Cancer-associated retinopathy (CAR) is a paraneoplastic syndrome most commonly associated with small-cell carcinoma of the lung, but also less frequently reported in patients with breast, endometrial, and other cancers. A paraneoplastic syndrome (PNS) is a secondary organ dysfunction occurring in a cancer patient at a site that is anatomically remote from the tumor. PNS is not due to a direct effect of the tumor itself or its metastases but caused by other mechanisms, commonly autoimmune mechanisms develop when malignant tumors express proteins, paraneoplastic antigens (PNA), which are normally present only in neurons. One retinal antigen implicated in the autoimmune mechanism of CAR is recoverin, a 23 kDa photoreceptor-specific calcium-binding protein modulating the activity of photoreceptor guanylyl cyclase. The anti-recoverin antibodies induced by the primary tumor may on contact with intraretinal recoverin initiate a photoreceptor degeneration and trigger photoreceptor death by apoptosis, thus causing blindness. Other circulating antibodies directed against a 46 kDa protein identified as retinol enolase and a 60 kDa retinal protein have been demonstrated in patients with clinically diagnosed CAR syndrome. In certain patients no specific antibody has been identified. This suggests that the CAR syndrome includes an heterogenous group of autoimmune conditions directed against various retinal proteins.

Keywords: Cancer-associated retinopathy, paraneoplastic, recoverin, autoimmune

INTRODUCTION

A paraneoplastic syndrome is a secondary organ dysfunction occurring in a cancer patient at a site that is anatomically remote from the tumor. Paraneoplastic syndromes are a complex of symptoms not due to a direct effect of the tumor itself, its metastases, opportunistic infections, metabolic abnormalities, vascular diseases, coagulopathies, complications of therapies, or malnutrition, but caused by immune or other mechanisms. Most paraneoplastic syndromes are associated with small-cell carcinoma of the lung (SCCL),
gynecologic malignancies, and breast neoplasms (ductal adenocarcinoma), while the symptoms frequently are in the form of abnormalities of the nervous system [paraneoplastic neurologic syndrome (PNS)]. PNS are rare neurological disorders that are related to cancer, even though the primary tumor and its metastases have not invaded the nervous system. PNS are thought to be autoimmune diseases that develop when malignant tumors express proteins, paraneoplastic antigens (PNA), which are normally present only in neurons. In one case PNS occurred in a patient with occult breast cancer and in a child with Langerhans cell histocytosis.

The abnormalities of PNS are frequently the result of autoimmune disorders, but may also be due to other mechanisms. In PNS associated with autoantibodies, these antibodies are targeted against antigens shared by tumor cells and nervous tissue (onconeural antigens). These immune responses subsequently will recognize the same antigens or epitopes occurring in other organs, e.g. in the nervous system. A second group of autoimmune PNS are monoclonal gammopathy syndromes associated with secretion of an antibody by the neoplasm. That some PNS are based on autoimmune reactions was first demonstrated for the Lambert-Eaton syndrome, which is a myasthenia-like disorder of the peripheral nervous system with autoantibodies that bind to presynaptic calcium channels at the neuromuscular junction. This binding disturbs the release of acetylcholine, thus leading to weakness of the proximal muscles and associated abnormalities. In another paraneoplastic syndrome, namely paraneoplastic cerebellar degeneration, autoantibodies were found against Purkinje cells in the cerebellum of a patient. The researchers subsequently also found Purkinje cell antigens in gynecologic tumors in the affected patient, but not in patients with similar tumors who did not have neurologic abnormalities.

Paraneoplastic syndromes affecting the visual system have also been reported, mainly consisting of cancer-associated retinopathy (CAR) and melanoma-associated retinopathy (MAR). CAR was first described by Sawyer et al. while MAR was first described in 1988 by Berson et al. The prevalence of CAR is unknown, with less than 100 reported cases worldwide, but CAR is still more common than MAR. The primary tumor most commonly associated with CAR (90%) is small SCLC, but it has also been less frequently reported in patients with breast, endometrial, and other cancers. MAR has been described only in association with cutaneous malignant melanoma, and not with melanomas affecting other tissues. In CAR, the visual loss frequently precedes the discovery of SCLC, while in MAR the retinopathy commonly presents after the melanoma is diagnosed, often at the metastatic stage.

In this review the characteristics and probable pathogenesis of the CAR syndrome will be discussed, with some notes on apoptosis due to antirecoverin antibodies.

**CLINICAL PICTURE**

CAR is characterized by a functional and progressive abnormality of the rods and cones, without any other neurological dysfunction. CAR should be suspected in patients who present with visual symptoms such as rapid unexplained visual loss and seeing shimmering lights, accompanied by a markedly attenuated electroretinogram (ERG). The chief complaint in CAR is unilateral or bilateral, symmetric or asymmetric, total or partial loss of vision, but night blindness may also be the presenting symptom. The visual loss occurs either gradually or in a stepwise pattern over weeks to months. Other symptoms may include visual shimmering, sparkling, or distortions. Examination shows poor visual acuity, impaired color vision, constriction of visual fields, and an afferent pupillary defect. Visual field defects most commonly consist of
central and/or ring scotomas. Histologically, there is severe loss of the inner and outer segments of the rods and cones, with widespread degeneration of the outer nuclear layer. The electroretinogram (ERG) is usually flat due to loss of the photoreceptors. The symptoms of CAR are in the majority of cases bilateral, although occasionally a time interval of several weeks may separate the symptomatology between the two eyes, which in turn commonly precedes the discovery of the primary tumor by several weeks to several months.²⁷,²⁸

The clinical presentation of CAR and MAR is rather similar, but the ERG pattern is different. In CAR, scotopic and photopic a- and b-waves are either absent or severely abnormal, whereas in MAR maximal combined rod-cone ERG responses are electronegative. As an apparent exception Goetgebuer et al.⁷ reported a case of CAR with electronegative ERG, leading the investigators to initially suspect MAR. However, the underlying malignancy turned out to be an oat-cell carcinoma in the right lung. The patient died of pneumonia 2 years after presentation.

Treatment of CAR with corticosteroids (methylprednisolone IV), immunoglobulins and plasmapheresis have presumably demonstrated their efficacy in reducing the titer of circulating antibodies and in maintaining effective vision until demise of the patient. However, spontaneous recovery of vision in CAR has not been reported. The prognosis ad vitam depends on the primary tumor.²⁷,²⁸

An old man with lung adenocarcinoma presenting with CAR at the age of 65 years was reported by Oohira.¹⁰ The patient had been followed-up for the last 15 years after the resection of the lung tumor in 1991. IgG of 23 kDa and 65 kDa, identified as antirecoverin and anti-heat shock cognate protein antibodies, respectively, were detected in his serum preoperatively, and the patient was treated with corticosteroids. The fundi and visual fields of both eyes gradually deteriorated over 13 years, but they had become stable in the last 2 years.

Espendar et al.¹¹ reported on a 66-year old women who had lumpectomy and chemotherapy for breast cancer in 1978 and serologically proven CAR and paraneoplastic optic neuropathy in 1988. Over an 8-year follow-up period after the initial diagnosis of CAR, the patient was successfully treated with alemtuzumab after therapeutic failures of respectively prednisone, plasmapheresis and cyclosporin. Alemtuzumab therapy allowed her to maintain fairly good visual function during the entire follow-up period, although she experienced some permanent decline in her visual fields and visual acuity after each of three episodes of CAR. However, it should be noted that her serum contained antibodies against the 40 kDa CAR antigen but not the 23 kDa CAR antigen. Alemtuzumab is a recombinant DNA-derived humanized monoclonal antibody that is directed against the 21-28 kDa cell surface glycoprotein CD52. CD52 is expressed on the surface of normal and malignant B and T lymphocytes, natural killer cells, monocytes, macrophages, and tissues of the male reproductive system.

**PATHOGENESIS**

As in other autoimmune PNS, CAR is apparently the result of an autoimmune process. In the serum of one patient with carcinoma of the cervix, antibodies against human retinal photoreceptors were found, whereas in two others their serum reacted with extracts of normal human, murine and bovine retinas, also with human and bovine choroid, but not with extracts of the lens or iris. There were only low background readings by sera of normal individuals or of cancer patients without disturbances of vision. These findings suggested that the antigen for this PNS was situated in the retina-choroidal complex.¹²

One of the first retinal antigens to be implicated in the autoimmune mechanism of the CAR syndrome was a 23 kDa protein...
identified as recoverin, a photoreceptor-specific calcium-binding protein modulating the activity of photoreceptor guanylyl cyclase.\(^{13,14}\) Recoverin is aberrantly expressed in more than 50% of 33 cancer cell lines.\(^{15}\) The anti-recoverin antibodies induced by the primary tumor may on contact with intraretinal recoverin initiate a photoreceptor degeneration and trigger photoreceptor death by apoptosis, thus causing blindness.\(^{16}\) Circulating antibodies directed against a 46 kDa protein identified as retinol enolase have been demonstrated in patients with clinically diagnosed CAR syndrome. Other autoantigens include the S-antigen (arrestin) and tubby-like protein 1 (TULP1) which is a molecule expressed in synaptic terminals of photoreceptor cells.\(^{17}\) Some reports have also implicated heat shock proteins (specifically heat shock cognate protein 70) in CAR autoimmunity. However, because both enolases and heat shock proteins are expressed in every tissue, it is difficult to attribute an organ-specific autoimmune disease to immunological activity with these ubiquitous antigens. In certain patients no specific antibody has yet been identified, suggesting that the CAR syndrome includes an heterogenous group of autoimmune conditions directed against various retinal proteins.\(^{18,19}\)

**Neuronal calcium sensors**

Calcium as a second messenger controls many biological processes and one of the mechanisms is by interaction with calcium-binding proteins. Among these proteins there is one class, the EF-hand superfamily, that shares a common calcium binding motif, namely the EF-hand. EF-hand proteins with regulatory roles are often termed calcium sensor proteins (CSP), comprising a heterogeneous class of proteins that includes calmodulin (CaM), neuronal calcium sensors (NCS), visininlike protein (VILIP), neurocalcin, hippocalcin, and the recently identified S100 family members. NCS are neuron-specific calcium sensor proteins, of which recoverin is the best-known example. Recoverin (also called S-modulin in the frog) is a myristoylated CSP expressed predominantly in vertebrate photoreceptor cells.

Calcium ion plays a critical role in the recovery phase of visual excitation and in adaptation to background light. The light-induced lowering of the calcium level in retinal rod outer segments restores the dark state by stimulating guanylate cyclase, decreasing the photosensitivity of the cGMP phosphodiesterase and switching the cGMP-gated channel to the high affinity form. Calcium-bound recoverin prolongs the photoresponse most likely by blocking the phosphorylation of photoexcited rhodopsin. This effect of recoverin is reversed by the light-induced lowering of the calcium level. The shortened lifetime of photoexcited rhodopsin, and hence of the phosphodiesterase, at low calcium concentrations promotes recovery of the dark state and contributes to adaptation to background light.\(^{20}\)

**Characteristics and effects of recoverin**

Recoverin, a relatively new member of the EF hand superfamily, was discovered in the early 1990s in the search for a soluble calcium-sensitive activator of guanylate cyclase. It is a 23 kDa calcium-binding protein present only in vertebrate photoreceptors, in certain other retinal neurons, and in pineal glands. The three-dimensional structure of recombinant unmyristoylated recoverin was elucidated and shown to be a compact protein made up of two domains separated by a narrow cleft. Each domain contains a pair of EF hands, which are the 29 residue helix-loop-helix motifs found in parvalbumin, troponin C, calmodulin, and other members of the superfamily. Thus there are four potential EF hand calcium-binding sites in the recoverin molecule, but only two (the second and the third EF hands) are capable of binding calcium ions. In the crystal form of recombinant unmyristoylated recoverin, a calcium ion is bound to EF hand 3, while EF
hand 2 can bind samarium but not calcium. The other two EF hands have novel structural features that prevent or impair calcium binding.

Recoverin can form a complex with rhodopsin kinase (GRK-1) in vitro, thus inhibiting phosphorylation of the visual receptor rhodopsin in a calcium-dependent manner. It has been confirmed recently that recoverin serves as a calcium-sensor of rhodopsin phosphorylation under physiological conditions. Recoverin is also found as an antigen in various neoplasms that are accompanied by CAR.\(^{(17,20,21)}\) The recombinant equivalent of this protein is now routinely used as the test antigen in the serological identification of recoverin hypersensitivity.\(^{(12)}\)

Investigations into the mechanism of cell damage caused by antirecoverin antibodies revealed an apoptosis-inducing activity on in vitro cultivated monolayers of rat retinal cells, and antibody-mediated retinal degeneration in vivo following intraocular injection of these immunoglobulins into the Lewis rat. This animal is genetically prone to the induction of autoimmune reactions in the eye and provides a useful model to demonstrate the pathological processes involved in immune-mediated retinal degeneration and the significance of inherited susceptibility.\(^{(12)}\)

To find out whether recoverin was able to induce the immunological mechanism leading to photoreceptor degeneration, Lewis rats were immunized with recoverin and subsequently showed a high titer of serum antibodies against recover-in, and also degeneration of immunocompetent T cells and photoreceptors.\(^{(12,17)}\)

Polans et al.\(^{(17)}\) proved that recoverin was expressed in the lung tumor of one patient with CAR, but not in similar lung tumors in patients without CAR. The immunodominant portion of recoverin was found by solid-phase immunoassay using overlapping heptapeptides that included the complete sequence of recoverin. The major determinant is formed by two linear strands of amino acids, namely residues 64-70 (Lys-Ala-Tyr-Ala-Gln-His-Val) and 48-52 (Gln-Phe-Gln-Ser-Ile). Residues 61-80 are also pathogenic, causing photoreceptor degeneration in Lewis rats immunized with these peptides. Interestingly, amino acid residues 64-70 promote both autoantibody binding and pathogenicity. Thus it is clear that recoverin as a nervous system antigen is in all probability responsible for the loss of vision in some cancer patients. While autoimmune diseases are widespread, CAR is one of the few autoimmune disorders where the specific self-antigen is known.\(^{(17)}\)

Tumor tissues in CAR patients selectively express recoverin, which will react with the patients’ autoantibodies. Thus there is the possiblity that the expressed recoverin, when released, will be able to trigger an autoimmune response resulting in retinal degeneration. In the study by Adamus et al. it was proven that antirecoverin antibodies enter the cell and subsequently cause cell death by apoptosis.\(^{(24)}\)

Other circulating antibodies directed against a 46 kDa protein identified as retinol enolase and a 60 kDa retinal protein have been demonstrated in patients with clinically diagnosed CAR syndrome. However, in certain patients no specific antibody has been identified. Therefore, this suggests that the CAR syndrome includes an heterogenous group of autoimmune conditions directed against various retinal proteins.

**Apoptosis as a cause of photoreceptor damage**

To evaluate the pathogenic effect of antirecoverin antibodies on retinal cells, in vitro experiments were performed using the immortalized rat retinal cell line E1A.NR3. This cell line contains cells expressing antigens specific for photoreceptors, bipolar cells and ganglion cells, including recoverin. It has been demonstrated that normal as well as specific antibodies may enter retinal cells. To find out the influence of antibodies on cell growth, the
following autoantibodies were used: five types of antirecoverin autoantibodies from CAR patients, antirecoverin antibodies of rats with experimental autoimmune uveoretinitis, and rabbit antirecoverin antibodies.\(^{22}\)

All of the above antirecoverin antibodies had a similar cytotoxic effect on E1A.NR3 cells, which effect depends on the number of antirecoverin antibodies administered and on time of exposure. The toxic effect was greater with high doses of antirecoverin antibodies and the number of viable cells decreased significantly after 48 hours. Normal nonspecific antibodies had no effect on cell viability with identical doses and time of exposure.

In a subsequent experiment E1A.NR3 cells were cultured in combination with antibody and complement. Human antirecoverin antibodies are of the IgG1 class and possess complement fixing ability. However, compared with cultures incubated with antirecoverin antibodies only, those with added complement showed no further cellular damage after 48 hours.

The possible expression of Fc receptors on the surface of E1A.NR3 cells was determined by an Fc rosetting assay using IgG-coated sheep erythrocytes. The assay results showed that the sheep erythrocytes did not form rosettes with the surface of E1A.NR3 cells. To test whether Fc receptors were induced by antibodies, the E1A.NR3 cells were initially incubated with antibody for 4 hours, then the rosette test was performed. In this case also no rosette formation was seen. These results indicate that E1A.NR3 cells do not express Fc-binding activity on their surface.

To ascertain whether antirecoverin antibodies are capable of influencing the growth of cells that do not express recoverin, antirecoverin antibodies were cultured together with three types of cells, i.e. human retinoblastoma cells Y79, rat pheochromocytoma cells PC12, and rat hyphophyseal tumor cells GH3. The proteins extracted from these cells were examined for the presence of recoverin by Western blot analysis using antirecoverin antibodies. None of these cell types expressed recoverin in culture, although retinoblastoma Y79 cells expressed recoverin mRNA. The cells had been cultured with a high dose of antirecoverin (300µg/mL) for 24 to 48 hours. By the immunoperoxidase method normal antibodies and antirecoverin antibodies were found within the cytoplasm of Y79, PC12 and GH3 cells. However, the results of MTT cytotoxic assays showed that antirecoverin antibodies did not affect the growth and viability of cells not expressing recoverin.

In a subsequent experiment, to the E1A.NR3 cell culture were added photoreceptor-specific monoclonal antibodies against another protein, i.e. arrestin, and incubated for 48 hours. Arrestin is expressed by E1A.NR3 cells. The results of culture with monoclonal antiarrestin antibody were similar with those of culture with antirecoverin antibody. After incubation with the highest dose of antibody, only 40 percent of the cells were still viable, compared with 100 percent viable cells in cultures with normal antibody.

In the CAR syndrome there are occasional inflammatory cell infiltrates in the diseased tissues, but inflammation of the retina is rarely reported. As there is no inflammation in CAR, it may be surmised that retinal degeneration in CAR develops as a result of a non-inflammatory process involving humoral immunity, i.e. the process of apoptosis.

Comparative analysis on E1A.NR3 cells cultured with specific antibody against recoverin, irrespective of cellular origin (human, rat, rabbit), showed changes in cell morphology, including shrinking of cell bodies, blebs, retraction of cell processes, and release of cells from the tissue culture plates. Cells cultured with control antibody on identical doses showed normal morphology. Because specific as well as normal antibody may enter the cell, the effect of antirecoverin antibodies must be due to specific antibody activity. Incubation of cells with antirecoverin antibodies
caused fragmentation of DNA into 200-bp integers and condensation of nuclear chromatin. DNA fragmentation was observed with all antirecoverin antibodies, both from patients' and animal sera. However, DNA fragmentation was not seen when the cells were incubated with normal antibody. Condensation of chromatin was analyzed using the fluorochromes Hoechst 33342 and propidium iodide. Addition of antirecoverin antibodies to the cells caused apoptosis, which was apparent from the numerous brightly-colored cell nuclei, most of which belonged to dead (pink-colored) cells. The number of apoptotic cells was on average 20 percent of the cell population, whilst in the control culture without added antibodies or with normal antibodies, only 1-2 percent of the cells were apoptotic, which may have been due to the natural cell cycle process.\(^{(16)}\)

A possible mechanism of antirecoverin-induced apoptosis of the photoreceptor cells is passage of the antibodies through leaks in the blood-retina barrier as a consequence of biochemical influences of the tumor, allowing internalization of the antirecoverin antibodies into the photoreceptor cells and access to the intracellular CAR antigen(s). The major result is that autoantibodies specific to recoverin penetrate into living retinal cells and trigger photoreceptor cell death through apoptotic mechanisms.\(^{(12,16)}\)

**RECOVERIN AS A PARANEOPLASTIC ANTIGEN WITHOUT PARANEOPLASTIC SYNDROME**

It might be assumed that autoantibodies against recoverin should be detected only in CAR patients. However, many paraneoplastic antigens have the capacity to induce low titer autoantibodies without any signs of paraneoplastic syndromes. In the process of raising antibodies against recoverin, Bazhin et al. found that immunized rabbits developed an immune response to recoverin at variable titers. Fundus examination and light microscopy of retinal sections detected retinal degeneration in rabbits with high titers of the antibody, while the eyes of rabbits with low titers did not differ from those of control animals. These findings led the researchers to screen serum samples of patients with lung cancers irrespective of the presence of CAR. Their search revealed 15 patients with SCLC among 99 individuals investigated (15%) and 9 of 44 patients with non-small cell lung cancers (20%) with relatively low titers of the autoantibodies in their sera, but without manifestations of CAR at the time of the serum sampling. Therefore they concluded that autoantibodies against recoverin can be detected in sera of patients with lung cancer without manifestation of paraneoplastic syndromes.

For these findings the investigators suggested the following explanations: (i) to overcome the blood-retinal barrier, the titer of the autoantibodies in the blood stream of patients should exceed a certain level. Otherwise the autoantibodies cannot penetrate into the cells, bind to the corresponding intracellular antigen and initiate apoptosis of the cells; (ii) the development of paraneoplastic syndromes might depend on the actual epitopes recognized by the autoantibodies. In the case of recoverin, autoantibodies from CAR patients mostly bind to residues 64-70 representing the EF-hand 2 domain and initiate apoptosis by blocking calcium-binding; (iii) The manifestation of CAR might well depend on a second event resulting in transmissibility of the blood–retinal barrier.\(^{(23)}\)

In CAR patients, anti-enolase autoantibodies may induce the apoptotic death of other retinal cells such as ganglion cells. Autoantibodies against retinal proteins from retinopathy patients with and without cancer had similar cytotoxic effects on retinal cells.\(^{(25)}\)

**CONCLUSIONS**

In CAR, certain tumors may express antigens that are normally present in the retina,
thus inducing autoantibodies against these retinal proteins. The identification of antiretinal antibodies is neither specific nor sensitive for the diagnosis of CAR. Analysis of immune-mediated vision loss is in its infancy, and a careful analysis and characterization of antiretinal antibody specificities will help in our understanding of the mechanisms and the diagnosis of patients with this form of vision loss. However, the pathogenic mechanisms of retinopathies are complex and our understanding of CAR is still incomplete.

REFERENCES


