The Cancer-Associated Retinopathy Antigen is a Recoverin-Like Protein

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Cancer-associated retinopathy (CAR) is a rare form of retinal degeneration that occurs in association with certain forms of cancer. CAR patients typically possess high titters of autoantibodies against a specific photoreceptor protein—the 23 kD retinal CAR antigen. The mechanisms involved in the vision loss experienced by CAR patients are not understood, but serologic studies indicate the process could include a series of autoimmune reactions directed at specific components of the retina. Because the retinal CAR antigen is the principal ocular autoantigen involved in the antibody response of CAR patients, characterizing it would contribute to the understanding of putative autoimmune involvement. Serum antibodies from CAR patients have been used to isolate the gene encoding the CAR antigen from a cDNA library of human retina. Nucleotide sequence analysis suggests that the CAR antigen shows approximately 90% homology to the published amino acid sequence of bovine recoverin. Invest Ophthalmol Vis Sci 33:2768-2772, 1992.

Paraneoplastic phenomena are secondary neurologic effects of cancer that involve a wide variety of central nervous system disorders affecting the brain, muscle movement, and vision. One of these phenomena is cancer-associated retinopathy (CAR), a rare secondary effect associated with certain forms of cancer. Although a variety of neoplasia can be involved, CAR is most frequently encountered with small cell (oat cell) carcinoma of the lung (SCCL), a neoplasm commonly associated with tobacco smoking. Small cell carcinomas, which are believed to originate from the Kulchitsky's cells located in the tracheobronchial mucosa, exhibit neuroendocrine properties that link them to the central nervous system. Small cell carcinomas are exceptional in their synthesis and release of a variety of small-molecular-weight, biologically active peptides, some of which have profound neurologic effects and may influence retinal function.

Although the biologic mechanisms involved in paraneoplastic degenerative retinopathies have not yet been defined, the peculiar antibody reactions encountered in these patients suggest autoimmunity as a contributing factor. One specific retinal protein, the retinal CAR antigen, has repeatedly been identified as a key component in the immunologic events that accompany paraneoplastic retinopathies.

To further evaluate the role of the 23 kD retinal CAR antigen in cancer-associated retinopathy, we have used serum antibodies from CAR patients to isolate the corresponding gene from a cDNA library of human retina. Sequence analysis shows high correlation with the photoreceptor protein recoverin. Recoverin is involved in the activation and regulation of guanylate cyclase, which influences rhodopsin function. The immunologic inactivation of recoverin may result in the photoreceptor loss characteristic of cancer-associated retinopathy.

Materials and Methods

Molecular Cloning and Sequencing

A lambda gt11 cDNA library of human retina (Clontech, Palo Alto, CA) was probed with high-titered serum antibody reactive with the retinal CAR antigen. A collection of eight positive clones was isolated. The insert from one was ligated into an M13 phage (Bethesda Research Laboratories, Gaithersburg, MD) and a “Bluescript” plasmid (Stratagene Cloning Systems, La Jolla, CA) to permit double and single strand, dual directional, dideoxy sequencing. Sequencing was performed at U.C. Davis and confirmed by Lark Sequencing Technologies Inc, Houston, Texas.
Recombinant CAR Antigen

Preparations of the recombinant form of the retinal CAR antigen (rec-CAR-Ag) were obtained through the IPTG-triggered expression of the CAR gene in liquid lysates of lambda gt11 isolates, replicating in the *Escherichia coli* host Y-1090.

Affinity Purification of Antibodies

Repeated Western blot (20 cm) transfers of liquid lysates allowed the rec-CAR-Ag to be resolved by antibody reactions as a single band on nitrocellulose. A collection of 10 strips containing the antigen was excised and used repeatedly to isolate related antibodies from whole sera obtained from CAR patients, according to the method described by Olmstead.16

Western Blot

Western blot assays were performed on bovine retina and liquid lysates expressing the recombinant protein using the conventional techniques we have previously described.4

Immunohistochemistry

Deparaffinized sections of rhesus monkey retina were exposed to CAR patients' antibodies affinity purified from the rec-CAR-ag, for 1 hr at room temperature. Antibody interactions were visualized using avidin-biotin conjugated to horseradish peroxidase reacting on diaminobenzidine (Vector laboratories, Burlingame, CA).

Results

A clone that expressed an immunoreactive β-galactosidase fusion protein of approximately 140 kD was isolated from the human cDNA retina library (Fig. 1A). The specificity of the antibody reaction with the rec-CAR-Ag was investigated by affinity purifying related antibodies from CAR patients' serum antibodies using the rec-CAR-Ag fusion protein and reapplying the isolated antibodies to Western blots of whole retina.16 The affinity-purified antibodies recognized only the CAR antigen and showed no reactivity with other retinal components when assayed at high (1:200) and low concentrations (1:1,000) (Fig. 1B). When assayed on Western blots of retina at high concentrations, the original CAR patient's serum recognized the 23 kD retinal CAR antigen and additional retinal polypeptides (Fig. 1C).

The cloned 1100 base pair nucleotide sequence (Fig. 2) contains an open reading frame (ORF) of 229 codons beginning at position 1 and extending through position 687. This ORF is in the same reading frame as the β-galactosidase fusion protein, approximately 18 kD larger than native β-galactosidase. This is consistent with the observed 140 kD immunoreactive fusion protein.

The 200 codon ORF that initiates at the first Met codon (position 88-90) and continues through position 687 encodes a predicted polypeptide (designated rec-CAR-Ag) that has extensive sequence homology with bovine protein recoverin15, differing at only 22 of 201 residues (Fig. 3). This suggests that the CAR antigen is recoverin or a closely related protein. Immunohistochemistry revealed specific localization of CAR antigen to the inner segments and nuclei of rods and cones in addition to the region of the outer plexiform layer where cone pedicles and rod spherules are found (Fig. 4). This localization is similar to the reported distribution of recoverin.15

Discussion

In previous studies, we reported a series of cancer patients who experienced rapid and unexplained vi-
sion loss. All showed high serum titers of antibodies reactive with the 23 kD retinal CAR antigen, but that differed in each patient according to the phase at which the blood sample was taken and the treatment received. All showed high serum titers of antibodies reactive with the 23 kD retinal CAR antigen, but possible that antibodies to the retinal CAR antigen develop secondary to other reactions initiated by toxic products of the cancer that damage the retinal pigment epithelium. Alternatively, the immunologic reactions that develop in CAR may originate from the host’s response to the neoplasm that has been shown in previous studies to incite a variety of immunologic responses, some of which may cross-react with the retina.

CAR reaction probably arises as an immunologic response to an unusual event during the development or proliferation of the malignant neoplasia. Either of two distinctly different initiating events could lead to the development of antibody specific for the CAR antigen: (1) synthesis in the tumor of a protein that is related antigenically to recoverin, resulting in autoimmunity on the basis of antigenic mimicry; or (2) the reaction to an unusual event during the development of antibodies to undamaged ocular tissue, it is possible that antibodies to the retinal CAR antigen might cross-react with the cancer itself, which may sensitize the host to the retinal CAR antigen through the aberrant production of an immunomodulation.

Fig. 2. Primary nucleotide sequence of human CAR cDNA and identification of the 200 codon rec-CAR-Ag ORF. Sequence determination used both single-strand and double-strand DNA templates for complete sequence determination of both strands of the cDNA fragment. The sequencing strategy and extent of sequence reactions are indicated at the bottom of the figure. Major restriction sites are E: Eco RI; P: Pst I; and K: Kpn I.
nologically similar antigen. Although antibodies to the retinal CAR antigen may not be the primary cause of vision loss, their presence exhibits a high degree of disease (cancer) specificity. So far, there have been no reports of correlates in other forms of retinopathies.

To initiate any putative autoimmune contribution to paraneoplastic retinopathy, components of an activated immune response to malignancy must be able to cross the blood-retinal barrier to influence ocular tissues. More diffusible components of the immune system, such as the interleukins, have been demonstrated in models to be highly organ selective and cell specific in their cytotoxicity.20 In cancer-associated retinopathy, these cytokines, mobilized in response to some yet to be defined stimulus, may initiate leakage of specific intraocular antigens to immune surveillance, contributing to the events that result in an autoimmune retinopathy.

It is important to appreciate that the proposal of photoreceptor leakage fails to consider that CAR patients do not exhibit comparable indications of hypersensitivity to other internalized photoreceptor components, such as rhodopsin or the retinal S-antigen, which are recognized as highly antigenic. However, a parallel may be found in animal models of experimental allergic uveitis, in which the predominant immunologic response is commonly overwhelmingly directed against the inciting antigen, with relatively less reaction with other photoreceptor components.21–23

CAR and other forms of cancer-related neuropathies, such as paraneoplastic cerebellar degenerations, have been reported to manifest prior to the diagnosis of cancer.2,3,6,24–26 The strong association of CAR anti-

![Fig. 3. Alignment of the predicted amino acid sequence of the human CAR antigen (rec-CAR-Ag) with the sequence of bovine recoverin (BoRec). Amino acid sequence is indicated by the single letter code, and identity of BoRec with the rec-CAR-Ag is indicated by a dash (-). Two regions indicated in boldface have extensive homology with the consensus Ca++ binding motif (En**nn*n*O*O*OG*; E = glutamic acid; n = nonpolar; = any residue; O = Ca++ chelating residue with oxygen in side chain; G = glycine; I = isoleucine, leucine, or valine).](#)

![Fig. 4. (A) Immunohistochemistry of affinity-purified CAR antibody on sectioned rhesus monkey retina. Antibody reactivity was restricted primarily to the inner segments and nuclei of rods and cones and to the region of the outer plexiform layer where cone pedicles and rod spherules are found. (B) Immunohistochemistry of affinity purified serum from an unrelated patient. The only antibody reactivity in the retina is confined to vessels where there is second antibody binding to the monkey serum (A, B: bar = 20 μm).](#)

body reactions with paraneoplastic vision loss invites its application to the immunologic screening of susceptible populations for the early detection and possible therapeutic management17 of specific forms of cancer.

Key words: recoverin, cancer-associated retinopathy, autoimmunity, recombinant CAR antigen

References


