Reviews

Latest Updates on Antiretinal Autoantibodies Associated With Vision Loss and Breast Cancer

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Cancer-associated retinopathy (CAR) is an uncommon paraneoplastic disorder of the retina that is frequently associated with breast cancer in pre- and postmenopausal women older than 50 years. In this review, we will give an update on the current knowledge regarding the association of antiretinal autoantibodies with the breast-CAR syndrome. Women with breast cancer and visual indications of CAR have a significantly increased incidence of autoantibodies (AAbs) against retinal proteins when compared to healthy women. The onset of visual loss in association with antiretinal AAbs peaks 2 to 3 years after the clinical diagnosis of breast cancer. Differences in severity of symptoms between women with or without antiretinal AAbs are evident, revealing more unfavorable presentation in seropositive women. The incidence of CAR in breast cancer is likely to rise as the survival time of patients with breast cancer increases; consequently, a prediction of breast-CAR based on autoimmunity to individual retinal antigens, or to panels of antigens (signatures), is clinically important.

Keywords: cancer-associated retinopathy, breast cancer, autoantibodies, retina, enolase

Cancer-associated retinopathy (CAR) is an uncommon paraneoplastic disorder of the retina that often leads to blindness in association with various systemic cancers, including breast cancer. An association between these cancers and retinal degeneration, located at a distance from the tumor site, was first recognized in the 1980s.[1–3] Patients with CAR typically present with rapid onset and progressive loss of vision and their medical history shows no obvious cause for the disorder on initial examination.[4] Breast cancer is the most common malignancy in women, with increasing incidence in Europe and North America.[5,6] According to the National Cancer Institute of the National Institutes of Health, approximately 12.3% of women will be diagnosed with breast cancer at some point in their lives (www.cancer.gov; provided in the public domain by the National Cancer Institute). Even though remote effects of breast carcinomas on the eye and other organs are infrequent, in some cases, ocular involvement can be the first sign of metastatic spread of cancer.[7] It has been reported that paraneoplastic complications of breast cancer are mainly associated with subacute cerebellar degeneration, paraneoplastic retinopathy, opsoclonus–myoclonus syndrome, lower motor neuron diseases, and stiff-man syndrome.[8] Paraneoplastic syndromes occur in 7% to 10% of patients with malignant neoplasms, yet the frequency of paraneoplastic retinopathy is unknown.[9] During the last 20 years, our laboratory has investigated patients with visual symptoms of CAR and occult malignancy, and the number of those individuals has been continuously increasing owing to growing physician awareness, improved diagnostic tools, and criteria. Recent findings have revealed that CAR syndrome in breast cancer (breast-CAR) is the fastest growing group of CAR syndromes in pre- and postmenopausal women older than 50 years, followed by lung-CAR and melanoma-associated retinopathy (Fig. 1A).[10] An increasing trend in the incidence of breast-CAR, based on data from our studies, is illustrated in Figure 1B. As the survival time

of women diagnosed with breast cancer increases, the incidence of CAR is likely to rise. Therefore, a prediction of breast-CAR or malignant disease, based on autoimmunity to an individual retinal antigen or to panels of antigens (signatures), is clinically important.

The discovery of autoantibodies (AAbs) against retinal proteins has revealed the compelling relationship between tumor immunity and visual loss.[11–14] It is believed that dysfunction of immunoregulation and cross-reactivity of the immune system against tumors and normal tissues play a role in the pathogenesis of paraneoplastic disease and represent an abnormal response of the immune system against normal tissues, either by the production of AAbs or T cells.[15,16] Some proteins that are normally restricted to retina, an immune-privileged site, are also ectopically expressed in some type of cancers, resulting in an immune response characterized by high titers of AAbs targeting antigens of the same apparent molecular weight in the tumors that are obtained from these patients.[17–19] These AAbs can also suppress the growth of the malignancy.[20] Moreover, rare inflammatory infiltrates of T cells, plasma cells, and macrophages are found both in the retina and in the cancer.[21] Studies of peripheral blood that was obtained from patients with CAR have documented the presence of recoverin-specific T cells, including CD8 cytotoxic T lymphocytes, in patients with retinal degeneration and anti-tumor immunity.[22,23] Unlike circulating proteins in the plasma, which are discarded by tumors, serum AAbs are detectable even when antigen expression is minimal; thus, they may have a positive diagnostic value. Although patients with paraneoplastic syndrome are typically unaware that they have cancer, with time they are found to have small carcinomas, including breast or ovarian carcinomas. In this review, we focus on the current status of antiretinal AAbs in breast cancer associated with loss of vision, which could be important in early diagnosis of CAR
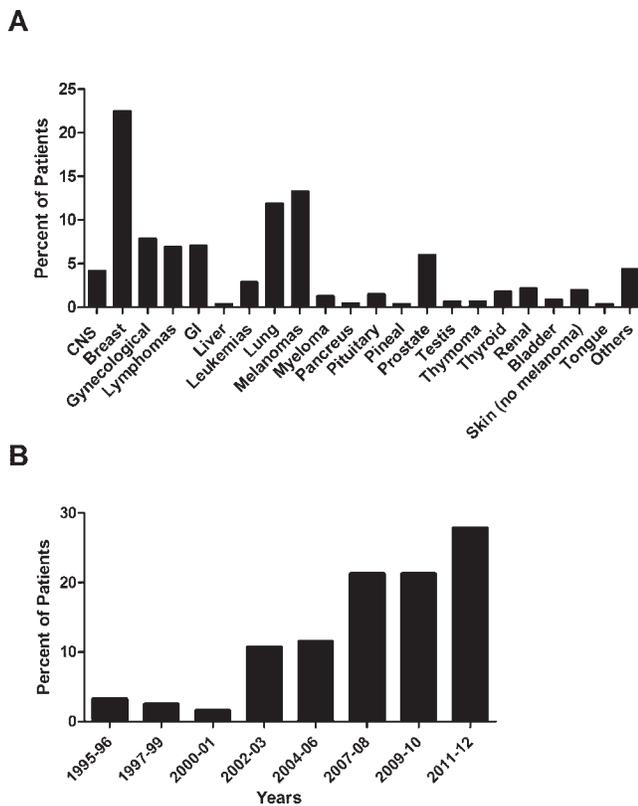
A

B

FIGURE 1. Breast–cancer-associated retinopathy. (**A**) Distribution of patients with different kind of cancers and ocular symptoms indicative of cancer-associated retinopathy studied at Ocular Immunology Laboratory during the past 20 years ($n = 546$). Note that breast cancer is the most frequent (22%) in this cohort as compared to other malignancies. (**B**) Increase in frequency of breast-CAR from 3% in 1995 to 28% in the last 2 years ($n = 122$). *y-axis* represents the percentage of patients with cancer.

and cancer, based on published literature and our unpublished findings.

BREAST CANCER AUTOIMMUNITY

The discovery of molecular targets in breast carcinomas is important for cancer biomarkers and also for therapy. Breast cancer is immunogenic and, as a result, multiple tumor antigens have been identified in patients.[24] Genetic mutations that cause the production of defective p53 tumor suppressor protein or the aberrant expression of specific mRNA-binding proteins are possible pathways to the initiation of cancer autoimmunity.[25] Changes in the structure or expression of certain self-proteins, occurring during tumorigenesis, suggest possible mechanisms by which the immune system could be primed to recognize tumor-associated epitopes as foreign. Those molecular changes manifest in the antigen upregulation, mutation, altered glycosylation, and changes in degradation that can carry those new antigens to the draining lymph nodes for amplification of the immune response and then entry into the systemic circulation.[26-28]

The initial expectation of biomarker research was the identification of specific AAbs that would help with an early diagnosis of breast cancer as well as reveal metastatic activity and a development of paraneoplastic disease.[29,30] It has also been assumed that mutated proteins that are predominant in carcinogenesis can elicit a strong autoantibody response by being recognized as *foreign*. However, most tumor antigens

recognized by AAbs are abnormally expressed wild-type proteins and not the products of mutated genes.[26] Those protein targets play an important role in various cellular pathways at different cellular locations: intracellular (nuclear or cytoplasmic) or on the plasma membrane.[31] Many tumor-specific AAbs have been identified in the sera of patients with breast cancer.[32] However, approximately 30% of women do not have detectable anti-tumor AAbs, or have AAbs that are not well characterized.[33] Also, some patients have AAbs that are specific for more than one type of tumor or one neurologic syndrome, suggesting a complex mechanism of autoantibody production.[34] Yet, no individual autoantigen or panel of antigens has been validated and incorporated for the early diagnosis of breast cancer.[24,29] Therefore, finding specific AAbs is important for an identification of novel diagnostic biomarkers for early diagnosis of cancer and also for CAR.[35]

CANCER-ASSOCIATED RETINOPATHY IN BREAST CANCER

The CAR syndrome is heterogeneous with diverse retinal manifestations, changes in retinal and optic nerve function, and the presence of AAbs with different antiretinal specificity.[36,37] Clinical symptoms and findings consistent with CAR include sudden onset of unexplained vision loss with progressive course, unexplained photopsias, night blindness, loss of peripheral or central vision, defects in visual field presenting central or paracentral scotomas, or a blind spot. Electroretinogram (ERG) findings are usually abnormal from the decrease in scotopic function to undetectable responses.[4,38,39] These "remote effects" of cancer are not due to metastases but likely represent immunological responses against tumor antigens, similar to those also present in a normal retina.[17,40] Retinal antigens are sequestered in immune-privileged eyes but the abnormal expression of similar or identical retinal proteins, such as recoverin, may occur in tumor cells and induce an autoimmune response, which manifests in the generation of AAbs and/or specific cytotoxic T cells.[41,42] It is not fully explained whether such immune responses automatically lead to paraneoplastic syndromes, to beneficial antitumor responses, or both.[43,44]

Paraneoplastic effects of breast cancer on the eye are uncommon.[10,45,46] One of the first cases of paraneoplastic ocular problems in breast cancer was reported more than 40 years ago by Peter Rudge on nonmetastatic manifestation of optic neuritis in a 49-year-old woman.[47] The author suggested a causal relationship between cancer of the breast and development of optic neuritis. The supporting evidence revealed that after the tumor surgery, which eliminated the source of antigens, the visual acuity dramatically improved and the swelling of the disc decreased. Later, in 1984, Klingele et al.[2] reported that a paraneoplastic retinopathy in a postmenopausal woman was a nonmetastatic remote effect of carcinoma that was characterized by a rapid visual deterioration, narrowing arterioles, and an extinguished ERG. The authors suggested that the syndrome represents an autoimmune disorder in response to cancer; however, specific AAbs were not determined.

Recently, we studied a cohort of 111 women with visual symptoms of CAR and a history of malignant breast cancer for the presence of antiretinal AAbs by Western blot. The onset of visual symptoms and discovery of AAbs corresponded to breast cancer, suggesting that AAbs are likely made before malignancy is clinically evident at the precancerous stage (Fig. 2). Only a small number of women (5%) developed visual problems before cancer was clinically diagnosed. It is important to point out that the latency time from the presentation of visual loss to finding of tumor may take several years.[48] For instance, a 76-year-old woman
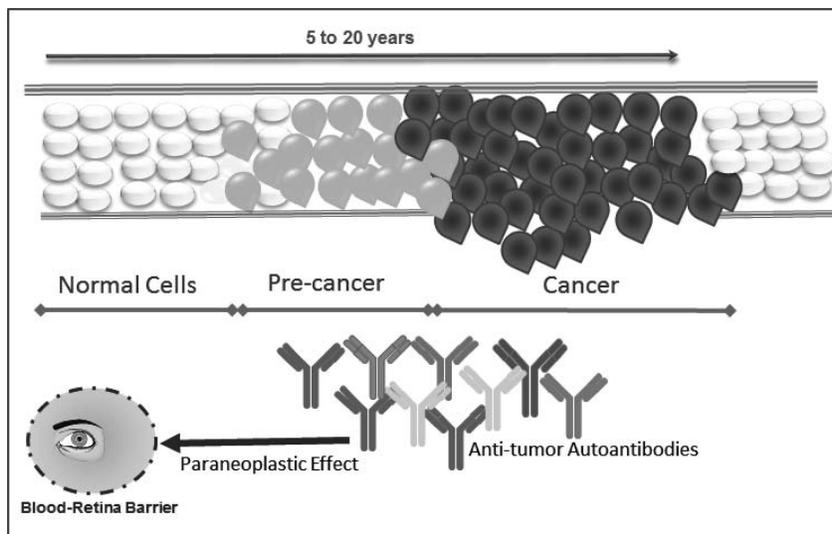
FIGURE 2. Schematic diagram of anti-tumor antibodies in formation process during carcinogenesis. Paraneoplastic autoantibodies may develop years before clinical presentation of breast cancer.

with a history of acquired night blindness and photosensitivity, associated with an enlarged blind spot, developed anti-enolase AAbs 4 years before clinical diagnosis of malignancy (Iannaccone A, Adamus G, unpublished communication, case report in preparation, 2014). The initial examination using ocular coherence tomography (OCT) showed a mild loss of photoreceptor cells and swelling of the retinal nerve fiber layer, and ERG showed a mild cone dysfunction; such presentation often correlates with the presence of anti-enolase AAbs.[39] Those symptoms and findings were highly suggestive of CAR, which eventually led to the diagnosis of breast malignancy 4 years later. Therefore, a regular follow-up in patients with CAR-like symptoms and antiretinal AAbs for tumor surveillance may be essential in suspected paraneoplastic disease. On the other hand, for most women, loss of vision associated with antiretinal AAbs peaked 2 to 3 years *after* the diagnosis of cancer. Figure 3 shows the latency time from finding cancer to the diagnosis of CAR and presence of antiretinal AAbs. The time from finding cancer to the presentation of visual symptoms was on average 4.6 years in seropositive women and 7.2 years in seronegative symptomatic patients, ranging from months to 20 years. One can speculate that in longer intervals of more than 10 years, AAbs and T-cell responses are involved in limiting the tumor growth rather than attacking the retina.
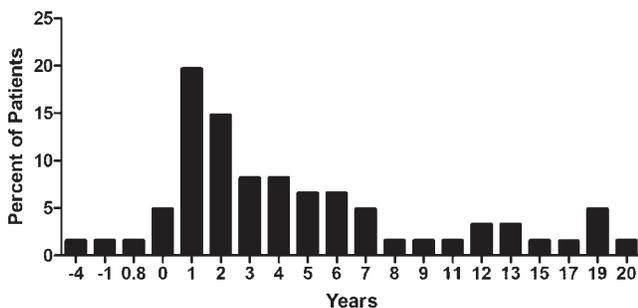

FIGURE 3. Latency time from the diagnosis of cancer to the manifestation of symptoms of breast-CAR and detection of antiretinal autoantibodies. Note that in some women visual symptoms and antiretinal autoantibodies precede the diagnosis of breast cancer by 4 years.

ANTIRETINAL AUTOANTIBODIES IN BREAST-CAR

In CAR syndrome, the autoantigens are aberrantly produced by cancer, and the same antigenic proteins may also be expressed in the retina, their usual location.[10,44,49] Antiretinal autoimmunity is triggered, preceding the diagnosis of the underlying malignancy when the tumor is too small to be clinically detected.[4,36] Thus far, the results from the studies on paraneoplastic syndromes agree that ocular paraneoplastic effects are not side effects of drugs used in treatment of breast cancer, because AAbs could be detected in serum years before finding malignancy, suggesting that the process leading to autoantibody formation occurs during the very early stages of tumorigenesis.[7,50-52] Therefore, the presence of serum AAbs against cross-reacting tumor-retina antigens in the context of clinical presentation may be an indication of the autoimmune paraneoplastic disease. Those AAbs usually belong to the IgG class with a relatively long lifetime that allows them to penetrate the tissue and, in time, induce adverse effects on the eye.[22,53] Figure 2 shows a diagram that illustrates a hypothetical model of the AAb formation process that may occur during the early stages of carcinogenesis before the manifestation of clinical symptoms of cancerous disease.

Autoantibodies against retinal proteins have been detected by using immunohistochemistry and immunoblotting (Western blot) in sera of many patients affected by CAR or other paraneoplastic neurologic diseases.[37,54-57] Autoantibodies found in breast-CAR often target important intracellular molecules involved in signal phototransduction, metabolic functions, and other key cellular processes.[30,58] In some cases, AAbs can be detected before clinical diagnosis of breast malignancy and before visual symptoms of CAR.[7,26,59]

Historically, recoverin (23-kDa calcium-binding protein) has been shown to be a target antigen in CAR syndrome.[21,60-62] However, the incidence of anti-recoverin AAbs in CAR is very low across different kinds of tumor (3%–5%).[10] Autoantibodies against retinal proteins other than recoverin are more frequently found in patients with breast carcinoma. Thus, the absence of anti-recoverin AAbs does not exclude the diagnosis of paraneoplastic retinopathy.[53,63] Moreover, it is important to point out that antiretinal AAbs, such as anti-enolase and anti-recoverin, have also been detected in patients with cancer but *without* clinical signs of CAR,[44,64] and it is not known whether individuals with antiretinal AAbs advance to paraneoplastic

TABLE 1. Clinical Findings in the Cohort of Seropositive and Seronegative Women With Breast Cancer and Their Correlation With Antiretinal Autoantibodies

Description	Seropositive Patients, % total, $n = 76$	Anti-retinal AAbs Association, kDa	P Value, Fisher's Exact Test	Seronegative Patients, % Total, $n = 35$
Visual acuity loss	33			15
Color vision loss	4	30	0.036*	4
Slow progressive loss of vision	7	46	0.013*	
		33	0.058	
Rapid progressive course	23	23	0.254	8
		62	0.273	
Symmetric vision loss	19	46	0.104	4
Asymmetric vision loss	16	50	0.191	1
Loss of peripheral vision	14			2
Loss of central vision	23	46		5
Visual field defect	33			8
Cone dysfunction (ERG)	36	46	0.343	12
Rod dysfunction (ERG)	31	50	0.052*	12
Optic nerve defect	13	46	0.143	1
Inflammation	7	45	0.020*	3

P values were determined by the Fisher's exact test performed to find significant statistical differences between seropositive and seronegative patients. ND, not determined.
* Statistically significant $P < 0.05$.

disease, and what regulates such a progression. The rare development of paraneoplastic disease caused by the autoimmune response could also be influenced by restricting mechanisms related to the blood–retina barrier (BRB) and the immune privileged status of the retina.[42]

Almost 20 years ago, our group first reported on the presence of serum AAbs against α-enolase in breast-CAR.[53] More recently, in 2007, Misiuk-Hojło et al.[59] examined a cohort of 295 women with diagnosed breast cancer, screening them for serum AAbs against retinal proteins by Western blot. They selected the six individuals with highest AAb titers for ophthalmic and neurologic examinations, of which two (~1%) presented symptoms characteristic of CAR. In addition to anti-enolase, they found AAbs against arrestin (48-kDa) and unidentified retinal antigens. The same group reported the case of a patient with high-titer anti-α-enolase AAbs and advanced breast carcinoma (T4d N0 M+ negative for estrogen and progesterone receptors) who presented with visual impairment 8 months after diagnosis of carcinoma. The symptoms included decreased dark vision in both eyes, photopsias, photosensitivity, prolonged glare following light exposure, and problems with reading.[65] Visual acuity was decreased (0.02 OD and 0.1 OS) and visual field analysis showed central scotoma, larger in the right eye. Electroretinogram findings were severely abnormal in the right eye. There was no further reduction in vision after chemotherapy and radiation. Because these findings were indicative of CAR, the authors suggested that anti-enolase AAbs were responsible for development of CAR.[59,65] Just recently, Eadie et al.[66] have reported the unusual case of a woman with localized foveal flattening and prior history of breast cancer 5 years earlier. The OCT findings show flattening of the foveal depression in both eyes with disruption of the inner retinal layers and depressed amplitudes within the macula in both eyes on multifocal electroretinogram. The patient was seropositive for antibodies against a heat shock protein of 70-kDa molecular weight.

CORRELATION OF AUTOANTIBODIES WITH SYMPTOMATIC COMPLAINTS

A key challenge to diagnose, monitor disease activity/severity, and predict response to therapy is identification of a relevant antibody biomarker. The manifestation of visual loss in association with antiretinal AAbs was evident in most women with breast cancer, and in the remaining symptomatic group, AAbs were not initially found (our unpublished studies). Importantly, differences in severity of symptoms between women with or without antiretinal AAbs were noticeable, revealing more unfavorable presentation in seropositive women (Table 1). A sudden onset of vision loss with progressive course was three times more frequent in seropositive patients than in the seronegative women. Symptomatic complaints indicated both rod and cone photoreceptor dysfunction. Rod dysfunction presented as nyctalopia, peripheral visual field loss with ring scotomas; and manifestation of cone-related problems included unexplained photopsia, photophobia, photosensitivity with reduced central vision, and impaired color vision. Abnormalities in the rod and cone photoreceptor function, as confirmed by ERG, were three times more frequent in patients seropositive for antiretinal AAbs. The central vision loss in seropositive women was evident more frequently than peripheral vision loss. Defects in visual field were often reported in seropositive women, showing central or paracentral scotomas, or a blind spot. Other noticeable fundus findings in breast cancer patients were retinal vasculitis, arteriole attenuation, occlusions, and cystoid edema. Approximately 13% of patients present with optic nerve problems (cupping of discs, discs pallor, disc atrophy).[37] In addition, their OCT has revealed a focal thinning of the retinal nerve fiber layer to bilateral foveal thinning, and in some cases, loss of outer retina, but most women initially had a normal-appearing retina despite profound visual and/or electrophysiological deficits.

Autoantibodies correlate with vision loss in women with breast cancer, and some of those specific AAbs coincided with signs of a particular retinal dysfunction (Table 1). For example, slow progressive course of retinal dysfunction that developed for years was significantly associated with anti-enolase (46-kDa) AAbs and anti–33-kDa retinal protein AAbs; such association with anti-enolase antibodies has been reported before.[39] Patients with signs of intraocular inflammation had antibodies against arrestin, a known uveitogenic retinal antigen.[67] The rod dysfunction as measured by the ERG was correlated with anti–50-kDa AAbs. A loss of color vision in breast-CAR was associated with anti-carbonic anhydrase II (30-kDa, CAII) AAbs.

TABLE 2. Significance of Specific Antibodies Present in Normal Individuals and in Breast-CAR Patients

Autoantigen	Normal, $n = 78$	CAR, $n = 111$	P Value Fisher's Exact Test	Accuracy of Marker, %	
				Sensitivity	Specificity
Recoverin	0	6	*0.0431	5	100
CAII	15	9	*0.0278	10	80
33-kDa	7	11	1	9	91
35-kDa	7	15	0.3681	13	91
40-kDa	5	13	0.3149	11	93
45-kDa	4	9	0.5635	8	94
Enolase	14	35	**0.0050	38	82
50-kDa	2	9	0.1276	8	97
62-kDa	6	13	0.4643	11	92

P values were determined by the Fisher's exact test performed to find significant statistical differences between normal subjects and CAR patients.
* Statistically significant $P < 0.05$, **$P < 0.01$.

Adamus and Karren[68] have shown that anti-CAII antibodies are potentially pathogenic and induce cellular damage by impairing the CAII cellular function through inhibiting its catalytic activity, decreasing intracellular pH, and increasing intracellular calcium, which, in effect, decreases retinal cell viability. Such AAbs could induce a selective loss of carbonic anhydrase–positive L/M cones responsible for color vison.[69] However, Table 2 and Figure 4 show that normal women had a higher incidence of anti-CAII AAbs than patients with breast-CAR. The significance of this finding is not clear, since this study has not determined the fine specificities of normal and CAR AAbs. It is possible that the site of the immunologic reaction of normal AAbs does not involve the active site of the enzyme.

Autoantibodies against α-enolase (46-kDa glycolytic protein) were found to be the most prevalent (32%) in breast-CAR followed by anti-35-kDa (13%), anti-40-kDa (12%), anti-50-kDa (11%), and anti-62-kDa (14%). Anti-recoverin AAbs were only found in 4.5% of CAR patients 1 to 15 years after diagnosis of breast cancer and none were present in women without CAR symptoms. Anti-enolase AAbs were approximately 2-fold more frequent in CAR, showing 32% seropositivity versus 18% in normal women without cancer or visual loss (Fig. 4). Recently, published studies have also shown a frequent presence of AAbs against enolase in the setting of gynecologic CAR,[70] as well as in various autoimmune diseases, suggesting that their presence in serum should be taken in the context of disease studied.[71] For instance, a decrease in the anti-enolase antibody level seems to be a common event for stage IV breast cancer and lung cancer when compared with the presence of those AAbs in healthy controls and patients with non–cancer-associated diseases.[72] Since the titer of anti-enolase AAbs was closely correlated with tumor progression, such AAbs could be potentially useful markers for monitoring the staging of breast cancer.

It is important to emphasize that more than one autoantibody exists in a patient. Therefore, multiple AAbs against various retinal antigens may create unique antibody patterns (antibody signatures). With a relatively low sensitivity, but high specificity, one could apply such a signature to determine which women may develop CAR and who is at higher risk of losing vision more progressively (Table 2). A positive result for antiretinal AAbs is consistent with antiretinal autoimmunity and suggests that a neoplasm might be present. However, the interpretation of such findings needs to be discussed in the context of visual problems. This is important because antiretinal AAbs have been found in other blinding diseases, including retinitis pigmentosa, age-related macular degeneration, diabetic retinopathy, and glaucoma; therefore, these conditions have to be excluded first before searching for malignancy.[73,74] By combining target antigens to a set of biomarkers, sensitivity and specificity would increase.[70] Moreover, multiple AAbs can augment autoimmune responses in CAR.[75] Also, multipotent antibody responses against multiple autoantigens are effective in tumor destruction, and therefore they usually do not induce clinical autoimmunity.[76] Several longitudinal cohort studies have shown that patients with autoimmune diseases may develop AAbs many years before they manifest clinical symptoms of disease, and the
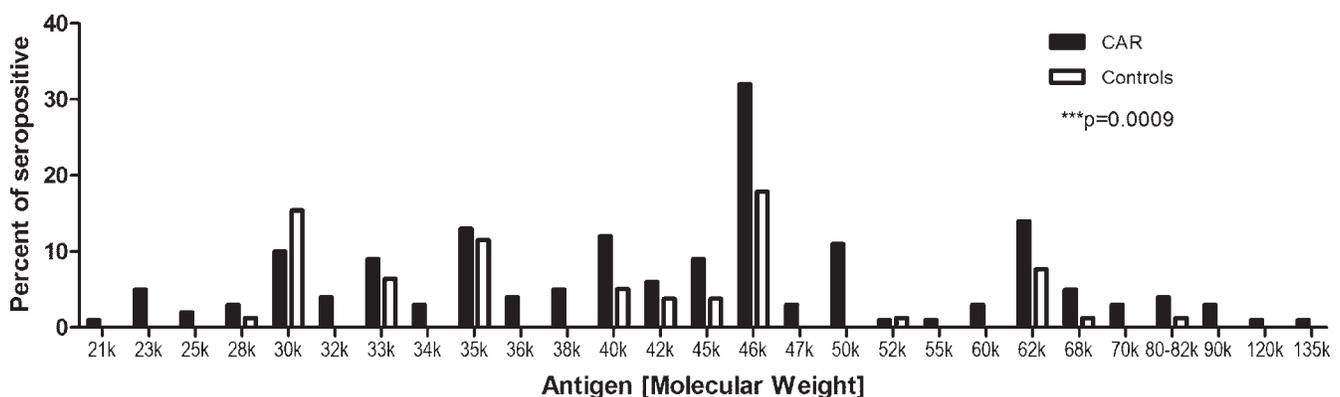

FIGURE 4. Distribution of antiretinal autoantibodies in breast-CAR group and controls. Patients with CAR developed antibodies against 27 different retinal proteins as opposed to 12 proteins by control subjects. x-axis shows retinal proteins marked by their molecular weights (k = 1000). y-axis represents the percentage of patients with AAbs reacting with specific proteins.
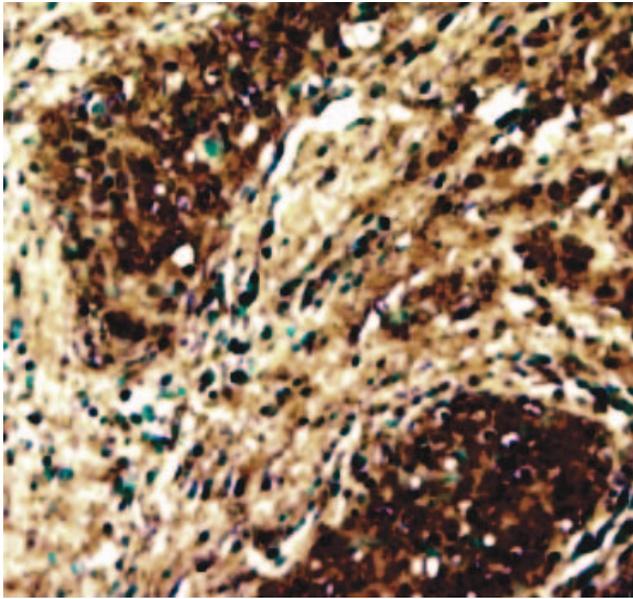
Figure 5. Immunostaining of ductal breast carcinoma with serum specific to anti-enolase autoantibodies. *Brown color* indicates anti-enolase positive cross-reactivity.

frequency of autoantibodies increases with age.[77] However, we found that the age of individuals did not contribute to the generation of more AAbs in both healthy, age-matched control subjects and individuals with CAR (not shown).

ANTIBODIES IN CONTROL SUBJECTS

A frequently raised question is about the true significance of antiretinal AAbs in CAR when AAbs of similar specificity can be also found in normal individuals without visual loss. The challenge is to determine which AAbs are pathogenic or benign, and what other factors are present that cause antiretinal antibodies to become pathologic. In general, AAbs are generated to provide a first line of defense against infections and contribute to the homeostasis of the immune system.[78] Healthy individuals have AAbs that are likely natural antibodies, primarily polyreactive IgM, and display a moderate affinity for antigens.[79,80] In contrast, long-lasting, high-affinity autoantibodies of the IgG class show pathologic properties related to alterations in cell clearance, antigen-receptor signaling, or cell effector functions.[43] Natural antibodies are produced mainly, if not exclusively, by a subset of long-lived, self-replenishing B cells, termed B-1 cells, that have a unique response pattern that minimizes the risk of autoimmunity.[78] B-2 cells express a repertoire that is explicitly selected against self-recognition and directed toward the generation of high-affinity antibody response to external antigenic stimuli. B-1 cells, whose repertoire is selected by recognition of autoantigen, maintain production of evolutionarily important antibody specificities that respond to pathogen-related, rather than antigen-specific, signals. The presence of AAbs highly specific for target organs in organ-specific autoimmune diseases, such as CAR, strongly suggests that AAbs are stimulated by inflammation in the target organ, although cross-reactivity with microbial antigens (molecular mimicry) cannot be excluded.[43] In the continuous presence of antigen and stimulation of immune responses, antiretinal AAbs in individuals predisposed to autoimmune diseases may be potentially responsible for development of autoimmune retinopathy.[81]

Autoantibodies to intracellular antigens can be stimulated by excessive stimulus from antigens released from dying cells, as well as by enhanced responses associated with intrinsic abnormalities in B or T cells. The expansion of many protective regulatory processes specific for patients older than 60 years, as well as the result of a damaged tissue process and elevated apoptosis, is a possible explanation for this higher autoimmunity without autoimmune disease.[82] The widespread presence of these naturally occurring, nonpathologic IgG autoantibodies presents a challenge in immunologic research.[80,83]

EVIDENCE FOR PATHOGENICITY OF ANTIRETINAL AUTOANTIBODIES

The mechanisms involved in the disruption of central tolerance, peripheral immune dysregulation, and alteration of self-antigens are the highlights of autoimmunity induced by cancer.[84] The immune response from both AAbs and T cells that recognize antigens expressed in tumors (eg, recoverin, enolase, carbonic anhydrase II), and at the same time in regions of the eye undergoing degeneration (photoreceptor cells), seems to cause the damage to the retina by triggering retinal cell death, retinal degeneration, and ultimately, loss of vision.[85–88] It has been postulated that tumor antigens mimic proteins expressed in the retina and induce the immune system to lose tolerance for these self-proteins.[14,17,18,22,44,49,89,90] Under inflammatory conditions, AAbs that are produced in the periphery may permeate the BRB by various cellular mechanisms and thus contribute to retinal autoimmunity, whereas under physiological conditions, the BRB is usually impermeable to antibodies.[91] If the origination of AAbs starts with the development of tumor, such AAbs should react with both the tumor and the retina. Figure 5 illustrates the immunoreactivity of breast-CAR AAbs against retinal enolase with ductal breast carcinoma. A cross-reactivity of AAbs with breast and retinal tissues is the basis for immunopathogenic course. Thus, CAR autoantibodies may play a double role, a positive role in fighting cancer and a negative role in killing retinal cells.

In paraneoplastic syndrome with highly immunogenic cancers, such as breast malignancy, more antigens are recognized as well as a greater number of epitopes (small peptide fragments that interact with antibodies) within individual autoantigens.[84] We have determined that CAR-related anti-enolase AAbs differ in their fine specificities from the control AAbs.[92] Even though anti-enolase AAbs of CAR patients usually bind to several epitopes on the enolase protein, there was a specific epitope that was not recognized by anti-enolase AAbs produced in normal subjects.

Immunoglobulin G1 and IgG3 autoantibodies can be found in patients with CAR.[53] Both subclasses can activate complement, but only weak complement reactivity is found in a few areas of the nervous system.[93] However, the ability of the IgG1 and IgG3 isotypes to bind Fc receptors may have played a role in the recruitment of these monocyte/macrophage cells to the injured tissue.[53,93,94]

Antiretinal antibodies can penetrate retinal cells in vitro and in vivo by the process called endocytosis.[85,86,95,96] Purified anti-recoverin or anti-enolase IgGs or their Fab fragments are equally cytotoxic for retinal cells in the presence and absence of complement, suggesting that complement does not play a major role in antibody pathogenicity.[86,95] Despite this, Thirkill[97] has reported that paraneoplastic antibodies against a 57-kDa protein inhibit the metabolic activity of RPE cells when complement is present in the cell culture. The role of complement in CAR needs to be further investigated.

Pathogenicity of AAbs occurs by blocking the function of the target antigen. Immunologic inactivation of an autoantigen

in the cell, such as recoverin and enolase, results in cell death, leading to photoreceptor degeneration.[40,96] In the case of anti-α-enolase antibodies, deregulation of the glucose metabolism in retinal cells has been observed, which leads to the apoptotic death of retinal cells after antibodies access retinal cells.[98] In fact, only CAR anti-enolase AAbs, but not enolase-negative sera or sera from healthy subjects, are cytotoxic.[10,92,96,98] They inhibit the enolase catalytic function, deplete ATP, elevate intracellular calcium, and lower intracellular pH, which leads to cell death. A similar mechanism may be in place in patients with circulating antibodies targeting different retinal proteins in association with retinal degeneration.[69,99]

Conclusions

Autoimmune breast-CAR is a heterogenic disease, and for that reason, different antiretinal antibodies frequently coexist in a single patient, creating antibody arrays related to the syndrome. Importantly, women with breast cancer and visual symptoms of CAR have significantly increased incidence of AAbs against the same retinal proteins, compared to healthy women. As in other autoimmune diseases, the diagnosis of breast-CAR cannot be made merely upon finding of AAbs but should be interpreted in the context of clinical findings. Autoantigens related to paraneoplastic autoimmunity can be diverse, and several determinants can be recognized within a single antigen as we showed for anti-enolase AAbs. Moreover, those AAbs reacted with the breast tumor and retina, indicating that there is a common antigen present in both breast and retinal tissue. The goal for future studies is to identify all target antigens and explain their relevance in pathogenicity of CAR.

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