



Autoantibodies against tumor-related antigens: Incidence and biologic significance

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ABSTRACT

In the last two decades, tumors have been found to evoke antigen-specific immune responses. However, the biologic role of spontaneous tumor-specific T-cell and antibody responses are still a matter of controversy. Paradoxically, cancer-related immunity has been suggested to promote tumor growth, to effectively suppress tumor progression, or to simply represent a clinically irrelevant epiphenomenon. In recent years, research has focused on tumor antigen-specific T cells, and little has been done to systematically evaluate the significance of spontaneously occurring tumor-associated autoantibodies. For this article, we screened the relevant literature on the incidence and significance of tumor-induced antibodies. We found that such spontaneous autoantibodies, targeting different antigens, are present at varying frequencies throughout a wide diversity of malignancies. In particular entities, these antibodies are already used or might be developed into diagnostic tools. Furthermore, autologous antibodies against some antigen families have a prognostic significance. Finally, tumor antigen-specific autoantibodies seem to be capable of disrupting tumor growth but, in certain instances, are also misused by the malignancy to evade immune control.

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1. Introduction

Rudolph Virchow's postulate of a tight link between inflammation and the development of cancer set the foundation for tumor immunology in 1863 [1]. Paul Ehrlich, by contrast, proposed in 1903 the elimination of nascent tumors by the human immune system [2]. Regardless of the biologic reality of these opposing theories, the observation that a tumor influences the immune system represents a major finding of the past century [3]. In the 1960s the cancer immunosurveillance paradigm of Burnet and Thomas implied for the very first time that tumor cells might bear a distinct pattern of antigens mimicking foreignness that would allow the immune system to break through self tolerance and to target the malignancy in a specific manner [1,4,5]. This hypothesis soon appeared to be contradicted by the early experiments of Rygaard and Povlsen [6] in nude mice that suggested an inability of the immune system to impede tumor growth. In contrast, more recent data from Smyth et al. [7] demonstrated that immunocompromised hosts are indeed more likely to develop spontaneous tumors than their healthy counterparts and that this effect is time dependent. To explain how some tumors could survive or even progress despite tumor-specific immune responses, Dunn and Schreiber introduced the three phases of cancer immunoediting: elimination, equilibrium and escape. Based on this model, tumors can be specifically

targeted and eliminated by the immune system, as suggested by the rejection of nonedited, allogeneic tumors in immunocompetent mice (elimination) [8]. If the immune system is not able to completely eradicate the nascent tumor, the tumor might persist but is contained by the immune system (equilibrium), or the immune system is overwhelmed and the tumor escapes immune control (escape) [3]. In opposition, Blankenstein et al. suggested that spontaneous tumors are indeed immunogenic, but that the resulting immune responses are unable to effectively limit tumor growth [9]. Although both views support the idea of an intricate interaction between the immune system and cancer, the truth about the biologic role of tumor-specific immune responses remains inconclusive, and none of the current hypotheses are able to arbitrate between the opposing points of view [9].

In the 1950s, Black et al. [10] first demonstrated the impact of the so-called "sinus histiocytosis" on the clinical course of breast cancer patients. Their work showed that the absence of secondary follicles and plasma cells from regional lymph nodes of tumor patients had a positive prognostic effect. This observation poured water on the wheels of those who believed in Virchow's idea of a tumor-promoting role of the immune system. At the same time, the model of Black et al. model can be considered as the beginning of humoral cancer immunology and as time went by and more sensitive serologic techniques became available, a large number of antigens targeted by antibodies were identified in a variety of malignant diseases [11].

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Since the mid-1990s [12] with the identification of cellular immunity as a major player in tumor immunity, an emphasis was put on T-cell immunology and on the development of vaccination strategies that tried to promote T-cell-driven immunity against tumor antigens presented in the context of HLA molecules [13]. Cancer-related autoantibodies were again sent to the background, and little effort was invested to systematically analyze their part in tumor immunology [14]. Accordingly, the biologic role of such immune responses remained in the dark [9], and it is still unclear whether autologous antibodies directed against tumor-specific or tumor-related antigens play a cancer-promoting role, whether they take part in a concerted immune attack against the malignancy, or whether they have no effect on tumor growth at all and simply represent an epiphenomenon of tumor persistence and/or progression [9].

In the present review, we summarize data explaining under which circumstances autologous antibodies against tumor-related antigens occur in cancer patients and whether they could be used as a marker for diagnostic or prognostic purposes. We highlight the quality of such serologic immune responses, and we try to answer the question of which biologic role(s) tumor-specific autoantibodies are likely to play.

2. Tumor-related autologous antibodies target various antigen families and can be found in most malignancies

In the past 40 years, researchers have identified a number of distinct tumor antigen families capable of evoking serologic immune responses in cancer patients [14]. Most antigens are tumor related but not tumor specific. They are frequently found at higher levels in tumors compared with normal tissue or are of increased immunogenicity in cancer patients [15]. In contrast, tumor-specific antigens constitute a rather small group of antigens, defined by Van den Eynden *et al.* as completely silent in most healthy tissues [15]. Tumor antigens can, in general, roughly be divided into nine subgroups: decoy proteins, stem cell antigens, viral antigens, oncogenic proteins, overexpressed proteins, frameshift antigens, nucleic acid-specific antigens, ganglioside-like antigens, and cancer-testis antigens.

2.1. Decoy antigens

Among the major issues of translational research in recent years was the systematic analysis of the antibody repertoire of cancer patients. These studies were mainly conducted to identify promising targets for T-cell-oriented immunotherapies [16] but also to discover novel, functionally relevant tumor-associated proteins [16]. Surprisingly, the largest group of antibody targets identified represented intracellular antigens belonging to the regular housekeeping contingent of both healthy and cancer cells [17]. These

proteins have miscellaneous biologic functions acting, for example, as enzymes and scaffold. Although they are capable of evoking specific antibody responses in various types of cancer, the biologic role and significance of these immune responses remains a matter of controversy [18].

Since 1995, when the serologic identification by recombinant expression cloning (SEREX) [16] methodology was established, almost all malignancies were screened for autoantibodies using cDNA libraries derived from autologous or allogeneic tumor mRNA (Table 1). In these analyses, the frequencies of antibody responses detected were highly inconsistent, varying from single patients to all samples analyzed [19–21]. Furthermore, antibody responses detected were often low-titered suggesting a relatively unspecific B-cell stimulation after necrotic release of tumor material rather than the establishment of an effective antibody-mediated anti-cancer immune response [18]. To some extent, those results might simply be based on a method-specific bias, as surface antigens require posttranslational modifications that are not commonly performed by the bacterial proteome [22]. However, according to the SEREX method analyzing the antigen repertoire, the main target of autoantibodies detected remains regular housekeeping genes and their mutated or wild-type proteins [17].

2.2. Stem cell antigens

During embryogenesis and development, stem cell antigens have an expression pattern restricted to stem and progenitor cells [23]. In later life, some of these antigens can be found in particular tissues, such as those of the central nervous system (CNS) [24]. As part of the regular human proteome, the healthy human immune system tolerates those antigens and does not mount a specific immune response [23]. Obviously, in some cancer types and autoimmune disorders, tolerance for stem cell antigens has been broken, as patients were able to develop specific B- and T-cell responses [23]. Well-described antigens in this setting belong to the Sry-like high-mobility-group box (SOX) family [25]. SOX proteins are a family of DNA-binding transcriptional factors, characterized by a highly conservative DNA-binding-domain (HMGbox). Twenty SOX-proteins have been described so far. They are mainly expressed during embryogenesis, and the majority are present within the developing central nervous system. Antibodies against SOX are not found in healthy donors [23] but in patients with small-cell lung cancer (SCLC) and Lambert Eaton myasthenic syndrome [23,24]. In SCLC, up to 43% [24] of all patients have antibodies against at least one of the SOX proteins. These seemed to be associated with the occurrence of paraneoplastic neurological syndromes [24]. However, the underlying mechanisms have not been elucidated, as experimental studies failed to support this hypothesis and as many patients will present with SOX antibodies without

Table 1
Overview of specific targets of antibody responses in cancer patients

Antigen family	Examples	Frequency	Incidence	Reference
Decoy antigens	Elongation factor 1, pyridoxal kinase, NMDA receptor	1%–100%	Small intestine, Colon, kidney, prostate, urothelial melanoma Skin, leukemia, lymphoma, sarcoma	[19, 21]
Stem-cell antigens	SOX	Up to 43%	SCLC, glioblastoma	[24]
Viral antigens	EBV, hepatitis B, HPV	30%	Lymphoma, nasopharyngeal cancer, hepatocellular cancer, cervical cancer, anal cancer	[26–28, 80]
Oncogenic antigens	HER2/neu, myc, cSrc, Bcr-Abl	10%–58%	Colon, breast, lung, chronic myeloid leukemia	[32–34, 104]
Overexpressed antigens	p53, DKK1, Survivin, BMI-1	10%–30%	Colon, lung, breast and hepatocellular, glioma and in several hematologic neoplasias	[36–38, 47]
Frameshift antigens	N/A	10%–30%	Prostate cancer	[48]
Nucleic acids	DNA, smooth muscle	20%	Lung cancer, colon cancer	[14, 105]
Gangliosides	(NeuAc)2-disialylparagloboside	10%	Melanoma, neural crest tumors	[105]
CTA	MAGE, GAGE, NYESO-1, SSX-2	1%–40%	Glioma, meningioma, head and neck, thyroid, esophageal, lung, gastric, hepatocellular, gallbladder, pancreas	[54]

any evidence for neurological symptoms [23]. Thus it remains unresolved whether SOX antibodies play a role in the development and/or progression of cancer and symptoms accompanying the malignancy [23].

2.3. Viral antigens

An increased incidence of virus-specific antibodies in cancer patients suggests a causal link between the malignancy and the respective viral infection [26]. Indeed, viruses, such as Epstein-Barr virus (EBV), human papillomavirus (HPV), hepatitis C virus (HCV), hepatitis B virus (HBV), and herpes simplex virus (HSV) are a now well-known oncogenic hit in the development of particular tumors such as those of lymphoma, cervical and nasopharyngeal carcinoma, or hepatocellular carcinoma [26–28]. As they interact with the immune system, those oncogenic viruses elicit specific antibody and T-cell responses [29]. In patients, virus-specific antibodies correlate with disease occurrence and are frequently found years before the first appearance of the malignancy [30]. In certain cancers, such antibody responses might even be used as diagnostic tools [30]. Although it is uncertain whether these antibodies play a role in the control of the respective malignancy or simply reflect virus load, they still provide important insights into the etiology of distinct tumor types.

2.4. Oncogeneic proteins

Oncogenic antigens are closely related to cancer pathogenesis [31] and define important characteristics of the malignant phenotype. Human epidermal growth factor 2 (Her2/neu), for example, is overexpressed in up to 20% of breast cancer patients and is associated with a worse prognosis, most probably based on an increased proliferation of the tumor cell and its augmented cancer cell invasion [32]. Approximately 10% of breast cancer patients and 14% of colon carcinoma patients develop autoantibodies against Her2/neu [32,33] and are for unknown reasons detectable in both, patients with Her2/neu-negative and -positive tumors. No data are available on the clinical impact of these antibodies [33].

Myc, another example of an oncogeneic protein, is involved in cell proliferation and has been found to be frequently overexpressed in lung cancer [34]. Approximately 13% of lung cancer patients develop autoantibodies against myc, but no data are available on the clinical impact of these humoral immune responses [34].

These findings demonstrate that, although more and more is known about the biologic role of oncogeneic proteins, it remains completely unclear whether autoantibodies directed against them have an impact on cancer progression and patient survival as well.

2.5. Overexpressed antigens

Although proteins belonging to this group of antigens are not strictly tumor-specific, they show a significant level of overexpression in tumors when compared with normal tissues [35]. Frequently, overexpressed antigens have been reported to be involved in the malignant phenotype (*i.e.*, DKK1, Survivin, BMI-1) [36–38]. Other members of this group of tumor antigens (*i.e.*, AFP, CEA) are potentially involved in tumor progression [39,40]. Alpha-fetoprotein (AFP), which is commonly used as a tumor marker, for instance, elicits antibody responses in approximately 25% of patients with hepatocellular carcinoma. The comparably high immunogenicity of this antigen might be based on its release into the peripheral blood [39]. However the occurrence of humoral immune responses against AFP does not seem to be tumor specific, as patients with Batten's disease, a neurodegenerative disorder in which autoantibodies are thought to play an important role, very often have such antibodies as well [41].

Mutations of p53 induce antibodies in colon, lung, breast, and hepatocellular carcinoma, as well as in gliomas and in several hematologic neoplasias [42–46], where their presence seems to correlate with both the mutation state and the overexpression of p53. Antibody frequencies are fluctuating between 10% and 30% of patients [43] and, accordingly, p53 antibodies rank among the most frequent targets of spontaneous antibody responses in cancer patients [43]. However, p53 autoantibodies have been reported in patients with inflammatory diseases [47]. Accordingly, the biologic meaning of p53-specific antibodies remains uncertain. Moreover, most studies have failed to find a clear correlation between the presence of autoantibodies directed against overexpressed antigens and the clinical outcome of the patient [47]. Accordingly, the significance of such humoral responses remains unclear.

2.6. Frameshift antigens

More recently, antigens resulting from frameshift mutations have been reported to be preferential targets of antibody responses in some cancer types [48]. These antigens result from somatic mutations in the genes' respective coding sequence that lead to a shift in the mRNA reading frame and, consequently, to variations in the amino acid sequence [49]. Frameshift products are frequently found in the form of truncated peptides, as mutations often introduce a novel stop codon [49]. Frameshift peptide-specific antibodies have been commonly detected in prostate cancer patients and even reached diagnostic significance [48]. Based on current data, antibody frequencies against frameshift antigens might range between 10% and 60% [48]. Importantly, this phenomenon is not restricted to the development of distinct mutations, introducing novel frameshift sequences but might also be linked to Yewdell's defective ribosomal products described as the backbone of immune responses during viral infections [50]. In this model, the increased immunogenicity of naturally occurring, irrelevant proteins might be mainly caused by alterations in the processing pathway of endogenous peptides in the malignant cells increasing their availability for the priming of the patient's immune system. Again, the biologic significance of frameshift-driven antibody responses remains uncertain.

2.7. Nucleic acid-specific antigens

Some cancer types, such as lung cancer induce paraneoplastic symptoms [51]. Phenomena such as myasthenia or Lambert Eaton syndrome appear to mimic autoimmune disorders and their misdirected immune responses [52]. As in the case of autoimmune disorders, frequent targets of this molecular mimicry are nucleic acids or nucleic acid-like structures [51]. Autoantibodies, such as anti-DNA, anti-Sm, and anti-RO, are often seen in cancer patients, even in those without paraneoplastic symptoms, and frequencies can be as high as 20% [14]. There is currently no answer to the question of whether such antibodies are simply an epiphenomenon or whether they represent markers of a causative event.

2.8. Ganglioside-like antigens

Some autologous antibodies in cancer patients were shown to be reactive to gangliosides and glycolipids, as summarized by Old *et al.* [53]. Unfortunately, some healthy donors were shown to react against those targets as well, and therefore the relation of these spontaneous antibody responses to the diagnosis of cancer remains unclear.

2.9. Cancer-testis antigens

The systematic analysis of humoral immune responses against malignancies contributed significantly to the identification and definition of the cancer-testis family of tumor antigens (CTA) [54]. CTA are a unique group of antigens the expression of which is

restricted to cancer cells and healthy testis [55]. This constantly growing group of antigens is of remarkable immunogenicity, generating both specific T cell as well as antibody responses in cancer patients [56]. In recent years, CTA received significant attention as their restricted expression pattern renders them exquisite targets for immunotherapies [57]. Currently, 204 CTA have been defined [54], most of them through specific antibodies found in sera from tumor patients. CTA, such as NY-ESO-1 or certain MAGE genes are the most prominent and well-characterized members [58,59]. Spontaneous antibody responses have been analyzed in many malignancies, such as esophageal, lung, or ovarian cancer for NY-ESO-1 and myeloma, melanoma, or lung cancer for the MAGE antigens [58,59]. Antibody frequencies (Table 1) range between 5% and 30% [58,59]; however, there are very few studies analyzing the occurrence of spontaneous antibody responses against a variety of CTA in larger groups of patients [54]. Furthermore, very little is known about the biologic relevance of CTA-specific immune responses. Accordingly, the fact that patients have progressive disease despite high-titered antibody responses, which often go along with CTA-specific T-cell immunity, has not sufficiently been explained [60].

In conclusion, spontaneous antibody responses against various antigens are frequently found in cancer patients. Their preferential targets, according to their antibody frequency, are decoy proteins as well as CTA. In addition, a growing body of evidence adds frame-shift peptides to this list. It is very likely that the number of known targets and frequencies of antibody responses will further increase with more sensitive techniques becoming available. However, it needs to be stressed that very little is known about the relevance of these antibodies for the immune control of cancer or about the role in the pathophysiology of the underlying malignancy.

3. Tumor antigen-specific autoantibodies have diagnostic relevance

Since the first observations of tumor-associated autologous antibodies, it has been investigated whether they could be used as an early disease marker in a minimally invasive diagnostic approach [61]. To be applicable as diagnostic markers, tumor-associated autoantibodies should only be present in cancer patients; they should be detectable in as many patients as possible; and they should ideally appear early in the course of the disease. Regarding the latter aspect, if immunosurveillance exists, tumor-specific immune responses seem likely to occur before a clinically apparent carcinoma [3].

Lubin *et al.* detected p53-specific antibodies almost 1.5 years before a clinically relevant lung cancer was diagnosed. All patients analyzed were classified high-risk, where the anti-p53 titers might be caused by p53 expression and alteration in occult precancer lesions, such as dysplasia and metaplasia. Based on their data, the authors suggested that p53-specific autoantibodies are always present at the time of diagnosis and never appear later in the course of the disease. If this is indeed the case, anti-p53 autoantibodies could be used as early markers of disease [62]. However, as in the case of lung cancer, only 30% of patients have anti-p53 antibodies, indicating that this approach might carry insufficient clinical sensitivity in this setting.

As a single cancer type potentially elicits antibody responses against myriad antigens, another approach is to combine several antibody responses in a diagnostic algorithm. For instance, the combined analysis of antibodies to p53, HER-2, IGFBP-2, and TOPO2 α increased both diagnostic specificity and sensitivity to up to 70% for breast cancer patients [63]. Recently, a review has assessed the value of autologous antibodies in breast carcinoma. Up to 75% of all breast carcinoma patients bear antibodies against at least one of the following antigens: p53, c-myc, NY-ESO-1, BRCA1, BRCA2, HER2, or MUC1. Altogether, specificity for breast cancer of 85% could be achieved, whereas sensitivity remained relatively low

at 64% [64]. In prostate cancer, autoantibodies against α -methyl-CoA-racemase have a higher sensitivity than PSA and a comparable specificity [65]. More strikingly, the combination of 22 phage-displayed antigens increased sensitivity to 88% and specificity to 82% for the diagnosis of prostate cancer [48].

Besides their tumor-specific occurrence, certain antibody responses could also be used in the discrimination of paraneoplastic from sporadic symptoms. In SCLC, SOX antibodies were able to discriminate between paraneoplastic and sporadic Lambert–Eaton syndrome [24]. In a setting where patients present with Lambert–Eaton syndrome, SOX antibodies might therefore represent a valuable tool to determine the etiology of this disease [24].

4. Tumor antigen-specific autoantibodies are of prognostic relevance in cancer patients

Since the observations of Black *et al.* on the negative prognostic impact of B-cell immunity in breast cancer patients [10], it has been considered likely that the humoral immune system might have an impact on the course of malignancies [2].

Breast carcinoma tissues are often infiltrated by cells of the innate and adaptive immune system [66], and CD20-positive B cells represent a significant subset of these cells [67]. In addition, more concise analyses identified a high rate of IgH somatic gene mutation and B-cell receptor rearrangements in a clonal way, suggesting an affinity-driven antibody maturation within the tumor tissue [68]. Interestingly, up to 70% of tumor-infiltrating B cells exhibit such a clonal pattern, in contrast to 7% in randomly chosen lymph nodes [67]. Altogether, these findings suggest the presence of B-cell-mediated immune responses within the tumor tissue rather than within loco-regional lymph nodes, at least in the case of breast cancer. Strikingly, patients showing a predominantly intratumoral B-cell response had a better overall and progression-free survival [22,69]. Earlier on, it had been observed that the removal of apparently tumor-free lymph nodes in patients with early breast cancer stages correlates with a lower recurrence rate [22]. In other words, the immune-driven survival benefit in breast cancer patients might stem from a better local tumor control by highly activated germinal centers within the tumor, whereas B cells within loco-regional lymph nodes have no impact or might even negatively influence the course of the disease [70].

The antigen p53 probably represents the best-studied tumor antigen with regard to the prognostic impact of tumor-associated antibody responses. Results of several small studies were inconclusive, varying between a missing effect and a negative influence of spontaneous anti-p53 antibodies on patient outcomes [71]. Interestingly, in hepatocellular carcinoma, it has been suggested that the presence of p53-specific antibodies increases overall survival when compared with antibody-negative patients [72]. However, a meta-analysis as well as studies analyzing larger cohorts clearly highlighted the correlation between p53-specific autologous antibodies and decreased overall and progression free survival in breast, lung, colon, and oral cancer [43,73].

Another well-studied example of antibody responses associated with a poor prognosis is found among CTA. Interestingly, antibodies against some CTA, such as NY-ESO-1, were primarily found in patients with advanced disease and poor prognosis [60]. Levels of NY-ESO-1-specific antibody responses seem to correlate with the volume of tumor tissue present in the patient's body [74]. Accordingly, spontaneous occurrence of NY-ESO-1 antibodies has particularly been found in multiple myeloma patients and melanoma patients who experienced relapse and/or progressive disease [74,75]. Moreover, in prostate cancer, NY-ESO-1 antibodies were associated with a lower overall survival, whereas no difference was found in patients with esophageal cancer [76,77]. These observations might, on one hand suggest that NY-ESO-1-specific antibodies merely represent markers of an increased tumor load and not a

biologically relevant immune response. Conversely, NY-ESO-1-specific antibodies might in fact exert a relevant antitumor effect by directly and/or indirectly suppressing tumor growth in earlier stages of the disease. As the disease progresses, these cancer-specific immune responses might lose their effect and are finally overwhelmed by the immunosuppressive effects of the cancer in its final stages [74]. This view is further supported by immune monitoring in patients vaccinated with CTA, in whom response to therapy and enhanced overall survival has been associated with both effective antibody and T-cell responses [78].

Several other antigens have been found to be associated with worse outcomes; among these antigens are antinuclear antibody (ANA), heat shock protein (Hsp) 90 or Laminin. Table 2 summarizes data regarding the association of occurrence of autoantibodies and survival; only studies that found a statistically significant association are shown. Importantly, no study has yet revealed how such antibodies impact on patient prognoses, and it is unclear whether autoantibodies themselves contribute to patient outcomes or are only a marker of clinical conditions.

Polymorphic epithelial mucin (MUC-1) is overexpressed by breast carcinoma and deficiently glycosylated on the cell surface [79]. In a study conducted to determine whether the presence of anti-MUC-1 antibodies could be a marker for advanced disease or disease progression, a clear link to earlier stages and a lower recurrence rate has been found among patients [79]. Based on these findings and *in vitro* data, it was hypothesized that spontaneously occurring anti-MUC-1 antibodies might bind to the surface of tumor cells uncovering adhesion molecules masked by the deficiently glycosylated MUC-1. According to this hypothesis, this would then lead to reduced invasion and metastasis, rendering MUC-1-specific antibodies part of an effective immune response against cancer cells.

The most impressive protective activity of specific antibodies against tumor antigens is seen in the case of HPV. Persistent HPV infection has been identified as an essential oncogenic event in the genesis of cervix carcinoma [80]. Spontaneous antibody responses against HPV antigens occur frequently, but are protective against reinfection only in a small number of patients [81]. It has been hypothesized that those responses are too low-titered to offer real protection against reinfection [81]. Recently, preventive vaccination against HPV has been included into the recommendations of the National Cancer Institute [82], and antibody responses induced are essential for the prevention of infection and cervical carcinoma [81]. Importantly, it needs to be stressed that HPV represents a viral antigen and that, in contrast to classical tumor antigens, antibody responses seem to be central to the effectivity of antiviral immunity. The situation might be completely different in the case of immediate antitumor responses in which T cells seem to play the most important role [83].

In addition, several specific autoantibodies, such as Tropomyosin, carcinoembryonic antigen (CEA), chronic myeloid leukemia (CML) 66 or SOX1 (Table 3) have been found to significantly correlate with better outcomes in cancer patients. Such data revealing a significant correlation are summarized in Table 3. It is noteworthy

that most studies, besides those of MUC-1, are retrospective single-center analyses and that the biologic roles of the respective antibodies remain largely unknown.

5. Spontaneous autoantibodies are able to drive an effective response against tumors

One of the first findings in the field of cancer serology consisted of the observation that autologous or allogeneic tumor cells were sometimes killed *in vitro* when serum of cancer patients was added to the culture medium [84]. Accordingly, in mice, transfer of antibodies from a mouse previously immunized with tumor was shown to provide an effective protection from tumor challenge in the recipient mice [85]. Since then, the process of elimination of antigen-expressing tumor cells by antibodies has comprehensively been explored. Roughly, it can be split of in an indirect and a direct pathway, where the first would promote mechanisms enhancing tumor antigen uptake and processing, later leading to an enhanced T-cell response, whereas the second would lead to a direct antibody-mediated attack against antigen-expressing tumor cells.

Antibodies might act as opsonins alleviating antigen uptake and processing of the target proteins by antigen-presenting cells. It is important to note that this mechanism is mediated by the Fc fragment and depends on Fc γ -receptor subtype (Fig. 1) [86]. Accordingly, antibodies can enhance dendritic cell (DC)-mediated antigen presentation to T cells [87]; this mechanism is highly dependent upon Fc γ R type IIIa [88], as it has the highest affinity to IgG1. Moreover, Fc γ R IIIa is essential to recruit natural killer cells to perform antibody-dependent cell-mediated cytotoxicity (ADCC) [89]. In addition, Fc γ R IIa can also drive antigen uptake upon IgG2 binding [90]. In lymphoma patients, vaccination-induced tumor specific antibodies enhanced effective antigen presentation through dendritic cells and correlated with an increased progression-free survival [88]. Moreover, it has been proposed that effective generation of tumor antigen-specific CD8⁺ T cells through cross-presentation requires the induction of tumor antigen-specific antibodies and the formation of immune complexes [91]. In fact, *in vitro* loading of tumor cells with a tumor-specific antibody leads to enhanced DC maturation and to an improved generation of tumor-specific CD8⁺ T cells [92]. Importantly, coating the tumor cells with an antibody induced spreading of T-cell specificity from the antibody's target to other tumor antigens [92].

By contrast, antibodies might also act directly through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), where the CH2 domain of the Fc fragment acts as a binding partner to C1q resulting in a starting point of the complement cascade [11]. From a functional point of view, the most effective complement activation is provided by the IgG1 subtype and, to a lesser extent, by IgG3 [69]. This might, to some degree, be related to the fact that IgG1 antibodies appear in the framework of a T_H1-type immune response, which is considered the most effective type of response directed against malignancies [85]. Accordingly, it seems that the antibody subtype is at least as important as sufficient titers of the tumor-specific antibodies [22], an idea that is supported by data from patients who received

Table 2

Overview of negative prognosis associated with the occurrence of autoantibodies against cancer-related antigens in cancer patients

Autoantibodies to	Number of patients	Tumor type	Impact	Reference
ANA	100	Breast cancer	Decreased recurrence-free survival	[106]
ANA	93	Gastric cancer	Decreased recurrence-free survival	[107]
Laminin	71	Breast cancer	Decreased overall survival	[108]
HSP90	327	Breast cancer	Decreased overall survival	[109]
p53	9489	Breast, gastric, colon cancer, NSCLC, oral cancer	Decreased overall survival	[110]
NY-ESO-1	207	Prostate cancer	Decreased overall survival	[76]
Nucleophosmin	100	Breast cancer	Decreased recurrence-free survival	[111]

Table 3

Overview of protective effects associated with the occurrence of autoantibodies against cancer-related antigens in cancer patients

Autoantibodies to	Patients (n)	Tumor type	Impact	Reference
CTSP-1	147	Prostate carcinoma	Increased recurrence-free survival	[112]
p53	130	HCC	Increased recurrence-free survival	[42]
Antineural	61	NSCLC	Increased overall survival	[113]
Cardiolipin	43	Colon carcinoma	Increased recurrence-free survival	[114]
Laminin-receptor	67	CLL	Increased recurrence-free survival	[93]
CML66	15	CML	Increased recurrence-free survival	[115]
GLEA3 and PHF3	62	Glioblastoma	Increased overall survival	[116]
Tropomyosin	55	Colon carcinoma	Increased recurrence-free survival	[117]
ds-DNA	55	Colon carcinoma	Increased recurrence-free survival	[118]
MUC1	30	NSCLC	Increased recurrence-free survival	[119]
MUC1	100	Ovarian cancer	Increased overall survival	[120]
Thomsen-Friedenreich	247	Gastric cancer	Increased overall survival	[121]
MUC5AC	30	Colon carcinoma	Increased overall survival	[122]
SOX1	90	SCLC	Increased overall survival	[123]
Carbo-anhydrase I	16	Hodgkin lymphoma	Increased recurrence-free survival	[124]
CEA	52	Breast cancer	Increased recurrence-free survival	[125]
Endostatin	95	Breast cancer	Increased overall survival	[126]
ANA	61	NSCLC	Increased overall survival	[113]

allogeneic stem cell transplantation further to induce a graft-versus-leukemia immune reaction. In transplanted patients with chronic lymphoid leukemia, for example, response to treatment correlated positively with the occurrence of high-titered IgG1 and IgG3 responses against the immature laminin receptor but not with IgG2 and IgG4 responses [93].

Overall, spontaneously occurring cancer-related autoantibodies seem to be capable of providing antitumor effects in both cancer patients and experimental models. However, these mechanisms are too often not effective enough to provide sufficient clinical responses, as cancer cells mount an entangled net of defenses and escape strategies to pass by the humoral system. The ability of tumors to hamper B-cell development, to mislead B-cell responses, to alter CDC, ADCC, and opsonin function, or to affect antibody-mediated signaling through Fc γ [94–96] will, in most cases, undermine a clinically positive effect of spontaneously occurring tumor-specific autoantibodies.

6. Conclusions

The diversity of the antibody repertoire directed against autologous tumor-related antigens illustrates the complex and heterogeneous nature of the human seromic repertoire or serome. In certain malignancies, the combination of a few targets of frequent occurrence is of diagnostic value. This approach is very attractive because of the lack of secondary prevention tools for many tumor types, although further studies, especially in a prospective manner are urgently needed to evaluate the real value of this method.

From a methodological point of view, broad screening for autoantibodies raises the need for appropriate positive controls. Although such controls are generally available for single antigens, approaches, such as SEREX, which screen for unknown antibody targets, often lack such a control [16], which hampers test results [18]. Another methodologic hurdle to the interpretation of serologic results is the wide variety of methods. Although ELISA allows quantitative assessment by titration, Western blot and proteomic analysis are rather qualitative approaches. Accordingly, a direct comparison between such methods proves to be difficult, as these methods provide additive but not interchangeable information [97].

Nevertheless, whereas some autoantibodies, such as NY-ESO-1-specific antibodies [76], are found only in cancer patients, MUC-1 and ANA antibodies are also found in healthy donors and persons with autoimmune diseases, respectively [79]. Although the occurrence of p53-specific antibodies in cancer-free donors has been suggested to represent a marker of a premalignant state [62], noth-

ing is known regarding the significance of autoantibodies directed against other cancer-associated antigens in healthy or asymptomatic donors [79].

Some autoantibodies are suggested to be a marker of progression or disease recurrence [74], whereas other tumor-associated autoantibodies seem to be related to a better response to therapy [98]. However, the presence of autoantibodies in patients without detectable tumor burden points to the idea that autoantibodies might simply be markers of particular outcomes and do not play causative roles [99]. Unfortunately, little is known regarding the behavior of such antibody responses over the course of the disease. In the vaccination setting, antibody responses have been routinely monitored over time, but comparable studies on spontaneously occurring antibody responses are scarce [100]. The few data available suggest that the occurrence of NY-ESO-1-specific antibodies might be tightly linked to disease progression [74], whereas anti-p53 antibodies have been suggested to be a permanent marker of disease, as antibody-positive patients remain positive throughout the course of their disease [43]. We believe that future studies should analyze, in a stringent manner, which clinical conditions contribute to the development of autoantibodies in cancer patients.

If cancer-associated autoantibodies indeed carry the potential to exert relevant antitumor effects, how can their presence be associated with a worse prognosis (Table 2)? One might argue for a simple failure of immunosurveillance that would explain how patients could bear tumor-specific antibodies before any tumor becomes detectable. In terms of immunoescape, an important body of evidence suggests antibody-driven tumor escape mechanisms. This point of view could provide an explanation for the decidedly worse outcome for many patients bearing tumor-associated antibodies. By contrast, for several antigens (Table 3), patients appear to experience a more favorable course of their disease if they are antibody positive. We believe that there are two possible explanations for such an association. First, the presence of autoantibodies directed against cancer-associated antigens might simply reflect the overall “fitness” of the patient, which in turn might have an effect on the clinical outcome. Second, the humoral immune response might indeed have an immediate effect on the malignancy, *i.e.*, by enhancing antitumor T-cell responses, as some studies have suggested [101].

In conclusion, data on the prognostic impact of autoantibodies in cancer patients encourage a coordinated analysis of the human serome. Such analyses could vastly improve the identification and selection of appropriate diagnostic and prognostic markers as well as of future targets for immunotherapies [102,103]. We be-

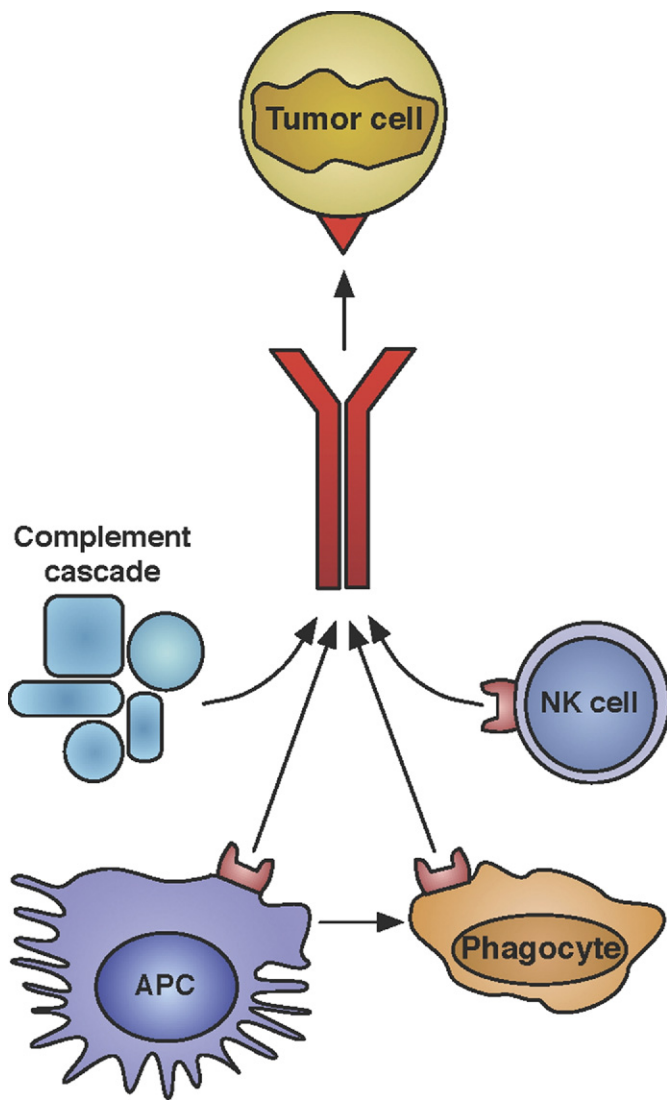


Fig. 1. Overview of tumor cell killing pathways mediated through antibodies: complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and opsonization.

lieve that spontaneously occurring or vaccination-induced antibody responses, and even monoclonal antibodies against a certain target, could very well increase the efficacy of tumor-specific T-cell responses, *i.e.*, in the framework of a vaccination with the full-length protein.

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