9 Tight Interplay Between Therapeutic Monoclonal Antibodies and the Tumour Microenvironment in Cancer Therapy

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Abstract

Therapeutic monoclonal antibodies (mAb) have changed the landscape of cancer therapy. With advances in the understanding of tumour biology and its microenvironment, different categories of mAbs have been developed; a first category is directed against tumour cells themselves, a second one comprises antibodies blocking the formation of neo-vasculature that accompanies tumour development, and, during the last decades, a third new category of immunomodulatory antibodies that target immune cells in the tumour microenvironment rather than cancer cells has emerged. In this chapter, we outline the main mechanisms of action of the different anti-tumour antibodies. We discuss the notion that, rather than passive immunotherapy that solely induces tumour cell killing, mAbs have multifaceted effects on the tumour microenvironment and could, qualitatively and quantitatively, reshape the immune infiltrate. We also discuss bystander effects of mAbs on the tumour microenvironment that should be carefully considered for the design of new therapeutic strategies.

Keywords

Immunotherapy · Monoclonal antibodies · Cancer therapy · Tumour microenvironment · IgG · Fab- and Fc-dependent mechanisms of action · Fc gamma receptors · Antibody-dependent cellular cytotoxicity · Antibody-dependent cellular phagocytosis · Innate immunity · Vaccinal effect · Long-term adaptive immunity · Immune checkpoints · Modulation of anti-tumour adaptive immunity · Bystander effects of monoclonal antibodies

9.1 Introduction

Forty years after their discovery by Milstein and Köhler, monoclonal antibodies are widely used for the treatment of cancer (Table 9.1). This success is partly due to the discovery of new therapeutic targets resulting from research advances in tumour biology and its microenvironment.

Lloyd Old and Ted Boyse's discovery of the first cell-surface differentiation antigens – used to

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Table 9.1 Approved or ongoing approval monoclonal antibodies in cancer regimen

International name	Class	Target	Indication	Approval year (EU/US)
Monoclonal antibodies targeting tumour-associated antigens				
Tositumomab-I131	Murine IgG2a	CD20	Non-Hodgkin lymphoma	NA/2003#
Rituximab	Chimeric IgG1	CD20	Non-Hodgkin lymphoma	1998/1997
Ibritumomab tiuxetan	Murine IgG1	CD20	Non-Hodgkin lymphoma	2004/2002
Ofatumumab	Human IgG1	CD20	Chronic lymphocytic leukaemia	2010/2009
Obinutuzumab	Humanized IgG1	CD20	Chronic lymphocytic leukaemia	2014/2013
Inotuzumab ozogamicin	Humanized IgG4 ADC	CD22	Acute lymphoblastic leukaemia	2017/2017
Moxetumomab pasudotox	Murine IgG1-immunotoxin	CD22	Hairy cell leukaemia	NA/2018
Blinatumomab	Murine bispecific antibody	CD19, CD3	Acute lymphoblastic leukaemia	2015/2014
Brentuximab vedotin	Chimeric IgG1 ADC	CD30	Hodgkin lymphoma, systemic anaplastic large cell lymphoma	2012/2011
Gemtuzumab ozogamicin	Humanized IgG4 ADC	CD33	Acute myeloid leukaemia	2018/2017; 2000#
Daratumumab	Human IgG1	CD38	Multiple myeloma	2016/2015
Isatuximab	Humanized IgG1	CD38	Multiple myeloma	2020/2020
Alemtuzumab	Humanized IgG1	CD52	Chronic myeloid leukaemia	2001#/2001#
Trastuzumab	Humanized IgG1	HER2	Breast cancer	2000/1998
Pertuzumab	Humanized IgG1	HER2	Breast cancer	2013/2012
Ado-trastuzumab emtansine	Humanized IgG1 ADC	HER2	Breast cancer	2013/2012
Dinutuximab	Chimeric IgG1	GD2	Neuroblastoma	2015/2015
Edrecolomab	Murine IgG2a	EpCAM	Colon cancer	1995#/NA
Catumaxomab	Rat/mouse bispecific mAb	EPCAM/CD3	Malignant ascites	2009#/NA
Elotuzumab	Humanized IgG1	SLAMF7	Multiple myeloma	2016/2015
Mogamulizumab	Humanized IgG1	CCR4	Sézary syndrome	2018/2018
Polatuzumab vedotin	Humanized IgG1 ADC	CD79b	Diffuse large B-cell lymphoma	2020/2019
Sacituzumab govitecan	Humanized IgG1 ADC	TROP-2	Triple-negative breast cancer	NA/2020
Monoclonal antibodies that interfere with tumour–stroma interactions				
Cetuximab	Chimeric IgG1	EGFR	Colorectal cancer	2004/2004
Panitumumab	Human IgG2	EGFR	Colorectal cancer	2007/2006
Necitumumab	Human IgG1	EGFR	Non-small-cell lung cancer	2015/2015
Bevacizumab	Humanized IgG1	VEGF	Colorectal cancer	2005/2004
Ramucirumab	Human IgG1	VEGFR2	Gastric cancer	2014/2014
Olaratumab	Human IgG1	PDGFRα	Soft tissue sarcoma	2016/2016
Monoclonal antibodies that exert direct immunostimulatory effects				
Ipilimumab	Human IgG1	CTLA-4	Metastatic melanoma	2011/2011
Nivolumab	Human IgG4	PD1	Melanoma, non-small-cell lung cancer	2015/2014
Pembrolizumab	Humanized IgG4	PD1	Melanoma	2015/2014
Cemiplimab	Human IgG4	PD-1	Cutaneous squamous cell carcinoma	2019/2018
Atezolizumab	Humanized IgG1	PD-L1	Bladder cancer	2017/2016
Avelumab	Human IgG1	PD-L1	Merkel cell carcinoma	2017/2017
Durvalumab	Human IgG1	PD-L1	Bladder cancer	2018/2017

Source: '*The Antibody Society*' (https://www.antibodysociety.org/resources/approved-antibodies/)
Withdrawn or marketing discontinued, *NA* not approved, *ADC* antibody-drug conjugate

distinguish lineage and functional subsets of leucocytes [1, 2] – led to the CD (cluster of differentiation) classification and the wide use of cell-surface markers to distinguish between normal and malignant cells. Anti-tumour antibodies can be broadly classified into three categories: 1. Antibodies targeting CD antigens expressed specifically by tumour cells of the hematopoietic lineage: lymphocytes (CD20, CD22 CD38) and myeloid cells (CD30, CD33) (Table 9.1). 2. Antibodies that are directed against tumour-associated antigens (HER2/neu, MUC1, CEA, EGFR) – a number of molecules overexpressed by tumour cells discovered using tumour genetics [3–7] (Table 9.1). 3. Antibodies targeting the tumour microenvironment (TME). Established tumours are complex tissues composed not only of tumour cells but also of stromal and mesenchymal cells, vasculature components and immune cells. Tumour-derived factors stimulate blood vessel growth that in turn sustains tumour progression leading to the hypothesis that anti-angiogenesis agents might be an effective anti-cancer strategy [8–11]. The isolation of vascular endothelial growth factor (VEGF) – an endothelial-cell mitogen and a key regulator of angiogenesis in the TME – and the demonstration that an anti-VEGF mAb inhibits tumour growth in different preclinical models, led to the development and approval in 2004 of bevacizumab (humanized IgG1 anti-VEGF mAb) for clinical use in cancer patients [11, 12]. Other antibodies blocking neo-vasculature formation that accompanies tumour development have been subsequently developed, notably anti-VEGFR2, -PDGFRα mAbs (Table 9.1).

Control of tumour growth is largely dependent on the quantity and quality of the tumour immune infiltrate [13, 14]. The role of immunity in the control of tumours, although suggested as early as 1957 by Burnet [15] has been neglected for a long time. Studies in 1957 clearly showed that tumours harbour immunological determinants capable of eliciting anti-tumour immunity and long-term immune memory [16]. Consistent with these observations, the team of Boon reported that autologous cytotoxic T-lymphocytes (CTL) from melanoma patients recognize self-peptides derived from MAGE-1 protein expressed on tumours [17]. MAGE-1 is the first member of a larger family of proteins called the cancer testis (CT) antigen family – expressed only in tumours and in germ cells – which has been widely used in vaccination assays to elicit anti-tumour T-cell immunity. The use of genetically modified mouse models of immunodeficiency revealed the key role of immune components in tumour growth control; such as IFN-γ signalling, perforin molecules and the T-cell compartment. These preclinical data have incited interest in the understanding of cancer surveillance [18]. From the concept of 'the three Es' of cancer immunoediting defined by Schreiber's group; elimination – corresponding to immunosurveillance of tumour growth by intratumoural immunity; equilibrium – representing the process by which immune attack induces the selection of resistant tumour cell variants; and escape – the process by which tumour cells escape immune control, came the finding that the immune system not only protects the host against tumour development but can also reshape the immunogenic phenotype of a developing tumour [18]. Studies performed on large cohorts of patients with cancer reveal correlations between the presence of tumour-infiltrating lymphocytes (TILs) and patient survival [13, 18, 19]. A favourable clinical outcome is often associated with the presence of tertiary lymphoid structures (TLS) – ectopic lymphoid formations that contain components required for the generation of an adaptive immune response including B-cell germinal centres, T-cell zones, mature dendritic cells and follicular dendritic cells [20]. These basic and clinical observations have paved the way to the development of a new category of immunomodulatory antibodies that target immune cells within the TME. Particularly, antibodies directed against regulatory receptors or immune checkpoint (ICP) molecules on immune cells have emerged over the last decade [21], as exemplified by the success of anti-CTLA-4 or anti-PD-1 antibodies in clinics. In the late 1990s, James P. Allison and T. Honjo (both awarded with the Nobel Prize in 2018) demonstrated, in preclinical tumour models, that the expression of inhibitory ICP on intratumoural T cells dampens

their anti-tumour activity and that blockade of PD-1/PD-L1 or CTLA-4 pathways using mAbs dramatically halts tumour development [22, 23]. These pioneering studies reveal anti-ICP mAbs as a promising strategy for specific tumour immunotherapy and have revolutionized the landscape of cancer therapy.

In this chapter, we will present the Fab- and Fc-dependent mechanisms of action of different categories of anti-tumour antibodies.

9.2 Direct Fab- and Fc-Dependent Mechanisms of Action of Therapeutic Monoclonal Antibodies in Cancer

Initially, it was thought that anti-tumour antibodies acted by rapidly recruiting blocking/killer mechanisms on tumour cells. Thus, use of mAbs was considered until recently more as passive immunotherapy based on their transient ability to block cancer cell activation and/or proliferation (i.e. by targeting growth receptors such as EGF-R or HER2/Neu (erbB-2)), to induce apoptosis (even marginal by HER2/neu, CD20), or to interfere with the adhesion of tumour cells (EpCAM) blocking the formation of metastases. However, the expression of mAb-targets on cancer cells and in the tumour area is not necessarily predictive of response to treatment. For example, although the presence of EGFR-positive tumour cells is a requirement for colorectal cancer patients to receive cetuximab and panitumumab (anti-EGFR mAbs), EGFR expression at the protein or mRNA level has not been correlated with treatment response [24], suggesting that the mechanism of activity of these mAbs may also be related to their effect on tumour infiltrating immune cells. Similarly, although the use of antibodies targeting the VEGF pathway has shown clinical benefits associated with a reduction in tumour blood vessel density, the direct neutralization of VEGF-driven vascular effects explains only part of their therapeutic effect. VEGF inhibitors, particularly bevacizumab, not only induce vessel normalization – associated with increased tumour blood perfusion, restoration of adhesion molecules on endothelial cells and improved influx of leucocytes into the tumour – but also activate and modulate the function of immune cells within the TME [12, 25]. Anti-VEGF and/or anti-VEGFR mAbs have an impact on the frequency of regulatory T cells and of tumour-infiltrating myeloid-derived suppressor cells (MDSCs) and reinvigorate dysfunctional DCs [12, 25].

Part of the therapeutic effects of anti-angiogenic antibodies can be triggered by Fc/FcγR interactions. Most of the marketed therapeutic antibodies are human IgG1 (either chimerized, humanized or fully human antibodies), the most efficient human IgG subclass, together with IgG3, in engaging FcγR and activating the complement cascade. Anti-tumour antibodies can trigger effector mechanisms leading to tumour cell death, such as complement-dependent cytotoxicity (CDC), antibody-dependent cell cytotoxicity (ADCC), antibody-dependent cell phagocytosis (ADCP). The activation of the classical pathway of complement through the binding of C1q to the Fc portion of mAbs and the recruitment of Fcγ receptors (FcγRs) expressed by NK cells, neutrophils, monocytes and macrophages leads to the formation and/or the release of effector molecules (membrane attack complex made of C5b-C9, perforin and granzymes, TNF-α, reactive oxygen intermediates (ROI), etc.) that induce cell death. ADCC and ADCP in myeloid cells through the engagement of FcγR are considered to play an important role in the in vivo efficacy of anti-tumour antibodies both in pre-clinical tumour models and in treated cancer patients [26]. Macrophages in tumour tissues are important for the efficacy of therapeutic antibodies thanks to their expression of different types of FcγR, enabling ADCP. Several studies provide evidence that macrophages are effector targets of therapeutic antibodies in cancer; in vitro human macrophages phagocytose tumour cells in response to

anti-CD20 (rituximab) and anti-HER2/neu mAbs (trastuzumab) [27–29] and, in vivo, macrophages have been associated with a better response to trastuzumab [30, 31]. Interestingly, all human IgG subclasses, including isotypes that exhibit low NK cell-mediated ADCC due to their poor binding to FcγRIIIa, have the potential to engage other FcγR (FcγRI and FcRγIIa) expressed in macrophages and to stimulate macrophage-dependent phagocytosis [32]. Significant correlations of FcγR polymorphisms with clinical outcome in patients treated with rituximab [33, 34], trastuzumab [35, 36] and cetuximab [37, 38], argue in favour of a role for FcγR$^+$ immune cells in the TME in the clinical response to mAb-based treatment. However, studies reveal no such associations in patients with breast cancer [39], raising the possibility of additional immune mechanisms that account for the clinical benefit of mAb-based immunotherapy. Notably, the duration and strength of the clinical responses following mAb treatment can be linked to the ability of tumour antigen-specific mAb to elicit adaptive cellular immunity via the activation of antigen presenting cells, as described later in this chapter.

FcγR/FcR interactions are also implicated in the anti-tumour activity of anti-ICP mAbs [40]. One underlying mechanism of the anti-tumour activity of anti-GITR, -OX40, -CTLA-4 and -TIGIT antibodies is through intratumoural depletion of regulatory T cells via FcγR$^+$ myeloid effector cells [40–46]. Consistent with these preclinical studies, melanoma patients with higher frequencies of FcγRIIIA$^+$ myeloid effector cells in the peripheral blood show higher response to ipilimumab treatment which is attributed to ADCC/ADCP mediated depletion of Treg in the TME [45]. Recently, Waight et al., reported an FcγR-dependent mechanism of action of anti-CTLA-4 mAbs that is independent of Treg depletion [46]. Engagement of activating FcγRIIIA on APCs by anti-CTLA-4, anti-TIGIT and anti-CD45RB antibodies mAbs improves T-cell activity by modulating both TCR and CD28 signalling [46].

9.3 Therapeutic Antibodies Reshape the Tumour Microenvironment

9.3.1 From Tumour Cell Destruction to the Triggering of Long-Term Adaptive Anti-tumour Immunity

In addition to the anti-tumour effects triggered by mAbs treatment on innate immunity, evidence suggests that these agents might also affect the local inflammatory and immune microenvironment [13]. Clinical data and in vivo animal models suggest that antibody treatment leading to tumour cell killing induces long-term anti-tumour responses by triggering target-specific adaptive memory responses, a phenomenon that has been termed the 'vaccinal' effect of antibody treatment [47]. Specific T- and B-cell responses are reported in cancer patients following therapy with anti-CA125 [48], anti-MUC1 [49], anti-HER2/neu [50, 51] and anti-EGF-R [52] mAbs. Studies in murine models also report that the therapeutic effect of anti-CD20 [53–56], anti-HER2/neu [57–60], or anti-EGF-R [61] mAbs depends on the induction of an adaptive immune response and on the presence of T cells. The anti-HER2/neu studies reveal an antibody-mediated mechanism in which danger signals activate both innate and T-cell-mediated immune responses [57–60]. A role for dendritic cells (DC) and macrophages at the tumour site in this vaccinal effect is supported by the ability of these cells to internalize – in an FcγR-dependent manner – exogenous IgG-complexed antigens (probably derived from tumour cell debris), and to present MHC II and MHC I-restricted peptides derived from these complexes [62–65]. In a human glioma model, FcγR-dependent engulfment of cetuximab-coated glial tumour cells by DCs leads to an increase in anti-tumour CD8$^+$ T cells [65]. Several studies demonstrate that upon mAb therapy, a cross-talk between NK cells and DCs can occur [52, 66, 67]. Cetuximab-activated NK cells result in enhanced cross-presentation of EGF-R-derived peptides to specific CTL [52].

Interestingly, it has been reported that human macrophages and DCs equally present tumour-associated antigens to $CD8^+$ T cells after phagocytosis of γ-irradiated melanoma cells [68]. One can thus hypothesize that phagocytosis of mAb-coated immune complexes by $Fc\gamma R^+$ macrophages also leads to an efficient activation of $CD8^+$ T cells. Nevertheless, the extent to which both APCs process and cross-present non-mutated tumour-associated antigens within the tumour microenvironment to prime T cells in situ has yet to be clarified. Non-mutated self-proteins overexpressed by tumour cells are universal target antigens to induce tumour-specific T-lymphocytes without the need to identify the mutanome of tumour cells. Recent results demonstrate that thymic deletion prunes but does not eliminate self-specific $CD4^+$ and $CD8^+$ T cells, and that some self-peptide-specific T cells can be detected at frequencies similar to T cells specific for non-self-antigens [69–72]. We also found that $CD4^+$ T cells against non-mutated human CD20-derived peptides are present in healthy donors and lymphoma patients [73]. While T-cell responses against these self-derived epitopes can be limited by a self-tolerant T-cell repertoire, it has been demonstrated that anti-CA125, anti-HER2/neu, anti-MUC1 and anti-EGFR mAb treatment can circumvent this tolerance as shown by the increase in frequencies of $CD4^+$ and/or $CD8^+$ T cells recognizing peptides derived from the target molecule in cancer patients [48–52].

9.3.2 Effects of Immunomodulatory mAbs on Lymphoid and Myeloid Compartments Within the Tumour Microenvironment

In the last decade, therapeutic mAbs directed against inhibitory checkpoints have changed the landscape of cancer therapy. Clinical studies have demonstrated that these antibodies can induce durable clinical responses even in patients with advanced cancer [74–76]. Of the many different checkpoint receptors, the cytotoxic T-lymphocyte antigen-4 (CTLA-4), as well as PD-1 and its ligands, PD-L1 and PD-L2, are most intensely studied. CTLA-4, expressed on T cells, is an early contributor to the development of immune tolerance. It negatively controls the priming and early antigen-dependent T-cell activation in lymphoid organs, and is also expressed in regulatory T cells (Treg). CTLA-4 inhibition is used with the aim of stimulating T-cell activation and, subsequently, anti-tumour immune responses. Ipilimumab, a human IgG1 anti-CTLA-4 mAb, which was the first anti-ICP mAb to demonstrate survival benefit for patients with metastatic melanoma, received Federal Drug Administration (FDA) approval for melanoma treatment in 2011 and is currently in clinical trials in various cancers, including lung, colorectal, bladder, renal and prostate cancer (https://www.cancer.gov/about-cancer/treatment/clinical-trials/intervention/ipilimumab?pn=4). PD-1 is a checkpoint inhibitor of T cells within peripheral tissues and the tumour microenvironment. PD-1 is also highly expressed in intratumoural Treg cells and might enhance the immunosuppressive activity of these cells. MAbs that target the PD-1/PD-L1 axis are approved for the treatment of patients with melanoma, cutaneous squamous cell carcinoma, non-small-cell lung cancer, bladder cancer and Merkel cell carcinoma (Table 9.1).

Overall changes in the tumour microenvironment during ICP therapy, both in preclinical models and in treated patients, have been comprehensively analysed through longitudinal gene expression studies as well as high-dimensional profiling approaches, such as mass cytometry and single-cell RNA sequencing [77–82]. Major changes in tumour- and immune-associated genes are reported in melanoma patients who exhibit clinical activity following ipilimumab (anti-CTLA-4 mAb) therapy [79]. A lower expression was observed for genes encoding tumour antigens (e.g. members of the MAGEA family, NY-ESO-1, MLANA), for genes involved in dermatological phenotype and functions (e.g. SOX10, MITF, two key transcription regulators in melanocytes, and tyrosinases TYR and TYRP1, involved in melanin synthesis) and for genes implicated in cell growth and differentiation (e.g. MYC, MXI1, IGF1R, CDK2, CCND1,

BIRC7, HRK and TNFRSF10B). By contrast, many IFN-γ-inducible genes and Th1-associated markers (e.g. PRF1, TAP1 and GZMB) increased after ipilimumab treatment, suggesting an accumulation of this type of T cells at the tumour site, which might play an important role in mediating the antitumour activity of ipilimumab [79].

It is generally assumed that ICP blockade (anti-PD-1, anti-CTLA-4 mAbs) can restore antitumour activity in dysfunctional infiltrating immune cells. Checkpoint inhibitors amplify preexisting T-cell responses, broaden the range of antigens being targeted by the T-cell repertoire, and induce T-cell-mediated immune responses against tumour neoantigens [82–85]. A whole-exome and transcriptome analysis in tumours from patients with advanced melanoma treated with nivolumab (anti-PD-1 mAb) shows that mutation and neoantigen load reduce from baseline in responding patients [77]. Interestingly, in responding patients, T-cell clones expand in proportion to the number of neoantigen mutations that disappear on therapy, suggesting an effective immune elimination of tumour cells containing non-synonymous mutations and neoantigens, and a selective pressure against the generation of antigenic mutations.

Analysis of changes in the TME in tumours of mice treated with anti-CTLA-4 and/or anti-PD-1 mAbs by mass cytometry and single-cell RNA sequencing demonstrates that anti-ICP mAbs induce both quantitative and qualitative changes in intratumoural $CD4^+$ and $CD8^+$ T cells as well as NK cell subsets. The dramatic reduction in Treg frequency and suppressive functions, and the remodelling of the $CD4^+$ and $CD8^+$ T-cell compartments, lead to increased expression of an anti-tumour effector gene signature (e.g. *Ifng, Gzmb*). This study also shows that anti-ICP therapy induces a shift towards a more activated $CD4^+$ T-cell compartment that expresses high levels of IFN-γ. T-cell activation markers are also altered: anti-CTLA-4 decreases the expression of TIM-3, LAG-3 and PD-1 in tumour neoantigen-specific $CD8^+$ T cells, while anti-PD-1 therapy decreases the expression of LAG-3 and PD-1 [80]. Recent work from the Allison group in murine tumour models and human melanomas show that the clinical activity of anti-CTLA-4 or anti-PD-1 mAbs relies on distinct effects on intratumoural T-cell subsets [81]. Both antibodies induce the expansion of specific tumour infiltrating T-cell subsets. Anti-PD-1 mAb predominantly expands exhausted tumour infiltrating $CD8^+$ T cells, while anti-CTLA-4, but not anti-PD-1, modulates the $CD4^+$ T-cell compartment, particularly by expanding an $ICOS^+$ Th1-like $CD4^+$ effector subset. Differences in the impact of the two mAbs on specific subsets of lymphoid cells are also reported in the work of Gubin et al. [80].

Recent studies suggest that durable clinical responses to immunotherapy also depend on bystander effects on T-cell subsets that do not express ICP molecules. Indeed, $PD\text{-}1^+$ $CD8^+$ T cells have limited potential to give rise to a long-lasting effector response due to their acquisition of a stable epigenetic state that cannot be reverted by ICP blockade [86–90]. In a preclinical model of colon cancer, Kurtulus et al. examined changes in the RNA profiles of intratumoural $CD8^+$ T cells after TIM-3/PD-1 blockade [91]. Two TIL populations with either high ($PD\text{-}1^+TIM3^+$) or low ($PD\text{-}1^-TIM3^-$) dysfunctional state acquired an effector profile following TIM3/PD-1 blockade. Interestingly, the $PD\text{-}1^-TIM3^-$ subset showed more profound changes than $PD\text{-}1^+TIM3^+$ subset. TIM3/PD-1 blockade increased the frequency of $PD1^-$ T-cell subsets bearing characteristics of effector and memory precursor-like cells, indicating that the treatment led to indirect changes in pre-existing populations in the TME. This memory-precursor-like subset requires the transcription factor Tcf7 and shares features with $CD8^+$ T cells that respond to checkpoint blockade in patients [91].

Intratumoural monocytes and macrophages also undergo striking remodelling following anti-ICP mAbs. While $CXC3CR1^+$ $CD206^+$ macrophages – CD206 is a marker of anti-inflammatory M2 macrophages – are present in progressively growing tumours in mice infused with control mAb, they dramatically reduce in response to anti-PD-1 and/or anti-CTLA-4 mAb therapy [80]. The therapy also leads to an accumulation of myeloid cells expressing high levels of *Nos2*

(iNOS), a marker of IFN-γ activated, pro-inflammatory macrophages. Indeed, IFN-γ production, as a consequence of T-cell reinvigoration following anti-ICP mAbs therapy, positively drives polarization of newly arrived monocytes towards iNOS-positive macrophages with anti-tumour activity [80]. In line with this observation, in patients with advanced melanoma treated with nivolumab (anti-PD-1 mAb) therapy, changes in macrophage-associated genes in tumours are associated with better clinical responses, suggesting that macrophages may play an important role in response to anti-ICP mAbs [77].

Several studies have shown that the tumour stroma can be a major target of anti-ICP therapy. As an example, clinical activity of agonist mAb anti-CD40 (developed to mimic CD40L engagement on T cells and to increase T-cell priming) can be a result of anti-CD40-dependent alteration of tumour stroma [92]. In a mouse model of pancreatic ductal adenocarcinoma, anti-CD40 mAb induces tumour regression by the recruitment and activation of circulating macrophages, which then translocate to tumour tissues and degrade the tumour stroma (displaying a decrease in collagen I content, consistent with degradation of the tumour matrix) [92]. Studies also reveal side effects leading to a cytokine storm and lethality, following systemic injection of CD40 agonist antibodies together with IL-2 in aged mice and young obese mice [93, 94]. In these mice, higher percentages of TNF^+-activated macrophages are detected in tissues following therapy as compared to young mice. This suggests a link between the hyper-inflammatory cytokine response to systemic immune stimulation and the increase in visceral fat observed in aged or young obese mice [94].

9.3.3 When Therapeutic mAbs are 'Not-So-Good Guys'

The CD40/CD40L story is an interesting case demonstrating that monoclonal antibodies are more than passive immunotherapy agents, and some of them may have multifaceted – beneficial or detrimental – effects on the tumour microenvironment and on anti-tumour immunity.

In a large clinical trial in metastatic colorectal cancer, the addition of cetuximab (anti-EGFR) to bevacizumab (anti-VEGF) plus chemotherapy resulted in decreased progression-free survival [95]. Pander et al. show that M2 macrophages present abundantly in colon carcinoma are activated by cetuximab-opsonized tumour cells, resulting in anti-inflammatory and tumour-promoting factors production, including IL-10 and VEGF. They suggest that this effect might explain the negative clinical effect of cetuximab in colon cancer [96]. In bevacizumab-resistant patient glioblastomas, the therapeutic mAb directly binds to the macrophage migration inhibitory factor (MIF) from the TME and blocks MIF-induced M1 polarization of macrophages, resulting in more M2 pro-tumoral macrophages [97]. Moreover, as VEGF increases glioma MIF production in a VEGFR2-dependent manner, bevacizumab-induced VEFG depletion down-regulates MIF in TME. Nevertheless, it should be noted that other studies in different microenvironments have reported beneficial effects of MIF down-regulation or deletion, including increased intra-tumoural effector $CD4^+$ and $CD8^+$ T cells [98, 99], reduced regulatory T cells [98], reduced MDSCs in the tumour [100] and higher numbers of activated DCs [99].

Moreover, mAbs, as therapeutic agents that actively reshape the microenvironment, could in some conditions induce immunosuppressive molecules. It has been reported that the numbers of $CD4^+$, $CD8^+$ T cells and $CD68^+$ macrophages expressing PD-L1 and VISTA inhibitory immune checkpoints increased in the prostate tumour microenvironment after ipilimumab therapy (anti-CTLA-4 mAb) [101]. This suggests that VISTA might represent a compensatory inhibitory pathway in ipilimumab-treated prostate cancer that is poorly responsive to immune checkpoint monotherapy. The authors also show that ipilimumab leads to an increase in $PD\text{-}L1^+$ and $VISTA^+$ macrophages expressing CD163 and ARG1, suggesting a shift towards an M2-like phenotype and function of these cells [101]. Furthermore, whereas antibody-dependent cellu-

lar cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) are two main mechanisms that critically contribute to the efficacy of anti-tumour therapeutic antibodies, a recent study reports that ADCP results in an immunosuppressive phenotype of tumour-associated macrophages with overexpression of inhibitory molecules PD-L1 and indoleamine 2,3-dioxygenase (IDO) [102]. Macrophages that undergo ADCP upon rituximab (anti-CD20) or trastuzumab (anti-Her2/Neu) mAbs treatment subsequently inhibit NK-cell-mediated ADCC and T-cell-mediated cytotoxicity in lymphomas and breast cancers. This study reveals a deleterious role of ADCP in macrophages that can be overcome with concomitant immune checkpoint blockade [102].

Interestingly, different studies also report that FcγR engagement by anti-ICP mAbs dampens anti-tumour activity of the mAbs. In a preclinical model, negative effects of FcγR recruitment was observed for two anti-PD-1 mAbs recognizing different epitopes on PD-1. The mechanism by which FcγR engagement reduces anti-tumour activity is different for the two mAbs. For one mAb, engagement of high affinity activating FcγRI results in the elimination of intratumoural $CD8^+$ effector cells. For the other mAb, the reduced activity relies on its binding to inhibitory FcγRIIB [103]. Anti-PD-1 mAbs (nivolumab, pembrolizumab and cemiplimab) are of the IgG4 isotype which has reduced ADCC and 'null' CDC. However, IgG4 binds to FcγRI and FcγRIIB, and these interactions can have clinical consequences. In vivo imaging studies reveal that a rat IgG2a anti-PD-1 mAb (that is used to mimic the biological property of human IgG4) can be captured from PD-1^+ T-cell surfaces by PD-1^- tumour-associated macrophages. This transfer limits anti-tumour efficacy of the therapeutic mAb [104]. More recently, hyperprogression observed in cancer patients treated with anti-PD-1 mAbs has been linked to the interactions of the mAbs with FcγR$^+$ M2 macrophages [105]. A possible role of inhibitory FcγRIIB is suggested by the authors of this work.

These different observations outline deleterious effects of mAbs on anti-tumour immunity, and should be carefully considered for the design of therapeutic strategies in cancer patients.

9.4 Concluding Remarks

Besides the direct impact on tumour growth, mAbs therapies can have remarkable effects on the network of cells within the TME, including (i) induction of long-term anti-tumour adaptive immunity by APC-mediated uptake and presentation of tumour antigens released upon cell death, (ii) durable modulation of the range of immune cells reactive against the tumour and (iii) overall reshaping of the myeloid and lymphoid compartments within the TME (Fig. 9.1a). Bystander effects of therapeutic mAbs can also occur, leading to deleterious inflammation and/or decreased anti-tumour immune responses (Fig. 9.1b). In this case, the underlying mechanisms should be carefully considered to overcome these negative effects with concomitant treatment to reduce inflammatory symptoms or by blocking additional inhibitory pathways.

Immunotherapies in patients with solid tumours include mAbs targeting tumour cells, the tumour vasculature and/or immune cells within the TME. Multiple immune evasion mechanisms can be used by tumours; immunosuppression or exhaustion in the TME, biological or physical barriers around the tumour that inhibit or prevent immune cell infiltration and poor antigen presentation due to a lack of antigens or of antigen-presenting cells. Thus, combinations of antibodies against different targets within the TME can circumvent the current limitations of single antibody therapies. Numerous mAb combinations are under investigation in clinical trials (i.e. antibodies against either different epitopes of the same molecule or different targets on the same tumour cell; anti-angiogenic antibodies combined with tumour-targeting or immunomodulatory mAbs; combinations of antibodies targeting different ICP molecules; anti-ICP mAbs combined with mAbs directed against cytokines, etc.) [106]. Bispecific or multispecific antibodies that simultaneously target tumour cells and immune effector cells are also being currently developed

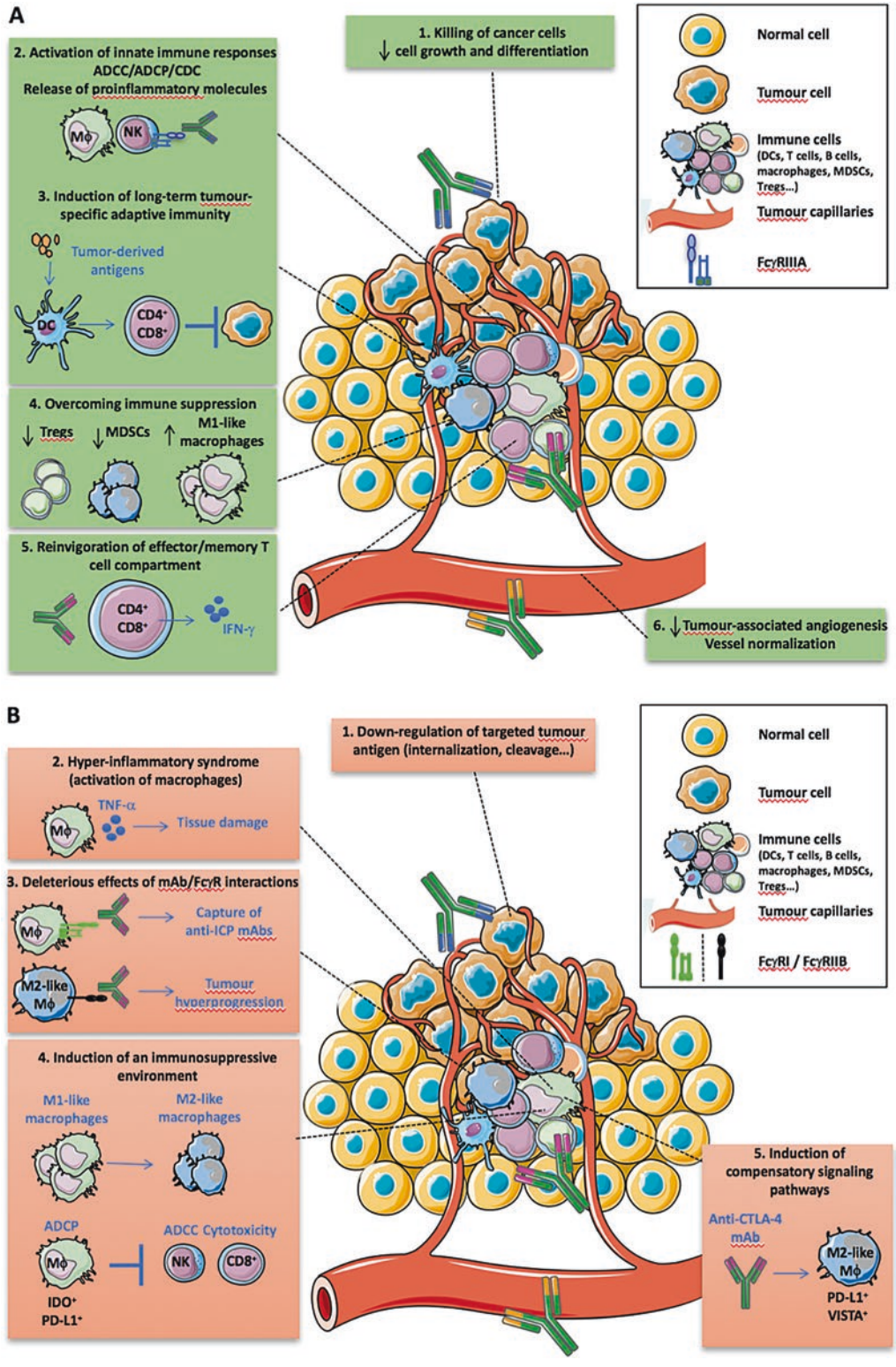

Fig. 9.1 Multifaceted effects of monoclonal antibodies on the tumour microenvironment and on anti-tumour immunity. Different categories of monoclonal antibodies (mAbs) have been developed for cancer therapy. A first category is directed against tumour cells themselves (in blue), a second one comprises antibodies blocking the formation of neo-vasculature that accompanies tumour development (in yellow) and a third category of immunomodulatory antibodies target immune cells in the tumour microenvironment rather than cancer cells (in pink).

for clinical use in patients with solid tumours [106]. These different combinations would exert wider therapeutic effects than a single therapeutic agent. Finally, the categorization of tumours according to the molecular and cellular composition of the TME would help to identify which tumour types are most likely to respond to different types of immunotherapies and to choose the appropriate combination of immunotherapies for each cancer.

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Fig. 9.1 (continued) These antibodies may have beneficial (**a**) or detrimental (**b**) effects on the tumour microenvironment and on anti-tumour immunity. (**a**) 1: Anti-tumour mAbs can block cancer cell activation and/or proliferation or directly induce apoptosis of tumour cells. 2: Besides these direct effects, mAbs recruit C1q molecule (belonging to the complement system) and innate cells expressing receptors for the Fc region of IgG (FcγR), such as NK cells and macrophages (Mϕ), leading to cell lysis and to the formation of tumour cell debris through complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), respectively. 3: Immature DCs then capture the resulting tumour-derived antigens, leading to the priming of self-reactive tumour-specific $CD4^+$ and $CD8^+$ T cells that can act back against tumour cells and eventually circumvent the pro-tumour immunosuppression. 4: MAbs can also have remarkable effects on the network of cells within the TME, including modulation of the frequencies of immunosuppressive cells – such as regulatory T cells (Tregs) or myeloid-derived suppressor cells (MDSCs) – or of anti-tumoural M1-like macrophages. 5: MAbs directed against immune checkpoint (e.g. anti-PD-1, or anti-CTLA-4 mAbs) can restore anti-tumour activity in dysfunctional infiltrating immune cells and induce a shift towards a more activated T-cell compartment that expresses high levels of IFN-γ. 6: Antibodies targeting the VEGF pathway induce a reduction and a normalization of tumour-associated blood vessels (**b**) Different bystander effects of therapeutic mAbs can also occur, leading to deleterious inflammation and/or decreased anti-tumour immune responses. 1: MAbs binding to tumour cells, could be followed by the down-regulation of targeted antigen. Interactions between mAbs and intratumoural macrophages can lead to deleterious effects including 2: hyper-inflammatory syndrome associated with tissue damages (e.g. for anti-CD40 mAbs); 3: decreased therapeutic activity of antibodies directed against immune checkpoint (anti-ICP mAbs) by FcγR-dependent capture of mAbs; or tumour hyperprogression following interactions between pro-tumoral M2-like macrophages expressing FcγR and anti-ICP mAbs. 4: By promoting the differentiation of intratumoural macrophages into pro-tumoural M2-like macrophages or by activating them, anti-EGFR or anti-VEGF mAbs can induce an immunosuppressive microenvironment. Furthermore, macrophages that undergo ADCP upon anti-CD20 and anti-Her2/Neu mAbs treatment can subsequently inhibit NK cell-mediated ADCC and T-cell-mediated cytotoxicity. These macrophages exhibit an immunosuppressive phenotype with overexpression of inhibitory molecules PD-L1 and indoleamine 2,3-dioxygenase (IDO). 5: MAbs blocking immunoregulatory molecules can induce inhibitory compensatory signalling pathways, as reported for anti-CTLA-4 mAb inducing an increased frequency of M2-like macrophages expressing PD-L1 and VISTA inhibitory ICP. (This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com)

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