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REVIEW

A systematic review of humoral immune responses against tumor antigens

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Abstract This review summarizes studies on humoral immune responses against tumor-associated antigens (TAAs) with a focus on antibody frequencies and the potential diagnostic, prognostic, and etiologic relevance of antibodies against TAAs. We performed a systematic literature search in Medline and identified 3,619 articles on humoral immune responses and TAAs. In 145 studies, meeting the inclusion criteria, humoral immune responses in cancer patients have been analyzed against over 100 different TAAs. The most frequently analyzed antigens were p53, MUC1, NY-ESO-1, c-myc, survivin, p62, cyclin B1, and Her2/*neu*. Antibodies against these TAAs were detected in 0–69% (median 14%) of analyzed tumor patients. Antibody frequencies were generally very low in healthy individuals, with the exception of few TAAs, especially MUC1. For several TAAs, including p53, Her2/neu, and NY-ESO-1, higher antibody frequencies were reported when tumors expressed the respective TAA. Antibodies against MUC1 were associated with a favorable prognosis while antibodies against p53 were associated with poor disease outcome. These data suggest different functional roles of endogenous antibodies against TAAs. Although data on prediagnostic antibody levels are scarce and antibody frequencies for most TAAs are at levels precluding use in diagnostic assays for cancer early detection, there is some promising data on achieving higher sensitivity for cancer detection using panels of TAAs.

Keywords Humoral immune response · Autoantibodies · Cancer · Tumor antigens

Introduction

Under physiological conditions, auto-tolerance prevents reactions of the immune system directed against self-antigens. Disturbance of self-tolerance can lead to autoimmune diseases that are characterized by chronic inflammation and/or tissue destruction [1]. It is well established that immune responses against self-antigens can occur in cancer patients and although they may sometimes result in autoimmune-disease-like disorders (paraneoplastic syndromes), the initiation of these immune reactions is most likely different from primary autoimmune diseases. Apart from a loss of self-tolerance that is considered important for the development of autoimmune diseases, immune responses in cancer patients may be initiated by alterations in the tumor itself that result in increased immunogenicity of self-antigens [2]. Several antigens have been identified that are expressed in tumor cells but not at all or only at very low levels in normal cells and might therefore function as tumor-associated antigens capable of priming the immune system to recognize tumor cells [3, 4].

An antigen-specific adaptive immune response may be composed of activated cytotoxic CD8+ T cells that are able to directly destroy antigen-expressing target cells, different

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CD4+ T cell subsets that modulate the strength, duration, and efficacy of the immune reaction, and B cells that produce antigen-specific antibodies (humoral immune response) [5].

Tumor-associated antigens can emerge through different mechanisms, such as coding DNA mutations that lead to new epitopes in expressed proteins (e.g., p53), post-translational modifications with immunological relevance (e.g., under-glycosylated MUC1), and altered tissue-specific expression patterns or levels that can lead to the exposure of antigens usually expressed solely in immuno-privileged sites (e.g., NY-ESO-1) [6–8].

Spontaneous adaptive immune responses have been found against many tumor antigens in cancer patients [9, 10]. The analysis of humoral immune responses against tumor antigens could yield diagnostic and prognostic markers and might lead to the exploration of new targets for immunotherapy. Our review summarizes the literature on humoral immune responses against tumor antigens with three major goals: (1) to summarize frequencies of antibodies against tumor antigens in patients with various cancer entities and healthy controls, (2) to summarize data on the diagnostic and prognostic impact of humoral immune responses against tumor antigens, and (3) to understand underlying biological mechanisms of humoral immune responses against tumor antigens and their potential relevance for cancer immunotherapy.

Currently, there are no standardized protocols or even certified assays to study humoral immune responses against tumor antigens. Likewise, there is no general agreement in which populations to study humoral immune responses against tumor antigens. Therefore, we have also put a strong focus on the technical aspects of measuring humoral immune responses with the goal to identify common sources of heterogeneity and to propose efforts to standardize these approaches.

Materials and methods

A systematic literature search of studies was performed published in Medline until 30 June 2008, using the keywords: (humoral immune response AND cancer), (humoral immune response AND tumor antigen), (autoantibodies AND tumor antigen). All studies that evaluated spontaneous humoral immune response against one or several tumor antigens in serum or plasma samples were included. In addition to papers identified in the primary search, reference lists of included articles were analyzed for additional manuscripts related to the topic. We only considered studies analyzing humoral immune responses against a-priori targets. Findings from proteomic analyses or tumor cDNA expression library-based serological studies (e.g., SERPA, SEREX) were excluded unless the findings were confirmed in independent immunoassays. Antibody reactivities observed in vaccination studies and case reports about single patients were not considered.

We included studies analyzing sera or plasma samples from patients with invasive cancers, precancers, benign diseases and healthy controls. We included self-antigens that could be assigned to one of the above described categories of tumor antigens. Studies on antinuclear antibodies (ANA) or antibodies against onconeural and viral antigens were excluded. Because there is no standardized nomenclature for tumor antigen names, we used the most common designation found in the literature. All antigen names were confirmed in the ExPASy database (http://www.expasy.org) to avoid using multiple antigen or protein names. Multiple protein names were found only for IMP3 (insulin-like growth factor II mRNA-binding protein 3), which was also named Koc [11–13]. For MUC1, different epitope regions have been analyzed, including core peptide epitopes (often simply named MUC1) and carbohydrate glycan-epitopes (e.g., TF and Tn). MUC1 is the only protein for which we separately report the results for different epitopes. Similarly, only for MUC1, Ig subclasses were consistently reported and are presented separately in our analysis. For other antigens, Ig subclasses were not systematically reported.

We analyzed the studies for antibody frequencies and associations between antibody presence and tumor stage or course of the disease as well as for the used serological method. We calculated weighted antibody frequencies stratified by tumor site for each antigen. Some studies did not report antibody frequencies at a specified cut-off value. These studies could not be included in the weighted average calculation but were assessed for findings on associations between antibody presence and tumor stage or prognosis.

Results and discussion

Prevalence of humoral immune responses against tumor antigens

The initial Medline search yielded 3,619 abstracts using the above-described search terms. Of these, 145 studies were identified which reported spontaneous humoral immune responses against one or several self-tumor antigens according to the above-described criteria (supplemental Table 1).

Humoral immune responses in cancer patients have been found against a wide variety of cellular and extracellular proteins. In total, 107 different tumor antigens were investigated in 145 studies (included in supplemental Table 1).

Most of the antigens identified were derived from overexpressed or mutated proteins. 42.0% (45/107) were cytoplasmatic proteins, 26.1% (28/107) were expressed predominantly in the nucleus (transcription factors, cell cycle regulators), 21.4% (23/107) were membrane-bound proteins (receptors, cell adhesion proteins), and 10.3% (11/107) were extracellular proteins (extracellular matrix proteins, secreted proteins).

Only 20 out of the 107 antigens were analyzed in more than one study, eight in five or more studies and three in ten or more studies. The antigens analyzed in five or more studies (by descending frequency) were p53 (37 studies, 17 tumor sites, 7,764 tested samples, including controls), MUC1 core peptide (13 studies, 7 tumor sites, 2,136 samples), NY-ESO-1 (13 studies, 11 tumor sites, 1,528 samples), c-myc (9 studies, 7 tumor sites, 2,968 samples), survivin (9 studies, 8 tumor sites, 2,132 samples), p62 (6 studies, 11 tumor sites, 2,773 samples), cyclin B1 (6 studies, 6 tumor sites, 2,353 samples) and Her2/neu (5 studies, 3 tumor sites, 771 samples). Table 1 shows the weighted antibody frequencies for antigens analyzed in five or more studies for the most frequently analyzed tumor sites (breast, colorectal, liver, and lung) as well as for healthy controls. For p53 antibodies, frequencies are also included for ovarian, esophageal, and oral cancer, because in these cancers the highest p53 antibody frequencies have been demonstrated repeatedly. Antibodies were detected in 0–69% (median 14%) of analyzed tumor patients, while for most antigens, antibodies were rarely found in healthy controls (0–3%). The most evident exception is the MUC1 core peptide where antibodies could be found in 53.0% (IgM) and 23.3% (IgG) of healthy controls (weighted average frequencies). It has to be noted that several studies used the antibody levels in the control group to define their cutoffs which could result in an overall low level of reactivity in healthy controls. Weighted average frequencies of antibodies against all antigens analyzed in more than one study are shown for all analyzed tumor sites in supplemental Table 2.

Methods used to measure humoral immune responses against tumor antigens

In total, 77.2% (112/145) of the studies on humoral immune responses against tumor antigens used ELISA assays, followed by western blotting (20.7%, 30/145 studies). Only 2.1% (3/145) of the studies used RIA or bead-based multiplex serology. The antigens used as targets for the immunoassays were derived from recombinant proteins in 75.9% (110/145) of the studies, followed by cell line extracts (11.7%, 17/145 studies) and synthetic peptides (9.7%, 14/145 studies). Tumor extracts were used in 2.8% (4/145) of the studies to detect serum antibodies (supplemental Table 1). Few studies validated their ELISA findings with an independent method, mostly using Western Blot or less frequently immunoprecipitation.

Most studies measured only IgG isotypes. For antibody responses against MUC1 epitopes, IgM antibodies were found more frequently than IgG in healthy controls, although the data are not completely consistent [14, 15]. The reported association of humoral immune responses against MUC1 with survival benefit in cancer patients was associated with IgG rather than with IgM responses in most studies [16–18].

Even within a tumor site, a wide range of antibody frequencies against the same tumor antigen have been reported in different studies. p53 antibodies in esophageal cancer patients were found in 60.0% (36/60) of tested patients in one study, while a second study reports p53 antibodies in only 7.0% (5/71) of esophageal cancer patients [19, 20]. Similarly, c-myc antibody frequency in hepatocellular carcinoma ranged from 1.3% (1/77) to 36.9% (24/65) [20, 21]. In addition to different assays used, various methods of normalization and to define cutoffs have been applied. Frequently, both background reactivity and cut-off were determined based on reactivity against a negative control antigen such as recombinant constructs only consisting of the tag, e.g., histidine or GST without the target antigen sequence or against albumin only [22, 23]. Other studies defined cutoffs by setting a threshold based on reactivity in normal control sera of various origins (e.g., threefold the standard deviation) [12, 21]. While one can assume that the targeted epitope plays an important role in determining antibody frequencies, this has only been demonstrated for the different MUC1 epitopes [24].

Association between antigen expression and humoral immune responses

For some antigens, a high correlation between antigen (over)expression and frequencies of humoral immune responses was found. Most data exist for p53, where antibody prevalence was associated with p53 overexpression or p53 mutations that lead to subsequent extensive accumulation of the protein in the cell [25–32]. Similarly, NY-ESO-1 antibody frequency was higher in patients with NY-ESO-1-expressing tumors (up to 83%) as compared to patients with NY-ESO-1 negative tumors (no antibody response) [33–35]. Goodell et al. [36] demonstrated a strong correlation between antibodies against Her2/neu and Her2/neu overexpression in breast cancer (82% antibodies in cases with strong expression vs. no antibodies in cases with weak expression). The finding that antibodies against MUC1 frequently occur in healthy individuals has been related to benign conditions such as inflammations that lead to the expression of hypo-glycosylated immunogenic MUC1 epitopes [37]. Against other antigens, antibodies were not

Table 1 Antibody frequencies in breast, colorectal, liver, lung cancer, and healthy controls against antigens analyzed in five or more studies

Antigen	Function	Localization	Tumor site	*n* studies	Samples analyzed	Antibody frequency (%) (weighted average)	Antibody frequency range (%)	References
c-myc	RNA splicing	Nucleus, cytoplasm	Breast	3	169	26.6	18.8–37.6	[12, 20, 21]
			Liver	4	349	20.1	1.3–36.9	[12, 13, 20, 21]
			Lung	4	331	12.4	9.1–17.7	[12, 20, 93, 94]
			Colorectal	3	154	11.7	4.4–15.4	[12, 20, 95]
			Healthy	7	1,017	1.0	0.0–3.4	[12, 13, 20, 21, 93–95]
Cyclin B1	Cell cycle control	Cytoplasm, nucleus	Lung	2	112	34.8	26.8–42.9	[12, 21]
			Colorectal	3	142	29.6	15.6–40.0	[12, 21, 95]
			Liver	2	207	13.0	12.3–13.4	[12, 13]
			Breast	1	64	4.7	–	[12]
			Healthy	6	911	1.4	0.0–2.4	[12, 13, 21, 95–97]
Her2/neu	Growth factor receptor	Membrane	Breast	2	124	26.6	21.2–55.0	[36, 98]
			Lung	1	104	12.5	–	[93]
			Healthy	2	150	2.0	2.0–2.0	[93, 99]
MUC1 (IgG)	Adhesion molecule	Membrane	Colorectal	1	56	48.2	–	[45]
			Breast	2	154	42.9	40.4–44.1	[14, 100]
			Lung	1	104	34.6	–	[100]
			Healthy	5	116	23.3	0.0–31.1	[14, 23, 45, 93, 101]
MUC1 (IgM)	Adhesion molecule	Membrane	Healthy	2	230	53.0	42.5–62.9	[14, 23]
			Breast	1	154	48.7	–	[14]
NY-ESO-1	Unknown	Cytoplasm	Lung	3	303	18.2	4.2–22.0	[35, 93, 102]
			Liver	2	281	8.9	2.2–12.2	[77, 103]
			Breast	1	26	7.7	–	[35]
			Healthy	4	169	0.6	0.0–2.0	[34, 35, 93, 103]
			Colorectal	1	25	0.0	–	[35]
p53	Cell cycle control	Nucleus, cytoplasm	Ovarian	5	486	45.5	24.8–59.5	[54, 55, 63, 75, 104]
			Esophageal	4	257	34.6	7.0–60.0	[20, 22, 62, 82]
			Oral	3	116	29.3	18.8–34.3	[19, 105, 106]
			Liver	5	364	12.9	9.1–25.0	[11–13, 20, 107]
			Breast	7	593	12.5	4.7–19.0	[12, 20, 26, 56, 60, 108, 109]
			Lung	5	439	9.1	7.1–26.8	[12, 20, 58, 93, 109]
			Colorectal	8	1,643	7.7	1.3–23.8	[12, 13, 20, 31, 57, 95, 108, 110]
			Healthy	17	1,136	2.0	0.0–6.3	[12, 13, 19, 20, 31, 55, 62, 93, 95, 97, 99, 104, 105, 108, 111–113]
p62	Insulin like growth factor II mRNA binding protein	Cytoplasm, membrane	Lung	2	140	19.3	17.9–21.4	[12, 13]
			Liver	4	299	7.7	10.7–17.7	[12, 13, 114, 115]
			Healthy	5	554	0.7	0.0–3.1	[12, 13, 97, 114, 115]
			Breast	2	162	0.0	5.1–7.8	[12, 13]
			Colorectal	2	110	0.0	9.2–11.1	[12, 13]
Survivin	Apoptosis inhibitor	Cytoplasm, nucleus	Lung	2	93	20.4	10.7–51.3	[12, 116]
			Breast	2	110	14.6	7.8–23.9	[12, 117]
			Liver	3	236	12.7	10.8–24.1	[12, 13, 118]
			Colorectal	1	45	4.4	–	[12]
			Healthy	6	538	0.7	0.0–2.5	[12, 13, 42, 97, 119, 120]

For p53 antibodies, frequencies are also included for ovarian, esophageal, and oral cancer. Protein function and cellular localization are from the ExPASy database (http://www.expasy.org)

detectable or only at low frequencies despite a strong overexpression of the antigen (Melan A, $p16^{INK4a}$) [35, 38]. The presence of antibodies in patients with antigen-negative tumors has been reported in some studies and could indicate either a loss of antigen-expression after immune priming and selection of antigen negative tumor cells or antigen expression in tissues that were not analyzed for expression, e.g., distant metastases [34, 39].

Association between tumor stage and humoral immune responses

Antibodies against several tumor-associated antigens such as p53, NY-ESO-1, survivin, and tyrosinase were found more frequently in advanced tumor stages which corroborates the importance of antigen load and duration of antigen exposure for the induction of humoral immune responses [39–43]. Higher incidence and titers of Her2/neu and MUC1 antibodies have been detected mainly at early stages [14, 23, 44, 45] (Table 2; supplemental Table 3) although Her2/neu antigen expression is known to increase in advanced metastatic disease [46, 47]. The loss of antibodies despite continued or even enhanced expression of the antigen may suggest that loss of antibodies leads to disease progression. However, these hypotheses have not been evaluated in prospective studies.

Diagnostic and prognostic relevance of humoral immune responses against tumor antigens

Several studies evaluated the use of humoral immune responses against tumor antigens as a serological biomarker for cancer. Antibodies against most tumor antigens are only found in 0–3% of healthy controls and seem to be highly specific for cancer, but average antibody frequencies against single antigens in tumor patients unselected for antigen expression rarely exceed 15% (Table 1; supplemental Table 2). Rare examples such as ECPKA and EpCAM have positivity rates of 70 or 90% in some cancer sites [48, 49]. Therefore, a reasonable clinical sensitivity for a diagnostic test cannot be expected for currently tested antigens. Recent studies show evidence that measuring antibodies against a panel of specific antigens could increase sensitivity substantially [12, 48, 50–53].

Specific associations between humoral immune responses against tumor antigens and prognosis could impact clinical management and might suggest a functional role of the immune response in tumor biology. Studies that have reported associations of antibody presence with survival are shown in Table 3 for the most frequent antigens and in supplemental Table 4 for all antigens.

p53

p53 is mutated in over 50% of all cancers such as esophageal, lung, colorectal, and ovarian and humoral immune responses can be observed that correlate to accumulation of the mutated protein in the cell [32]. Poor survival of p53-antibody positive patients has been demonstrated in many studies for various tumor sites [19, 31, 54–63]. This association might reflect the higher antigen load in more advanced stages that have a worse prognosis. It has also been speculated that specific p53 mutations are associated with antibody production and that p53 antibodies might reflect specific mutations that lead to a more aggressive tumor [26, 64]. Furthermore, two mechanisms explaining a tumor promoting role of p53 antibodies in tumor immunosurveillance have been described: Antibodies directed against p53 could inhibit antigen uptake by antigen presenting cells through the formation of immune complexes

Table 2 Studies reporting an association of humoral immune responses with tumor stage for tumor antigens analyzed in five or more studies

Antigen	Result	Tumor site(s)	Reference(s)
Her2/neu	Higher antibody prevalence in early stages	Breast	[44]
MUC1		Breast, gastric, colorectal, ovarian	[14, 23, 45]
NY-ESO-1	Higher antibody prevalence in advanced stages	Esophageal	[39]
p53		Colorectal, oral, gastric, breast, lymphoma, esophageal, ovarian	[19, 31, 57, 59–63]
Survivin		Head and neck	[42]

Table 3 Studies reporting an association of humoral immune responses with prognosis for tumor antigens analyzed in five or more studies

Antigen	Results	Tumor site(s)	Reference(s)
MUC1	Antibodies associated with survival benefit	Breast, gastric, lung, ovarian, pancreatic	[14, 16–18, 23, 81]
NY-ESO-1	Antibodies associated with poor prognosis	Prostate	[40]
p53		Ovarian, breast, colorectal, lung	[54–58]

[65–67]. There is also evidence for an immune inhibitory effect of cytokines secreted by B cells that might result in suppression of effective cytotoxic T cell responses directed against p53 [68].

Only very few studies analyzed antibodies in individuals with a premalignant tumor stage and even less studies were designed prospectively to evaluate the risk of cancer development in antibody positive patients. Although antibodies against p53 could be found in patients with premalignant oral lesions and in heavy smokers with risk for the development of lung cancer [54, 69–71], the clinical utility of p53 antibody serology in monitoring high risk individuals needs to be evaluated in prospective studies. So far, there are only anecdotal reports of anti-p53 immune responses in a prospective setting [72–74].

For several tumor sites a decline or even disappearance of p53 antibody levels few weeks after surgical tumor removal has been demonstrated, which is in line with the hypothesis that constant stimulation of the immune system by the antigen is necessary to maintain high antibody levels [62, 73, 75, 76]. Disappearance of NY-ESO-1 antibodies after tumor resection has also been demonstrated [33, 77]. These findings led to the suggestion that antibody serology might be a tool for detection of disease recurrence. However, the predictive value of antibodies for tumor recurrence has not been demonstrated in prospective studies yet.

MUC1

The epithelial mucin MUC1 is overexpressed in over 90% of all adenocarcinomas, such as breast, colorectal, pancreatic and ovarian cancers and associated with an aggressive behavior of the tumor [78]. Altered glycosylation patterns of MUC1 may induce immune responses on the cellular and humoral level and tumor vaccines and monoclonal antibodies based on this antigen are tested in clinical trials [37]. The presence of MUC1 antibodies has been associated with improved survival in early stage patients with ovarian, gastric, lung, pancreatic or breast cancer [14, 16–18, 45, 79–82]. It is difficult to demonstrate a causal relationship between antibody presence and longer survival, as the development of antibodies in longer survival patients may simply reflect protracted exposure to the antigen. Tumor immune surveillance is most likely significantly affected by cell-mediated immunity, thus functional modulations cannot be attributed to humoral responses alone [83, 84]. Increased expression of MUC1 in adenocarcinomas may affect cellular adhesion and metastasis by interfering with integrin- and cadherin-mediated adhesion [85]. Some data suggest a functional role of MUC1 antibodies by binding MUC1 proteins and inhibiting their tumor invasive functions [86]. MUC1 containing immune complexes are frequently found in cancer patients [87].

Hypoglycosylated MUC1 derivates may also be generated by inflammations and other prevalent benign conditions in otherwise healthy individuals [37]. Because MUC1 antibodies are repeatedly found in healthy controls, it is conceivable that an immune response could be primed that confers a protective effect for the development of tumors. Cramer et al. [88] showed a protective effect of humoral immune responses against MUC1 on the development of ovarian cancer.

Her2/neu

Her2/neu is overexpressed in 30% of all adenocarcinomas, such as breast, colorectal, and ovarian cancers and associated with poor disease outcome [89]. The monoclonal recombinant anti-Her2/neu antibody Trastuzumab is successfully used to treat Her2/neu overexpressing breast cancers [90]. Interestingly, although no clinical effects in terms of survival benefit have been reported for endogenous Her2/neu antibodies, it has been shown that these antibodies can modulate the transforming properties of Her2/neu signaling toward an anti-proliferative state, similar to what has been demonstrated for the therapeutic Her2/neu antibody [91]. It can be speculated that the levels of spontaneously induced endogenous Her2/neu antibodies are insufficient to confer relevant growth inhibitory effects. In support of this hypothesis, high titers of endogenous Her2/neu antibodies induced by vaccination with peptides were shown to improve survival [90, 92].

Conclusions and perspective

Antibodies against tumor antigens are frequently found in the serum of cancer patients and have been analyzed in many studies during the last years. However, the biological and clinical relevances of humoral immune responses against tumor antigens have not been systematically evaluated so far. Two questions are especially important when analyzing humoral immune responses against tumor antigens: (1) How are humoral immune responses against tumor antigens generated and how is tumor biology modulated by these immune responses? (2) Can antibodies against tumor antigens be used as serological biomarkers for cancer early detection, post-therapy surveillance or estimation of prognosis?

Overall, the mechanisms by which tumor proteins become immunogenic and induce humoral and/or cellular immune responses remain largely unknown. Both cellular and humoral immune responses have been found independently from each other and also against different epitopes. Based on the data summarized in this review, a common functional role of humoral immune responses against tumor antigens is very unlikely. Some antibodies directed against

tumor-associated antigens seem to be mere markers of exposure to the antigen without functional relevance. These immune responses might be used for cancer early detection, prognosis, and post-treatment surveillance. Data on prediagnostic antibody levels are scarce so that it is currently unclear what the predictive value of prediagnostic humoral immune responses might be. Even in blood samples obtained at diagnosis, antibody frequencies for most tumor antigens are low precluding their use in diagnostic assays for cancer detection; however, there is some promising data on achieving higher sensitivity of antibody serology for cancer detection using panels of tumor antigens. Also, by analyzing the antibody spectrum against tumor-associated antigens, it might be possible to detect cancer subtypes with specific molecular and/or immunologic features that could be relevant for novel therapeutic approaches.

Humoral immune responses against p53 have been shown to be associated with poor prognosis. While this might be primarily related to increased exposure with the antigen, antibodies might also reflect p53 mutations that lead to a more aggressive tumor behavior. Also, it cannot be excluded that direct immune-modulatory mechanisms affect tumor biology. Associations of antibodies with favorable prognosis were repeatedly reported for MUC1 antibodies and may be related to interference of antibodies with MUC1-induced tumor growth promoting mechanisms. A similar cancer-suppressive mechanism has been suggested for endogenous antibodies directed against Her2/neu. Based on these observations, it may be suggested that cancer vaccines targeting these antigens should be designed to induce not only cytotoxic cellular immune responses, but also CD4 T cell and B cell responses.

Further studies using combination of antigens will be necessary to identify appropriate antigen panels and evaluate the suitability of antibody serology for diagnostic purposes. Additional efforts in this field should cover more systematically dynamic changes of antibody levels during the course of disease, especially associations with therapy response and survival. The functional role of humoral immune responses against tumor antigens needs to be further analyzed in the context of cellular immune responses and especially in cancer immunotherapy settings. Future data should be generated using more standardized methods, especially with respect to data normalization and threshold definition for antibody positivity.

References

1. Kamradt T, Mitchison NA (2001) Tolerance and autoimmunity. N Engl J Med 344:655–664
2. Fonseca C, Dranoff G (2008) Capitalizing on the immunogenicity of dying tumor cells. Clin Cancer Res 14:1603–1608
3. Smyth MJ, Godfrey DI, Trapani JA (2001) A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2:293–299
4. Turk MJ, Wolchok JD, Guevara-Patino JA et al (2002) Multiple pathways to tumor immunity and concomitant autoimmunity. Immunol Rev 188:122–135
5. Delves PJ, Roitt IM (2000) The immune system. First of two parts. N Engl J Med 343:37–49
6. Houghton AN (1994) Cancer antigens: immune recognition of self and altered self. J Exp Med 180:1–4
7. Jäger E, Jäger D, Knuth A (2003) Antigen-specific immunotherapy and cancer vaccines. Int J Cancer 106:817–820
8. Renkvist N, Castelli C, Robbins PF et al (2001) A listing of human tumor antigens recognized by T cells. Cancer Immunol Immunother 50:3–15
9. Finn OJ (2008) Cancer immunology. N Engl J Med 358:2704–2715
10. Rescigno M, Avogadri F, Curigliano G (2007) Challenges and prospects of immunotherapy as cancer treatment. Biochim Biophys Acta 1776:108–123
11. Himoto T, Kuriyama S, Zhang JY et al (2005) Analyses of autoantibodies against tumor-associated antigens in patients with hepatocellular carcinoma. Int J Oncol 27:1079–1085
12. Zhang JY, Casiano CA, Peng XX et al (2003) Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. Cancer Epidemiol Biomarkers Prev 12:136–143
13. Zhang JY, Megliorino R, Peng XX et al (2007) Antibody detection using tumor-associated antigen mini-array in immunodiagnosing human hepatocellular carcinoma. J Hepatol 46:107–114
14. Mensdorff-Pouilly S, Verstraeten AA, Kenemans P et al (2000) Survival in early breast cancer patients is favorably influenced by a natural humoral immune response to polymorphic epithelial mucin. J Clin Oncol 18:574–583
15. Croce MV, Isla-Larrain MT, Capafons A et al (2001) Humoral immune response induced by the protein core of MUC1 mucin in pregnant and healthy women. Breast Cancer Res Treat 69:1–11
16. Kurtenkov O, Klaamas K, Rittenhouse-Olson K et al (2005) IgG immune response to tumor-associated carbohydrate antigens (TF, Tn, alphaGal) in patients with breast cancer: impact of neoadjuvant chemotherapy and relation to the survival. Exp Oncol 27:136–140
17. Kurtenkov O, Klaamas K, Mensdorff-Pouilly S et al (2007) Humoral immune response to MUC1 and to the Thomsen-Friedenreich (TF) glycotope in patients with gastric cancer: relation to survival. Acta Oncol 46:316–323
18. Hamanaka Y, Suehiro Y, Fukui M et al (2003) Circulating anti-MUC1 IgG antibodies as a favorable prognostic factor for pancreatic cancer. Int J Cancer 103:97–100
19. Ralhan R, Nath N, Agarwal S et al (1998) Circulating p53 antibodies as early markers of oral cancer: correlation with p53 alterations. Clin Cancer Res 4:2147–2152
20. Looi K, Megliorino R, Shi FD et al (2006) Humoral immune response to p16, a cyclin-dependent kinase inhibitor in human malignancies. Oncol Rep 16:1105–1110
21. Koziol JA, Zhang JY, Casiano CA et al (2003) Recursive partitioning as an approach to selection of immune markers for tumor diagnosis. Clin Cancer Res 9:5120–5126
22. Ralhan R, Arora S, Chattopadhyay TK et al (2000) Circulating p53 antibodies, p53 gene mutational profile and product accumulation in esophageal squamous-cell carcinoma in India. Int J Cancer 85:791–795
23. Richards ER, Devine PL, Quin RJ et al (1998) Antibodies reactive with the protein core of MUC1 mucin are present in ovarian cancer patients and healthy women. Cancer Immunol Immunother 46:245–252

24. Graves CR, Robertson JF, Murray A et al (2005) Malignancy-induced autoimmunity to MUC1: initial antibody characterization. J Pept Res 66:357–363
25. Green JA, Mudenda B, Jenkins J et al (1994) Serum p53 autoantibodies: incidence in familial breast cancer. Eur J Cancer 30A:580–584
26. Davidoff AM, Iglehart JD, Marks JR (1992) Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers. Proc Natl Acad Sci USA 89:3439–3442
27. Komiya T, Hirashima T, Takada M et al (1997) Prognostic significance of serum p53 antibodies in squamous cell carcinoma of the lung. Anticancer Res 17:3721–3724
28. Kressner U, Glimelius B, Bergstrom R et al (1998) Increased serum p53 antibody levels indicate poor prognosis in patients with colorectal cancer. Br J Cancer 77:1848–1851
29. Hammel P, Leroy-Viard K, Chaumette MT et al (1999) Correlations between p53-protein accumulation, serum antibodies and gene mutation in colorectal cancer. Int J Cancer 81:712–718
30. Bourhis J, Lubin R, Roche B et al (1996) Analysis of p53 serum antibodies in patients with head and neck squamous cell carcinoma. J Natl Cancer Inst 88:1228–1233
31. Lechpammer M, Lukac J, Lechpammer S et al (2004) Humoral immune response to p53 correlates with clinical course in colorectal cancer patients during adjuvant chemotherapy. Int J Colorectal Dis 19:114–120
32. Soussi T (2000) p53 Antibodies in the sera of patients with various types of cancer: a review. Cancer Res 60:1777–1788
33. Jäger E, Stockert E, Zidianakis Z et al (1999) Humoral immune responses of cancer patients against "Cancer-Testis" antigen NY-ESO-1: correlation with clinical events. Int J Cancer 84:506–510
34. Maio M, Coral S, Sigalotti L et al (2003) Analysis of cancer/testis antigens in sporadic medullary thyroid carcinoma: expression and humoral response to NY-ESO-1. J Clin Endocrinol Metab 88:748–754
35. Stockert E, Jäger E, Chen YT et al (1998) A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 187:1349–1354
36. Goodell V, Waisman J, Salazar LG et al (2008) Level of HER-2/neu protein expression in breast cancer may affect the development of endogenous HER-2/neu-specific immunity. Mol Cancer Ther 7:449–454
37. Karsten U, Mensdorff-Pouilly S, Goletz S (2005) What makes MUC1 a tumor antigen? Tumour Biol 26:217–220
38. Reuschenbach M, Waterboer T, Wallin KL et al (2008) Characterization of humoral immune responses against p16, p53, HPV16 E6 and HPV16 E7 in patients with HPV-associated cancers. Int J Cancer 123:2626–2631
39. Akcakanat A, Kanda T, Koyama Y et al (2004) NY-ESO-1 expression and its serum immunoreactivity in esophageal cancer. Cancer Chemother Pharmacol 54:95–100
40. Fossa A, Berner A, Sophie DF et al (2004) NY-ESO-1 protein expression and humoral immune responses in prostate cancer. Prostate 59:440–447
41. Wadle A, Kubuschok B, Imig J et al (2006) Serological immune response to cancer testis antigens in patients with pancreatic cancer. Int J Cancer 119:117–125
42. Chang JT, Wong FH, Liao CT et al (2004) Enzyme immunoassay for serum autoantibody to survivin and its findings in head-and-neck cancer patients. Clin Chem 50:1261–1264
43. Fishman P, Merimski O, Baharav E et al (1997) Autoantibodies to tyrosinase: the bridge between melanoma and vitiligo. Cancer 79:1461–1464
44. Disis ML, Pupa SM, Gralow JR et al (1997) High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. J Clin Oncol 15:3363–3367
45. Nakamura H, Hinoda Y, Nakagawa N et al (1998) Detection of circulating anti-MUC1 mucin core protein antibodies in patients with colorectal cancer. J Gastroenterol 33:354–361
46. Traina A, Agostara B, Marasa L et al (2006) HER2/neu expression in relation to clinicopathologic features of breast cancer patients. Ann N Y Acad Sci 1089:159–167
47. Ferretti G, Felici A, Papaldo P et al (2007) HER2/neu role in breast cancer: from a prognostic foe to a predictive friend. Curr Opin Obstet Gynecol 19:56–62
48. Nesterova M, Johnson N, Cheadle C et al (2006) Autoantibody biomarker opens a new gateway for cancer diagnosis. Biochim Biophys Acta 1762:398–403
49. Kim JH, Herlyn D, Wong KK et al (2003) Identification of epithelial cell adhesion molecule autoantibody in patients with ovarian cancer. Clin Cancer Res 9:4782–4791
50. Qin S, Qiu W, Ehrlich JR et al (2006) Development of a "reverse capture" autoantibody microarray for studies of antigen-autoantibody profiling. Proteomics 6:3199–3209
51. Philip R, Murthy S, Krakover J et al (2007) Shared immunoproteome for ovarian cancer diagnostics and immunotherapy: potential theranostic approach to cancer. J Proteome Res 6:2509–2517
52. Lu H, Goodell V, Disis ML (2008) Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer. J Proteome Res 7:2168
53. Ran Y, Hu H, Zhou Z et al (2008) Profiling tumor-associated autoantibodies for the detection of colon cancer. Clin Cancer Res 14:2696–2700
54. Abendstein B, Marth C, Müller-Holzner E et al (2000) Clinical significance of serum and ascitic p53 autoantibodies in epithelial ovarian carcinoma. Cancer 88:1432–1437
55. Mayerhofer K, Tempfer C, Kucera E et al (1999) Humoral p53 antibody response is a prognostic parameter in ovarian cancer. Anticancer Res 19:875–878
56. Volkmann M, Sinn HP, Gaugel D et al (2002) Anti-p53 in breast cancer: concordance of different assay procedures and association with p53 antigen expression. Oncology 63:297–305
57. Tang R, Ko MC, Wang JY et al (2001) Humoral response to p53 in human colorectal tumors: a prospective study of 1, 209 patients. Int J Cancer 94:859–863
58. Lai CL, Tsai CM, Tsai TT et al (1998) Presence of serum anti-p53 antibodies is associated with pleural effusion and poor prognosis in lung cancer patients. Clin Cancer Res 4:3025–3030
59. Qiu LL, Hua PY, Ye LL et al (2007) The detection of serum anti-p53 antibodies from patients with gastric carcinoma in China. Cancer Detect Prev 31:45–49
60. Sangrajrang S, Arpornwirat W, Cheirsilpa A et al (2003) Serum p53 antibodies in correlation to other biological parameters of breast cancer. Cancer Detect Prev 27:182–186
61. Jezersek B, Rudolf Z, Novakovic S (2001) The circulating autoantibodies to p53 protein in the follow-up of lymphoma patients. Oncol Rep 8:77–81
62. Shimada H, Takeda A, Arima M et al (2000) Serum p53 antibody is a useful tumor marker in superficial esophageal squamous cell carcinoma. Cancer 89:1677–1683
63. Vogl FD, Stickeler E, Weyermann M et al (1999) p53 autoantibodies in patients with primary ovarian cancer are associated with higher age, advanced stage and a higher proportion of p53-positive tumor cells. Oncology 57:324–329
64. Scott N, Sagar P, Stewart J et al (1991) p53 in colorectal cancer: clinicopathological correlation and prognostic significance. Br J Cancer 63:317–319
65. Qin Z, Richter G, Schüler T et al (1998) B cells inhibit induction of T cell-dependent tumor immunity. Nat Med 4:627–630
66. Gumus E, Erdamar S, Demirel G et al (2004) Association of positive serum anti-p53 antibodies with poor prognosis in bladder cancer patients. Int J Urol 11:1070–1077

67. Manson LA (1991) Does antibody-dependent epitope masking permit progressive tumour growth in the face of cell-mediated cytotoxicity? Immunol Today 12:352–355
68. Tan TT, Coussens LM (2007) Humoral immunity, inflammation and cancer. Curr Opin Immunol 19:209–216
69. Imai H, Ochs RL, Kiyosawa K et al (1992) Nucleolar antigens and autoantibodies in hepatocellular carcinoma and other malignancies. Am J Pathol 140:859–870
70. Trivers GE, Cawley HL, DeBenedetti VM et al (1995) Anti-p53 antibodies in sera of workers occupationally exposed to vinyl chloride. J Natl Cancer Inst 87:1400–1407
71. Lubin R, Schlichtholz B, Teillaud JL et al (1995) p53 antibodies in patients with various types of cancer: assay, identification, and characterization. Clin Cancer Res 1:1463–1469
72. Schlichtholz B, Legros Y, Gillet D et al (1992) The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. Cancer Res 52:6380–6384
73. Lubin R, Zalcman G, Bouchet L et al (1995) Serum p53 antibodies as early markers of lung cancer. Nat Med 1:701–702
74. Schlichtholz B, Tredaniel J, Lubin R et al (1994) Analyses of p53 antibodies in sera of patients with lung carcinoma define immunodominant regions in the p53 protein. Br J Cancer 69:809–816
75. Angelopoulou K, Diamandis EP, Sutherland DJ et al (1994) Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. Int J Cancer 58:480–487
76. Hammel P, Boissier B, Chaumette MT et al (1997) Detection and monitoring of serum p53 antibodies in patients with colorectal cancer. Gut 40:356–361
77. Korangy F, Ormandy LA, Bleck JS et al (2004) Spontaneous tumor-specific humoral and cellular immune responses to NY-ESO-1 in hepatocellular carcinoma. Clin Cancer Res 10:4332–4341
78. Yonezawa S, Goto M, Yamada N et al (2008) Expression profiles of MUC1, MUC2, and MUC4 mucins in human neoplasms and their relationship with biological behavior. Proteomics 8:3329–3341
79. Parmigiani RB, Bettoni F, Grosso DM et al (2008) Antibodies against the cancer-testis antigen CTSP-1 are frequently found in prostate cancer patients and are an independent prognostic factor for biochemical-recurrence. Int J Cancer 122:2385–2390
80. Bachelot T, Ratel D, Menetrier-Caux C et al (2006) Autoantibodies to endostatin in patients with breast cancer: correlation to endostatin levels and clinical outcome. Br J Cancer 94:1066–1070
81. Hirasawa Y, Kohno N, Yokoyama A et al (2000) Natural autoantibody to MUC1 is a prognostic indicator for non-small cell lung cancer. Am J Respir Crit Care Med 161:589–594
82. Shimada H, Kuboshima M, Shiratori T et al (2007) Serum antimyomegalin antibodies in patients with esophageal squamous cell carcinoma. Int J Oncol 30:97–103
83. Boon T, van der BP (1996) Human tumor antigens recognized by T lymphocytes. J Exp Med 183:725–729
84. Finn OJ (2007) Human tumor immunology at the molecular divide. J Immunol 178:2615–2616
85. Wesseling J, van der Valk SW, Hilkens J (1996) A mechanism for inhibition of E-cadherin-mediated cell–cell adhesion by the membrane-associated mucin episialin/MUC1. Mol Biol Cell 7:565–577
86. Agrawal B, Krantz MJ, Reddish MA et al (1998) Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nat Med 4:43–49
87. Gourevitch MM, Mensdorff-Pouilly S, Litvinov SV et al (1995) Polymorphic epithelial mucin (MUC-1)-containing circulating immune complexes in carcinoma patients. Br J Cancer 72:934–938
88. Cramer DW, Titus-Ernstoff L, McKolanis JR et al (2005) Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev 14:1125–1131
89. Menard S, Casalini P, Campiglio M et al (2004) Role of HER2/neu in tumor progression and therapy. Cell Mol Life Sci 61:2965–2978
90. Jager D, Knuth A (2005) Antibodies and vaccines—hope or illusion? Breast 14:631–635
91. Montgomery RB, Makary E, Schiffman K et al (2005) Endogenous anti-HER2 antibodies block HER2 phosphorylation and signaling through extracellular signal-regulated kinase. Cancer Res 65:650–656
92. Disis ML, Schiffman K (2001) Cancer vaccines targeting the HER2/neu oncogenic protein. Semin Oncol 28:12–20
93. Chapman CJ, Murray A, McElveen JE et al (2008) Autoantibodies in lung cancer: possibilities for early detection and subsequent cure. Thorax 63:228–233
94. Yamamoto A, Shimizu E, Ogura T et al (1996) Detection of autoantibodies against L-myc oncogene products in sera from lung cancer patients. Int J Cancer 69:283–289
95. Chen Y, Lin P, Qiu S et al (2007) Autoantibodies to Ca^{2+} binding protein Calnuc is a potential marker in colon cancer detection. Int J Oncol 30:1137–1144
96. Ersvaer E, Zhang JY, McCormack E et al (2007) Cyclin B1 is commonly expressed in the cytoplasm of primary human acute myelogenous leukemia cells and serves as a leukemia-associated antigen associated with autoantibody response in a subset of patients. Eur J Haematol 79:210–225
97. Shi FD, Zhang JY, Liu D et al (2005) Preferential humoral immune response in prostate cancer to cellular proteins p90 and p62 in a panel of tumor-associated antigens. Prostate 63:252–258
98. Disis ML, Calenoff E, McLaughlin G et al (1994) Existent T-cell and antibody immunity to HER-2/neu protein in patients with breast cancer. Cancer Res 54:16–20
99. McNeel DG, Nguyen LD, Storer BE et al (2000) Antibody immunity to prostate cancer associated antigens can be detected in the serum of patients with prostate cancer. J Urol 164:1825–1829
100. Chapman C, Murray A, Chakrabarti J et al (2007) Autoantibodies in breast cancer: their use as an aid to early diagnosis. Ann Oncol 18:868–873
101. Snijdewint FG, Mensdorff-Pouilly S, Karuntu-Wanamarta AH et al (1999) Cellular and humoral immune responses to MUC1 mucin and tandem-repeat peptides in ovarian cancer patients and controls. Cancer Immunol Immunother 48:47–55
102. Türeci O, Mack U, Luxemburger U et al (2006) Humoral immune responses of lung cancer patients against tumor antigen NY-ESO-1. Cancer Lett 236:64–71
103. Nakamura S, Nouso K, Noguchi Y et al (2006) Expression and immunogenicity of NY-ESO-1 in hepatocellular carcinoma. J Gastroenterol Hepatol 21:1281–1285
104. Hogdall EV, Hogdall CK, Blaakaer J et al (2002) P53 autoantibodies in sera from Danish ovarian cancer patients and their correlation with clinical data and prognosis. APMIS 110:545–553
105. Kaur J, Srivastava A, Ralhan R (1997) Serum p53 antibodies in patients with oral lesions: correlation with p53/HSP70 complexes. Int J Cancer 74:609–613
106. Castelli M, Cianfriglia F, Manieri A et al (2001) Anti-p53 and anti-heat shock proteins antibodies in patients with malignant or pre-malignant lesions of the oral cavity. Anticancer Res 21:753–758
107. Volkmann M, Muller M, Hofmann WJ et al (1993) The humoral immune response to p53 in patients with hepatocellular carcinoma is specific for malignancy and independent of the alpha-fetoprotein status. Hepatology 18:559–565

108. Coomber D, Hawkins NJ, Clark M et al (1996) Characterisation and clinicopathological correlates of serum anti-p53 antibodies in breast and colon cancer. J Cancer Res Clin Oncol 122:757–762
109. Lubin R, Schlichtholz B, Bengoufa D et al (1993) Analysis of p53 antibodies in patients with various cancers define B-cell epitopes of human p53: distribution on primary structure and exposure on protein surface. Cancer Res 53:5872–5876
110. Angelopoulou K, Stratis M, Diamandis EP (1997) Humoral immune response against p53 protein in patients with colorectal carcinoma. Int J Cancer 70:46–51
111. Creaney J, McLaren BM, Stevenson S et al (2001) p53 autoantibodies in patients with malignant mesothelioma: stability through disease progression. Br J Cancer 84:52–56
112. Labrecque S, Naor N, Thomson D et al (1993) Analysis of the anti-p53 antibody response in cancer patients. Cancer Res 53:3468
113. Selter H, Schmidt G, Villena-Heinsen C et al (1999) Humoral immune response to p21WAF1/CIP1 in tumor patients, non-tumorous patients and healthy blood donors. Cancer Lett 137:151–157
114. Zhang JY, Zhu W, Imai H et al (2001) De-novo humoral immune responses to cancer-associated autoantigens during transition from chronic liver disease to hepatocellular carcinoma. Clin Exp Immunol 125:3–9
115. Zhang JY, Chan EK, Peng XX et al (2001) Autoimmune responses to mRNA binding proteins p62 and Koc in diverse malignancies. Clin Immunol 100:149–156
116. Yagihashi A, Asanuma K, Kobayashi D et al (2005) Detection of autoantibodies to livin and survivin in Sera from lung cancer patients. Lung Cancer 48:217–221
117. Yagihashi A, Ohmura T, Asanuma K et al (2005) Detection of autoantibodies to survivin and livin in sera from patients with breast cancer. Clin Chim Acta 362:125–130
118. Yagihashi A, Asanuma K, Kobayashi D et al (2005) Autoantibodies to survivin in patients with chronic hepatitis and hepatocellular carcinoma. Autoimmunity 38:445–448
119. Anderson KS, Ramachandran N, Wong J et al (2008) Application of protein microarrays for multiplexed detection of antibodies to tumor antigens in breast cancer. J Proteome Res 7:1490–1499
120. Soling A, Plugge EM, Schmitz M et al (2007) Autoantibodies to the inhibitor of apoptosis protein survivin in patients with brain tumors. Int J Oncol 30:123–128