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Serologic Laboratory Findings in Malignancy

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Serologic findings such as antinuclear antibodies (ANA), usually found in systemic rheumatic diseases, have been known for decades to occur in patients with various cancers.[1,2] These and other early reports on ANAs in cancer sera[3–8] and the remarkable work responsible for the well-recognized value of autoantibodies for the diagnosis of autoimmune diseases (ADs) such as systemic lupus erythematosus (SLE), scleroderma, and dermatomyositis (DM)/polymyositis (PM)[9–13] suggested that autoantibodies could also be potential diagnostic and prognostic biomarkers for cancer. Biomarker discovery using genomics[14–21] and proteomics[22,23] led to the identification of a multitude of autoantibodies in various types of cancer, some of which appeared to be potential biomarkers for the diagnosis and prognosis of cancer.[16–21] Other approaches using antigens known to be involved in carcinogenesis showed that autoantibodies can also be used as potential diagnostic biomarkers.[24–26] However, ANAs in cancer sera were for a long time regarded as epiphenomena without any clinical significance. The interpretation of the significance of autoantibodies in cancer sera remained controversial, because although there are examples of autoantibodies commonly found in the ADs such as anti-DNA, anti-Sm, anti-RNP, and other antibodies, [27–31] a large number of the autoantibodies found in cancer sera do not recognize the autoantigens classically associated with the ADs.[15–17,22] These conflicting data were generally interpreted as an indication of the nonspecificity of autoantibodies in cancer sera. However, exceptions were known to occur, and a relatively small but steadily increasing group of autoantibodies recognizing the same antigens have been reported in both cancer and autoimmune sera. It is well known that patients with cancer may develop rheumatic, neurologic, and other symptoms generally thought to result from reactivity of autoantibodies with autoantigens located in tissues other than the primary tumor. The discussion of the clinical features as well as the serologic findings in cancer patients with paraneoplastic syndromes,[32–34] as well as those in cancer developing in patients with rheumatic diseases,[35–38] are outside of the scope of this review. Space limitations preclude the discussion of other important serologic findings in cancer sera, such as the cytokines, which have been covered in several publications.[39–41]

This review discusses serologic laboratory findings in malignancy of interest to rheumatologists as well as oncologists, from both the practical and basic viewpoints. Because of the potential significance of the use of autoantibodies for the screening and diagnosis of solid tumors, the article reviews primarily the work intending to identify

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diagnostic biomarkers for cancer and the number of autoantibodies common to the systemic ADs and cancer. Hundreds of autoantibodies recognizing tumor-associated antigens (TAAs) have been reported and reviewed in the past, and by necessity their discussion in this review is limited. The serologic findings in the sera of patients with solid tumors and hematological malignancies that the clinician may encounter in the practice of medicine are also reviewed. Finally, in view of the provocative recent data on the diagnostic and prognostic value of autoantibodies in cancer sera obtained using genomics and proteomics methodologies and the increasing recognition of an important role of B cells in the anticancer immune response,[42–47] the potential significance of the prominent autoantibody response in cancer sera is discussed.

AUTOANTIBODIES AS POTENTIAL DIAGNOSTIC BIOMARKERS OF SOLID TUMORS

Cancer sera contain antibodies that react with a unique group of autologous cellular antigens called TAAs. Many studies have demonstrated that single antibody specificities recognize their corresponding autoantigens in a range from 10% to 20%, which is not satisfactory for diagnostic purposes, and there is general agreement that panels of autoantibodies are superior to single autoantibody markers as potential diagnostic markers in cancer.[15,16,18–21,24,48,49] Autoantibody panels with relatively high sensitivity and specificity have been reported for several cancers.[16,18–21] However, the levels of sensitivity and specificity achieved thus far do not seem sufficiently high to be useful in the clinical arena. Further work will be necessary to improve the accuracy of autoantibody panels to levels that could be helpful for the clinician. This task is a challenging one that will involve the refining of the reported diagnostic panels, and should culminate in the prospective validation of such panels with independent collections sera from cancer patients and controls. Validation is a prerequisite for any diagnostic autoantibody panel before its use in the clinical laboratory and its introduction in clinical trials. Problems encountered by these studies include the selection and recruiting of large numbers of cancer patients and controls required to achieve statistical significance. Multiple studies have found that potential TAAs manifest reactivity with sera from control donors. On this basis, the validation of antigen-antibody systems with cancer-related serologic profile is a complex task requiring a large number of sera from cases and controls. Not only should the numbers of control sera be sufficient for statistical evaluation of the data on autoantibody reactivity, but the choice of controls is of utmost importance. The use of control subjects drawn from the population at risk for a given cancer is probably the preferred method, because data showing statistical significance obtained using convenience controls may not stand using real-life controls. Many studies have used high-throughput microarray or proteomics platforms that are labor intensive and very complex, and although highly adequate for biomarker discovery, these are not suitable for use at the clinical laboratory level. Thus, once a diagnostic instrument based on autoantibodies has achieved sufficiently high accuracy to identify cases and controls at the biodiscovery level, other more flexible and easy-to-use platforms such as specific enzyme-linked immunosorbent assays (ELISAs), or other platforms based on bioluminescence, will probably be used to test the diagnostic panels in clinical studies. There is presently a great deal of interest in these studies that promise to promote the early diagnosis of cancer.

Several different approaches have been used thus far for biomarker discovery. Some studies were based on the construction of microarrays using collections of proteins known to be involved in carcinogenesis, which are recognized as autoantigens in various malignancies.[24,48,49] These microcollections are hybridized with sera from cancer patients and controls using high-throughput autoantigen microarray technology or ELISA platforms. Using this approach, Zhang and colleagues[24] reported that a mini-array of multiple TAAs would enhance antibody detection and could be a useful approach for cancer detection.

These investigators used full-length recombinant proteins expressed from cDNAs encoding c-myc, p53, cyclin B1, p62, Koc, IMP1, and survivin in a diagnostic mini-array. Enzyme immunoassay was used to detect antibodies in sera from 6 different types of cancers. Antibody frequency to any individual TAA was variable but rarely exceeded 15% to 20%. With the successive addition of TAAs to a total of 7 antigens, there was a stepwise increase of positive antibody reactions up to a range of 44% to 68%, showing the advantages of using a panel of TAAs rather than single specificities. Sera from patients with breast, lung, and prostate cancer showed separate and distinct profiles of reactivity, suggesting that uniquely constituted antigen mini-arrays might be developed to distinguish between some types of cancer. It was proposed that detection of autoantibodies in cancer sera can be enhanced by using a mini-array of several TAAs as target antigens. Chapman and colleagues[48] used a quality-controlled, semi-automated indirect ELISA to test a panel of 7 antigens comprising several well-recognized cancer-associated proteins including the c-myc oncogene, p53, HER2, MUC1, NY-ESO-1, CAGE, and GBU4-5. These proteins were selected because they are known to be involved in the carcinogenic process, to be aberrantly expressed on the cell surface of solid tumors, or to induce autoantibody responses in cancer sera. One of these antigens, GBU4-5 encoding a DEAD box domain, is of interest because DEAD box–containing proteins are involved in RNA processing, ribosome assembly, spermatogenesis, embryogenesis, and cell growth and division. Using this approach Chapman's group reported elevated levels of autoantibodies to at least 1 of the 7 antigens in the panel, in 76% of lung cancer (LC) patients tested, with a specificity of 92%. There was no significant difference between the detection rates in the LC subgroups. More recently, this group confirmed the value of an autoantibody panel as a diagnostic tool in 3 cohorts of patients with newly diagnosed LC, and potentially able to identify patients at high risk of LC. Autoantibody levels were measured against a panel constructed with p53, NY-ESO-1, CAGE, GBU4-5, annexin 1, and SOX2. This panel demonstrated a sensitivity/specificity of 36%/91%, 39%/89%, and 37%/90% in the 3 cohorts of LC patients with good reproducibility. The advantages of this approach are that the proteins tested in the mini-arrays are known to be involved in important aspects of carcinogenesis, that the antigens printed are recombinant proteins, and that the platforms used could be more easily adapted for cancer detection in the clinical laboratories. The disadvantages of this approach are that the choice of antigens is arbitrary and that the antigens tested are common to several cancers and not necessarily specific for the tumor of interest. This objection is not a serious one, because reactivities shared by several cancers may contribute to a diagnostic panel showing high specificity for the tumor. The expectation using this approach is that the addition of new antigens will be able to produce a diagnostic panel with sufficiently high accuracy to be useful in the clinical arena.

Other investigators have attempted to identify TAAs recognized by serum antibodies using proteomics[22,23] or genomics[14,15] methodologies. The use of proteomics has led to the identification of a large group of autoantigens recognized by cancer sera. An example of this approach is the report of Brichory and colleagues,[50] who implemented a proteomics approach for the identification of tumor antigens that elicit a humoral response. These investigators used 2-dimensional polyacrylamide gel electrophoresis to simultaneously separate several thousand individual cellular proteins from tumor tissue or tumor cell lines. Separated proteins were transferred onto membranes, and sera from cancer patients were screened individually by Western blot analysis for antibodies that react against separated proteins. Proteins that specifically reacted with sera from cancer patients were identified by mass spectrometric analysis. The investigators reported the identification of autoantibodies reacting against a group of 4 25-kDa proteins identified as PGP 9.5 in 9 of 64 sera. Their findings suggested that ectopic expression of PGP 9.5 and release into the serum are associated with a humoral response detectable in a subset of LC patients.

The genomic approach uses immunoscreening cDNA libraries constructed from mRNA isolated from tumor tissues or from established cancer cell lines to identify TAAs targeted by autoantibodies.[14–21] Immunoscreening expression libraries has been used for several decades, and since the report of Carlsson and colleagues,[51] some changes in the original procedure have been introduced. Immunoscreening cDNA expression libraries using SEREX (Serologic analysis of cDNA expression libraries)[14] resulted in the identification of a broad spectrum of candidate tumor antigens. [14,15] SEREX analysis involves immunoscreening of expression libraries with autologous patients' serum, identification of gene products encoded by positive clones, analysis of mRNA expression, and evaluation of the seroreactivity of autoantigen panels using sera from cases and controls. Two main strategies commonly used for the determination of serologic profiles of antigens identified by biopanning cDNA libraries are a small-scale conventional serologic survey, also called petit serology, and an ELISA using purified recombinant proteins as substrate.[15] Petit serology directly uses crude phage lysates and requires large volumes of sera individually pre-adsorbed with *Escherichia coli* phage lysates. The disadvantages of petit serology are that large volumes of sera from large numbers of patients and controls are difficult to obtain, the procedure is labor intensive, and it does not easily lend itself to high throughput. Determination of serum autoreactivity by ELISA requires purified recombinant proteins as substrate. The system is more simple and manageable because the substrate is devoid of phage particles and can be quite robust. Moreover, ELISA could be more easily adapted for use in clinical trials than autoantigen microarrays. Subsequently, many studies used array-based methods using different formats and detection principles.[52–58] Lagarkova and colleagues[58] described SMARTA (serologic mini-arrays of recombinant tumor antigens), an improved version of allogeneic screening protocol for testing SEREX-defined recombinant clones using serologic mini-arrays in 96-well format. This method was thought to be useful for extensive serologic analysis of a small panel of preselected recombinant antigens, providing a desirable balance between labor-intensive conventional screening as proposed by classic SEREX and expensive robot-assisted autoantigen microarray analysis.[58] Modifications of the immunoscreening procedure used to identify the potential TAAs have been proposed,[16,59] including the use of cDNA libraries prepared with mRNA from heterologous cancer donors, and the selection of cloning sera containing high-titer IgG antibodies. These and other modifications intend to allow the identification of autoantigens relevant to the process of carcinogenesis, which could contribute to a diagnostic panel with high sensitivity and specificity useful in the clinical setting. Using autoantigen microarray methodology, the amplified colonies identified by immunoscreening are printed as a microarray on treated glass slides and hybridized with sera from cancer patients and controls. Following this procedure, the authors have reported a 12-phage breast cancer predictor group constructed with phage inserts recognized by sera from patients with breast cancer and not by noncancer or autoimmune control sera. Several autoantigens including annexin XI-A, the p80 subunit of the Ku antigen, ribosomal protein S6, and other unknown autoantigens were found to significantly discriminate between breast cancer and noncancer control sera. In addition, sequences identical to annexin XI-A, nucleolar protein interacting with the FHA domain of pKi-67, the KIAA1671 gene product, ribosomal protein S6, elongation factor-2, Grb2-associated protein 2, and other unknown proteins could distinguish ductal carcinoma in situ from invasive ductal carcinoma of the breast, and appear to be potential biomarkers for the diagnosis of breast cancer.[16,17] In further work, biopanning a T7 cDNA library of breast cancer proteins with breast cancer sera identified a small group of expression sequence tags with identity to the oncogene Bmi-1 and other proteins, having in common their ability to participate in regulatory processes such as self renewal and epigenetic chromatin remodeling.[60]

In aggregate, the serologic markers for the diagnosis of cancer reported thus far with antibody-based methods, though promising to revolutionize the fields of screening and early

diagnosis of cancer, have not been definitively validated and exhibit limited specificity and sensitivity, insufficient for diagnostic or prognostic purposes in the clinical arena. Thus there is an urgent need to develop and, more importantly, to validate biomarkers with higher accuracy, which alone or in combination with other available screening methods, such as mammography in breast cancer[61] or low-dose helical computed tomography in LC,[62] might significantly improve the likelihood of detecting cancer at an earlier stage.

AUTOANTIBODIES COMMON TO AUTOIMMUNE DISEASES AND MALIGNANCIES FOUND IN CLINICAL PRACTICE

Antinuclear Antibodies

Antinuclear antibodies in malignancies have been reported for decades,[1,2] and this subject has been reviewed in the past.[35,63,64] Forty years ago, it was first suggested that the prevalence of ANAs is increased in patients with malignancies, particularly in breast cancer.[65] Subsequently, multiple case reports confirmed that ANAs are commonly found in sera of cancer patients,[1,2] and many studies involving large numbers of cancer-patient sera and noncancer controls have shown that ANAs are frequently identified in the sera of patients with neoplasms.[66–68] Immunofluorescence using HEp-2 cells became the gold standard for ANA determination in the clinical laboratory, and multiple techniques to detect ANAs have evolved during these 4 decades.[69–73]

In the practice of medicine, positive ANA tests are frequently reported in the general population, and their interpretation is often perplexing because no apparent cause of this finding is evident when the patient does not have a systemic AD. It has been thought for a long time that the frequency of autoantibodies increases with age. However, in the study of Li and colleagues,[74] age was not related to ANA positivity in healthy subjects who were negative for current or past ADs. It has been suggested that humans, as a species, may be predisposed to autoimmunity.[75] The influence of sex has been noted, because several works reported that ANA-positive tests are significantly more frequent in healthy females than in healthy males.[76,77] In this context, women are known to be more susceptible to some ADs such as SLE, rheumatoid arthritis (RA), Hashimoto thyroiditis, and primary biliary cirrhosis. This propensity of females to develop autoimmune processes has been also found in animal models of ADs.[78] It is not surprising that ANA test positivity is more frequent in females than in males, because it has been reported that women develop more robust immune responses than men.[79,80] The hormonal basis for sex differences in ADs that make women more at risk for a variety of ADs may also be pertinent to the pathogenesis of some solid tumors such as breast cancer. It has been reported that autoantibodies are typically present many years before the diagnosis of SLE (unpublished data), and the authors have made similar observations in patients with scleroderma and Hashimoto thyroiditis.[81] Relevant to the interpretation of a positive ANA test in a healthy person are the reports that autoantibodies can be detected in cancer sera many years before the clinical diagnosis of cancer.[5,82,83] Similarly, an unknown number of subjects who are at risk for neoplasia and will eventually develop cancer may have positive ANA tests, contributing also to the tip of the autoimmunity iceberg.[75] The implication of these findings is that many healthy subjects in the general population, who will eventually develop systemic ADs or cancer, may present positive serology for ANAs. In a survey of ANAs in a rheumatology practice, Shiel and colleagues[84] reported that in 2.9% of all patients with ANAs and no established diagnosis referred to a rheumatologist for evaluation, a neoplasia was found. The authors speculate that an unknown proportion of healthy persons who have the predisposition to develop an AD but never reach the clinical diagnostic threshold, and others who have premalignant changes but will or will not develop cancer, may also present with autoantibodies of

unknown cause. It has been reported that up to 20% or more of otherwise healthy people can express ANAs. This interesting subject has been recently discussed.[74,75]

An increasing number of autoantibody specificities have been reported in the sera from cancer patients.[15–17,24,27–31,49] Imai and colleagues reported that patients with hepatocellular carcinoma (HCC), or gastrointestinal, lung, and ovarian cancers had autoantibodies to nuclear and nucleolar antigens detected by immunofluorescence on cell substrates. The frequency of ANAs was significantly higher in patients with HCC than in patients with chronic hepatitis or liver cirrhosis. A higher percentage of nucleolar fluorescence was detected in sera from patients with HCC, and 3 of these nucleolar antigens were identified as NOR-90, nucleolus organizer region doublet polypeptides of 93 and 89 kDa involved in RNA polymerase I transcription; fibrillarin, a 34-kDa protein of the nucleolar U3 ribonucleoprotein particle that is engaged in preribosomal RNA processing; and nucleophosmin/protein B23, a 37 kDa polypeptide that is associated with ribosome maturation and cellular proliferation. These antigens are nucleolar components that are engaged in some aspect of ribosome biosynthesis. Autoantibodies to these nucleolar antigens have also been found in systemic ADs, and they do not represent autoimmune reactions unique to cancer. The investigators suggested that these antibodies might reflect reaction pathways related to immune responses that are antigen driven.[67] The report of Imai and colleagues is a classic example of the potential of autoantibodies to contribute significantly to patient care. In some patients with liver cirrhosis who developed HCC they observed seroconversion to ANA positivity, and a marked increase in titer and/or a change in antibody specificity preceding or coincident with clinical detection of HCC. These changes in ANA titer and/or specificity showed a close temporal relationship with transformation from long-established chronic liver disease to HCC.

Shoenfeld and colleagues[27,28] reported anti-DNA antibodies, anti-histone, and anti-Sm-RNP in the sera of patients with monoclonal gammopathies.[29] Anti-dsDNA autoantibodies were also reported in patients with colorectal adenocarcinoma.[30] Despite these isolated reports, in aggregate the literature on autoantibodies in cancer has not consistently demonstrated the ANA specificities characteristic of the systemic ADs such as SLE, scleroderma, or DM in cancer sera. This finding may simply reflect molecular differences between the autoantigens involved in cancer and those characteristically involved in the systemic ADs.

Antiphospholipid Antibodies

There is growing evidence on the association of antiphospholipid antibodies (aPL) with malignancies.[85] The antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by a combination of arterial and/or venous thrombosis, recurrent fetal loss, often accompanied by a mild to moderate thrombocytopenia, and elevated titers of aPL.[86] aPL are directed predominantly against self protein phospholipid complexes. aPL reported in cancer sera include lupus anticoagulant (LAC), anticardiolipin antibodies (ACL), and α2-glycoprotein I. Conflicting results have been published on the association of aPL and the prevalence of thrombotic events. The prevalence of aPL in cancer sera is variable. In the report of Zuckerman and colleagues,[87] 22% of cancer sera were ACL positive compared with 3% of healthy controls. Patients with ACL-positive sera, mainly those with high titers, had a significantly higher rate of thromboembolic events than ACL-negative cancer patients. Of interest, the levels of aCL decreased 3 months after the initiation of successful treatment of cancer and remained negative during a 12-month follow-up period.[87] LAC was reported in 58% of patients with lung adenocarcinoma, and the investigators found a strong association of thrombosis with LAC but not with ACL in cancer patients.[88] Other studies demonstrated an increased prevalence of aPL in various malignancies, without increase of the risk for thrombosis.[89,90]

Lossos and colleagues[91] found ACLs in 68% of sera from patients with acute myeloid leukemia (AML) and an increase in their titers during AML relapses. However, the presence of ACL was not associated with an increased risk of thromboembolism. The investigators suggested that ACL could be a useful marker to assess relapses and disease activity.

Font and colleagues reported that the prevalence of aPL was higher in cancer patients with venous thromboembolism (VTE) than in patients without VTE and healthy subjects. The aPL positivity persisted in only 4 out of 21 patients, suggesting that aPL may not be pathogenic in the development of VTE observed in patients with solid malignancies.[92]

APS can be associated with ADs or with chronic infections,[93,94] and it has been observed that in these patients the aPL titers wax and wane throughout the course of the disease, but usually fail to disappear. However, when APS is associated with hematological malignancies, aPL have been shown to disappear after proper treatment. [85] Because diminishing the antigenic load may influence the aPL levels, this suggests that the antibody response might be triggered by tumor antigens.[87]

OTHER AUTOANTIBODIES COMMON TO CANCER AND AUTOIMMUNE RHEUMATIC DISEASES

There have been multiple reports of autoantibodies common to cancer and autoimmune rheumatic diseases which have been reviewed.[63,64] Here, the authors discuss only a few examples of this interesting association. p53 autoantibodies in cancer sera have been known to occur for 3 decades.[95] Crawford and colleagues described antibodies against human p53 in 9% of sera from breast cancer patients. Later, Caron de Fromentel and colleagues[96] found that anti-p53 antibodies were present in sera of children with cancer, in 21% with B-cell lymphomas, and in 12% with a wide range of tumor types. These studies remained largely unnoticed until the discovery in the early 1990s that the P53 gene is the most common target for molecular alteration in almost every type of human cancer, and subsequently the occurrence of p53 antibodies in cancer sera was confirmed, suggesting the possible value of p53 and other autoantibodies for the diagnosis of cancer. This subject has been comprehensively reviewed.[97] These autoantibodies do not have diagnostic specificity because they have been found in patients with various cancer types including lung, pancreas, bladder, breast, and ovarian cancers.[97] p53 is a nuclear transcription factor playing an important role in the control of cell proliferation and apoptosis. The p53 tumor suppressor protein arrests the cell cycle primarily at the G1 phase or induces apoptosis in response to cellular DNA damage, thus allowing DNA repair.[98] For these and other reasons, p53 has been called the “guardian of the genome.”[99] The molecular process leading to the generation of p53 antibodies, in particular their association with mutations, has been studied in more detail than for any other antigen/antibody system in cancer sera.[100] These antibodies seem to result from the strong immunogenicity of the p53 protein, and although they may be associated with P53 gene missense mutations, p53 antibodies may react with epitopes in the wild-type protein. Those antibodies developing in patients with P53 mutations react with immunodominant epitopes and not necessarily with epitopes in the mutated part of the molecule.[100] Moreover, some patients with tumors having P53 mutations and expressing high levels of the mutant protein may not develop p53 antibodies. Autoantibodies against p53 have been detected in the sera of patients with several ADs including type 1 diabetes, thyroid disease, SLE, systemic sclerosis, overlap syndromes, and other rheumatic diseases.[101–104] The clinical value of anti-p53 antibodies in malignancies remains a subject of debate, but consistent results have been reported in breast, colon, head and neck, and gastric cancers, in which p53 antibodies have been associated with high-grade tumors and poor survival.[97,105–108] These reports suggest a potential prognostic value for p53 autoantibodies. The involvement of p53 in early stages of carcinogenesis is suggested by the

finding of p53 antibodies months to years before the clinical diagnosis of cancer.[63,109] In agreement with this possibility, anti-p53 antibodies were found in the sera of workers exposed to vinyl chloride who later developed angiosarcoma of the liver, and in the sera of heavy smokers who eventually developed LC.[99] All these findings suggest that anti-p53 and other autoantibodies are potential biomarkers for the early detection of cancer. In breast cancer, it has been possible to detect the reappearance of these antibodies 3 months before the detection of a relapse. These autoantibodies are of the IgG class, indicating a secondary response after a prolonged immunization before the diagnosis of the disease.[97] Based on these studies, the authors speculate that autoantibodies may in the future be found to be helpful in the identification of healthy subjects at high risk for cancer, bearing premalignant changes.

Autoantibodies to c-myc have been reported in sera from patients with cancer and with ADs such as SLE, scleroderma, and DM.[100–112] The c-myc protein is a phosphorylated nuclear protein closely associated with the nuclear matrix.[113] Autoantibodies to c-myc have been reported in sera from patients with breast[111] and colorectal cancers,[113] and full-length recombinant c-myc tested in a mini-array has been shown to contribute to the sensitivity and specificity of a diagnostic autoantigen panel.[49]

Anti-Ku antibodies have been reported in cancer and autoimmune sera from patients with the scleroderma-polymyositis overlap syndrome.[114] DM and PM are inflammatory disorders characterized by muscle inflammation and a tendency to develop internal malignancy.[115] Autoimmunity is thought to play a critical role, and several characteristic autoantibodies have been described.[116,117] It is clear that the availability of biomarkers predicting the development of neoplasia in these patients would be very helpful for the clinician. Anti-Ku antibodies have been further reported in a small number of patients with several systemic ADs including SLE, scleroderma, and RA.[118] The heterodimeric Ku protein, composed of 86-kDa (Ku80) and 70-kDa (Ku70) subunits, is the DNA-targeting component of DNA-dependent protein kinase, which plays a critical role in mammalian DNA double-strand break repair[119] through the nonhomologous end-joining pathway.[120] The authors have reported antibodies to the p80 subunit of Ku antigen in sera of breast cancer patients.[16,17] The heterodimeric Ku protein has been widely implicated in tumor biology.[121] The finding of an autoimmune reaction directed toward the Ku antigen in the sera of cancer patients suggests that the molecular changes leading to autoimmunity of proteins involved in DNA repair may be important in breast carcinogenesis.

Anticollagen antibodies are common findings in the sera from patients with RA, SLE, relapsing polychondritis, and other autoimmune connective tissue disorders.[121–123] The authors' laboratory reported that autoimmunity to collagen antigens occurs frequently in patients with LC before initiation of therapy.[3–8] The prevalence of anticollagen antibodies was found to vary between 12% and 28% depending on the type of collagen, and overall, 43% of LC sera were positive for one or more collagen antigens. Subsequently the authors have found anticollagen antibodies with specificity for type I α2 chain in the sera of patients with breast cancer [unpublished data]. In the light of the recognized role of stromal proteins in the development and progression of cancer,[124,125] the authors speculate that anticollagen antibodies in cancer sera may reflect an autoimmune response to collagen macromolecules in the tumor stroma. Because autoantibodies to collagen macromolecules have been reported in the sera from patients with RA and SLE, the finding of anticollagen antibodies in lung and breast cancers and probably in other solid tumors is reminiscent of the findings in the systemic ADs.

Antibodies to annexin XI-A,[126] RPA32,[127] and elongation factor-2[128,129] have been reported in cancer sera[5,16,17] and autoimmune sera.[130–133] Annexin XI is a member of the

annexin superfamily of Ca^{2+} and phospholipid-binding, membrane-associated proteins implicated in $Ca2^{+}$-signal transduction processes associated with cell growth and differentiation. Annexin XI may have a role in cellular DNA synthesis and in cell proliferation as well as in membrane trafficking events such as exocytosis, and has been found to be identical to a 56-kDa antigen recognized by antibodies in 3.9% of patients with systemic ADs.[130,131] The authors' laboratory has reported antiannexin XI-A antibodies in 19% of women with breast cancer and in 60% of sera from women with ductal carcinoma in situ of the breast.[16] The authors have also reported a prevalence of anti-RPA32 in 11% of breast cancer sera.[5] A parallel was found between breast cancer and ADs in reference to serum reactivity to annexin XI-A and RPA32, because the frequency of these antibodies in breast cancer sera (11%–19%) is substantially higher than in the systemic ADs such as SLE and Sjögren syndrome, which has been estimated to be 2% to 3% and 3.9%, respectively. It is pertinent that both SLE and Sjögren syndrome are known to be associated with a tendency to develop lymphoid malignancies.[134,135] There are reports on the cancer-predicting ability of several members of the large annexin family that are suspected to be involved in the process of carcinogenesis.[136–138] The authors have also reported that elongation factor 2 (EF-2) is recognized as an autoantigens by breast cancer sera.[16,17] EF-2 is phosphorylated by a calmodulin-dependent protein kinase, CaM K III, which is selectively activated in proliferating cells.[139] Of interest, Alberdi and colleagues[133] reported a cross-reaction between anti-dsDNA antibodies from patients with SLE and EF-2, and demonstrated in vitro that this interaction could lead to cellular dysfunction, as evidenced by inhibition of protein synthesis, suggesting a direct pathogenic role for cell penetrating anti-dsDNA antibodies. Therefore, it is possible that the antibodies to RPA32, annexin XI-A, and EF-2 and other as yet unknown autoantigens in the sera of a small proportion of patients with systemic ADs may represent early markers of malignancy. The possibility of these autoantibodies being useful markers to identify patients with rheumatic diseases at risk of developing cancer could be investigated in prospective studies.

SIGNIFICANCE OF AUTOANTIBODIES IN CANCER SERA

The development of autoantibodies is the consequence of breakdown of immunologic tolerance, but their presence is not exclusive of autoimmune conditions.[63] Autoantibodies have been for years considered to be epiphenomena probably related to the breakdown and release of tumor proteins. Although the interpretation of positive serologic findings in cancer sera remains controversial, the significance of the autoantibodies observed in cancer can be viewed through the prism of the humoral autoimmune response in the autoimmune diseases.[7–9] Indeed, many of the features characterizing the autoantibody response in the ADs are mimicked by the humoral response in cancer sera. Although mutated proteins can elicit an autoantibody response and mutations are a prominent feature in carcinogenesis, the majority of the TAAs recognized by antibodies in cancer sera are abnormally expressed wild-type proteins and not the products of mutated genes. Several longitudinal cohort studies have shown that patients with ADs may develop autoantibodies many years before they manifest clinical symptoms.[81] Similarly, autoantibodies in cancer sera many appear many years before the diagnosis of cancer,[5,82,83] suggesting that the process leading to autoantibody formation in patients with cancer occurs during the very early stages of tumorigenesis. Frenkel and colleagues analyzed the sera of 169 women who were healthy at the time of blood donation for the presence of antibodies to 5-hydroxymethyl-2′-deoxyuridine, an oxidized DNA base, using ELISA. Sera collected 6 to 72 months before these women were discovered to have breast cancer showed significantly elevated levels of this antibody. The investigators suggested that this autoantibody potentially can serve as a marker for increased risk of breast cancer, because relatively high serum levels were also detected in otherwise healthy women with a first-degree family history of breast cancer and in women with the diagnosis of benign conditions.[83] Many of the cellular proteins

recognized as autoantigens by serum antibodies are involved, as suggested by Tan,[9] in fundamental cellular functions such as DNA replication and transcription. This association has been confirmed in many studies.[7–9,15,17] The mechanisms that trigger humoral autoreactivity in cancer patients is complex and not completely understood, but seems to be the consequence of abnormal self-antigen expression by tumor cells and of the development of an inflammatory reaction within the tumor microenvironment.[31,140,141] Many recent studies on the significance of infiltrating lymphocytes in tumor tissue have provided evidence that B-cell autoreactivity is extremely important in cancer,[42–47] and together with the plethora of autoantibody specificities cloned by immunoscreening cDNA expression libraries[14–19,24,48,49] or by proteomics[22] found to be associated with cancer, suggest an antigen-driven humoral immune response. In agreement with this possibility, there is evidence that the majority of the autoantibodies detected in cancer sera found to be associated with diagnosis of the neoplasia are of the IgG class of immunoglobulins.[15–17]

As has been demonstrated in the systemic ADs, autoantibodies in cancer sera may have diagnostic and prognostic value and have the potential to detect cancer early, when the treatment has the best chance to affect tumor behavior. In support of this possibility, immunopathologic studies of premalignant disease have shown molecular alterations that have been associated with autoreactivity to cancer-associated proteins.[142]

The cancer stem cell hypothesis[143–145] may be relevant to the interpretation of autoantibody tests in cancer sera. This hypothesis would have important implications for biomarker discovery, because it suggests that a small subset of tumor-initiating cells or stem cells is responsible for cancer initiation and recurrences. Malignant tumors are heterogeneous and antigen diverse, and an undetermined portion of the TAAs identified by autoantibodies, although indeed tumor associated, have probably originated in the bulk of the nontumorigenic but as yet antigenic cells. It is likely that a biomarker discovery approach targeting the cancer stem cell compartment may in the future yield diagnostic and prognostic panels with the highest accuracy. Also, most reported studies have emphasized the potential diagnostic and prognostic value of autoantibodies against antigens in cancer epithelial cells, whereas autoantibodies identifying stromal tumor autoantigens, which are also potentially valuable diagnostic and prognostic markers, have not received a great deal of attention.

An ever increasing number of autoantibodies are being reported in numerous diseases of seemingly different etiology, including type 1 diabetes and many other diseases in which the common denominator seems to be autoimmunity.[146–149] There are important lines of evidence suggesting that autoantibodies in cancer sera are not epiphenomena and that they can significantly contribute to the early diagnosis and prognosis of cancer. Moreover, the study of tumor-associated humoral autoimmunity may offer novel insights into the early events driving cancer.

SUMMARY

Autoantibodies are extremely promising diagnostic and prognostic biomarkers of cancer, and have the potential to promote early diagnosis and to make a large impact by improving patient outcome and decreasing mortality. Moreover, autoantibodies may be useful reagents in the identification of subjects at risk for cancer, bearing premalignant tissue changes.

Great efforts are being made in many laboratories to validate diagnostic panels of autoantibodies with high sensitivity and specificity that could be useful in a clinical setting. It is likely that prospective studies of sufficiently large cohorts of patients and controls using high-throughput technology may allow the identification of biomarkers with diagnostic significance, and perhaps of discrete antigen phenotypes with clinical significance.

The identification of TAAs may also be essential for the development of anticancer vaccines, because autoantibodies found in cancer sera target molecules involved in signal transduction, cell-cycle regulation, cell proliferation, and apoptosis, playing important roles in carcinogenesis. On this basis, molecular studies of antigen-antibody systems in cancer promise to yield valuable information on the carcinogenic process. TAAs identified by serum antibodies in cancer sera can be natural immunogenic molecules, useful as targets for cancer immunotherapy.

An important problem encountered in the practice of medicine is the identification of healthy individuals in the general population who unknowingly are at high risk of developing cancer. For the rheumatologist, a related problem is the identification of those patients with rheumatic diseases who are at high risk for developing a malignant process. These problems encountered in the fields of cancer and the rheumatic diseases can in the future be helped by new diagnostic instruments based on antibodies. The need for promoting the early diagnosis of cancer is a recognized major public health problem in need of significant research support for the validation of multiple promising but inconclusive studies, with the intention of producing diagnostic panels of autoantibodies in various types of cancers. Cancer developing in patients with rheumatic diseases is also an important problem requiring prospective long-term follow-up studies of patients with rheumatic diseases, particularly because some of the new biologic therapies seem to increase the cancer risk. It is possible that a panel of autoantibodies common to patients with cancer and the rheumatic diseases may prove to be of value in the identification of those patients with ADs at high risk for neoplasms.

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