Cells (NKT Cells)

NKT cells represent a particular subset of T lymphocytes, which express both T cell markers, such as the αβ T-cell receptor (TCR) and associated CD3 complex, and NK cell markers, such as NK1.1 [43]. These cells recognise glycolipids presented by the MHC class I-like molecule CD1d and are believed to play an important role at the interface between the innate and adaptive immune responses to infection and malignancy [44]. Two main subtypes of NKT cell are recognised, with Type I NKT cells expressing an invariant α-TCR chain and being implicated in anti-tumour immunity, whilst Type II NKT cells express a variety of TCR molecules (in addition to CD1d) and appear to have a more immune inhibitory role [43]. The presence of these immune effector cells within tumours has been examined in a number of different malignancies, including, in the paediatric setting, neuroblastoma. Type I NKT cells were found in 53% of 98 untreated primary stage 4 neuroblastoma samples [45] and their infiltration correlated with favourable outcome, with expression of the chemokine CCL2 and with absence of MYCN amplification (indicating less aggressive disease). Subsequent investigations have confirmed that expression of CCL2 is repressed in MYCN amplified tumours, leading to a failure of NKT cell infiltration and potentially contributing to tumour immune escape [46]. Neuroblastoma (and most other tumour) cells do not themselves express CD1d, the molecular target for NKT cells, suggesting indirect mechanisms for the anti-tumour effects of infiltrating NKT cells. Recent data suggest that the target of NKT cells may, in fact, be $CD1d^+$ cells within the tumour stroma, particularly tumour-associated macrophages [47].

Tumour-Associated Macrophages

Macrophages represent a further important cellular component of the tumour stroma. Far from being mere bystanders to tumour development, there is increasing evidence that tumour-associated macrophages (TAMs)

promote and facilitate tumour growth [48, 49]. Of key importance is the concept of distinct macrophage phenotypes, mirroring the dichotomy between T_H1 and T_H2 T helper cells and type I and type II immune responses. Classical or M1 macrophage activation occurs in the context of bacterial infection or in response to IFN-γ and leads to enhanced antigen presentation and increased production of IL-23, IL-12 and reactive oxygen species. M1 macrophages are associated with a type I inflammatory response leading to enhanced cytotoxicity and anti-tumour effects. By contrast, M2 macrophages, induced by signals such as IL-4, IL-10, IL-13 and glucocorticoids, have a very different phenotype. These M2 macrophages contribute to tissue remodelling and repair, and to angiogenesis; functions which promote tumour growth and metastasis [50].

Growing evidence suggests that TAMs are active in facilitating tumour progression [51]. For example, in a murine model of breast carcinoma, transgenic expression of the macrophage growth factor, colony stimulating factor (CSF-1), in mammary epithelium leads to enhanced macrophage infiltration and acceleration of the progression to carcinoma, whilst transgenic mice lacking CSF-1 showed delayed development of invasive carcinoma (although an unchanged incidence of primary tumours) and reduced numbers of tumour-associated macrophages [52]. In human breast cancer, CSF-1 expression also correlates with macrophage infiltration, more frequent metastases and poorer outcomes [53]. An association between extensive TAM infiltration and poor prognosis has been demonstrated in carcinoma of the breast, prostate, cervical and bladder, and in non-small cell lung cancer and glioma [54, 55].

In the paediatric field, a recent report has demonstrated a similar association between TAMs and poor outcome in neuroblastoma. Song *et al.* [47] examined 129 cases of untreated stage 4 MYCN non-amplified neuroblastoma and demonstrated a clear correlation with the expression in tumour samples of TAM-associated genes and reduced 5-year event-free survival. Interestingly, there was no such correlation for lymphocyte markers. The presence of macrophages appears directly to contribute to enhanced neuroblastoma cell proliferation through, at least in part, the secretion of IL-6, a cytokine that promotes neuroblastoma growth and enhances bone marrow invasion [47]. As discussed above, there is in neuroblastoma some evidence for a model in which tumour-promoting TAMs, rather than tumour cells themselves, are the key cellular target of tumour-infiltrating NKT cells.

At least in the specific context of stage 4 MYCN non-amplified neuroblastoma, there is therefore clear evidence of a tumour-promoting role for TAMs. Since macrophage infiltration appears to be an important feature of the immune environment of paediatric tumours [30] - perhaps even more so than in adult cancers – the role of TAMs in the progression of paediatric cancers is potentially of great significance and further investigations are clearly warranted.

Tumour-Infiltrating Dendritic cells

Myeloid dendritic cells (mDCs) are, like macrophages, derived from monocytes and are the main antigen presenting cells of the immune system. Dendritic cell infiltrates correlate with favourable outcome in various adult cancers, including cervical [56], oesophageal [57], colorectal [58] and transitional cell carcinoma [59], suggesting that these cells may have anti-tumour effect through presenting tumour antigens to immune effector cells, such as cytotoxic lymphocytes. The situation is complicated, however, since antigen presentation by immature dendritic cells (that is in the absence of appropriate co-stimulatory signals) can lead to the induction of T cell tolerance. Furthermore, in some cancers (such as breast carcinoma), the presence of a different subtype of dendritic cells, plasmacytoid (pDCs), has been shown to correlate with *worse* outcome [60]. These pDCs are able to induce Tregs and promote tolerance to tumour antigens [61], thereby inhibiting the anti-tumour immune response. Thus, dendritic cells cannot simply be seen as stimulators of the immune response, rather they integrate a wide variety of signals which may be either tolerogenic (e.g. IL-10, TGF-β and signals from regulatory T cells) or immunogenic (e.g. TLR ligands and signals from T_H cells). Ultimately, the response they induce following antigen presentation may then lead either to tolerance or to an active anti-tumour immune response [62, 63].

Few data concerning dendritic cell infiltration in paediatric tumour types are available. As discussed previously, a comparison of adult-type carcinomas and paediatric tumours (sarcomas and blastomas) demonstrated marked differences in the nature of the leucocytic infiltration, with paediatric tumours containing very few dendritic cells (identified by S100, CD1a, CD83, CD123 or DC-LAMP staining) [30]. This finding is in line with an earlier study showing significantly reduced numbers of mDC precursors in the blood of paediatric cancer patients [64]. Systemic defects in dendritic cell function appear to be a feature of many types of cancer [65]. For example, decreased numbers of circulating ($CD11c^+$) mDCs have been demonstrated in patients with breast, lung or head and neck cancer [66, 67], and these DCs have a reduced ability to stimulate T cell proliferation *in vitro* [66]. These DC defects in cancer patients likely relate, at least in part, to abnormal differentiation from DC precursors [65]. A failure of differentiation leads to reduced numbers of DCs, and increased numbers of immature myeloid cells (myeloid-derived suppressor cells), which themselves have immuno-inhibitory properties, as discussed further below.

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a heterogenous group of immature, myeloid lineage cells which exert profound inhibitory effects on T-cell responses [68]. They have a variable phenotype, but in humans are typically $Lin^-HLA\text{-}DR^-CD33^+$ or $CD11b^+CD14^-CD33^+$; there is no human equivalent of GR1 which serves as a marker of MDSCs in mice. MDSCs inhibit T lymphocyte, and possibly also NK and NKT cell, activity through a variety of mechanisms, including pathways involving arginase and nitric oxide synthetase (NOS). They further inhibit the anti-tumour immune response through induction of Tregs and promotion of a T_H2 helper/M2 macrophage response, as well as directly promoting angiogenesis [69, 70]. To date, the vast majority of work on MDSCs has been performed in murine cancer models and relatively few data are available on the numbers or roles of these cells in human cancer patients or within human tumours. Diaz-Montero *et*

al. [71] showed increased numbers of circulating MDSCs ($Lin^{-/Lo}$, HLA DR^-, $CD33^+$, $CD11b^+$) in adult patients with a range of solid tumours, with the greatest number of MDSCs in those with advanced disease. MDSCs have been isolated from the blood of patients with renal cell carcinoma [72] and non-small cell lung cancer [73], and from both blood and tumour samples in patients with hepatocellular carcinoma [74]. At present there are, to our knowledge, no data on MDSCs in paediatric cancer patients.

TUMOUR IMMUNE ESCAPE MECHANISMS

The cellular immune environment within the tumour is complex and may comprise cells which possess both anti-tumour and tumour-promoting properties. Thus, whilst infiltrating cytotoxic lymphocytes may represent a beneficial immune response against the tumour, this may be inhibited by Tregs or MDSCs; whilst TAMs may directly promote tumour growth and metastatic spread. It is becoming increasingly apparent that the interactions between tumour cells and the immune system may be of critical importance in tumour initiation and progression, and that many tumours exploit mechanisms by which they are able to avoid immune detection or inhibit the anti-tumour immune response. These mechanisms, considered in more detail below, include intrinsic signalling pathways such as STAT3 and NF-κB; cytokines such as TGF-β; metabolic pathways including indoleamine 2,3-dioxygenase (IDO), cyclooxygenase (COX2) and nitric oxide synthase (NOS); loss of expression or shedding of immune mediators such as MHC class I and NKG2D; and, of particular relevance to neuroblastoma, expression and secretion of gangliosides that are themselves immunomodulatory.

STAT3

As discussed in the introduction, intracellular signalling pathways implicated in oncogenesis have also been demonstrated to contribute to modulating the immune microenvironment. Amongst the intracellular signalling molecules that fulfil this dual role is the transcription factor STAT3 (signal transducer and activator of transcription 3). STAT3 is constitutively activated in many human cancers, either as a result of cytokine or growth factor signalling (for example, *via* IL-6, IL-10, EGF, VEGF, IGF, PDGF or HGF) or through direct activation, for example by oncogenes such as SRC and ABL [75]. As discussed below, there is also evidence that in alveolar RMS, STAT3 can be activated by the PAX3-FKHR fusion protein [76]. STAT3 activation regulates transcription of multiple downstream targets, with anti-apoptotic, mitogenic and pro-angiogenic effects, and thus directly promotes tumour growth; for example, STAT3 activation is required for SRC-mediated transformation of fibroblasts [77]. However, there is also growing evidence that STAT3 activation has significant effects on the local immune environment, serving to inhibit the anti-tumour immune response (Fig. **2**). In an important paper, Wang *et al.* [78] demonstrated that STAT3 activity in tumours inhibits local inflammation, dendritic cell differentiation and activity, and T cell-mediated immunity. STAT3 expression within tumour cells leads to the secretion of multiple anti-inflammatory factors (including in particular IL-10, IL-6 and VEGF) which have direct effects on components of the immune system and also trigger a 'feed forward' loop in which STAT3 becomes activated in immune cells [79]. STAT3 activation in dendritic cells inhibits functional maturation (such as upregulation of MHC class II) and blocks the differentiation of immature myeloid cells into DCs [78]. STAT3 activation has also been implicated in reducing cytotoxicity mediated by CD8+ T cells, neutrophils and NK cells, and in promoting the proliferation of regulatory T cells [79].

Thus, it appears that STAT3 activation plays a central role in mediating tumour immune escape through activation of multiple signalling pathways in both cancer cells and infiltrating immune cells, making STAT3 inhibition a potentially exciting therapeutic strategy [80]. In patient samples, constitutive STAT3 activation has been demonstrated in many adult solid cancers including breast, colon, prostate, lung, pancreatic, pituitary, gastrointestinal, ovarian, cervical and melanoma, as well as in lymphoma and leukaemia [81]. Phosphorylated (active) STAT3 has also been demonstrated in glioma, with increased numbers of pSTAT3-expressing cells in higher grade tumours [82]. There was a trend towards poorer outcomes in those with higher levels of STAT3 activation, although this only reached statistical significance in the small subgroup of patients with anaplastic astrocytomas. In paediatric tumours, STAT3 activation has been demonstrated in a proportion of rhabdomyosarcoma and osteosarcoma tumour specimens, whilst inhibition of STAT3 (using a dominant-negative construct or small molecule inhibitor) induced apoptosis in human sarcoma cell lines in which STAT3 is constitutively activated [83]. Activated STAT3 has also been demonstrated in a subset of Ewing's sarcoma tumour specimens [84], although somewhat surprisingly given the pro-oncogenic and anti-immune roles of STAT3 outlined above, high levels of pSTAT3 were associated with better outcomes in this population. The potential role of STAT3 in the most common paediatric extra-cranial solid tumour, neuroblastoma, remains to be elucidated. It has recently been demonstrated that neuroblastoma cells (both *in vitro* and in an *in vivo* mouse model) are receptive to IL-6 signalling, leading to STAT3 activation and hence cellular proliferation and *in vivo* tumour growth [85]. The authors suggest that IL-6 produced by bone marrow stromal cells in response to signals from neuroblastoma cells may have a paracrine effect in promoting the growth and survival of neuroblastoma bone marrow metastases. It remains to established whether this proposed phenomenon has generally applicability to neuroblastoma in patients.

PAX3-FKHR Fusion Protein

Many sarcomas and leukaemias have chromosomal translocations leading to the production of novel fusion proteins [86]. Amongst those recognised in paediatric tumours are the t(2;13)(q35;q14) and t(1;13)(p36;q14) translocations, giving rise (respectively) to PAX3-FKHR or PAX7-FKHR fusion proteins, which are characteristic of alveolar rhabdomyosarcoma (ARMS). Whilst PAX3-FKHR has been shown to contribute directly to malignant transformation and tumour proliferation [87, 88], there is also evidence for a role in immune modulation. *Via* an interaction with STAT3, PAX3-FKHR leads to the production by ARMS tumour cells of secreted proteins, including IL-10, which inhibit local dendritic cell activation

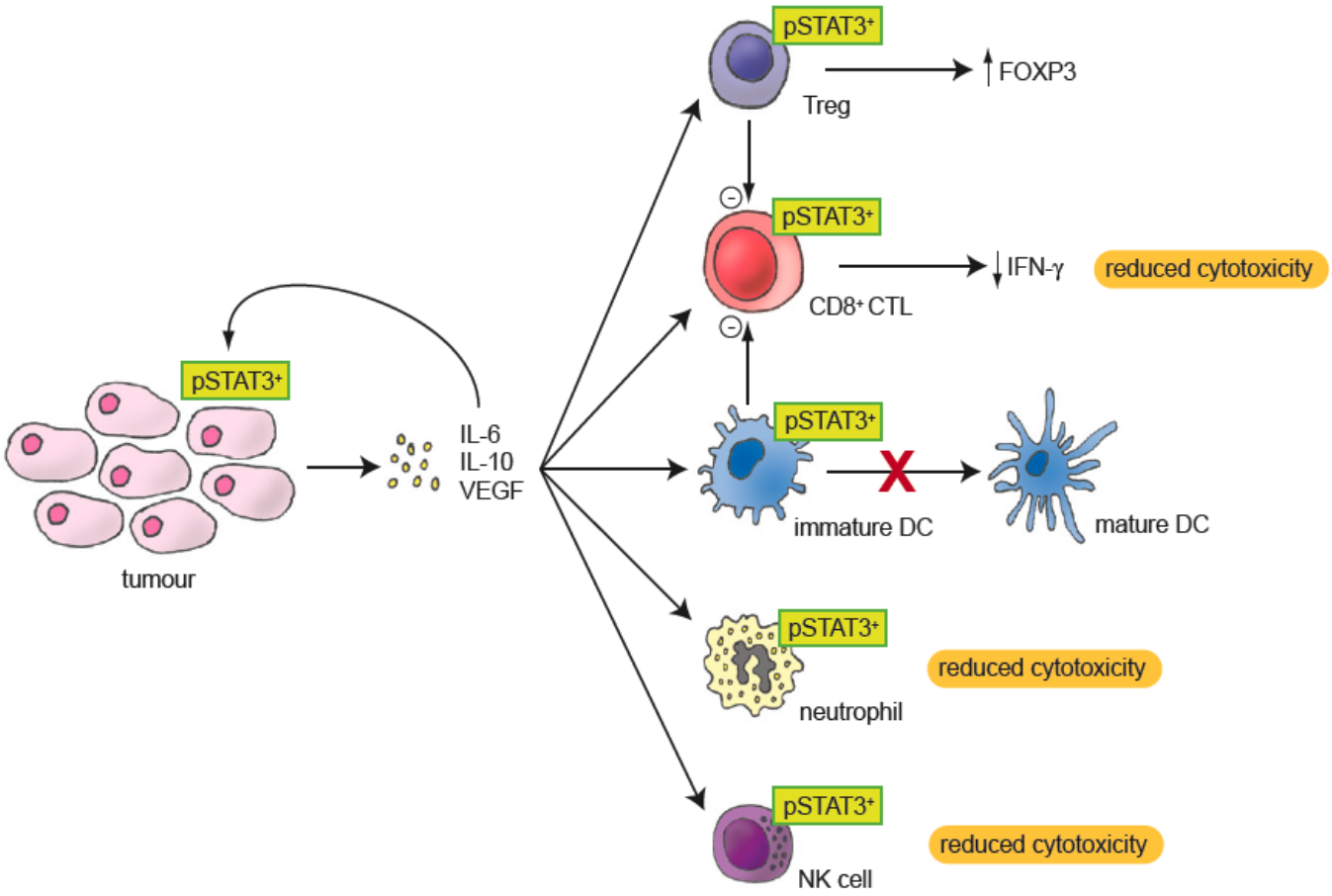

Fig. (2). STAT3 mediates pleiotropic pathways in the tumour microenvironment, including immune escape mechanisms. In STAT3 activated tumours, phosphorylation of STAT3 ($pSTAT3^+$) may occur in both tumour cells and in tumour-associated myeloid cells and lymphocytes. STAT3-dependent production of immune inhibitory cytokines from tumour cells signals to surrounding haemopoeitic cells to activate STAT3, which in turn generates an immune inhibitory environment *via* multiple effects.

[76]. In a mouse model, tumours expressing PAX3-FKHR showed inhibition of DC maturation and decreased infiltration by neutrophils and macrophages. Expression of the PAX3-FKHR fusion protein was also associated with loss of MHC class I expression, a potential further mechanism of immune evasion.

Nuclear Factor-κB (NF-κB)

Another important example of the integration of oncogenic and immune/inflammatory pathways at the level of a transcription factor is the nuclear factor-κB (NF-κB) family. Like STAT3, NF-κB signalling may contribute to tumour development through expression both in tumour cells and infiltrating immune cells. Within tumour cells, NF-κB activation leads to the upregulation of anti-apoptotic genes (such as BLC2) and has been shown to be important in malignant transformation in both mouse models of inflammation-induced cancer and in human tumours (for comprehensive reviews, see [89, 90]). Meanwhile, within immune cells the results of NF-κB signalling include the expression of pro-tumour cytokines (e.g. IL-6 and TNF-α) and angiogenic factors. Within tumour-associated macrophages, NF-κB has been shown to contribute to the switch from the anti-tumour M1 phenotype towards the tumour-supporting M2 phenotype [91, 92]. At present data concerning NF-κB signalling within paediatric tumours are very limited, although the literature does contain a number of reports examining the roles of NF-κB in paediatric cancer cell lines. For example, in the retinoblastoma cell lines Y79 and WERI-Rb1 inhibition of NF-κB leads to enhanced apoptosis, suggesting a role for NF-κB signalling in tumour proliferation and survival [93]. By contrast, in the neuroblastoma cell lines SH-SY5Y and IMR32, NF-κB mediates doxorubicin-induced cytotoxicity [94]. Intriguingly, recently published work by Wang *et al.* [95] suggests that NF-κB signalling may have a role in the development of rhabdomyosarcoma. They propose a model of tumourigenesis in which dysregulated activity of NF-κB and its downstream target gene, Ying Yang 1 (YY1), leads to repression of the micro-RNA, miR-29. Without miR-29, YY1 is left uncontrolled, thereby impairing differentiation into mature skeletal muscle and ultimately leading to the development of rhabdomyosarcoma.

The above discussion relates to the classical ('canonical') NF-κB signalling pathway which involves activation of the inhibitor of NF-κB (IκB) kinase-β (IKKβ), followed by phosphorylation-induced degradation of the inhibitor IκBα and ultimately nuclear translocation of dimers comprising RelA (also known as p65) and p50 (NF-κB1). There is, however, also evidence for an alternative ('non-canonical') pathway, involving activation of IKKα and nuclear

translocation of RelB-p52 (NF-κB2) dimers [96]. It is thought that signalling *via* these two alternative NF-κB pathways may have divergent effects on the immune response, such that whilst classical signalling has pro-inflammatory effects, non-canonical NF-κB signalling leads to resolution of early inflammatory responses and the promotion of tolerance to self, or the development of adaptive immunity to foreign antigens [97]. For example, non-canonical NF-κB signalling appears to have a role in the induction of the immuno-modulatory enzyme indoleamine 2,3-dioxygenase (IDO) in plasmacytoid dendritic cells and in the consequent generation of regulatory T cells [97].

Transforming Growth Factor β (TGF-β)

The peritumoural immune environment is hugely complex and many different cytokines may impact on the differentiation, migration and function of immune cells; transforming growth factor β (TGF-β) represents perhaps one of the most important of these cytokines. The role of TGF-β in tumour development is complicated [98]. Early in tumour development, TGF-β appears to act as a tumour suppressor, inhibiting the proliferation of malignant cells; for example, *in vitro* TGF-β inhibits the proliferation of neuroblastoma cell lines [99]. Subsequently, these cells acquire mutations in the TGF-β receptor or downstream signalling pathway and become refractory to the growth-inhibitory effects of TGF-β. Nevertheless, tumour cells may continue to over-express TGF-β, and at later stages in tumour development this serves to promote tumour growth through enhanced angiogenesis and inhibition of the anti-tumour immune response [100, 101] (Fig. **3**). TGF-β inhibits the cytotoxic effects of $CD8^+$ CTLs and NK cells through down-regulation of IFN-γ, perforin and granzyme B, whilst enhancing the differentiation and proliferation of regulatory T cells. Through inhibitory effects on dendritic cell maturation, TGF-β suppresses effective antigen presentation, whilst inhibition of $CD4^+$ T cell differentiation into T_H1 or T_H2 cells limits the availability of pro-inflammatory cytokines. TGF-β acts as chemoattractant for neutrophils, but inhibits their activation such that they are unable to kill FAS-ligand expressing tumour cells. This FAS-ligand then enhances tumour growth in its own right by inducing death in FAS-expressing cells including, in particular, tumour-infiltrating T lymphocytes [100]. TGF-β expression also contributes to the immune inhibitory effects of myeloid-derived suppressor cells (MDSCs), discussed above [102].

The expression of TGF-β, its receptors and components of the downstream signalling pathway have been extensively investigated in human cancers [103]. Assessment of TGF-β signalling in tumour samples is, however, particularly challenging due to cross-talk between tumour and stromal cells and the variety of TGF-β ligands and receptors. High levels of expression of TGF-β have been associated with advanced tumour stage in colorectal, gastric, prostate and non-small cell lung cancer, and also correlate with increased angiogenesis and poor prognosis [103]. Paediatric-specific data are more limited, although in a small study of paediatric small round blue cell tumours, McCune *et al.* [104] demonstrated by immunohistochemistry expression of TGF-β 1 and 3 in the majority of rhabdomyosarcoma specimens examined. Expression in Ewing's sarcoma was variable, whilst in neuroblastoma TGF-β 1 and 3 were detected in differentiating ganglioneuroblastoma, but not in poorly differentiated neuroblastoma specimens. More recently, loss of expression of type III TGF-β receptor was demonstrated (by RT-PCR) in stage 3 or 4 neuroblastoma, but not in localised tumours [105]. In Ewing's sarcoma, which frequently has chromosomal translocations involving the EWS gene, the fusion proteins EWS-FLI1, EWS-ERG and EWS-ETV1 have all be shown to suppress transcription of the type II TGF-β receptor, contributing to a loss of tumour sensitivity to TGF-β [106, 107].

Indoleamine 2,3-Dioxygenase (IDO)

Another mechanism through which tumours may create a state of immune tolerance is through the enzyme indoleamine 2,3-dioxygenase (IDO), which catabolises the essential amino acid tryptophan. In a groundbreaking paper, Munn and Mellor demonstrated that IDO plays a crucial role in preventing fetal rejection, through tryptophan metabolism and inhibition of T cell activity [108]. Since then, there has been growing interest in the potential role of IDO in modulating the immune response to cancer. As previously discussed, whilst dendritic cells are potentially able to initiate an anti-tumour immune response through cross-presentation of tumour antigen to T cells, in the absence of appropriate 'danger' signals, or in the presence of specific stimuli (such as IL-10 and TGF-β), they may instead trigger T cell tolerance. A growing body of literature [63] suggests that IDO expression by dendritic cells plays a crucial role in determining this tolerogenic phenotype, leading to inhibition of effector T cell development and promoting the generation of regulatory T cells (Fig. **4**). Accumulations of IDO^+ $CD123^+$ plasmacytoid dendritic cells have been identified in the tumour-draining lymph nodes of patients with malignant melanoma [109], leading to the suggestion that recruitment of IDO^+ immunosuppressive dendritic cells to tumour-draining lymph nodes might contribute to the induction of tolerance to tumour antigens [63] - although others have disputed this [110, 111].

In addition to its expression by components of the immune system (particularly dendritic cells), IDO may also be expressed within tumours themselves. Uyttenhove *et al.* [112] demonstrated in a mouse model that IDO transfection of normally immunogenic tumour cells prevents their rejection in pre-immunised mice, correlating with a failure of recruitment to the tumour site of specific anti-tumour T cells. Thus, IDO expression by tumour cells appears to contribute to tumour immune escape. Although IDO expression has been detected in a wide range of carcinomas [112], the results from a more detailed analysis of the expression in particular tumour types and correlations with outcome are considerably more complicated [110]. In colorectal carcinoma, for example, high levels of IDO expression within the tumour correlate with a significant reduction in infiltrating $CD3^+$ T cells, although this does not clearly translate to a poorer overall survival [113]. In endometrial carcinoma, increased IDO expression within the tumour again correlates with a reduced infiltration of T cells ($CD3^+$ and $CD8^+$) and also $CD57^+$ NK cells, and with impaired progression-free survival [114]. By contrast, in renal cell carcinoma, increased IDO expression correlated with

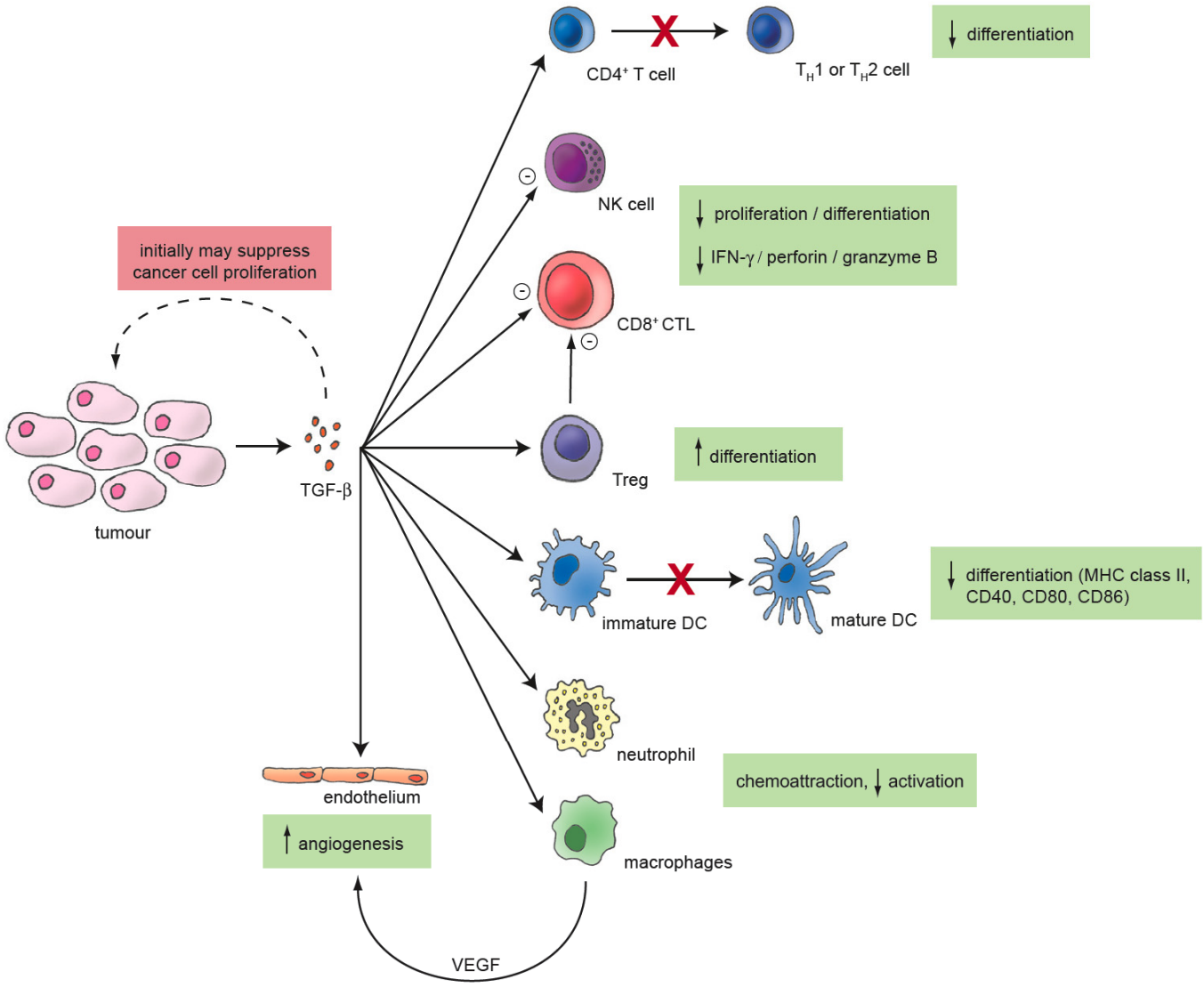

Fig. (3). Immune inhibitory pathways induced by transforming growth factor beta (TGFβ). Although TGFβ can function as a tumour suppressor, downregulation of its receptor on tumour cells allows for loss of this negative feedback loop. Continued TGFβ production may promote multiple immune inhibitory effects on surrounding cellular components of the immune response.

prolonged survival [115]. In these tumours, IDO expression was seen almost exclusively in endothelial cells of newly formed intratumoural blood vessels, rather than by tumour cells themselves. Meanwhile, in non-small cell lung cancer, intratumoural IDO expression is due to eosinophil infiltration rather than direct expression by tumour cells [116].

The functional role of IDO expression by stromal cells remains to be elucidated although it is possible that tryptophan metabolism by IDO^+ cells may directly limit tumour growth through either tryptophan depletion or the generation of toxic metabolites (similar mechanisms as are suggested for the T cell suppressive effects of IDO). At present there are very few data concerning the patterns of expression and potential roles of IDO in paediatric cancers. Shahlaee *et al.* [116] have reported IDO expression in paediatric Hodgkin's lymphoma, although possible correlations with anti-tumour immune response or clinical outcome were not investigated in this pilot study.

Cyclooxygenase (COX2) and Prostaglandin-E2 (PGE_2)

Another metabolic pathway with potential significance for both tumour growth and the immune response involves the inducible isoform of cyclooxygenase, COX2, which catalyses the synthesis of prostaglandins from arachidonic acid. COX2 is expressed in many types of tumours and appears to play important roles in angiogenesis, tumour cell invasiveness and apoptosis [117]. In the paediatric setting, COX2 appears to be expressed in Wilms' tumours [118] and medulloblastoma [119], as well as osteosarcoma, Ewing's sarcoma and rhabdomyosarcoma [120]. Although Dickens *et al.* [121] reported no correlation between COX2 expression and clinical outcome in these paediatric sarcomas, a more recent publication suggests that in patients with osteosarcoma and lung metastases, increased levels of COX2 expression do indeed correlate with poor outcome [122].

COX2 expression within tumours may affect multiple downstream pathways; of particular relevance to this review are the potential immunomodulatory effects of prostagladin-

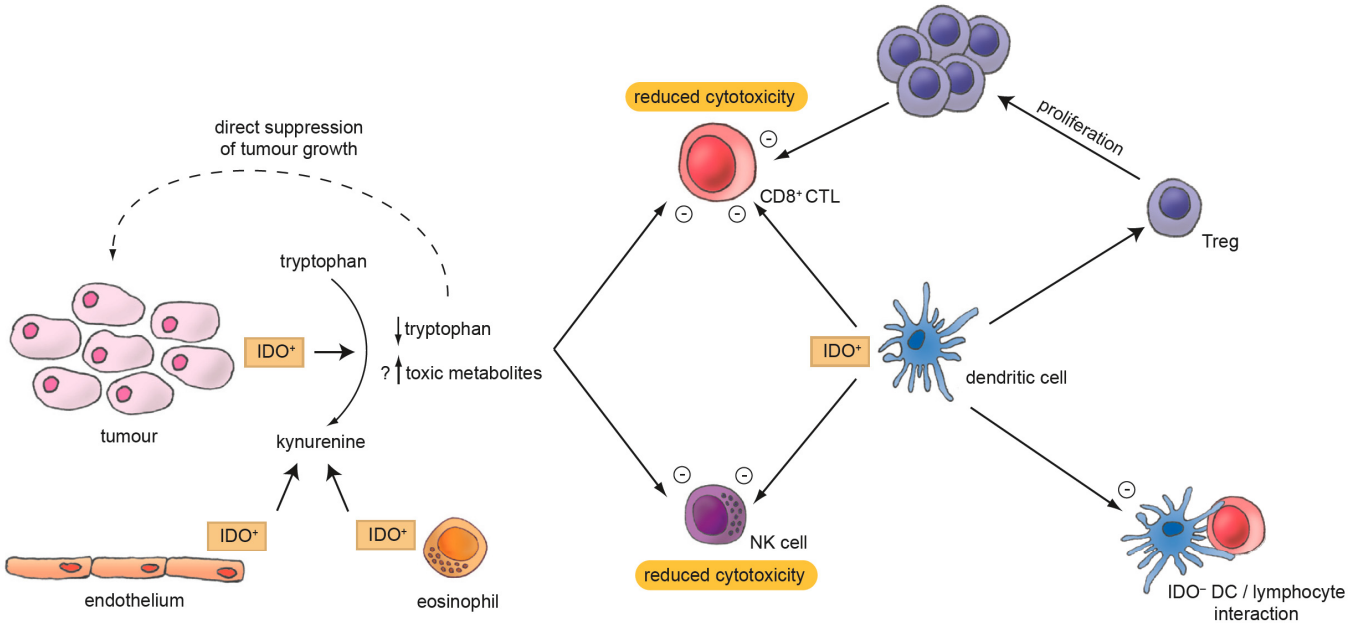

Fig. (4). Indoleamine 2,3-deoxygenase (IDO) mediates multiple pathways of immune inhibition. Current concepts of how IDO mediates immune inhibition in the tumour microenvironment involve its production by both tumour cells and (plasmacytoid) dendritic cells in tumour-draining lymph nodes. IDO catalyses the metabolism of tryptophan to kynurenine and the biological effects of IDO may result from either local tryptophan depletion or from the effects of metabolites. More recent data points additionally to IDO production in tumour endothelial cells or eosinophils in some tumour types.

E2. Evidence from both murine cancer models and human breast cancer patients suggests that PGE_2 signalling may contribute to the predominant T_H2-type immune response often seen in cancer patients. In a murine model of lung cancer, blocking COX2 led to tumour regression associated with increased lymphocytic infiltration, increased levels of IL-12 and IFN-γ (T_H1 cytokines) and decreased levels of IL-10 (T_H2 response) [123]. In breast cancer patients, high levels of PGE_2 within tumours have been linked with impaired T cell and dendritic cell function, decreased serum levels of T_H1 cytokines such as IFN-γ, IL-2 and IL-12, and increased levels of T_H2 cytokines including IL-10 and IL-4 [124]. *In vitro*, PGE_2 has been shown to induce FOXP3 expression and enhance regulatory T cell function [125] and in dendritic cells causes upregulation of IDO, although it appears that a further signal may be required for the enzyme to become functionally active [126]. PGE_2 therefore has the potential to inhibit the immune response to malignancy *via* effects on a number of different immune cells, including CTLs, DCs, Tregs and $CD4^+$ T helper cells, and consequently, there is growing interest in the possible clinical benefits of selective COX2 inhibitors in cancer patients [117].

Arginase and Nitric-Oxide Synthase (NOS)

The metabolism of the amino acid arginine involves other potentially important pathways which may impact on both cancer cell proliferation and the immune response [127]. Arginase metabolises arginine to produce ornithine which, through conversion to polyamines, may enhance tumour cell proliferation. Alternatively, metabolism by nitric oxide synthase (NOS) produces nitric oxide (NO) which has a wide range of downstream effects that may either inhibit or promote tumour development [128]. Nitric oxide contributes to the cytotoxicity of macrophages and NK cells, enhancing their anti-tumour effects and also promotes the survival and maturation of dendritic cells. However, NO may also promote tumour growth through the promotion of angiogenesis and tissue invasion, and inhibition of the anti-tumour immune response. Nitric oxide inhibits the proliferation of T lymphocytes, altering IL-2 receptor-mediated signalling within T cells, and also appears to make an important contribution to the T cell inhibitory effects of myeloid-derived suppressor cells (MDSCs). These MDSCs may also express arginase leading to a local depletion of arginine and further contributing to impaired T cell proliferation and enhanced apoptosis [127].

Inducible NOS has been demonstrated in many human tumours (including oesophageal, lung, prostate, bladder, breast, pancreatic and colorectal carcinomas) and its expression frequently correlates with tumour stage, microvessel density and poor outcome [128]. There are few published reports examining expression of iNOS in paediatric tumours and at present it is unclear whether the correlations with outcome seen in adult carcinomas can be generalised. Exploring the potential role of NOS in paediatric CNS malignancies, Kao *et al.* [129] examined levels of nitric oxide in CSF samples and tissue expression of iNOS by immunohistochemistry. They demonstrated significantly higher levels of CSF NO in patients with CNS tumours (including glioma, ependymoma and medulloblastoma) compared to controls (patients with epilepsy or hydrocephalus). Positive immunostaining for iNOS was seen in most tumour samples examined (although not in control samples) and iNOS was predominantly expressed by infiltrating macrophages. Cobbs *et al.* [130] also demonstrated increased NOS expression in astrocytic tumours and a positive correlation with tumour grade. In this

study, however, NOS was expressed mainly by tumour cells and endothelial cells, with a lower level of expression by infiltrating macrophages. Nitric oxide synthase has also been demonstrated in osteosarcoma (of the jaw), with expression correlating with higher grade, more advanced tumours [131]. Details of the expression and potential roles of NOS and other components of the arginine metabolic pathways in paediatric tumours are not currently available.

Loss of MHC Class I Expression

Tumours may express a variety of antigens which can serve as targets for cytotoxic T cells. Although CTL responses can be initiated by dendritic cells by cross-presentation of exogenous antigen (e.g. taken up from dying tumour cells) in the context of MHC class I, tumour antigens may also be directly presented to the immune system by tumour cell surface class I or II MHC molecules. Amongst the various mechanisms utilised by tumours for immune escape, it is therefore perhaps unsurprising that loss of functional MHC class I appears to be important [132]. Complete loss of MHC class I expression would tend to make cancer cells targets for NK cell mediated killing (through loss of 'self recognition') and tumours may therefore exhibit a wide variety of altered MHC class I phenotypes without complete loss of expression [133]. These changes appear common in many adult cancers, including cervical, breast, colorectal and laryngeal carcinomas [133]. In the paediatric setting, there is also evidence that loss of MHC class I is important in neuroblastoma. Elevated MYCN expression causes loss of MHC class I expression in neuroblastoma cells through reduced activation of the MHC class I gene enhancer [134]. A study utilising patient-derived tumour samples demonstrated an association between reduced MHC class I expression (at the mRNA level) and MYCN amplification, whilst MHC expression appeared normal in tumours without MYCN amplification [135]. A more recent study using immunohistochemistry to assess MHC class I and II expression in neuroblastoma samples showed apparent complete absence of expression of both classes of MHC molecule in all 17 tumours examined [136]. A similar immunohistochemical study showed loss of expression of a variety of components of the antigen-processing machinery in 24 stroma-poor neuroblastoma samples [137]. Loss of functional MHC class I has also been demonstrated in astrocytoma, with loss of expression of β2-microglobulin, TAP1 and LMP2 (all components of the antigen-presenting machinery) correlating with increasing tumour grade [138].

NKG2D Ligands

The NKG2D receptor is an important activating receptor on NK cells, with engagement of this receptor alone sufficient to trigger NK-mediated cytotoxicity. It is also expressed by $CD8^+$ T cells and by γδ T cells, where it serves to provide a co-stimulatory signal [139]. The major ligands for NKG2D in humans are MICA and MICB (MHC class I chain-related proteins) and ULBP (UL-16 binding protein). These are cell-surface proteins which share structural and sequence homology with MHC class I proteins [140]. Expression of NKG2D ligands is tightly regulated in normal tissues, but is frequently increased in response to cellular stress, infection, or during tumourigenesis. Thus, increased expression by tumour cells of NKG2D ligands may trigger an early innate NK-mediated response. There is evidence that escape from NK killing in established tumours can be mediated by repressed expression of the NKG2D ligands themselves, or shedding of the proteins from the tumour cell surface [140]. For example, in an examination of paediatric neuroblastoma samples, although expression of MICA, MICB and ULBP mRNA was detectable in most tumours, MICA protein could not be detected by immunohistochemistry in any primary tumours [141]. However, a soluble form of MICA was detected in the serum of most patients examined, at levels which were significantly higher than in normal controls. In *in vitro* assays, this soluble MICA lead to downregulation of NKG2D on $CD8^+$ T cells and to reduced NK cell cytotoxicity [141]. MICA expression has recently been examined in osteosarcoma patients. In contrast to neuroblastoma, MICA protein could be detected by immunohistochemistry in the majority of osteosarcoma tumour samples and was generally expressed at much higher levels than in benign bone tumours or normal bone tissue [142]. Albeit in only a minority of cases (5 of 16), there were increased levels of circulating soluble MICA and tumours from these patients appeared to have lower MICA expression than other tumours, suggesting possible 'shedding' of cell surface MICA as a means of immune escape.

Tumour-Associated Gangliosides

Gangliosides are membrane-associated glycosphingo-lipids which have important regulatory roles during embryogenesis and have also been implicated in tumour development. Particular gangliosides, which show restricted patterns of expression in normal tissue, may be expressed at high levels by tumour cells (e.g. GD3 by melanoma) and are implicated both in tumourigenesis and as mediators of metastatic spread [143]. There is also evidence that gangliosides secreted by tumour cells can modulate the immune response and, in particular, act to inhibit dendritic cell differentiation and function. Neuroblastoma (and other neuroendocrine) tumour cells ubiquitously express the ganglioside GD2, whilst expression in normal tissues is restricted to neurons. Thus, GD2 is an attractive antigen for neuroblastoma immunotherapy strategies [144], including humanised anti-GD2 monoclonal antibodies such as ch14.18 [145], or GD2-directed cytotoxic lymphocytes [146]. In addition to its cell surface expression, there is evidence that GD2 is shed from neuroblastoma cells, with increased circulating levels of GD2 in patients with advanced stage neuroblastoma [147]. In patients over the age of 1 year with stage 4 disease, high circulating levels of GD2 are associated with more rapid disease progression [148]. More recently it has been demonstrated *in vitro* that dendritic cell differentiation and function is inhibited by gangliosides derived from neuroblastoma [149] and also from melanoma [150]. In adult non-small cell lung cancer specimens, high levels of the GM3 ganglioside correlate with decreased infiltration by $CD83^+$ mature dendritic cells [151] suggesting a similar anti-DC phenomenon. Undoubtedly, however, the role of gangliosides in individuals tumours is complex and individual molecules may have significantly different effects on the immune system. In neuroblastoma, for example, whilst GD2 expression is associated with more aggressive disease, other gangliosides (GD1b, GT1b and GQ1b) are

found at highest levels in low-risk tumours with favourable outcomes [152]. The possible biological roles of these gangliosides are yet to be established.

CONCLUSIONS

Recent years have seen something of a renaissance of interest in the interplay between cancer development and the immune system. It is clear that tumour cells do not grow in isolation and that their interactions with components of their microenvironment, including supporting stroma, endothelium and immune cells are of considerable importance in the development and progression of malignancy. There is a growing appreciation that the immune cells within tumours are far more than mere bystanders but rather contribute to the balance between tumour progression or regression. It is increasingly apparent that immune cell infiltration (for example, by $CD8^+$ cytotoxic lymphocytes, macrophages or dendritic cells) can be of prognostic significance, and that the exact phenotype of these cells and the balance between pro- and anti-inflammatory signals is of crucial importance. Furthermore, different tumours may employ a range of methods to avoid immune detection or to inhibit or tolerise the anti-tumour immune response (for example, down-regulation of MHC class I, activation of STAT3 signalling, expression of IDO). In the future, alongside identifying molecular pathways which directly contribute to tumour proliferation, it is likely that a detailed knowledge of the immune avoidance pathways activated in a particular tumour will be of importance in determining relevant treatment strategies and immunotherapy approaches. Although data relating specifically to paediatric cancers are rather limited, it is likely that similar principles apply. There is therefore a compelling need for a more detailed understanding of the patterns of immune cell infiltration in paediatric cancers and a better understanding of the immune avoidance mechanisms these tumours may employ. Finally, whilst the principles of immune avoidance are likely to be relevant to both adult-type carcinomas and paediatric cancers, it is important to consider the differences in the origins of adult versus paediatric cancers. Many adult carcinomas develop in the context of chronic inflammation, whilst this is probably much less relevant to the development of paediatric cancers. Thus, the role of the immune system and the interactions between the developing tumour and immune system may differ significantly between the two groups. Considerable care must therefore be taken in directly translating the results of studies in the adult population; it is important that paediatric tumours and the immune response in paediatric cancer patients are explored in their own right.

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