

# Pro- and anti-tumour effects of B cells and antibodies in cancer: a comparison of clinical studies and preclinical models

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**Abstract** The primary immune role of B cells is to produce antibodies, but they can also influence T cell function via antigen presentation and, in some contexts, immune regulation. Whether their roles in tumour immunity are similar to those in other chronic immune responses such as autoimmunity and chronic infection, where both pro- and anti-inflammatory roles have been described, remains controversial. Many studies have aimed to define the role of B cells in antitumor immune responses, but despite this considerable body of work, it is not yet possible to predict how they will affect immunity to any given tumour. In many human cancers, the presence of tumour-infiltrating B cells and tumour-reactive antibodies correlates with extended patient survival, and this clinical observation is supported by data from some animal models. On the other hand, T cell responses can be adversely affected by B cell production of immunoregulatory cytokines, a phenomenon that has been demonstrated in humans and in animal models. The isotype and concentration of tumour-reactive antibodies may also influence tumour progression. Recruitment of B cells into tumours may directly reflect the subtype and strength of the anti-tumour T cell response. As the response becomes chronic, B cells may attenuate T cell responses in an attempt to decrease host damage, similar to their described role in chronic infection and autoimmunity.

Understanding how B cell responses in cancer are related to the effectiveness of the overall anti-tumour response is likely to aid in the development of new therapeutic interventions against cancer.

**Keywords** B cells · Antibodies · Tumour models · Clinical correlations

## Abbreviations

Breg	Regulatory B cell
CXCR3	CXC chemokine receptor 3
DMBA	7,12-Dimethylbenz[a]anthracene
FcR	Fc receptor
FcεRI	High affinity IgE receptor Fc epsilon RI
FcεRII	Low affinity IgE receptor Fc epsilon RII
Flt3L	FMS-like tyrosine kinase 3 ligand
GRP	Gastrin-releasing peptide
HSV1-TK	Herpes simplex virus 1 thymidine kinase
MCA	Methylcholanthrene
PSA	Prostate-specific antigen
SCC	Squamous cell carcinoma
SEREX	Serological identification of antigens by recombinant expression cloning
Th1	T helper type 1
TILs	Tumour-infiltrating lymphocytes
TPA	Tetradecanoyl phorbol acetate
VCAM-1	Vascular cell adhesion protein 1

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## Clinical evidence

Correlations between the presence of tumour-infiltrating B cells and patient survival have been extensively reviewed recently [1], and only a brief summary will be provided here. Most studies demonstrate a positive association. It is

not clear from the clinical observations if this association is specifically related to beneficial effects of B cell functions such as antigen presentation, antibody production, or a combination of both. B cells may also be recruited into tumours in response to IFN- $\gamma$  production [2, 3] and thus serve as a surrogate marker of an effective T cell-mediated anti-tumour response.

### Associations between B cell infiltration and patient survival

Immunohistochemical analysis has indicated that B cell density in tumours and/or lymph node metastases correlates with an improved prognosis in melanoma [4], head and neck cancer [5] and ovarian cancer [6]. In general, fewer B cells are present in visceral metastases [7]. Interestingly, CD20<sup>+</sup> tumour-infiltrating lymphocytes (TILs) are often colocalised with CD8<sup>+</sup> TILs, which may reflect a micro-environment rich in IFN- $\gamma$ , recently reported to serve as a B cell tissue attractant via upregulation of CXC chemokine receptor 3 (CXCR3) [2] and/or vascular cell adhesion protein 1 (VCAM-1) [3]. This interpretation is consistent with the improved prognosis for ovarian cancer infiltrated with both B and T cells, compared with T cells alone [6] and the positive effects of intratumoural injections of recombinant human IL-12, a driver of IFN- $\gamma$  production and switching to Th1-associated serum antibodies such as human IgG1 [8]. An alternative explanation is that the presence of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and B cells, often organised into tertiary lymphoid structures, reflects the chronic nature of the anti-tumour immune response, and is analogous to that seen in chronic infectious and autoimmune diseases [9]. This interpretation is supported by the oligoclonal nature of B cell infiltrates and the presence of isotype switched, somatically mutated Ig transcripts, suggesting that tumour-infiltrating B cells have been selected for specific recognition of tumour antigens [6].

Bioinformatic analysis quantifying transcripts associated with B cells has proven effective in predicting prognosis in some breast cancer subtypes [10]. Thus, the combination of a “high B cell and low IL-8 gene signature” derived from a metagene expression prediction analysis was associated with a good prognosis in a subgroup of triple negative breast cancer patients [10]. B cell gene expression signatures identified using mRNA sequencing in primary breast cancer tissue also correlated with increased metastasis-free and progression-free survival in basal-like and HER2-enriched breast cancer, as well as in immunoreactive ovarian cancer [11]. In another study, expression of the immunoglobulin kappa C gene was as effective as the entire B cell metagene in predicting improved metastasis-free survival in a large series of breast, non-small cell lung carcinoma and colorectal cancer patients [12]. In cases in

which B cell receptor or antibody diversity was examined, the presence of isotype switching and somatic hypermutation indicated that B cells within the tumour microenvironment had received CD4<sup>+</sup> T cell help [6, 11]. Taken together, these studies suggest that B cell involvement in the tumour response is a positive clinical indicator, either as a reflection of a type I T cell response, or via intrinsic B cell functions.

### Effects of B cell depletion

While B cell-depleting antibodies such as rituximab are commonly used as cytotoxic agents in human B cell-related malignancies, few studies have targeted human B cells as a means of modulating the immune response against non-B cell tumours. Studies in renal cell carcinoma, melanoma [13] and colorectal carcinoma [14] reported no major clinical benefit. In a study in which B cells were depleted as an adjunct to chemotherapy for B cell lymphoma, overall survival was increased despite a concurrent increase in the rate of secondary solid tumour development in B cell-depleted patients [15]. The long-term effects of B cell depletion remain to be established definitively.

### Mouse models examining the role of B cells in anti-tumour immunity

#### T cell anti-tumour responses in B cell-deficient mice (Table 1)

Studies conducted in B cell-deficient mice have generally suggested that B cells inhibit rather than enhance spontaneous anti-tumour immunity. Early studies using chronic administration of anti-mouse IgM to deplete B cells reported suppression of the growth of subcutaneous methylcholanthrene (MCA)-induced T-10 tumour cells [16], as well as a longer time to tumour development in the MCA-induced skin carcinogenesis model [17]. Subsequent studies using genetically manipulated B cell-deficient mice such as  $\mu$ MT<sup>-/-</sup> (also known as Igh-6<sup>-/-</sup>) and JH<sup>-/-</sup> confirmed that the absence of B cells restricted tumour growth in a variety of tumour models. These included TS/A, an implantable mammary adenocarcinoma, J558L, a plasmacytoma, and CMS-5, a fibrosarcoma, all of which were rejected by  $\mu$ MT<sup>-/-</sup> but not wild-type mice vaccinated with irradiated tumour cells [18]. Increased proliferation of CD4<sup>+</sup> T cells and cytotoxic activity of CD8<sup>+</sup> T cells from  $\mu$ MT<sup>-/-</sup> mice was observed in response to immunisation with irradiated TS/A cells in this study, and the effect could be inhibited by adoptive transfer of B cells but not immune serum, suggesting direct effects of T-B collaboration on T cell function [18]. Vaccination with adenoviral vectors

**Table 1** Enhanced anti-tumour responses in B cell-deficient mice

Role of B cells	Model	Type of deficiency	Immune response	References
Negative	T-10 fibrosarcoma	Chronic depletion	Spontaneous	[16]
Negative	MCA-induced carcinogenesis	Chronic depletion	Spontaneous	[17]
Negative	TS/A mammary carcinoma J558L plasmacytoma CMS-5 fibrosarcoma	Genetic knockout	Vaccinated with irradiated tumour cells	[18]
Negative	B16 melanoma	Genetic knockout	Vaccinated with adenovirus expressing tumour antigen	[19]
Negative	EL4 thymoma MC38 colon carcinoma B16 melanoma	Genetic knockout	Spontaneous	[20]
Negative	EL4 thymoma MC38 colon carcinoma EMT6 breast carcinoma	Genetic knockout	Spontaneous	[21]
Negative	EMT-6 breast carcinoma	Genetic knockout	Spontaneous	[22]
Negative	D5 melanoma EL4gag thymoma	Genetic knockout	Spontaneous	[23]
Negative	PDSC5 and WDSC SCC lines	Genetic knockout	Spontaneous	[24]

expressing melanoma antigens prevented the growth of B16 melanoma in  $\mu\text{MT}^{-/-}$  but not wild-type mice and was associated with increase in the magnitude and longevity of the tumour-specific effector T cell response [19]. Shah et al. [20] showed that the growth of MC38 colon carcinoma, EL4 thymoma and B16 melanoma was retarded in unvaccinated  $\mu\text{MT}^{-/-}$  mice, and once again the effect was abrogated by adoptive transfer of B cells. Increased granzyme B and IFN- $\gamma$  expression and increased cytotoxic activity against MC38 targets were observed in T cells from  $\mu\text{MT}^{-/-}$  tumour-bearing mice in this study [20]. The inhibitory effect of B cells on the T cell response to MC38 tumour was dependent on B cell expression of OX40L, suggesting a role for T-B collaboration and Th2 differentiation in the suppression of T-dependent anti-tumour effects [21]. Adoptive transfer of wild-type B cells also reversed inhibition of EMT-6 mammary tumour growth in  $\mu\text{MT}^{-/-}$  mice [22]. In this case, the effect was associated with recruitment of regulatory T cells (Tregs) and B cells to the tumour and was independent of IL-10 [22]. In contrast, Inoue et al. [23] concluded that IL-10 production by B cells was a major cause of decreased IFN- $\gamma$  production and increased growth of EL-4 gag thymoma and D5 melanoma, but not MCA304 sarcoma, in wild type compared with  $\mu\text{MT}^{-/-}$  mice. Finally, growth of 2 orthotopic squamous cell carcinoma (SCC) cell lines was reduced in  $\text{JH}^{-/-}$  mice and correlated with increased CD8<sup>+</sup> T cell infiltration [24].

In contrast to anti-tumour responses, T cell responses to immunisation with protein antigens are reduced in  $\mu\text{MT}^{-/-}$  mice [25–27], and the effect is most pronounced for Th2 differentiation [28]. Reduction in priming to tumour antigens in chronically B cell-depleted animals has been shown for a Friend leukaemia virus-induced leukaemia [29].

### Abnormalities in B cell-deficient mice

While the field has gained a greater understanding of T cell and B cell tumour biology using the  $\mu\text{MT}^{-/-}$  mice system, it is important to remember that, from a clinical perspective, B cell-deficient mice do not model the situation in patients, whose B cell compartment remains relatively intact prior to and during cancer progression. Mice rendered genetically deficient in B cells manifest several secondary immune abnormalities that may contribute to their tumour-resistant phenotype. Dendritic cell production of the T helper type 1 (Th1) cell-stimulating cytokine IL-12 is increased in  $\mu\text{MT}^{-/-}$  mice [30]. In addition to the direct Th1-augmenting effects of IL-12 on T cells, enhanced IL-12 production may decrease B cell germinal centre reactions, reducing antibody affinity and the effect of normal B cell transfer into  $\mu\text{MT}^{-/-}$  mice [31]. T cell receptor diversity is also substantially reduced in  $\mu\text{MT}^{-/-}$  mice, reflecting the loss of thymic B cells [32]. B cell-deficient mice also have defects in myeloid cell subsets, including specific macrophage populations within the spleen [33].

### B cell depletion models (Table 2)

Given the immune abnormalities in mouse models with lifelong B cell deficiency, it may be more useful to assess the roles of B cells in anti-tumour immunity using acute B cell depletion. The majority of such models use antibody depletion, often combined with chemotherapy or vaccination, and suggest that B cells make a positive contribution to tumour clearance in a therapeutic setting. In a B16F10 melanoma model, acute B cell depletion using an anti-CD20 mAb before tumour challenge decreased the number

**Table 2** Modification of anti-tumour responses by B cell depletion

Role of B cells	Model	B cell manipulation	Immune response	References
Positive	B16 melanoma	Acute anti-CD20	Spontaneous	[34]
Positive	B16 melanoma	Acute anti-CD20	Therapeutic inhibition of CD73	[35]
Positive	GL26 glioblastoma	Acute anti-CD20 depletion before tumour challenge	AD-TK + Flt3L vaccination	[36]
Positive	FBL leukaemia	Chronic anti-IgM	Transfer of immune T cells	[29]
Negative	AB12 mesothelioma TC1 and LKR lung cancer EL4 thymoma	Acute anti-CD20	Spontaneous	[38]
Negative	TC1 lung cancer	Acute anti-CD20	Ad.E7 vaccination	[38]

**Table 3** Evidence for regulatory B cells

Role of B cells	Model	B cell manipulation	References
Negative	DMBA-/TPA-induced carcinogenesis	Genetic knockout	[40]
Negative	BL3750 lymphoma (CD20 <sup>+</sup> ) in WT or CD20 <sup>-/-</sup> hosts	Acute anti-CD20	[41]
Negative	4T1 breast carcinoma	Genetic knockout	[42]
Negative	TRAMP prostate tumour	B cell transfer	[43]
Negative	TRAMP prostate tumour Myc-CaP prostate cancer	Genetic knockout	[45]

of CD4<sup>+</sup> and CD8<sup>+</sup> T cell effector memory cells and the production of TNF- $\alpha$  and IFN- $\gamma$ , while subcutaneous tumour burden and lung metastases were increased [34]. A second study in the B16 model used anti-CD20-mediated B cell depletion to demonstrate that tumour regression in response to inhibition of CD73 was at least partly B cell dependent [35]. In the orthotopic GL26 model of glioblastoma in which tumour-protective T cell responses were induced by treatment with an adenovirus vector encoding the cytotoxic molecule herpes simplex virus 1 thymidine kinase (HSV1-TK) plus the cytokine FMS-like tyrosine kinase 3 ligand (Flt3L), overall survival was reduced by anti-CD20 depletion of B cells or anti-CD49d/anti-LFA depletion of marginal zone B cells [36]. Survival of mice that rejected primary tumours was severely affected if B cells were depleted prior to secondary challenge, indicating the importance of B cells for memory responses [36]. In addition, tumour control was also decreased in  $\mu$ MT<sup>-/-</sup> mice, in contrast to the results described above. It is possible that this difference is due to different effects of B cells in spontaneous anti-tumour responses, compared with those resulting from vaccination. By using Prdm<sup>fllox/fllox</sup>CD-19<sup>Cre/+</sup> mice in which antibody-secreting B cells expressing Blimp-1 are absent, Candolfi et al. elegantly showed that B cell enhancement of the response to vaccination was not dependent on antibody production and was thus likely to reflect antigen presentation by B cells. In a model of Friend murine leukaemia virus-induced leukaemia, chronic B cell depletion via an anti- $\mu$  mAb decreased CD4<sup>+</sup> and CD8<sup>+</sup>

T cell priming and cytotoxicity in response to vaccination with irradiated tumour cells or vaccinia virus-encoded antigen [29]. However, survival of tumour-bearing mice after treatment with cyclophosphamide and adoptive transfer of T cells from tumour-vaccinated mice was unaffected by the presence of B cells, suggesting that B cell involvement was limited to the priming phase of the vaccination response. This positive effect of B cells was reproduced in  $\mu$ MT<sup>-/-</sup> and CD19<sup>-/-</sup> mice vaccinated with tumour antigen-loaded exosomes [37].

In contrast to the studies described above, acute B cell depletion with an anti-CD20 mAb was reported to improve control in multiple tumour models by Kim et al. [38]. The effect was greater when B cell depletion was combined with vaccination with an adenovirus encoding a tumour antigen.

### B cells with regulatory function (Table 3)

Over the last decade, researchers have gained a greater understanding of the regulatory roles of B cells in chronic inflammation, autoimmunity and cancer. Regulatory B cell (Breg) function has been extensively reviewed previously [39], and only those aspects relevant to anti-tumour responses will be discussed briefly below. The ability of Breg cells to interfere with protective anti-tumour immune responses has been shown to involve production of IL-10 in a chemical carcinogenesis (7,12-dimethylbenz[a]anthracene/tetradecanoyl phorbol

acetate (DMBA/TPA)) model of SCC [40] and in the BL3750 lymphoma model [41]. TGF $\beta$ -dependent, IL-10-independent induction of Foxp3 expression in CD4<sup>+</sup> T cells was dependent on Bregs in the 4T1 model of murine breast cancer [42].

Secreted products from B cells that do not have a Breg phenotype may also have effects on tumour growth in vivo. In the case of prostate cancer, promotion of castration-resistant progression is at least partly dependent on a specific response of prostate epithelial cells to lymphotoxin derived from B cells [43, 44]. A recent report also implicated TGF $\beta$ -dependent B cells (plasmablasts) expressing PD-L1 and secreting IgA and IL-10 in resistance of prostate cancer to oxaliplatin chemotherapy [45]. These studies suggest that many different tumour-promoting mechanisms may be utilised by B cells in tumour models.

### Summary of B cell studies

B cell infiltration in human cancer is generally associated with an improvement in prognosis. Many mechanisms have been postulated but little direct functional evidence is available. A negative effect of B cells on spontaneous T cell responses in tumour-bearing animals has been convincingly demonstrated, whereas B cells enhance the anti-tumour response in many murine studies of therapeutic vaccination. Overall, acute B cell depletion models may reflect human disease and therapeutic approaches more accurately than models reliant on lifelong genetic deficiency of B cells.

## Antibodies in tumour immunity

### Differences in the arrangement of the IgH locus in humans and mice

Human and mouse antibody isotypes differ in both their chromosomal arrangement and function. In the human IgH locus, the constant region genes (in order moving distally from the V, D and J segments) encode IgM, IgD, **IgG3**, **IgG1**, IgA1, **IgG2**, **IgG4**, IgE and IgA2. The corresponding order in mice is IgM, IgD, **IgG3**, **IgG1**, **IgG2b**, **IgG2a**, IgE and IgA. Because isotype switching removes all upstream C regions, sequential gamma chain switching in response to persistent antigen in humans will result in production of IgG4, an isotype that has been linked to regulatory effects [46]. In contrast, the most distal murine gamma chain gene is IgG2a, one of the most pro-inflammatory isotypes [47]. This may be an important consideration when comparing chronic anti-tumour responses in mouse and man, along with the much shorter time frame in animal models.

### Correlations between serum antibody isotype distribution and patient survival

Human IgG4<sup>+</sup> B cells were reported to accumulate in melanoma tissue and their presence correlated with local IL-10 production, but not with a change in patient survival [48]. However, a relative increase in total (not tumour specific) serum IgG4 in patient serum was shown to correlate with decreased survival in this study, while there was a trend towards a positive correlation between serum IgG1 (essentially equivalent to murine IgG2a in its functions) and increased survival [48]. IgG4 was also shown to inhibit IgG1-dependent monocyte killing of tumour cells in vitro [48]. In a second study, an increase in total serum IgG4 was seen in metastatic melanoma compared to primary disease, and high titres were more likely in advanced disease [49].

There is also evidence that IgE may be involved in tumour clearance. In patients with pancreatic cancer, serum IgE was significantly increased and specific antibody-dependent cytotoxicity against pancreatic cancer cells could be demonstrated in vitro [50]. Enhanced survival of patients with glioma has also been associated with increased serum levels of IgE [51]. IgG4 has been shown to inhibit IgE-dependent pro-inflammatory functions mediated via IgE receptor Fc epsilon RI (Fc $\epsilon$ RI) and IgE receptor Fc epsilon RII (Fc $\epsilon$ RII) [52], suggesting a possible mechanism for the poor outcomes associated with high serum IgG4.

### Effects of human tumour-reactive antibodies

Numerous reports have identified tumour-specific antibodies in the serum of cancer patients [53]. Most anti-tumour antibody detection methods, including serological identification of antigens by recombinant expression cloning (SEREX), were designed to detect switched IgG antibodies, indicative of CD4<sup>+</sup> T cell involvement and possibly Treg induction directed against the same tumour antigen [54]. Relatively few studies have attempted to assess the effects of spontaneous tumour-specific antibody responses on patient survival. In a study of pancreatic cancer, the serum titre of antibodies specific for MUC1, a membrane-tethered mucin glycoprotein whose glycosylation is frequently altered in tumour cells, served as a prognostic marker of increased survival, independent of tumour stage [55]. In melanoma, no association between prognosis and antibody to at least one of a large panel of tumour antigens was found, although for a minority of individual tumour antigens, the prognosis was worse for patients with detectable serum antibody, excluding those with metastatic disease [56]. In colorectal carcinoma patients who had undergone complete resection, a correlation between tumour-specific antibody and worse prognosis was reported [57]. In



**Table 4** Evidence for tumour antigen-specific antibody effects

Role of antibody	Model	Antibody isotype	Additional therapy	Reference
Positive	B16 melanoma	IgG2a	Nil	[59]
Positive	B16 melanoma	IgG2a > IgG2b (not IgG1, IgG3)	Nil	[60]
Positive	B16 melanoma	IgG2a	Nil	[61]
Positive	B16 melanoma	IgG2a	Anti-VEGF receptor	[62]
Positive	MCA205 fibrosarcoma D5G6 melanoma	IgG2b	Chemotherapy or irradiation	[63]
Positive	HCC-97H human hepatocellular carcinoma	Unspecified	Nil	[64]
Positive	B16 melanoma	IgG2a	Peptide vaccine	[65]
Positive	RM1 prostate carcinoma (GRP-dependent growth)	Unspecified	GRP-vaccine	[66]
Positive	AsPC-1 human pancreatic carcinoma	Human IgG1	Nil	[67]
Positive	Human ovarian carcinoma	IgE (not IgG1)	Transfer of human lymphocytes	[68]
Positive	TSA-LACK mammary carcinoma	Human IgE	Vaccination, humanised Fc $\epsilon$ RI $\alpha$ -transgenic	[69]
Positive	CT26 prostate carcinoma expressing human PSA	Human IgE	Vaccination, humanised Fc $\epsilon$ RI $\alpha$ -transgenic	[70]
Negative	K14-HPV16 transgenic SCC	IgG1, IgG2a	Nil	[76]
Negative	K14-HPV16 transgenic SCC	IgG1, IgG2a	Nil	[77]

contrast, serum Abs produced by infiltrating B cells could not be detected in ovarian cancer [6]. In advanced melanoma patients treated with the CTLA-4-reactive mAb ipilimumab, antibodies specific for the tumour testis Ag NY-ESO-1 correlated with clinical benefit, although the effect could not be separated from that of enhanced CD8<sup>+</sup> T cell involvement [58].

#### Tumour-reactive antibodies in mouse models (Table 4)

To pinpoint the precise effects of tumour-specific antibodies, as opposed to tumour-specific B cells, hybridoma technology has been used to generate mAbs for administration in murine tumour models. Early studies using therapeutic administration of TA99, a mouse IgG2a isotype mAb specific for the melanoma Ag gp75, demonstrated positive effects against subcutaneous and metastatic B16F10 tumours [59]. Subsequent studies comparing the effect of antibodies of the same specificity but different isotypes indicated that IgG2a, and to a lesser extent IgG2b, protected mice from lung metastases after intravenous tumour cell inoculation, while IgG1 and IgG3 did not [60]. This phenomenon is due to the intrinsic functions of different Fc receptors (FcR) and has recently been reported to involve neutrophil-mediated cytotoxicity [61]. In addition, mAbs directed to tumour Ags may have different effects in vivo compared to in vitro due to changes in Ag expression. For example, gp75 is poorly expressed on the surface of cultured B16 cells, but is highly expressed in vivo [62].

Tumour-specific antibodies are particularly effective in metastasis models. These include the commonly used B16F10 melanoma model [60], MCA 205 fibrosarcoma and D5 melanoma [63] and a human HCC-97H hepatocellular carcinoma in nude (T cell-deficient) mice [64]. Several laboratories have also reported efficacy of antibodies against subcutaneous tumours. When combined with adjuvanted peptide vaccination, administration of TA99 was able to eradicate a proportion of subcutaneous B16F10 melanomas [65]. A vaccine against gastrin-releasing peptide (GRP) protected against growth of GRP-expressing RM-1 prostate cancer subcutaneous tumours and lung metastases, and immune serum could transfer partial protection [66]. In immunodeficient animal models, administration of purified anti-tumour antibodies was able to inhibit the growth of human pancreatic and colorectal cancer lines [67] and human ovarian cancer xenografts [68]. Recent studies of the anti-tumour effects of human IgE have made use of transgenic mice expressing the human high affinity Fc $\epsilon$ RI required for IgE isotype-dependent functions [69]. A human IgE specific for prostate-specific antigen (PSA) slowed the growth of CT26 prostate cancer cells expressing human PSA [70].

In many cases, the effects of B cells and antibody in mouse models have not been separated using techniques such as serum transfer or specific inactivation of antibody production. Studies that demonstrated an effect against metastases include that of Sorrentino et al., who showed that growth of B16F10 lung metastases was enhanced in  $\mu$ MT<sup>-/-</sup> mice, as were concentrations of IL-10 and

TGF $\beta$  in bronchoalveolar lavage [71]. Adoptively transferred B cells were protective, especially after activation with CpG-ODN [71], and this effect was also seen in T cell-deficient hosts [36]. In the 4T1 breast cancer model, tumour-specific IgG antibody produced after in vitro stimulation of in vivo primed B cells was shown to mediate in vitro complement-dependent tumour cell lysis [72]. In a second mammary carcinoma model, targeting human HER2/*neu* to B cells via a CD19 single chain variable fragment led to mixed IgG1, IgG2a and IgG2b antibody responses capable of activating complement and inhibiting human breast cancer cell growth in vitro [73]. Administration of the B cell-targeted fusion protein also inhibited the in vivo growth of subcutaneous D2F2/E2 mammary carcinoma cells expressing human HER2/*neu* [73]. In a second study in the D2F2/E2 model, administration of a human anti-HER2/*neu* IgE mAb prolonged survival of mice expressing the appropriate human Fc receptor, Fc $\epsilon$ R1 $\alpha$ , after intraperitoneal administration of D2F2/E2 tumour cells [74].

Recently, it has been suggested that the in vivo concentration of tumour-reactive antibodies may significantly affect whether they contribute to tumour progression or clearance [75]. These studies in the MC38 colon carcinoma model showed that polyclonal antisera specific for the Neu5Gc tumour Ag enhanced tumour growth at low concentrations but were inhibitory at higher concentrations. Higher avidity antibodies required lower concentrations for inhibitory effect.

### Antibody effects during cancer initiation

In a well characterised mouse model of virally induced SCC, immune complexes containing viral antigen were shown to be the primary drivers of chronic inflammation, which in turn strongly predisposed to malignancy [76]. Malignant transformation was dependent on the presence of both antibody and FcR $\gamma$ , which contributed directly to chronic skin inflammation [77] and a shift in macrophage phenotype from M2 to the more pro-inflammatory M1 [78]. Interestingly, the predominant isotype deposited in pre-neoplastic skin was mouse IgG1, which is poorly bound by activating Fc $\gamma$ Rs, and is not generally regarded as a pro-inflammatory isotype. Thus, the lower amounts of highly pro-inflammatory mouse IgG2a may be the major drivers of carcinogenesis in this model. While antibodies have a clear role in progression in this mouse model of SSC, B cell infiltration of tumours was not observed, in contrast to human head and neck SSC where the presence of peritumoural B cells correlated with increased survival [8]. Whether this difference is related to differences in species, oncogenic drivers or the stage of cancer progression remains to be determined.

### Summary of antibody studies

Overall these data suggest that tumour-specific antibodies may have diverse roles in anti-tumour immunity. The relative amounts of each antibody isotype and their affinity, the stage of tumour progression and the tumour cell surface Ag expression are all factors that should be considered when evaluating the efficiency of antibody protection in tumour models. Antibody-dependent effects may also be influenced by the number and type of in situ FcR-expressing cells.

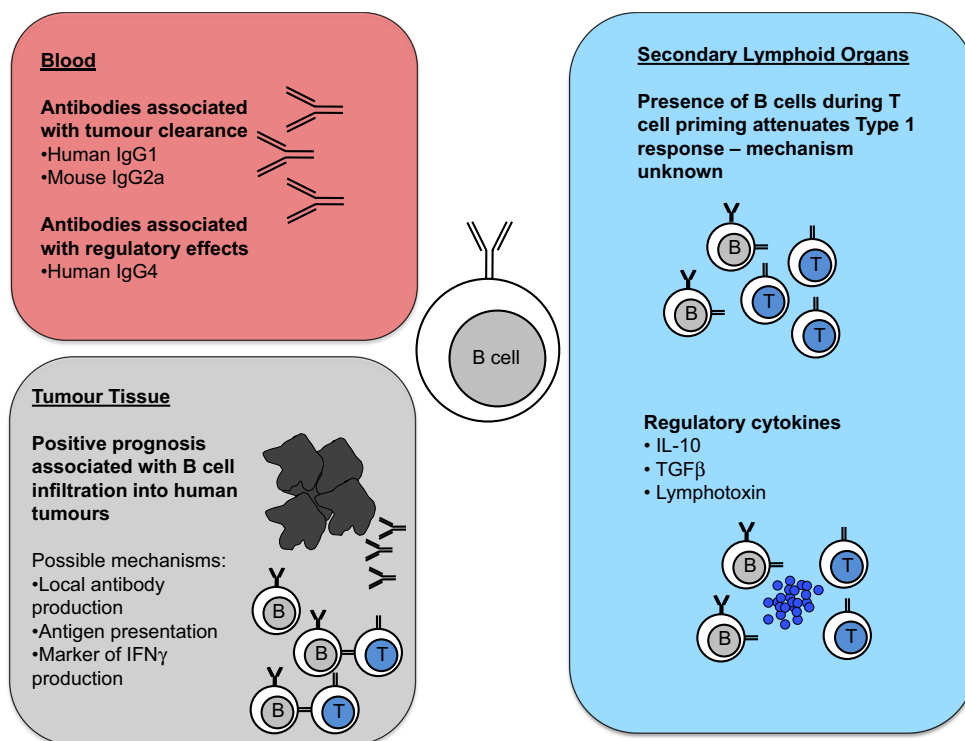
It is likely that the isotype and concentration of tumour-specific antibodies reflect the strength and status of the CD4<sup>+</sup> T cell response. Antibody may play an atypical role in driving malignant transformation in some cancers, but at the later stages of cancer progression, antibodies appear to have similar effects to those in other chronic immune responses.

### Conclusions

At first glance, it appears that B cells play fundamentally different roles in spontaneous anti-tumour immunity in mouse and man. They are generally associated with improved outcomes in clinical disease, and yet often produce worse outcomes in mouse models. Many hypotheses have been advanced to explain this apparent paradox, including differences between human and mouse immune systems, differences between the responses to naturally occurring and transplantable tumours and the artificiality of genetically modified mouse models that affect B cell viability and function.

Our view is that the differences between the conclusions drawn from human and mouse studies can be accommodated within a single model if the problem of anti-tumour immunity is considered from the wider perspective of how normal immune responses are regulated (Fig. 1). Despite the current emphasis on distinct types of immune response (Th1, Th2, Th17) and on responses involving only a single arm of the adaptive response (CD4<sup>+</sup> vs CD8<sup>+</sup> T cells, CD8<sup>+</sup> T cells vs B cells), most natural immune responses include a mixture of cell types, cytokines and differentiation states. It is highly unusual to see immune responses that do not simultaneously involve antigen-specific responses by CD4<sup>+</sup>, CD8<sup>+</sup> and B cells (although not all may contribute equally to pathogen clearance, autoimmunity or tumour control). When patients make spontaneous immune responses to their tumour, it is highly likely that all three cell types are involved. Whether or not all cell types are then recruited into primary tumour or metastases is not necessarily a direct reflection of their initial involvement in the anti-tumour response. Indeed, the presence of CD4<sup>+</sup>, CD8<sup>+</sup> and B cells within tertiary lymphoid follicles

**Fig. 1** Mechanisms by which B cells affect tumour growth



in tumours such as melanoma is likely to reflect chronicity of response, which is also associated with the presence of ectopic lymphoid tissue in chronic anti-pathogen responses and autoimmunity [79]. Not only is the immune response in most mouse tumour models of much shorter duration, but laboratory studies usually measure the functional immune response in lymph nodes and spleen, rather than documenting infiltration of different immune cells into the tumour.

Mouse models in which B cells are present when T cells are first primed against the tumour usually show detectable immune deviation away from the type 1 response that is highly effective in clearing most tumours. This phenomenon is not restricted to anti-tumour responses, but is a general property of T cell priming in the context of active T-B collaboration in both mouse and man. Published reports have focussed on those examples where this degree of immune deviation has significant effects on tumour growth in mice. However, this does not imply that effective anti-tumour immune responses cannot also be mounted by normal immune systems containing B cells—only that they may have been even more effective had B cells been artificially absent during priming.

In patients who have already made an anti-tumour response at presentation, the question of whether the response would have been stronger if B cells had not been present is not clinically relevant. How preclinical studies of B cells inform therapeutic options at this stage then involves two questions: are B cells currently exerting a positive or negative effect in patients who have already

mounted an anti-tumour response and will B cells exert a positive or negative effect in relation to future therapy?

Interestingly, in mouse models in which anti-tumour responses are modulated by vaccination, most studies have shown that B cell depletion has a negative effect, which may be related to enhanced B cell-dependent presentation of vaccine rather than naturally tumour-derived antigen. Acute B cell depletion during chronic anti-tumour responses is also detrimental in most cases. While this appears to run counter to the concept of Breg effects in cancer, B cell depletion removes both positive and negative effects of different B cell subsets. It is not yet clear whether targeted depletion of regulatory but not conventional B cells would be more beneficial and whether this will be possible in the clinic.

The effects of anti-tumour antibody in both mouse and man are dependent on isotype and rely on the same immune components, including FcRs and accessory cells such as neutrophils, monocytes and macrophages. The major species difference is that repeated isotype switching within IgG subclasses in humans generates IgG4, an isotype with regulatory function rather than the pro-inflammatory IgG2a isotype generated in mice. However, the relative importance of this difference in tumour immunity is unclear, particularly as IgG4 is a relatively minor component of human serum IgG.

In summary, there is little evidence to support B cell depletion as an immunoenhancing therapy in human non-B cell cancer. While priming of type 1 immune responses that



produce IFN- $\gamma$  and cytotoxic function is more efficient in the complete absence of B cells, evidence from many different models indicates a positive role for B cells in maintaining and enhancing anti-tumour immunity in both mouse and man.

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#### Compliance with ethical standards

**Conflict of interest** The authors have no conflicts of interest.

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