Part One
Tumor-associated Antigens (TAAs): Subclasses of TAAs

Tumor-Associated Antigens. Edited by Olivier Gires and Barbara Seliger

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T Cell Antigens in Cancer

Annette Paschen

1.1
Introduction

The immune system has the ability to discriminate between normal and malignant cells. Studies in different animal tumor model systems demonstrate that innate and adaptive immune cells cooperate to eliminate cancer cells [1]. Clinical observations such as the increased rate of tumor formation in immune-compromised individuals and the spontaneous, though rare, regressions of tumors also indicate the presence of anti-tumor activity in the human immune system [2, 3]. Of the different cellular effectors involved in anti-tumor immunity, cytotoxic $CD8^+$ T lymphocytes (CTLs) are of particular interest due to their ability to specifically and effectively kill autologous tumor cells [4–7], leading to increased efforts by scientists and clinicians to also exploit this anti-tumor potential for cancer immunotherapy.

How do cytotoxic $CD8^+$ T lymphocytes distinguish between normal and malignant cells? Initially, T lymphocytes screen target cells for their protein composition. During the continual turnover of cellular proteins (antigens) small peptide fragments are generated, which are sampled and exposed on the cell surface by major histocompatibility complex (MHC) class I molecules. CTLs monitor these degradation products (epitopes) using their T cell receptors (TCRs). Due to mutational events and epigenetic alterations, tumor cells differ from normal cells in their protein composition and degradation products. The emerging aberrant antigen epitope repertoire presented on MHC class I molecules can be recognized by autologous CTLs leading to the specific killing of the tumor cells [4–7].

Before acquiring cytotoxic effector function, naïve $CD8^+$ T lymphocytes must be primarily activated (T cell priming). A few studies in mouse tumor models demonstrated that malignant cells upon migration to peripheral lymphoid organs have the ability to directly prime antigen-specific $CD8^+$ T cells [8]. More frequently, T cell priming has been described to be dependent on the activity of a specific cellular mediator, the dendritic cell (DC). Briefly, DCs internalize tumor antigens in the periphery, migrate to the draining lymph nodes and present the processed antigens to

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the resident naïve T cell repertoire. In addition to this antigen stimulus, DCs provide accessory signals (e.g. cytokines, co-stimulatory molecules) known to be of importance for the effective priming of $CD8^+$ T cells [9].

Fragments of the sampled and processed antigens are not only exposed on MHC class I, but also on MHC class II molecules of the DCs, the latter being recognized by $CD4^+$ T cells. Upon binding to specific peptide–MHC class II complexes $CD4^+$ T cells become activated and express the surface molecule CD40 ligand (CD40L). Interaction of CD40L with CD40 on DCs strongly enhances their $CD8^+$ T cell priming capacity [10–12]. In addition to primary T cell activation, helper $CD4^+$ T cells are also required for the maintenance of antigen-specific $CD8^+$ T cell responses as well as for the activation of antigen-specific B cells and subsequent antibody production [13–15]. Consequently, therapeutically effective anti-tumor immunity may be dependent on the activation of both antigen-specific cytotoxic $CD8^+$ T cells and helper $CD4^+$ T cells.

1.2 Generation of T-cell Epitopes

MHC class I molecules are composed of a β_2-microglobulin subunit that non-covalently associates with the polymorphic heavy chain containing the peptide binding groove. Several hundreds of human heavy chain alleles are known (listed at http//www.anthonynolan.com/HIG/index.html), albeit that specific alleles are preferentially expressed in certain population groups (e.g. the MHC class I molecule HLA-A*0201 is present in about 50% of Caucasians). Each individual can express a maximum of six different alleles, and the peptide binding motifs have been identified in some cases. The peptide ligands in general consist of 9 to 10 amino acids (aa), though exceptionally long variants have also been described [16, 17]. Binding of the peptide ligands is a prerequisite for stable surface expression of MHC class I molecules.

The major source of MHC class I peptides are endogenous proteins degraded by the proteasome in the cytosol. The proteasome is a multi-catalytic complex composed of several subunits. The three subunits β1, β2, and β5, mediate its standard catalytic activity. In response to IFN-γ, this subunit composition is changed, leading to the formation of the so-called immunoproteasome containing the catalytic subunits β1i (LMP2), β2i (MECL-1), and β5i (LMP7). Standard and immunoproteasome are characterized by different cleavage preferences [18]. It has been demonstrated that some tumor antigen epitopes are dependent on the activity of the standard proteasome in order to be efficiently generated whereas others require the immunoproteasome [19, 20]. The proteasome generates the correct C-terminus of the majority of MHC class I ligands, albeit that a few exceptions have been described [21, 22]. Recent evidence has established a peptide splicing property of the proteasome which leads to the generation of a $CD8^+$ T cell epitope from two discontinuous fragments of a long precursor peptide. By splicing peptides, the proteasome generates additional diversity to the pool of antigenic peptides *in vivo* [23].

Peptides generated in the cytosol are translocated via the transporter associated with antigen processing (TAP) into the endoplasmic reticulum (ER) where loading onto MHC class I molecules takes place. In principle, the proteasome can generate peptides of the correct size that directly fit into the groove of MHC class I molecules. However, the majority of peptides are produced as N-terminal-extended precursors that require additional processing by cytosolic and/or ER-localized peptidases before binding to the MHC class I groove can occur [24].

MHC class I peptides are not exclusively generated from endogenous proteins, but can also be derived from endocytosed exogenous antigens via different intracellular pathways, collectively known as cross-presentation pathways [9]. Until recently, cross-presentation was described as a specific feature of professional antigen presenting cells (pAPCs), such as macrophages and especially DCs. Interestingly, Godefroy *et al.* demonstrated that CTLs from a melanoma patient recognized a cross-presented epitope on autologous tumor cells generated from an exogenous protein after receptor-mediated internalization [25]. However, the relative contribution of this pathway to the overall generation of MHC class I peptides by tumor cells remains to be elucidated.

Unlike MHC class I molecules, MHC class II αβ heterodimers present ligands of 12 to 26 amino acids in length. Constitutive MHC class II presentation is restricted to a few cell types, mainly pAPCs as B cells, macrophages and DCs characterized by high endocytic activity. Exogenous proteins internalized via endocytosis (phagocytosis, receptor-mediated endocytosis, pinocytosis) are a major source of the peptides presented by MHC class II molecules. Unfolding and proteolysis of the internalized antigens as well as loading of the fragments onto MHC class II molecules occur in the late endosomes and lysosomes of the cell [26]. However, MHC class II molecules can also present peptides derived from endogenous proteins originating from different cellular compartments including the cytosol. These antigens access the endosome/lysosome by different pathways. For instance cytoplasmic proteins and organelles can be engulfed by autophagosomes, which then fuse with lysosomes for protein degradation and MHC class II loading [26]. Although constitutive surface exposure of MHC class II molecules is normally restricted to pAPCs, some tumors show *in situ* MHC class II expression. In the case of melanoma this expression is associated with prolonged patient survival [27].

1.2.1
Subclasses of Tumor-associated T-cell Antigens

A report characterizing the first human tumor antigen recognized by CTLs from a melanoma patient and designated MAGE-1 was published in 1991, while the first $CD8^+$ T cell epitope was described in 1992 [4, 28]. In the following years data concerning human tumor-associated T cell antigens and their epitopes continuously increased (detailed information available at http://www.cancerimmunity.org/links/databases.htm). Although the antigens are heterogenous in nature, they can be categorized by their expression pattern as unique antigens, cancer testis antigens, differentiation antigens, and overexpressed antigens.

1.2.1.1 Unique Tumor Antigens

Altered proteins originating from gene mutations and fusion proteins arising from chromosomal aberrations in tumor cells are the source of neo-antigens which are recognized by $CD4^+$ and $CD8^+$ T cells [29–42]. Since the accumulation of genetic alterations is a hallmark of cancer, it can be assumed that each tumor expresses multiple of these unique antigens, which are truly tumor-specific and accordingly not present in any normal tissue. Consequently, tolerance mechanisms acting on all self-reactive T cells do not affect the unique antigen-specific T-cell repertoire, i.e. high affinity TCRs specifically recognizing unique tumor antigens are neither deleted in the thymus during lymphocyte maturation nor deleted or anergized in peripheral lymphoid and non-lymphoid tissues [43–47].

For the majority of unique antigens described so far, expression is restricted to the tumor cells of the patients from whom they have been isolated [33, 34, 37, 38], whereas only a few of these antigens are shared by different tumors of the same histology. The shared expression of these antigens, such as the mutated BRAF, K-RAS and CDKN2A, is attributable to their biological function, which is known to be of importance for tumor formation and maintenance indicating that high antigen stability is advantageous for the tumor [31, 35, 36]. In addition to mutations, the activity of cryptic promoters or the partial and thus incomplete splicing of RNA in tumor cells can provide another source of 'shared unique' antigens [32, 48].

With respect to the tumor-specific expression and the recognition by high affinity T cells, 'shared unique' T cell antigens fulfill the criteria of ideal target structures for cancer immunotherapy. However, their therapeutic targeting would still be limited to a very small subset of patients due to the peptide–MHC restriction. For instance, a mutated antigen differing from the wild-type protein by a single amino acid might only give rise to a single neo-epitope presented by only one MHC allele. Researchers might overcome this limitation within the next years by screening cancer cells for shared genetic alterations [49]. This will lead to the prediction of new potential antigens, whose presentation by the tumors and recognition by T cells has then to be validated.

In the case of some malignancies, an association with viral infections has been described. Antigens expressed by the virus provide another source of unique tumor antigens. For instance, infections of B cells with Epstein Barr Virus (EBV) can result in the formation of B-cell malignancies. Such tumor cells can be recognized by T cells directed against EBV antigens [50, 51]. Similarly, infection of cervix epithelial cells with human papilloma virus (HPV) can induce the outgrowth of cervical carcinoma. The viral oncoproteins, E6 and E7 expressed in tumor cells, function as T cell antigens [52, 53].

1.2.1.2 Cancer Testis Antigens

In addition to mutations, cancer cells are characterized by epigenetic alterations that induce expression of otherwise silenced genes. For instance, demethylation which occurs in many tumor cells, elicits the transcription of a specific set of genes whose products belong to the group of cancer testis antigens (CTAs) [54]. These antigens are detectable in many tumors of different histology, but not in normal tissues except for MHC-negative testicular germ cells and placental trophoblasts. Due to this highly

restricted expression pattern CTAs might also be classified as tumor-specific, comparable to unique tumor antigens. However, medullar thymic epithelium cells have been demonstrated to express the RNA of different CTA. Thus, the possibility that the CTA-specific TCR repertoire is affected by thymic deletion cannot be completely excluded [43, 44, 55].

The CTA group encompasses several antigen families including the well-known family of MAGE antigens. So far, this family has been found to consist of nine antigens, which are known to be recognized by specific $CD4^+$ and $CD8^+$ T cells [4, 20, 28, 56–60]. Also, members of the SSX antigen family have been demonstrated to be targets of T helper cells and CTL responses [61–63]. Another well-characterized CTA is NY-ESO-1. This antigen was initially defined as an antibody target structure recognized by sera from cancer patients suffering from a variety of malignancies and was subsequently verified as an antigen that could be detected by $CD4^+$ and $CD8^+$ T cells [64–67]. Based on their highly specific and broad expression profile, CTAs seem to be well suited to cancer therapy and thus have been targeted in many different clinical trials on cancer immunotherapy [68].

1.2.1.3 **Differentiation Antigens**

These antigens are expressed in malignant and normal cells of the same lineage. Thus, the expression of melanoma differentiation antigens (MDAs) is also detectable in normal melanocytes. Due to this expression pattern, central as well as peripheral tolerance mechanisms act vigorously on antigen-specific T cells [43–47, 55, 69] suggesting that the remaining specific T-cell repertoire is of low affinity. However, there is evidence that even for these antigens self-tolerance is incomplete. MDA-specific T cells of high affinity have been isolated from the blood and tumors of melanoma patients and the adoptive transfer of *ex vivo* expanded, activated autologous T cells into patients mediated regression of metastatic tumors [70–73]. Furthermore, in *vitiligo* (= hypopigmentary skin disorder) MDA-specific CTLs are involved in the auto-immune destruction of normal skin melanocytes indicating a breakdown of natural tolerance. T cell-mediated local elimination of melanocytes can also sometimes be observed in melanoma patients [74, 75]. Several MDAs such as Melan-A/MART-1, gp100, tyrosinase, tyrosinase-related protein 1 (TRP1) and TRP2 have been described as specific targets of T helper cells and CTLs [5–7, 76–79]. In addition to melanoma, T cell differentiation antigens are known to be present in other malignancies, for example CEA in cases of gut carcinoma and PSA in prostate carcinoma [80, 81]. Targeting differentiation antigens in clinical trials, as has frequently been the case, is associated with the risk of inducing autoimmunity. In cases where the normal cells (e.g. melanocytes) are dispensable autoimmune toxicity is tolerable.

1.2.1.4 **Overexpressed Antigens**

In comparison to normal cells, tumor cells downregulate the expression of some gene products, whereas others are strongly overexpressed. Peptides derived from several overexpressed proteins are recognized by specific $CD4^+$ and $CD8^+$ T lymphocytes [16, 25, 82–89]. The distribution of these antigens in normal tissues is

heterogenous, with some antigens being expressed in a few normal tissues, while others can be detected ubiquitously. T cells responding to these antigens ignore normal cells *in vitro*, most probably due to the very low level of antigen expression. Again it can be assumed that central and peripheral mechanisms act vigorously on the antigen-specific T cell repertoire in order to maintain self-tolerance [43–47]. This tolerance may be circumvented in the case of several hundred-fold overexpression and the subsequent increase in the presentation of a given antigen [90].

Overexpressed antigens such as Her2/neu, MUC1, PRAME, survivin, and telomerase not only exhibit a heterogenous distribution pattern in normal tissues, but also show a heterogenous pattern in terms of their biological function [82–89]. In some cases this function is known to be important for the survival of cancer cells (e.g. the anti-apoptotic activity of survivin), suggesting highly stable expression of the antigen in tumor cells.

Within the group of overexpressed antigens the cell surface antigen, mucin MUC1 has some specific features. The glycosylation pattern of the MUC1 protein expressed on tumor cells and normal cells is different. Interestingly, helper $CD4^+$ T cells are capable of recognizing glycosylated MUC1 peptide epitopes and of discriminating different glycosylation patterns, thereby distinguishing between modified peptides originating from cancer cells and normal cells [69, 91]. There is accumulating evidence that peptide epitopes carrying different types of post-translational modifications can be recognized specifically by $CD4^+$ and $CD8^+$ T cells [92]. Very recently, phosphopeptides have been eluted from MHC class I molecules of tumor cells. These modified peptides, the majority of which are derived from the aberrant phosphorylation of signaling proteins, can be recognized by $CD8^+$ T cells and might represent new targets for cancer immunotherapy [93].

Do patients' T cells preferentially recognize antigens from any of the four subclasses described above? Several studies have demonstrated that T lymphocytes isolated from one cancer patient respond to multiple tumor antigens of different subclasses [37, 94, 95]. Lennerz *et al.* carried out a comprehensive analysis on the specificity of T cells obtained from the peripheral blood of a melanoma patient at different time points during disease progression and demonstrated that in this individual the predominant anti-tumor immune response was due to T cells responding to unique antigens [37]. In contrast, by analyzing the T-cell infiltrate of a regressing melanoma metastasis Coulie and colleagues demonstrated that the different established T cell clones specifically recognized CTAs and MDAs [95].

1.3 Identification of T-cell Antigens and their Epitopes

In order to identify tumor-associated T-cell antigens/epitopes, researchers followed very different strategies. The major approaches, of which there are several variants, are presented in subsequent chapters in this book (for detailed information see Chapters 3 and 4 on SEREX, and Proteomex and AMIDA respectively).

The classical strategy employed to define antigens/epitopes is based on the use of tumor cell lines and autologous T cells as screening tools, thus restricting its application to only a few malignancies such as melanoma. Initially, T cells isolated from the tumor or the peripheral blood of the cancer patient are co-cultured with autologous tumor cells for epitope sensitization and induction of proliferation. Subsequently, stimulated T cells are used to screen HLA-matched target cells transfected with the tumor cDNA expression library for the presence of the antigen. Specific killing of the transfectants indicates synthesis and processing of the tumor antigen. The antigen-specific T cells are then co-incubated with target cells expressing only truncated antigen fragments in order to determine the epitope-containing region. Subsequently, overlapping synthetic peptides covering the antigen fragment of interest are loaded onto the target cell in order to define the minimal T cell epitope. Several members of all four subclasses of antigens were characterized using this strategy, which is still the major approach for defining patient-specific unique $CD4^+$ and $CD8^+$ T cell antigens/epitopes [4–7, 16, 25, 28–30, 32–35, 37–39].

Alternatively, peptides eluted from MHC molecules and subsequently characterized by liquid chromatography in combination with mass spectroscopy, can be employed to define potential T-cell epitopes and their corresponding antigen sources. In several studies peptides were eluted from MHC class I molecules of tumor cells [58, 93, 96], and in another approach ligands were isolated from MHC class II molecules of dendritic cells pulsed with tumor lysate [97]. In any case, the immunogenicity of the identified MHC ligands has to be proven by sensitizing (priming) T cells against the corresponding synthetic peptides *in vitro* and the expression profile of the corresponding antigen source in normal and malignant cells has to be determined.

More recently, researchers exploited antibodies from the sera of cancer patients as a tool for the identification of potential B and T cell antigens. According to the SEREX technology antibodies are used to screen tumor-derived cDNA expression libraries for target proteins. These proteins should also be recognized by helper T cells, since antibody production by plasma cells is dependent on the helper function of antigen-specific $CD4^+$ T cells [64, 98]. In another more rational approach potential T-cell targets are selected based on their gene, RNA and/or protein expression profile in tumor cells assuming that specifically expressed and overexpressed tumor proteins should function as T-cell antigens [49, 99]. In any case, T-cell recognition of the candidate proteins either after selection by serological techniques or based on their expression profile, must be demonstrated.

In order to prove the recognition of potential antigens by T cells, the reverse immunology approach can be applied. First, the protein of interest is screened by predictive computer algorithms for peptides that might bind to a specific MHC molecule. Predicted candidate sequences are synthesized and analyzed for their MHC binding ability. High affinity binders are then loaded onto DCs for *in vitro* priming of autologous T cells. Finally, peptide-reactive T cells are employed to demonstrate the generation and presentation of the corresponding epitope either by tumor cells expressing the target antigen endogenously (in the case of $CD8^+$ T cells) or by antigen-loaded pAPCs (in the case of $CD4^+$ T cells) [99].

Although many tumor-associated antigens and epitopes were defined using the reverse immunology strategy, a major disadvantage of this approach is that, following extensive T cell culture, only a few predicted peptides can be verified as true epitopes. To partially overcome this limitation, different strategies for peptide pre-selection prior to *in vitro* T-cell sensitization were developed. *In vitro* digestion of polypeptides encompassing the potential epitope using purified proteasomes can be undertaken to narrow down the spectrum of predicted candidate peptides [86, 100]. Furthermore, mice transgenic for HLA class I and II molecules can be employed for pre-selection of candidate sequences. Peptides recognized by murine T cells in response to vaccination are chosen for subsequent *in vitro* sensitization of human T lymphocytes, this procedure has been applied to peptides derived from the MAGE-A4 and SAGE ($CD8^+$ T cell epitopes) as well as TRP-2 and gp100 ($CD4^+$ T cell epitopes) antigens [60, 79, 101, 102].

Of the different approaches described above, combinations are currently applied to define patients' individual tumor-associated T-cell antigen repertoire [103]. Although techniques and experimental strategies are continuously improving, definition of tumor antigens and epitopes, especially comprehensive personalized analyses, is still a challenging, however mandatory task.

1.3.1 T-cell Antigens for Cancer Immunotherapy – How are Candidates Selected?

The identification of the first tumor-associated antigens and their epitopes in the early 1990s initiated a new area in clinical cancer immunotherapy. In principle two treatment concepts can be distinguished: (1) antigen-specific vaccination (active immunotherapy) in order to induce and boost the anti-tumor activity of specific $CD4^+$ and $CD8^+$ T cells within the tumor host and (2) adoptive T-cell therapy (passive immunotherapy) for elimination of cancer cells by antigen-specific, *ex vivo* expanded and adoptively transferred autologous CTLs.

Over the past 15 years specific immunotherapy (in the case of solid tumors) has mainly been applied to melanoma patients receiving different types of vaccines [68], consisting of synthetic peptide epitopes and recombinant tumor antigens combined with adjuvants or of recombinant viral vectors as well as antigen-loaded DCs. Unfortunately, therapeutic effects were only detectable in very few individuals, and similar observations were also reported in adoptive T-cell transfer studies with specificity for the gp100 antigen [68, 104]. However, it has recently become very clear that in order to be effective T cell-based therapy should be used with strategies that overcome immune suppression induced by the tumor. Via an inhibitory network of different mechanisms the tumor counteracts an effective anti-tumor immune response: malignant cells release immune inhibitory factors such as PGE-2, IL-10, and TGF-β. Furthermore, the tumor microenvironment is enriched with immune-suppressive cells, e.g. regulatory $CD4^+CD25^+$ T cells and myeloid suppressor cells [1, 105]. Thus immune suppression must be reversed, as was demonstrated impressively in recent clinical trials on adoptive T cell therapy: 50% of melanoma patients showed objective therapeutic responses when treated with autologous

in vitro expanded tumor-reactive T cells after non-myeloablative chemotherapy, which eliminates immune-suppressive cells and in addition stimulates the supportive activity of innate immune cells [71, 73, 106]. However the removal of immune barriers strongly increases the risk of inducing fatal autoimmunity. Consequently, T-cell antigens for cancer immunotherapy need to be selected very carefully by taking the following criteria into consideration:

1. *Specificity of antigen expression*: antigens/epitopes should only be targeted if induction of severe autoimmune responses by antigen-specific T cells can be excluded.

2. *T cell affinity*: antigens/epitopes should only be targeted if high affinity T cells are known to exist in the patient. Preclinical studies have demonstrated that CTLs with high affinity for their specific peptide–MHC complexes are superior in eradicating tumor cells [107]. Interestingly, the affinity of a TCR for its peptide–MHC complex can be enhanced by amino acid substitutions in the peptide sequence. Such altered MHC ligands in contrast to the native epitope, strongly activate specific T cells that can cross-react with the wild-type sequence exposed on tumor cells [108], though a certain risk of inducing non-cross-reactive T cells cannot be excluded. On the other hand, even the low affinity tumor-specific T-cell repertoire of cancer patients might be exploited for adoptive immunotherapy, in cases where the T cells have been genetically engineered *in vitro* to express high affinity tumor antigen-specific TCRs [109].

3. *Stability of the peptide–MHC complex*: a recent preclinical *in vivo* study clearly demonstrated that the poor immunogenicity of a self-tumor antigen is not necessarily the consequence of central and peripheral tolerance mechanisms acting on the specific T-cell repertoire, but can be due to the low affinity of the antigen epitope for its specific MHC class I molecule [110]. Again amino acid substitutions can improve the binding of the peptide ligand to the MHC molecule. Such peptide analogs are currently under intense clinical investigation in vaccination therapies [111], but again the risk of inducing suboptimal T cells (see above) has to be considered [112].

4. *Antigen stability, processing, and expression level*: based on the cancer immunoediting theory defined by Schreiber and colleagues, the immune system will select tumor cells that by different mechanism resist T-cell attack [1, 113]. In accordance with this model, loss of antigen expression in human tumors has been described [105, 113]. In order to impede the generation of antigen loss variants it has been suggested that antigens which are important for the oncogenic process should be targeted; alternatively, multiple antigens should be selected for therapy.

   The generation of antigen epitopes is controlled by the activity of the proteasome in addition to cytosolic and ER-localized peptidases [18, 21, 24]. Tumor cells differ in terms of the expression of specific catalytic and regulatory proteasome subunits and peptidases, resulting in the presentation of a divergent epitope repertoire [114, 115]. Thus, specific tumor antigen epitopes should only

be targeted if cancer cells are known to present the epitopes effectively, otherwise even T cells of high therapeutic potential cannot act on their target cells [116, 117].

Ideally the antigen of interest should be expressed at high level in the tumor cells. As indicated by recent preclinical *in vivo* studies, high expression of the tumor antigen will allow T cells to kill cancer cells and in addition, cells in the tumor stroma that internalize and cross-present the tumor antigen, leading to an effective elimination of well-established tumors [118].

1.4 Conclusions

So far, more than 100 tumor-associated T-cell antigens have been characterized, the majority of which was identified by employing T cells and tumor cells from melanoma patients as screening tools. Several of these antigens are shared by tumors of different histology. However, since each tumor entity is characterized by a specific signature of genetic and epigenetic alterations, it has to be assumed that its unique antigen pattern still needs to be defined. In future clinical trials antigen-specific immunotherapy should be combined with strategies to overcome tumor-induced immune suppression. Whether under conditions of reduced immune inhibition the specificity of T cells directed against self-antigens (CTA, differentiation antigens, overexpressed antigens) is sufficiently selective to prevent fatal autoimmune disease, still needs to be determined in well-designed clinical studies. On the other hand, complete remission of solid tumors in advanced patients, though rare, can be achieved by 'simple' vaccination [68]. Understanding the molecular and cellular mechanisms behind this process might pave the way for inducing effective tumor immunity without creating severe autoimmunity.

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