Role of the Immune System in Cancer Development and Therapeutic Implications

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KEY POINTS

- The humoral as well as the cellular immune system play important roles in the control of cancer.
- Therapeutic efficacy of immunological approaches is limited by a restricted availability of tumor-specific antigens. Presently cancer/testis (CT), activation markers, differentiation, amplification, mutational antigens, and danger signals serve as tumor-associated target structures.
- The efficacy of cytokine therapies and cell-based approaches (T cells, dendritic cells, natural killer [NK] cells) is presently tested in preclinical and in clinical trials.
- Presently, several monoclonal antibodies have been approved by the US Food and Drug Administration (FDA) for the treatment of cancer. The target structures of these antibodies include CD20 for non-Hodgkin's lymphoma (NHL) and B-NHL; CD33 for CD33 positive acute myeloid leukemia, epithelial cell-adhesion molecule (EpCam) (expired 2000), vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) for colorectal cancer, ErbB2 (human epidermal growth factor receptor 2 [HER2]) for breast cancer, and CD52 for B-cell chronic lymphocytic leukemia (B-CLL). Numerous other antibodies are currently being tested in clinical trials.

Abstract

This chapter elucidates immunological aspects in cancer therapy. For many years, the impact of the immune system in cancer immunity was a matter of debate. Nowadays, it is generally accepted that an active immune system can monitor, edit, and destroy malignantly transformed cells in vitro and in established tumor mouse models. However, the capacity of the immune system to fight human tumors is limited, as human tumors are highly individual, complex, and dynamic systems that have the capacity to modulate anticancer immune responses and to affect the tumor microenvironment. Here, we describe the development of innovative immunological strategies from a preclinical stage to clinical application. In the last decade, especially humanized monoclonal antibodies (mAb) have emerged as promising pharmaceutical tools ("magic bullet") for the treatment of cancer in combination with radio- and/or chemotherapy.

7.1 Introduction

Immune homeostasis is a fine balance between the induction of immune responses that defend against foreign pathogens and the suppression of immune responses for the maintenance of self-tolerance to prevent autoimmune diseases. Since tumor cells develop from the host's own tissue they might be considered as being "self" by the immune system, and this makes the generation of an efficient immune defense against cancer difficult. However, Rudolf Virchow (1863) succeeded in detecting infiltrating leukocytes in tumor tissues (overview in Mantovani et al. 1992). A few years later, William Coley (1890) associated the presence of fatal bacterial infections with the induction of antitumor immune responses in patients with partially resected tumors (Coley 1893). Based on these earlier findings, Paul Ehrlich postulated the "virulent capacity of tumors" in 1909.

The substantial progress that has been made in the treatment of cancer using radio- and chemotherapy led to reduced attention about the involvement of the immune system in the control of cancer for several decades. However, with an increase in the understanding of the molecular mechanisms of immune recognition and regulation came accumulating evidence that the immune system plays a crucial role in the control of cancer. Nowadays, it is generally accepted that an active immune system can monitor, edit, and destroy malignantly transformed cells in vitro and in established tumor mouse models (Smyth et al. 2001; Dunn et al. 2002). However, the capacity of the immune system to fight against cancer in humans is limited, as human tumors are highly individual, complex, and dynamic systems, which have the capacity to modulate anticancer immune responses and to affect the tumor microenvironment.

Nevertheless, correlative relationships between altered immune function, tumor development, and antitumor immune responses (Dunn et al. 2002, 2004) have been observed in spontaneous human tumors.

Spontaneous remission is defined as a complete or partial, temporary, or permanent disappearance of all or at least some relevant tumor parameters in the absence of any proven medical intervention. For a variety of different cancer entities such as colon cancer (Beechey et al. 1986), mammary carcinomas (Larsen et al. 1999), malignant melanoma (Mackensen et al. 1994), acute myeloid leukemia (AML) (Tzankov et al. 2001), and liver metastases of a non-small cell lung (NSCLC) carcinoma (Kappauf et al. 1997), spontaneous remissions have been documented. Furthermore, immunocompromised individuals such as those with human immunodeficiency virus (HIV) infection are more susceptible to lymphomas and Karposi's sarcomas (Boshoff and Weiss 2002). Together with promising results derived from xenograft and syngeneic tumor mouse models, which have demonstrated the capacity of humoral (antibody) and cellular immune responses to eradicate established tumors, these findings further support the concept of cancer immunosurveillance or immunoediting (Dunn et al. 2004).

7.2 Immune System

The ability of the immune system to effectively respond to tumors is dependent on the following assumptions:

- Tumor cells differ from normal cells.
- The immune system can recognize these differences.
- The immune system is in an active state and capable in generating an effective and protective immune response.

These prerequisites indicate that cancer immunoediting is a dynamic process that involves both the tumor as well as the immunocompetent effector system. The efficient eradication of tumors in a living organism requires crosstalk between leukocytes of the innate and

adaptive arms of the immune system, which reside in different immunological compartments. It has been shown that the cytokine interferon-gamma (IFN-γ), and the cytolytic effector molecules perforin and granzyme are secreted by cells of the innate and adaptive immune system, which contribute to the host's immune defense against cancer. Following uptake into tumor cells, intracellular located granzyme B initiates apoptosis via the activation of procaspases 3, 7, 10 inactive cytosolic inhibitor of caspase-activated DNase (ICAD), and the disruption of the membrane potential of mitochondria, which causes the release of cytochrome c into the cytosol. The situation of the host's immune defense is complicated by the fact that throughout evolution, tumors have adopted strategies to interfere with and to overcome the immune system. These immune escape mechanisms involve the downregulation of major histocompatibility complex class I (MHC I) and costimulatory molecules, the loss of tumor-specific antigens, the stimulation of inhibitory receptors expressed on effector cells, the stimulation of the growth of inhibitory CD4/CD25 double-positive regulatory T cells (T_{regs}), and the secretion of inhibitory molecules such as serpin-protease inhibitors, which interfere with the apoptosis cascade. For example, about 60% of metastases express significantly reduced levels of MHC class I on their cell surfaces. These findings indicate that a better understanding of the interaction between immune cells, tumor cells, and the tumor microenvironment and their consequences will guide the development of more effective approaches for controlling and successfully treating cancer.

7.3 Tumor Markers

As mentioned earlier, a key barrier to the generation of protective anticancer immune responses is that, in contrast to pathogens, tumors are not typically seen as being "foreign" by the host's immune system. It is therefore essential to identify and characterize tumor-specific antigens/peptides that can be used for the development of innovative immunotherapeutic strategies. Recent approaches include the serological analysis of recombinant cDNA expression libraries (SEREX) (Chen et al. 1997), differential gene expression analysis, and T-cell epitope cloning (TEPIC), using samples obtained from patients with cancer (Boon and van der Bruggen 1996; van den Eynde and Boon 1997). These methods have identified antigens that can be grouped into various categories including cancer/testis, activation, differentiation, amplification, mutational antigens, danger signals such as membrane-bound heat shock proteins (HSPs), and pathogens (Table 7.1).

7.3.1 Cancer/Testis Antigens

The expression pattern of cancer/testis (CT) antigens in healthy human individuals is restricted to germline tissues such as testis and placenta. Nevertheless, a high proportion of melanoma, bladder cancer, lung, esophageal, and ovarian tumors show a surface positive phenotype in a lineage nonspecific fashion (Boon et al. 1997; Van den Eynde and Boon 1997; Old and Chen 1998). The expression of these antigens frequently maps to genes on the X chromosome. The CT antigens are also linked to the unique class of differentiation antigens that have the capacity to elicit a cellular and humoral immune response. Representative CT antigen members that belong to multigene families are summarized in Table 7.1.

7.3.2 Activation Antigens

Mucins (MUC-1, 2, 3, 4, 11, 12, 13) are a family of highly glycosylated proteins that can be grouped into the activation antigens. They are predominantly found on mammary, ovarian, and pancreatic carcinomas (Boon and van der Bruggen 1996). Weakly glycosylated members of this protein family are expressed on healthy epithelial cells. Membrane location of MUC-1 and MUC-4 is achieved through a hydrophobic membrane-spanning domain that mediates plasma membrane retention (Singh et al. 2004). A mouse monoclonal antibody (mAb) directed against CD227 is able to detect the membrane-bound form of MUC-1.

7.3.3 Differentiation Antigens

The following members of the differentiation antigens including tyrosinase, gp100, and Melan-A/Mart-1 are expressed on normal melanocytes. Prostate-specific antigen (PSA) is found on the cell surface of healthy prostate tissue. Compared with normal tissues, the expression density of these and other antigens including carcino-embryogenic antigen (CEA), alpha-1-fetoprotein, and epithelial cell-adhesion molecule (EpCam) in tumors is highly increased. Due to the lack of tumor-specificity of these antigens, it is important to note that

Table 7.1. Categories of tumor-associated antigens

Antigen category	Antigens
CT	Melanoma associated antigen (MAGE-1, -2, -3) (Boon and van der Bruggen 1996) NY-ESO-1 = LAGE-1 (esophageal cancer, ovarian cancer) (Odunsi et al. 2003; Jager et al.1999) B-melanoma antigen (BAGE)
Activation	MUC-1, 2, 3, 4, 11, 12, 13
Differentiation	CEA α-1-Fetoprotein EpCam Tyrosinase (Brichard et al. 1993) Melan-A/Mart-1 (Coulie et al. 1994) Glycoprotein (gp100) (Kawakami 1995) PSA
Amplification	Her2/neu proto-oncogen (c-erb-B2; Cheever et al. 1995) p53 (Scanlan et al. 1998) Preferentially expressed antigen in melanoma (PRAME) Aldolase A
Mutational	Human leukocyte antigen allele type A2 (HLA-A2) CDK4 (Wolfel et al. 1995) β-Catenin (Robbins et al. 1996) Caspase 8 (Mandruzzato et al. 1997) Melanoma-ubiquitous mutated (MUM-1) Mutated p53 (Gnjatic et al. 1998)
Damage signals	HSP70, HSP 72, HSP 90 Gp 96
Pathogens	Human papilloma virus (HPV) types 16 and 18 (Tindle 1996) Epstein-Barr virus (EBV) (Lennette et al. 1995) Human T-cell lymphotropic virus type I (HTLV-1; leukemia) HHV-8 *Helicobacter pylori* bacteria (chronic gastritis and stomach carcinoma)

an efficient immune response against these tissue-specific antigens can also affect normal tissues. One well-known example is the destruction of normal melanocytes by cytotoxic Melan-A–specific T cells, which can cause vitiligo in the healthy skin of melanoma patients. A list of differentiation antigens with a high expression on tumors is shown in Table 7.1.

7.3.4 Amplification Antigens

This group of antigens is ubiquitously and widely expressed in normal tissues but highly overexpressed in tumor cells. Important representatives of this group are Her-2/neu, which is predominantly overexpressed on adenocarcinoma of the colon, mammary, ovarian, pancreatic, and lung carcinomas and p53, PRAME, and aldolase A, which are overexpressed in lung carcinomas (Coulie et al. 1999; Gure et al. 2000).

7.3.5 Mutational Antigens

Although this group of antigens—mostly peptides—are ubiquitously expressed in normal tissues, they are expressed in a mutated form in many tumors. In general, each tumor exhibits an individual pattern of mutation, and the resultant antigenic profile is therefore considered as being tumor-specific and unique (Wang and Rosenberg 1999; Renkvist et al. 2001). Most mutations are point mutations that are translated into individually mutated proteins. Since these mutations cause

severe changes in the activity of the encoded proteins, these antigens affect the oncogenic potential of the tumor. Examples of mutational antigens that are found in a variety of different tumor entities are listed in Table 7.1.

7.3.6 HSPs

HSPs were firstly discovered in 1962 (Ritossa 1962) as a set of evolutionary conserved molecules whose expression is highly inducible not only by a variety of different stress stimuli such as elevated temperatures, irradiation, heavy metals, cytostatic drugs, amino acid analogue, glucose deprivation, oxidative stress, but also by inflammation or viral and bacterial infections. Under physiological conditions, HSPs are required for cell differentiation and antigen processing for proper protein folding of nascent polypeptides, for transport of proteins along membranes, and for prevention of protein aggregation (Morimoto 1991; Pierce 1994). In contrast to normal tissues, malignantly transformed cells such as tumors have been found to overexpress HSPs in the cytosol, which might cause the translocation of them into the plasma membrane and into the extracellular milieu. Members of the HSP70 and HSP90 families are present on the plasma membranes of a number of different tumor entities (Multhoff et al. 1997; Shin et al. 2003) where they act as danger signals for the innate (Schmitt et al. 2007) and adaptive cellular immune system. T cells have been found to recognize HSP-chaperoned immunogenic peptides that are cross-presented by antigen-presenting cells (APCs) (Srivastava et al. 1998). In contrast, natural killer (NK) cells have the capacity to recognize membrane-bound Hsp70 on tumors, even in the absence of immunogenic peptides. Since the corresponding normal tissues lack an HSP membrane expression, the presence of HSPs on the plasma membrane is considered as a tumor-specific antigen (unpublished observation). HSPs that are predominantly found on tumor cell surfaces and in the extracellular space are Hsp70, Hsp72, and a major stress inducible member of the HSP70 family, Gp96 (glucose-related protein 96), an endoplasmic reticulum (ER)-residing member of the HSP90 family.

7.3.7 Pathogens

A small proportion of tumors (2–5%) are initiated by viral infections, which causes a transformation of human cells (Coulie et al. 1999). Human papilloma virus type 16 and 18 are associated with cervical carcinomas (Bontkes et al. 2000; Youde et al. 2000, Rudolf et al. 2001), Epstein-Barr virus infections with Burkitt's lymphomas, human T-lymphotropic virus (HTLV-1) with T-cell lymphomas, and human herpes virus 8 (HHV-8) infections with Karposi's sarcoma. A chronic bacterial infection of the stomach with *Helicobacter pylori* has been associated with gastritis and with gastric tumors.

7.4 Preclinical Immunotherapeutic Approaches

A better understanding of the molecular basis of the immune homeostasis and its regulatory mechanisms has re-attracted many researchers to the concept of augmenting the antitumor responses. An emerging number of newly identified tumor-associated antigens (TAA) including differentiation, mutational, amplification, CT, danger signals that have been identified using expression libraries, differential gene expression analysis (Chen et al. 1997), T-cell epitope cloning (Boon and van der Bruggen 1996), and bioinformatics (Scanlan et al. 2000) have also advanced this field. The following section aims to summarize immunoediting and immunotherapeutic concepts including nonspecific cytokine therapies, specific antibody, and cell-based concepts that have been tested successfully in animal models. The proof of principle and the in vivo efficacy of some of these have already been demonstrated in first human clinical trials.

7.4.1 Cytokines

Cytokines, also termed as interleukins, lymphokines, or chemokines, are small (8–30 kDa) signaling proteins and glycoproteins that are predominantly produced by hematopoietic cells. Their main function is to recruit and stimulate the immune system against pathogens and to support differentiation and developmental processes during embryogenesis. Cytokine-based immunostimulation is believed to have the potential to treat established primary tumors and distant metastases. One of the earlier immunological approaches aimed on the broad, nonspecific stimulation of the adaptive (T lymphocytes) and innate (NK cells) immune system by the administration of high doses of recombinant interleukin 2 (IL-2) (Rosenberg 1986). Large-scale production of interferons, using recombinant DNA technology in 1983 enabled the first systematical evaluation

of appropriate dose, route, and schedule for application in humans. Meanwhile, cytokines have an established role in therapy of malignant melanoma and renal cell carcinoma as described later in this chapter.

7.4.2 Antibodies

The development of the technology for producing mAb from hybridoma cells (Kohler and Milstein 1975), for which Kohler, Jerne, and Milstein were awarded the Nobel Prize in Physiology and Medicine in 1984, led to the hypothesis that the "magic bullet" against cancer has been found. Despite their high degree of specificity and affinity, the development of clinically applicable antibodies for the treatment of cancer has proven to be more complex than was originally anticipated. Patients that had been treated with the first generation of murine mAb developed a human–anti-mouse antibody response (HAMA) against the therapeutic agent, and this drastically limited the therapeutic success. Nowadays, therapeutic antibodies are humanized either by grafting CDR (complimentarity-determining regions) onto human antibodies, or by creating chimeric antibodies by transferring the murine Fab antigen–binding variable region onto a human constant Fc portion. Approval has already been achieved for the clinical application of humanized monoclonal antibodies, which are directed against CD20 (Rituxan, MabThera, Zenapax, Zevalin, Bexxar) for the treatment of non-Hodgkin's and cutaneous B-cell (Kerl et al. 2006) lymphomas, CD33 (Mylotarg) for the treatment of CD33-positive myeloid leukemia, CD52 (Campath, Mabcampath) for the treatment of B-cell chronic lymphocytic leukemia (B-CLL), EpCam (Panorex) for the treatment of colorectal cancer, ErbB2 (Herceptin) for Her-2 overexpressing breast cancer, and vascular endothelial growth factor (VEGF) (Avastin) and epidermal growth factor receptor (EGFR) (Erbitux, Vectibix) for the treatment of colon carcinoma.

Naked, unconjugated antibodies kill their tumor targets by different mechanisms including antibody dependent cell-mediated cytotoxicity (ADCC) (Steplewski et al. 1985), complement-dependent cytotoxicity (CDC) (Houghton et al. 1983), and by the direct induction of apoptosis via death receptor targeting (Contassot et al. 2007). Alterations of signal transduction (Trauth et al. 1989), blocking of ligand-receptor interactions (Yang et al. 1999), and the prevention of the enzymatic cleavage of cell surface proteins (Baselga et al. 2001) can also be involved. The antibody Apomab, which is directed against the death receptor DR5, augments apoptosis of colorectal, NSCLC, and pancreatic model tumor cell lines by clustering of DR5 at the cell surface and thus stimulating a death-inducing signaling pathway involving caspase 8 and Fas-associated cell death (Adams et al. 2008). Effector cells of the innate immune system (NK cells, monocytes, macrophages) expressing Fc-gamma (Fc-γ) receptors such as low (CD16)-, intermediate (CD32)-, and high (CD64)-affinity Fc receptors can mediate ADCC after antibody binding to the tumor targets. Another possibility is to use antibodies as vehicles to more specifically deliver toxic compounds such

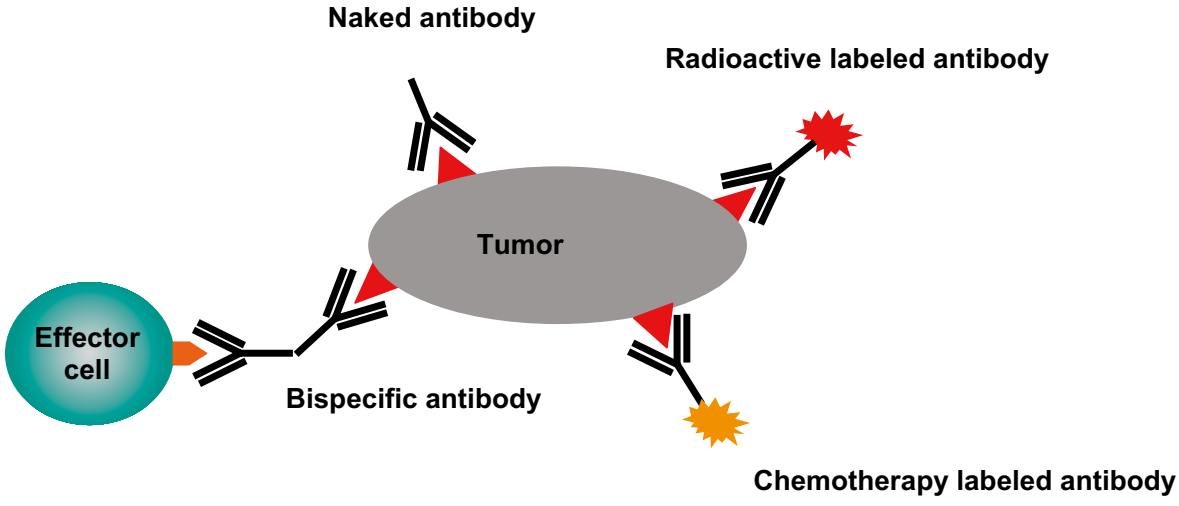

Fig. 7.1. Principles of tumor cell kill by antibodies: bispecific antibody-mediating effector-tumor cell interaction, naked antibody mediating antibody dependent cellular cytotoxicity (ADCC), radioactive labeled antibody mediating targeted internalization of radionuclides, and chemotherapy-labeled antibody-mediating targeted internalization of cytostatic drugs

as radionuclides and/or chemotherapeutics directly to the tumors. The principles of antibody-mediated tumor kill are schematically illustrated in Fig. 7.1.

Despite these promising strategies, the clinical outcome of antibody-based therapies is still limited by a number of factors including their relatively short in vivo half-life and clearance from the host's body, the insufficient degree of glycosylation of humanized antibodies, variations in the affinity and avidity of the humanized antibodies, and the low amount of tumor-specific or tumor-associated antigens.

7.4.3 Cell-Based Therapies

Adoptive cell-transfer therapies have developed into potent treatments for patients with highly immunogenic tumors including metastatic melanoma (Dudley and Rosenberg 2007). Current studies are aimed at improving GMP (good manufacturer practice) methods for generating and administering appropriate lymphocyte populations in future clinical trials and improving the resilience of antitumor immunity in tumor patients.

7.4.3.1 Dendritic Cells

Dendritic cells (DCs) can be subdivided in two developmental lineages, the myeloid and the lymphoid (Steinman and Inaba 1999). DCs control the activity of B lymphocytes, T lymphocytes, and NK cells (Banchereau and Steinman 1998). As professional APCs, their primary task is to capture foreign antigens from the periphery, process and maturate them into peptides, and present them on MHC molecules to naïve T cells. In the absence of essential costimulatory signals that are concomitantly delivered, T-cell activation is insufficient. Apart from their activating function, DCs are also able to tolerize the immune system against self-antigens in order to avoid autoimmune reactions (Turley 2002). The migratory capacity of DCs is regulated by chemokines. The expression of the chemokine receptor CCR7 promotes the migration of immature DCs to inflamed tissues and that of mature DCs to the draining lymph nodes, where the antigen is presented to naïve T cells (Sallustro and Lanzavecchia 2000).

Although lymphodepletion by chemotherapy and total body irradiation can reduce the absolute number of APCs, it also has been shown to promote their maturation into an active state, as indicated by an upregulation of CD86 and MHC class II antigens in a mouse model (Zhang et al. 2002). Irradiation has also been found to stimulate secretion of the inflammatory cytokine IL-12 by DCs, which subsequently activates T cells and NK cells. The maturation of DCs and their capacity for antigen cross-presentation is also enhanced by the secretion of tumor-necrosis factor (TNF), IL-1, and IL-4 and by the presence of "danger signals" such as lipopolysaccharide (LPS) and/or HSPs (Asea and Stein-Streilein 1998). DCs pulsed with tumor lysates (Nair et al. 1997), tumor protein extracts (Ashley et al. 1997), and/or synthetic peptides can generate protective immunity to subsequent tumor challenge in tumor mouse models. The requirements for GMP-grade production of the cell products presently limit the applicability of this therapeutic approach in human patients.

7.4.3.2 T Cells

The term *immunosurveillance*, which characterizes the important role of T cells in generating an antitumor immune response, was established in 1967 by Burnet. Generally, T lymphocytes can be grouped roughly into CD4 T helper and CD8 cytotoxic T cells. T cells, composing between 60 and 80% of the peripheral blood lymphocyte (PBL) pool, recognize their targets via the T-cell receptors (TCRs), but only after primary stimulation by APCs such as monocytes, macrophages, or DCs (Lanzavecchia and Sallusto 2001). The enormous heterogenicity of TCRs is obtained by variable-diversity-joining gene recombination and crossover events. APCs present processed foreign peptides to CD8 T cells in the context of MHC class I and to CD4 T cells in the context of MHC class II molecules. As indicated above, an effective, long-lasting T-cell stimulation requires concomitant costimulation via interactions between essential costimulatory molecules such as B7 on APCs and CD28 on responding T cells.

Tumor-specific cytotoxic lymphocytes (CTL) play a crucial role in the immunotherapy of cancer (Gattinoni et al. 2006) by directly targeting and killing tumor cells that express appropriate antigens for which they are specific, whereas CD4 T cells provide help for these events via the secretion of pro-inflammatory cytokines such as IL-2. Although IL-2 is a growth factor for T and NK cells, which promotes expansion and cytotoxic function of effector cells, it is also essential for the maintenance of peripheral self-tolerance (Furtado et al. 2002). Non-mutated self-antigens expressed by tumors primarily serve as target antigens for CD8 CTLs. Adoptive cell transfer therapies involve ex vivo activation

and expansion of tumor-reactive T-cell populations that are then transferred into patients. Although the immunoreconstitution with ex vivo expanded tumor-infiltrating lymphocytes (TILs) (Wang and Rosenberg 1999) has shown some success, more recent data indicate that the adoptive transfer of TILs after non-myeloablative, but lymphodepleting systemic chemotherapy is superior with respect to tumor regression. It thus appears that the removal of the host's immune system increases the efficacy of the adaptive cell transfer. One explanation for this might be that the depletion reduces the number and activity of endogenous immunoregulatory T-cell populations such as CD4/CD25 double-positive T_{regs} (Ni and Redmond 2006). These cells might compete with CD8 T cells for activating cytokines and/or the availability of APCs and thereby suppress antitumor immune responses. T_{regs} are characterized by an upregulated expression of the transcription factor fork-head box P3 (FoxP3) protein and by a constitutively high expression of the IL-2 receptor alpha chain (CD25), the glucocorticoid-induced TNF-receptor related protein (GITR), the cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), and in the case of humans, low levels of CD127. A number of studies suggest that T_{regs} are involved in the control of antitumor immune responses, and these cells have been shown to accumulate in tumor lesions, where they inhibit the function of tumor infiltrating cytotoxic T cells (Antony et al. 2005). However, in addition to T_{regs} (Zhang et al. 2005), $CD11b^{+}Gr1^{+}$ myeloid suppressor cells (MSCs), NK cells, and natural killer T (NKT) cells (Kronenberg 2005) have been found to exert immunosuppressive functions. Apart from the arginine metabolism, the exact mechanisms of action of these cells have yet to be elucidated (Bronte and Zanovello 2005). Another reason could be that homing of lymphocytes is improved following depletion of the host's immune cells.

7.4.3.3 NK Cells

NK cells, also formerly termed large granular lymphocytes (LGLs), are specialized cells of the innate immune system that exert their function against pathogen-infected and tumor cells as a first line of defense. NK cells, which compose about 5 to 20% of circulating lymphocytes (Trinchieri 1989), can stimulate the immune system indirectly by the release of high amounts of IFN-γ, or mediate a direct cytotoxic response via the secretion of perforin and granzymes or via FAS–FAS ligand interaction. The discrimination of self and non-self by NK cells is regulated by a fine balance of activating (short intracellular immunoreceptor tyrosine-based activation motifs [ITAM]) and inhibiting (long intracellular immunoreceptor tyrosine-based inhibition motifs [ITIM]) receptors. These receptors can be grouped into the following main receptor families: immunoglobulin like receptors with specificity for *HLA* alleles; C-type lectin receptors NKG2D, CD94, NKG2A, NKG2C; and natural cytotoxicity receptors (NCRs) NKp30, NKp44, NKp46. The cytokines IL-2 and IL-15 are crucial to the survival, expansion, and differentiation of NK cells (Koka et al. 2003). NK cells play key roles in the crosstalk between the innate and adaptive immunity (Degli-Esposti et al. 2005; Pulendran and Ahmed 2006). Knockout mice for recombinant activating gene 2 (*RAG-2*), perforin, interferon gamma, or STAT-1 or NK deficient mice are more susceptible to the development of tumors than their wild-type counterparts (Dunn et al. 2004; Smyth et al. 2001 b). NK cells kill their susceptible targets by releasing cytotoxic granules containing granzymes and/or perforin via interactions with death-inducing ligands (TRAIL, FAS ligand) through the secretion of inflammatory cytokines (IFN-γ, TNF-α), and T-cell recruiting chemokines such as RANTES, MIP1-α, MIP1-β), and via antibody dependent cellular cytotoxicity (ADCC) (Smyth et al. 2001a; Robertson 2002). In contrast, cytokines such as IL-2, IL-12, IL-15, IL-18, IL-21, IL-23, and IL-27 augment NK cell–mediated tumor activities (Ma et al. 2006a; Smyth et al. 2004). Remarkably, alloreactive NK cells have been shown to prevent graft versus host disease (GvHD) by eliminating the recipient antigen-specific DCs in a mouse acute myelogenous leukemia (AML) model system (Ruggeri et al. 2002; Miller et al. 2005).

7.4.4 mAbs

mAbs, targeting tumor-specific antigens, can initiate ADCC via their Fc part and with the help of activated NK cells, macrophages, granulocytes, and the complement system. Experimentally, mAbs such as trastuzumab, rituximab, and anti-EGF receptor have been shown to induce ADCC. Macrophages and granulocytes express both activating and inhibitory Fc receptors, whereas NK cells present only the low affinity activating Fc-γ receptor (CD16). The interaction of NK cells with Fc-γ ligand initiates the release of IFN-γ, TNF-α, and T-cell-recruiting chemokines. This release can be enhanced by the addition of pro-inflammatory cytokines such as IL-2 and IL-15 (Parihar et al. 2002).

Other approaches for enhancing antibody-mediated NK cell activity involve the use of oligodeoxynucleotides (ODN) containing unmethylated CpG motifs, which mimic bacterial DNA. The Toll-like receptor 9 (TLR-9) has been identified as the receptor for CpGs by TLR-9 knockout mouse systems. The use of bacillus Calmette-Guerin (BCG) (Brandau et al. 2001; Suttmann et al. 2006) as an adjuvant is another method for a nonspecific stimulation of NK cells via the secretion of IL-12 and IFN-γ by monocytes. More recently, defensins, a family of cysteine-rich cationic polypeptides that are constitutively expressed by epithelial cells, have been found to attract immature DCs and thus induce signaling through TLR-4. The presence of NK cells and CD8-positive T cells is a prerequisite for their antitumor activity (Ma et al. 2006b). Last, but not least, HSP70 or peptides derived thereof, acting as classical danger signals, have been found to activate NK cells against Hsp70 membrane–positive cancer cells in vitro (Gastpar et al. 2005), in tumor mouse models (Stangl et al. 2006), and in a clinical phase I trial (Krause et al. 2003). The mechanism of tumor cell lysis has been characterized as a perforin-independent, granzyme B-mediated apoptosis (Gross et al. 2003).

7.5 Role of Immunotherapy in Clinical Practice

From the large pool of potential immunotherapeutics until today, only a few have made their way to clinical application. This section summarizes the clinically relevant immunotherapies in their typical fields of application.

7.5.1 Malignant Melanoma

Immunotherapy has a long history in malignant melanoma, which is considered a highly immunogenic tumor. However, two large trials testing the efficacy of adjuvant IFN-γ showed no advantage for patients with high-risk primary tumors or lymph node metastases (Southwest Oncology Group [Meyskens et al. 1990]; European Organization for Research and the Treatment of Cancer, unpublished). A phase II study on IFN-β as an adjuvant for melanoma demonstrated possible advantages and led to the initiation of a randomized study whose results have not been published so far. IFN-α is the first substance that has shown a significant advantage in prospective randomized trials. IFN-α2a and IFN-α2b differ by two amino acids and can be regarded as equivalent on the basis of their effectiveness. Low-dose IFN-α (3 million IU subcutaneously, three times weekly for 18–24 months) should be offered all patients with primary melanoma thicker than 1.5 mm and no indication of lymph node involvement, on the basis of three studies that showed a significant increase in the recurrence-free survival time (Grob et al. 1998; Pehamberger et al. 1998; Cameron et al. 2001).

A variety of randomized studies with different IFN-α dosages as an adjuvant has been conducted in patients with lymph node metastases. The clearest results are available for IFN-α2b, using a high-dose regimen (initiation: 20 million IU/m^2 intravenously daily day one to five every week for 4 weeks, maintenance: 10 million IU/m^2 subcutaneously three times weekly for 11 months). The first prospective randomized study showed an incidence in the recurrence-free survival prolonging disease free and overall survival (Kirkwood et al. 1996).

Table 7.2. IFN-α in malignant melanoma

Treatment concept	Tumor extension	Scheme	Effect
Adjuvant	Primary tumor >1.5 mm thickness, no lymph node involvement, R0 resection	Low dose	Prolonged RFS
	Positive lymph nodes, R0 resection	High dose	Prolonged RFS Prolonged DFS Prolonged OS
Palliative	Inoperable recurrent tumor Metastasized tumor (stage IV)	IFN-α combined with chemotherapy	Objective response Unchanged OS

RFS recurrence free survival, *DFS* disease free survival, *OS* overall survival

A follow-up confirmatory trial testing high-dose IFN vs. lower-dose IFN vs. observation was not able to confirm the earlier results (Kirkwood et al 2000). A third trial comparing high-dose IFN vs. a vaccine was terminated early because a clear disease-free and survival advantage for the IFN arm was evident early. On the other hand, IFN treatment was associated with higher toxicity compared with the vaccine arm (Kirkwood et al. 2000).

Based on these studies, IFN-α was introduced as standard adjuvant therapy for stage III resected melanoma. However, toxicity remains an issue. Flu-like syndromes including fever, chills, headache, malaise, myalgias, arthralgias, and fatigue acutely occur during therapy with interferons and diminish over time with continued daily or alternate daily administration. Vigorous hydration is essential, as patients tend to become dehydrated.

Inoperable recurrent tumors, inoperable regional metastases, and distant metastases (stage IV) are the major indications for systemic chemotherapy and chemoimmunotherapy in malignant melanoma. Many studies have evaluated the effectiveness of cytokine monotherapy in patients with advanced disease. Both IFN-α as well as IL-2 can achieve remission rates comparable with that of cytostatic agents (Keilholz et al. 1997). Treatment with IL-2 resulted in prolonged complete remissions in 5% of patients (Dillman et al. 1997). The combination of cytostatic agents and cytokines leads to an increase in the objective response rate similar to polychemotherapy, but no improvement of overall survival (Falkson et al. 1998; Bajetta et al. 1994; Smith et al. 1992). The tolerability of chemotherapy is reduced by IFN-α as well as by IL-2. As treatment in such situations is primarily palliative, the effect of any regimen on the quality of life must be carefully considered. As a first-line treatment, single-agent therapy is recommended, as polychemotherapy or biochemotherapy do not show significant advantages for prolongation of survival and are more toxic (Table 2.2).

Peptide immunization, vaccination with dendritic cells and hybrid vaccines, adoptive transfer of T cells, and immunization with naked and packaged DNA have been tested in phase I studies only and should only be used in clinical trials (Garbe et al. 2008).

7.5.2 Renal Cell Carcinoma

Metastatic renal cell carcinoma (RCC) has been notoriously resistant to conventional chemotherapy. In the early 1980s, the observation of spontaneous remissions in RCC led to a search for therapeutic agents with potential to improve the immunologic response against RCC tumor cells. Early trials used in vitro stimulation of T cells with IL-2 to produce lymphokine-activated killer (LAK) cells that were co-administered with high-dose IL-2. However, it was later recognized that the therapeutic effect resided predominantly with high-dose IL-2, and the use of LAK cells was abandoned (Rosenberg et al. 1993). However, the utility of high dose IL-2 is limited by its toxicity. Side effects include fever, chills, lethargy, diarrhea, nausea, anemia, thrombocytopenia, eosinophilia, diffuse erythroderma, hepatic dysfunction, confusion, and in approximately 5% of patients, myocarditis. IL-2 can lead to a capillary leak syndrome, leading to fluid retention, hypotension, and respiratory distress syndrome. Early high-dose studies were associated with 2–4% mortality. These patients require intensive supportive care. Mortality rates could be decreased to less than 1% in experienced treatment centers.

In spite of toxicity, the response to high-dose IL-2 treatment in metastatic RCC may be spectacular with long-lasting CRs in individual cases. However, overall responses are achievable in only about 20% of patients, and complete long-lasting responses occur in only about 5% (Fyfe et al. 1995). In a National Institutes of Health trial that randomized patients to receive high-dose IL-2 or a dose that was 10 times lower, a significantly higher response rate with high-dose IL-2 than with low-dose intravenous IL-2 (21 versus 13%) was seen, but no overall survival difference and a higher morbidity as anticipated were found (Yang et al. 2003). This was confirmed in a multi-institutional phase III trial testing intravenous high-dose IL-2 or low-dose subcutaneous IL-2 plus IFN-α (response rates were 23.2 vs. 9.9%), while there was no significant difference in overall survival (17 vs. 13 months). As expected, there were more grade 3 and 4 toxicities in the high-dose IL-2 arm (McDermott et al. 2005). It can be concluded that high-dose IL-2 is an acceptable therapy for patients with little or no comorbidities and excellent performance status, for whom the possibility of long-term CR is worth the complexity, risk, and acute toxicity of the treatment. How to best sequence or combine IL-2 with newer drugs is unknown. In phase II studies, recombinant IFN-α was reported to induce response in RCC in up to 29% of cases. However, in contrast to IL-2, IFN-α alone has no curative potential, and CRs are rare and of short duration. In a randomized trial comparing IFN-α with medroxyprogesterone acetate, IFN-α treatment was associated with a longer survival time, although the benefit was minimal (median survival time, 8.5 versus 6 months), and patients treated with IFN-α had a lower quality of life (Medical Research Council 1999).

A large study of 425 patients evaluated the activity of low-dose IL-2 in combination with IFN-α, as well as each agent alone. IFN-α or IL-2 alone had low response rates, but the response rate for the combination was significantly higher ($p < 0.01$), with significantly improved 1-year event-free survival ($p = 0.01$). However, no difference in overall survival was seen (Negrier et al. 1998).

In the future, new biologic agents might play a more important role than unspecific immunomodolators in RCC. Seventy-five percent of all RCC are clear-cell RCC. These are characteristically associated with loss of function of the von Hippel-Lindau (*VHL*) gene, resembling a constitutively activated hypoxic response resulting from upregulation of the hypoxia factor (HIF). HIF activation results in upregulation of genes encoding VEGF, transforming growth factor (TGF), Met, stromal cell-derived factor (SDF)-1 and chemokine receptor CXCR4, among others.

Small-molecule multikinase inhibitors that target VEGF receptors (sunitinib and sorafenib) have a favorable toxicity profile and can prolong time to progression and preserve quality of life when used in newly diagnosed or previously treated patients. Lately, sunitinib malate has been shown to be more effective than IFN-α in a large multicenter phase III trial (median progression-free survival 11 vs. 5 months) (Motzer et al. 2007).

IFN-α does not improve survival or relapse-free survival as an adjuvant. A phase III study treating patients with pT3-4a and/or node-positive RCC was not able to show a benefit of low dose IFN-α given daily for 5 days every 3 weeks for up to 12 cycles compared with postoperative observation (median survival 7.4 years in the observation arm and 5.1 years in the treatment arm, median recurrence-free survival 3.0 years in the observation arm and 2.2 years in the interferon arm) (Messing et al. 2003). Also, a similar study using high dose IFN-α showed no benefit (Clark et al. 2003). Those results were confirmed in a prospectively randomized clinical trial to investigate the role of adjuvant immunochemotherapy in high-risk patients with RCC. Two hundred and three RCC patients were stratified into three risk groups: patients with tumor extending into renal vein/vena cava or invading beyond Gerota's fascia (pT3b/c pN0 or pT4 pN0), patients with locoregional lymph node infiltration (pN^+), and patients after complete resection of tumor relapse or solitary metastasis (R0). There was no relapse-free survival benefit, and the overall survival was inferior with an adjuvant 8-week-outpatient, sc-rIL-2/sc-rIFN-α2a/iv-5-fluorouracil (5-FU)–based immunochemotherapy compared with observation (Atzpodien et al. 2005). In summary, there is no role for immunomodolators in the adjuvant treatment of RCC.

7.5.3 Hematologic Malignancies

IFN is an effective treatment in hairy cell leukemia (Quesada et al. 1986). Nine complete and 17 partial responses were documented by bone marrow core biopsies. Peripheral blood hematologic indices improved or normalized in all patients. Previously untreated patients showed significantly higher complete remission rates than did patients who had undergone splenectomy. Therapy was well tolerated, and most patients experiencing tumor remission also reported an improved quality of life. Another study found similar results in a small population of patients, with an overall response rate of 93% (Foon et al. 1986). On IFN treatment, peripheral blood counts returned to normal levels. This study also assessed NK cell activity and immunologic surface markers, and noted normalization of both parameters after therapy. Today, new nucleoside analogs show better results. Yet, IFN-α is still the first option in recurrences or if there are contraindication against nucleoside analogs.

A significant survival benefit of more than 89 months in a phase II trial in patients with chronic myelogenous leukemia suggests that IFN is effective in this disease as well (Allan et al. 1995; Ohnishi et al. 1995). This survival advantage was independent of cytogenetic improvement with IFN, which was also noted. 7 to 8% of the patients showed complete remission with IFN-α monotherapy. Guilhot et al. (1997) were able to show better results with combinations of IFN-α and cytosinarabinoside.

In non-Hodgkin's lymphoma (NHL), post–stem cell transplant IL-2 has shown activity. Low-dose IL-2 was also evaluated in combination with histamine, but no differences in response were observed compared with IL-2 alone.

7.6 Clinical Use of mAbs

7.6.1 Naked Antibodies

More than 200 mAbs have been tested in clinical studies, but the number of clinically relevant antibodies remains limited (Table 2.3). The first mAb that received US Food and Drug Administration (FDA) approval is rituximab, which is a chimeric antibody directed against the surface antigen CD20 on B lymphocytes, expressed on most B-cell NHL and subtypes of acute

lymphatic leukemias (ALL). In combination with polychemotherapy, rituximab is used for primary therapy of follicular NHL and diffuse large B-cell NHL as well as for maintenance therapy in recurrent follicular B-NHL after successful induction chemotherapy. Chemoimmunotherapy with rituximab is standard in therapy of primary and recurrent mantle cell lymphoma (Tobinai et al. 2006; ToBinai 2007). Rituximab might also be successful in combination with chemotherapy in CLL and in Burkitt's lymphoma, improving progression-free and overall survival.

Alemtuzumab is a humanized antibody directed against CD52 on B and T lymphocytes, and monocytes, macrophages, eosinophilic granulocytes, and NK cells. It is approved for clinical application in fludarabine-refractory CLL. In those patients, remission rates of 40% can be achieved. Interestingly, alemtuzumab has been shown to be especially effective for bone marrow manifestations of CLL. The role of alemtuzumab in primary therapy of CLL is not yet clear. Further studies will evaluate whether the efficacy of alemtuzumab in recurrences can be enhanced. Promising results were seen with alemtuzumab-chemoimmunotherapy in periphery T-cell lymphoma (Ravandi and O'brien 2006). In contrast to rituximab, therapy with alemtuzumab is accompanied by heavier infusion-associated complications such as fever, shivering, dyspnea, or exanthema, and a higher rate of infectious complications.

Metastasized human epidermal growth factor receptor 2 (HER2)-expressing breast cancer treatment was the first indication for trastuzumab, a HER2-specific humanized monoclonal antibody. HER2 is a receptor tyrosine kinase of the EGFR family that is overexpressed in 25–30% of all breast cancer patients. Overexpression of HER2 leads to enhanced cell proliferation. A phase III study combining trastuzumab with first-line chemotherapy showed prolonged progression-free and overall survival (Lin and Rugo 2007). It has also been approved as monotherapy for chemotherapy refractory metastasized breast cancer (Ligibel and Winer 2002). In addition, efficacy of adjuvant chemotherapy can be significantly enhanced by trastuzumab (Colomer 2005).

The chimeric mAb cetuximab is directed against EGFR. EGFR plays an important role in pathogenesis and progression of solid tumors such as colorectal cancer, NSCLC, and head and neck tumors. Binding of cetuximab to EGFR hinders the activation of intracellular tyrosine kinases and the following signal transduction pathway. The antibody also induces direct lysis of the tumor cells. A multicenter phase II study (BOND-1) was able to show that combination of irinotecan with cetuximab could overcome irinotecan resistance. In 23% of the patients, tumor remission, and in 30% stable disease was reached (Saltz 2005). Cetuximab is now used for therapy of metastasized colorectal carcinoma in combination with irinotecan after progression with irinotecan monotherapy. In a phase III study of locally advanced head and neck tumors, the combination of cetuximab with radiotherapy significantly prolonged survival (Bonner et al. 2007). In metastasized NSCLC, a phase II study showed that combination of cisplatin, vinorelbin, and cetuximab leads to a significant survival benefit compared with chemotherapy with cisplatin and vinorelbin alone (Lilenbaum 2006).

Bevacizumab is a VEGF-specific humanized mAb. Binding to VEGF inhibits tumor angiogenesis. It is ap-

Table 7.3. mAbs in clinical use

Generic name	Target antigen	Structure	Application
Rituximab	CD20	Chimeric IgG-1κ	B-NHL Mantle cell lymphoma CLL B-precursor ALL
Alemtuzumab	CD52	Humanized IgG-1 κ	CLL Peripheral T-cell lymphomas
Trastuzumab	HER2	Humanized IgG-1 κ	Breast cancer
Cetuximab	EGFR	Chimeric IgG-1 κ	Head and neck cancer Colorectal carcinoma NSCLC
Bevacizumab	VEGF	Humanized IgG-1 κ	Colorectal carcinoma NSCLC

IgG immunoglobulin G

proved in combination with irinotecan and 5-FU for first-line therapy of metastasized colorectal carcinoma. Patients with contraindications for irinotecan can be successfully treated with 5-FU and bevacizumab. In primary therapy of advanced NSCLC, the addition of bevacizumab to carboplatin and paclitaxel leads to enhanced progression-free and overall survival (Sandler et al. 2006; Lyseng-Williamson and Robinson 2006). Contraindications are squamous cell histology and brain metastases because of enhanced risk of heavy bleeding.

7.6.2 Radioimmunoconjugates

With the help of immunoconjugates, cytotoxic substances such as radioisotopes, cytokines, enzymes, or toxins can specifically be targeted to the tumor cells by the monoclonal antibody. Only two radioimmunoconjugates have approval for therapy, ^{90}Y–ibritumomab tiuxetan and ^{131}I-tositumomab. Both are directed against CD20 and are used for recurrent or refractory follicular B-NHL after therapy with rituximab. The radioimmunoconjugates might also be successful in therapy of transformed follicular NHL and primary diffuse large cell B-NHL.

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