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# Autoantibodies against retinal proteins in paraneoplastic and autoimmune retinopathy

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## Abstract

**Background:** Autoimmune retinal degeneration may occur in patients who present with sudden or, less commonly, subacute loss of vision of retinal origin, associated with an abnormal ERG, through the action of autoantibodies against retinal proteins. Often the patients are initially diagnosed with or suspected of having a paraneoplastic retinopathy (PR), such as cancer-associated retinopathy (CAR). However, there is limited information on the occurrence, the specificity of autoantibodies in these patients, and their association with clinical symptoms.

**Methods:** Sera were obtained from 193 retinopathy patients who presented with clinical symptoms resembling PR or autoimmune retinopathy (AR), including sudden painless loss of vision, typically associated with visual field defects and photopsias, and abnormal rod and/or cone responses on the electroretinogram (ERG). Sera were tested for the presence of anti-retinal autoantibodies by Western blot analysis using proteins extracted from human retina and by immunohistochemistry. Autoantibody titers against recoverin and enolase were measured by ELISA.

**Results:** We identified a higher prevalence of anti-retinal autoantibodies in retinopathy patients. Ninety-one patients' sera (47.1%) showed autoantibodies of various specificities with a higher incidence of antibodies present in retinopathy patients diagnosed with cancer (33/52; 63.5%; p = 0.009) than in retinopathy patients without cancer (58/141; 41.1%). The average age of PR patients was 62.0 years, and that of AR patients was 55.9 years. Autoantibodies against recoverin (p23) were only present in the sera of PR patients, autoantibodies against unknown p35 were more common in patients with AR, while anti-enolase (anti-p46) autoantibodies were nearly equally distributed in the sera of patients with PR and those with AR. In the seropositive patients, the autoantibodies persisted over a long period of time – from months to years. A rebound in anti-recoverin autoantibody titer was found to be associated with exacerbations in visual symptoms but not in the recurrence of cancer. When compared to sera from healthy subjects, autoantibodies against retinal proteins from both groups of patients were cytotoxic to retinal cells, indicating their pathogenic potential.

**Conclusions:** These studies showed that patients with sudden or subacute, unexplained loss of vision of retinal origin have anti-retinal antibodies in a broad range of specificity and indicate the need for autoantibody screening. Follow-up tests of antibody levels may be useful as a biomarker of disease activity associated with worsening of vision. Moreover, the heterogeneity in autoantibody specificity may explain the variation and complexity of clinical symptoms in retinopathy patients.

## Background

The causes of acquired retinal diseases are poorly understood, although some patients appear to have an autoimmune component contributing to the pathogenicity [1,2]. In these patients, serum antibodies have been associated with loss of vision, but the precise role of the autoantibodies has not been fully established. Indeed, the occurrence, relative frequency, and specificity of these autoantibodies are unknown.

Paraneoplastic retinopathies (PR), including cancer-associated retinopathy (CAR), in which retinal degeneration occurs in the presence of systemic cancer, have been the most intensively studied group of autoimmune retinopathies. Patients with CAR possess autoantibodies that react with retinal proteins, including recoverin (23 kDa) and α-enolase (46 kDa) [3,4]. Other autoantibodies against retinal proteins have also been reported, such as neurofilament proteins, heat-shock protein 70, TULP1 protein, 40-kDa insoluble protein, and others, but their role in the pathogenicity of acquired retinal diseases has not been established [3,5-9]. Autoantibodies binding to bipolar cells have been linked to the melanoma-associated retinopathy (MAR) syndrome [10-12]. However, autoantibodies with the same specificity have recently been described in a CAR patient [13]. Autoantibodies with other specificities were also found in the sera of patients with MAR [11,14]. In addition, anti-recoverin antibodies have been reported in patients with non-cancer retinopathy [15,16] and in some patients without visual symptoms, who have small-cell carcinoma of the lung [17].

In recent years, retinopathies without underlying neoplasm at the time of testing and enigmatic retinopathies with an abnormal electroretinogram (ERG) have been described, in which patients share certain clinical and immunological features of PR [15,18-20]. If autoantibodies are detected, and if cancer is not detected on initial evaluation or does not occur within the next several months after the detection of autoantibodies, the term autoimmune retinopathy (AR) is used. For example, autoantibodies of AR patients label the inner plexiform layer [18] or Müller cells (described as a 35-kDa retinal antigen) in the retina [19]. Findings from these reports of individual case indicate that AR demonstrates diverse clinical and immunological features. The relative incidence of anti-retinal autoantibodies associated with retinopathy is unclear.

We believe that circulating autoantibodies against retinal antigens may contribute to retinal dysfunction and degeneration, especially in those patients who do not have a genetic (familial) basis for their retinopathy. We undertook the current study to examine the occurrence and the specificity of autoantibodies in a cohort of 193 retinopathy patients presenting with sudden or less commonly, subacute loss of vision and abnormal ERG in association with diagnosed or suspected cancer, and to identify any clinical-immunological associations.

## Methods

### *Patients and source of antibodies*

We collected blood samples from 193 patients who presented with clinical symptoms that resembled PR (including CAR syndrome) or AR. These patients presented with sudden or, less commonly, subacute painless loss of vision, typically associated with visual field defects and photopsias. They were all examined by ophthalmologists who diagnosed retinal disease of unexplained origin and referred them for this study. The findings of abnormal rod and/or cone responses on the ERG often triggered the suspicion of PR or AR. Patients who had ocular trauma, active eye inflammation, retinal infection, retinal detachment, retinal surgery, or a family history of retinal degeneration were excluded from the study. Sera were prepared from blood and stored at -80°C prior to immunological tests. Samples were collected between 1993 and 2002 from patients primarily from US eye clinics: Portland, Oregon (59 patients); Chicago, Illinois (26 patients); Iowa City, Iowa (27 patients); Madison, Wisconsin (17 patients); and other US clinics, Canada, Europe, and Australia (64 patients). The subjects represented 82 men and 111 women, ranging between 19 and 82 years (mean age: 58.8 years). The patients were divided into two groups: 52 with PR, who presented CAR and MAR symptoms with confirmed systemic cancer, and 141 with non-paraneoplastic AR, whose cancer screening by the referring physician was negative. For controls, we used the sera from 79 healthy age- and sex-matched volunteer subjects (47 men and 32 women) who had no vision loss at the time of collection.

Investigations were performed according to the guidelines of the "Declaration of Helsinki". The Oregon Health & Science University Institutional Review Board approved the project and informed consent was obtained at the time of blood collection.

### *Antibody testing*

#### *Western blotting*

Initial screening of sera was performed using retinal proteins that were extracted from a human retina, with 1% octyl glucoside in phosphate/saline buffer (PBS), pH 7.2. The proteins were separated by SDS-gel electrophoresis on a 10% gel and transferred to an Immobilon membrane (Millipore, Bedford, Massachusetts). Individual strips containing retinal proteins were blocked with 10% normal goat serum, 1% bovine serum albumin in PBS for 1 hr, and then probed with 1:100 diluted serum (1 hr) followed by a 1-hr incubation with anti-human IgG (H and L chain) conjugated to alkaline phosphatase (Sigma, St.

Louis, MO). Color reaction was developed by adding the phosphatase substrate until dark bands, appeared in comparison to the positive control. Blots were run and examined in a masked fashion. As a positive control, we used a reference human serum containing anti-recoverin antibodies diluted 1:100. As a negative control, we omitted serum and applied only a secondary antibody.

#### *Immunocytochemistry*

The second specificity test was performed using human-donor retina embedded in sucrose and frozen in OCT medium. Ten μm cryosections were fixed in 2% paraformaldehyde or were left unfixed. The sections were treated with a blocking solution (1% BSA, 1% normal goat serum in PBS) for 1 hr followed by a 20-minute incubation with 0.25 % Triton-X-100 to permeabilize the tissue. Then a 1:100 diluted serum from a patient was added for 1 hr. The sections were washed and then incubated with a 1:2000 diluted biotinylated anti-human IgG (H and L chain) for 1 hr. After the sections were washed, a 1:5000 diluted streptavidin HRP was added for 30 minutes. All reagents were purchased from Zymed (San Francisco, CA). Color reaction was developed by adding a DAB peroxidase substrate (PIERCE, Rockford, IL). Controls consisted of retinal sections that were treated with either a 1:100 diluted serum from a control (negative control) or a 1:100 diluted serum from a CAR patient with anti-recoverin autoantibodies (positive control); or a secondary antibody only. Sections were evaluated for positive cell labeling in a light microscope by a masked observer.

#### *ELISA for anti-recoverin and anti-enolase antibodies*

Microtiter plates were coated with 100 ng/well of bovine recoverin or α-enolase in 0.1 M Tris-HCl buffer, pH 9.0 overnight at room temperature. After blocking with 1% BSA in PBS for 1 hr, 100 μl of serially diluted patients' serum was added to each well, followed by a 1-hr incubation with anti-human IgG conjugated to peroxidase (Zymed). Color reaction was developed 30 min later after incubation with the peroxidase substrate (2,2'-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid) in 0.1 M citrate-phosphate buffer, pH 4.5, containing 3% $H_2O_2$ and measured at 405 nm using a Bio-Rad Microplate Reader.

### *Cytotoxicity assay*

To test the cytotoxic effect of antibodies from patients, we used a colorimetric MTT cytotoxicity test as described by Adamus et al. [21]. Twenty-eight random samples of sera were taken from each group of subjects for analysis. Serum antibodies were purified by ammonium sulfate precipitation, and the purity was checked by SDS-gel electrophoresis. E1A.NR3 retinal cells at a density of $10^4$ cells/well in 200 μl DMEM medium were grown with purified serum IgG from patients and controls, at 150 μg/ml for 48 hrs.

### *Statistical analysis*

Significant differences between groups were assessed using the nonparametric Mann-Whitney U test and Chi-square test. A $p$ value <0.05 was considered significant.

## Results

### *Antibody specificity to retinal antigens*

We determined the occurrence of autoantibodies against retinal antigens (anti-retinal antibodies) in 193 patients who presented with painless loss of vision, photopsia, and abnormal ERG. Of the 193 sera, 91 (47.1%) showed the presence of anti-retinal antibodies as measured by Western blot analysis of human retinal extracts and by immunocytochemical analysis of sections of human retinas. In the majority of cases, Western blotting was sufficient to identify anti-retinal antibodies. However, anti-bipolar cell antibodies, which were negative on the blot, could be identified only by immunocytochemistry (data not shown). Typically, a serum demonstrated immunostaining of a single protein band on the blot. When the serum was suspected to react with known proteins – such as recoverin, arrestin, α-enolase, rhodopsin, transducin, carbonic anhydrase, phosphodiesterase, and IRBP – its reactivity was confirmed by the additional incubation of serum with a purified protein on a blot in a separate experiment.

In our studies, 27% of patients were diagnosed with cancer, including small cell carcinoma of the lung, endometrial carcinoma, colon carcinoma, breast carcinoma, ovarian carcinoma, skin melanoma, and others (Table 1 and 2). The average age of patients with cancer was 62.0 years, and the average age of patients without diagnosed cancer was 55.9 years ($p$ = 0.007). There was a higher incidence of antibodies present in those patients diagnosed

**Table 1: Occurrence of anti-retinal antibodies in patients with paraneoplastic retinopathies and autoimmune retinopathies**

| | |
|---|---|
| Total Patients | 193 |
| Total women | **111** |
| Total men | **82** |
| Patients with cancer | 52 |
| Patients without cancer | 141 |
| Patients with Ab | 91/193 (47.2 %) |
| Total Women with Ab | 58/111 (63.7 %) |
| Total Men with Ab | 33/82 (36.3 %) |
| PR Patients with Ab | 33/52 (63.5%) |
| Women with PR and Ab | 20/33 (60.6 %) |
| Men with PR and Ab | 13/33 (39.4 %) |
| AR Patients with Ab | 58/141 (41.1%) |
| Women with AR and Ab | 38/58 (65.5 %) |
| Men with AR and Ab | 20/58 (34.5 %) |

Ab – antibodies; PR – paraneoplastic syndrome; AR – Autoimmune retinopathy

with cancer (33/52; 63.5%) than in patients with retinopathy without cancer (58/141; 41.1%)(p = 0.009). In both patients with paraneoplastic retinopathy and those with retinopathy without cancer, more women than men possessed anti-retinal antibodies (38/58; 65.5% in AR, and 20/33; 60.6% in PR) (Table 1). Considering how many proteins are present in the retina, the heterogeneity in antibody recognition was relatively low. Figure 1 shows the distribution of autoantibody specificity among retinal proteins grouped according to their molecular weight. The most frequently observed antibodies among the patients were the antibodies against retinal α-enolase (28/91), recoverin (12/91), and p35 (15/91). Antibodies against recoverin (p23) were present only in the sera of patients with PR (12/53). Table 2 shows the antibody specificity in patients with cancer. We observed that antibodies against unidentified p35 were more common in patients with AR, while anti-enolase (anti-p46) reactivity was found nearly equally in the sera of patients with PR and those with retinopathy without cancer. Proteins of other molecular weights were also found to react with antibodies from patients, but their occurrence was lower (Figure 1). These proteins included antibodies with confirmed specificity against carbonic anhydrase II (30 kDa), rhodopsin (40 kDa), arrestin (48 kDa), phosphodiesterase (PDE; 88 kDa), and other retinal proteins of unknown identity.

**Table 2: Autoantibody specificity in patients with retinopathy and systemic cancer**

| Patient # | Sex | Age | Cancer | Antibody Specificity |
|---|---|---|---|---|
| P403 | F | 62 | Endometrium Ca | Recoverin [29]; see Fig. 3 |
| P626 | F | 73 | Endometrium Ca | Recoverin |
| P593 | F | 46 | Cervical Ca | Enolase |
| P587 | F | 81 | Ovarian Ca | p35, p39, p46, p58 |
| P405 | M | 62 | SCCL | Recoverin |
| P407 | M | ?? | SCCL | Recoverin |
| P409 | M | 64 | SCCL | Recoverin; see Fig. 3 |
| P536 | F | 75 | SCCL | Recoverin |
| P619 | M | 85 | SCCL | Recoverin |
| P634 | M | 84 | Benign lung tumor | Recoverin |
| P622 | M | 68 | Lung adenocarcinoma | p35 |
| P402 | M | 84 | Colon Ca | Recoverin |
| P692 | M | 58 | Colon | p35 |
| P559 | F | 52 | Colon Ca | Bipolar cells [13] |
| P624 | F | 47 | Colon Ca, skin Ca | p41 |
| P596 | M | 66 | Bladder Ca | Enolase |
| P568 | M | 78 | Skin melanoma | Enolase |
| P578 | F | 44 | Skin melanoma | Enolase |
| P474 | F | 35 | Skin melanoma | Enolase |
| P618 | F | 35 | Skin melanoma | p35=transducin β [14] |
| P566 | M | 59 | Skin melanoma | p35 |
| P614 | M | 45 | Skin melanoma | p40 |
| P570 | M | 70 | Squamous cell Ca of the skin | Recoverin |
| P462 | F | 65 | Breast Ca primary, Recurrent Breast Ca | Enolase |
| P488 | F | 85 | Breast Ca | Enolase |
| P529 | F | 58 | Breast Ca | Enolase |
| P726 | F | 74 | Breast Ca | Enolase |
| P718 | F | 64 | Breast Ca in situ | Recoverin; see Fig. 3 |
| P547 | M | 49 | Kidney Ca | p60 |
| P711 | M | 64 | Tongue Ca, lung sarcoidosis | p58 |
| P561 | F | 55 | Cutaneous B-cell Lymphoma | Enolase |
| P651 | F | 81 | Adenocarcinoma of unknown primary discovered in a femoral lymph node | Arrestin |
| P708 | F | 73 | Metastatic adenocarcinoma spread to liver of unknown primary | p60 |
| P717 | F | 75 | Metastatic Ca spread to head of unknown primary | Enolase |

SCCL – small-cell carcinoma of the lung; Ca – carcinoma

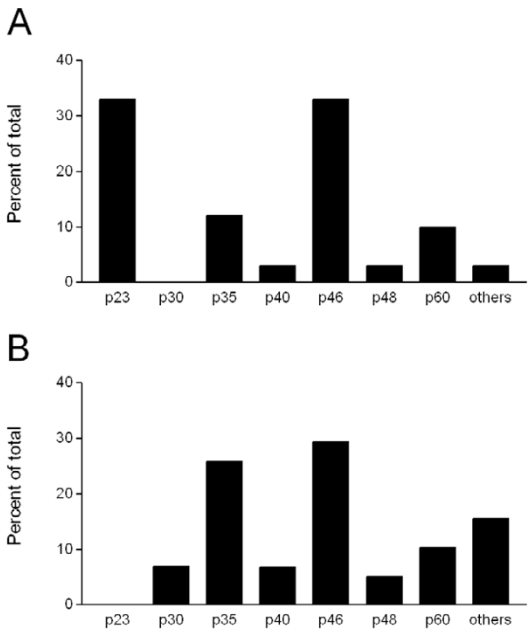


**Figure 1**
Distribution of autoantibody specificities in patients' sera. (A) Paraneoplastic retinopathy, (B) Autoimmune retinopathy. Bars represent the percent of antibody-positive patients with a specific antigenic protein. Anti-recoverin antibodies (p23) were present only in the sera of patients with paraneoplastic retinopathy.

In the control group of 79 sera from healthy subjects without visual loss (47 men, 32 women), we found 15 sera with anti-retinal antibodies, but in contrast to the retinopathy patients, they reacted with multiple retinal proteins on the blot. In the control group, carbonic anhydrase (a protein of 30 kDa) was commonly recognized (5/15).

### *Antibody cytotoxicity*

To study the role of antibodies in the pathogenicity of retinopathy, we randomly selected 28 seropositve samples from each of the 3 groups of patients: retinopathy patients with and without cancer, and control subjects. Purified serum IgGs were tested for their cytotoxic effect on cultured retinal cells. Figure 2 shows that after a 48-hour incubation, the cell survival rate was significantly lower in retinal cells grown in the presence of sera from patients with PR (62.5%; $p < 0.0001$) and AR (68.3%; $p = 0.0003$) than retinal cells grown in the presence of non-specific sera from control subjects (86.5%).

### *Follow-up studies*

We obtained multiple serum samples from the follow-up clinic visits of 40 patients, of whom 31 were initially seropositive and 9 were seronegative. The repeat samples were obtained to either monitor worsening symptoms or to determine the effectiveness of treatment on the antibody level. Samples that initially tested negative by Western blotting and immunocytochemistry remained negative 1 to 2 months later. In the seropositive patients, the antibodies persisted over a long period of time – from months to years. Some patients underwent diverse immunomodulatory treatments, including systemic steroids, immunoglobulin infusion (IVIG), or Rituxan (monoclonal antibodies against B cell CD20), in an attempt to improve visual symptoms and to reduce the level of anti-retinal antibodies, but these treatments had varying results. Table 3 summarizes the data from the seropositive patients. Figure 3 shows the fluctuation of anti-recoverin antibody levels in 3 patients tested in follow-up visits over a long-term. The level of anti-recoverin antibodies changed over time, and the rebound in antibody titers was associated with exacerbations in visual symptoms but not with a recurrence of cancer. In one CAR patient (Fig. 3A), anti-recoverin antibodies remained at a non-measurable level for almost 5 years, and then with the worsening of symptoms, the level considerably increased. However, an extended work-up for cancer was negative. Similarly, the reoccurrence of anti-recoverin antibodies in other patients was not associated with metastastic events (example in Fig. 3B). Treatment for cancer drastically decreased anti-recoverin antibodies (Fig. 3C), which was a result of the immune cells damage by radiation. However, anti-recoverin antibody levels slowly increased when the immune system recovered, suggesting that cancer treatment has no direct effect on the level of these antibodies.

## Discussion

This study shows that autoantibodies are associated with autoimmune retinopathies. Autoimmune retinopathy demonstrates diverse clinical and immunological features and by expanding the inclusion criteria of patients we were able to demonstrate that (i) retinopathy patients with and without cancer, who had unexplained visual loss, had a high prevalence of autoantibodies against retinal proteins. In our subject population, about half had anti-retinal autoantibodies, underscoring their frequency and their potential pathogenic role. Even though not all patients had antibodies against known autoantigens, such as recoverin (23 kDa) and retinal α-enolase (46 kDa), a more general role for autoantibodies can be proposed based on our findings. Moreover, such high occurrence of antibodies indicates a need for testing to help with diagnosis. (ii) An initial seropositive test would be helpful in diagnosing cancer. (iii) By including the clinical follow-up seropositive and seronegative patients to establish the

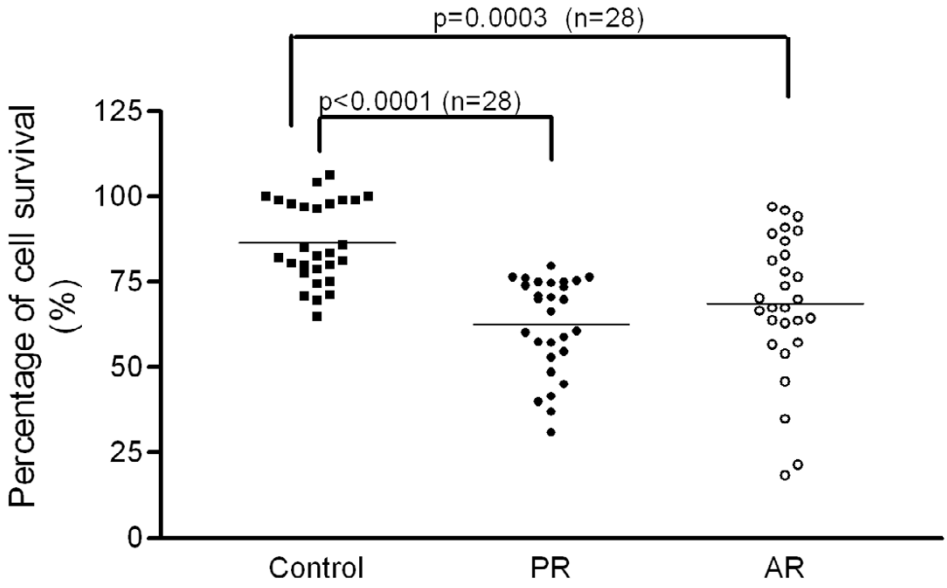


**Figure 2**
The effect of antibodies from patients with retinopathy on the survival of retinal cells. E1A.NR3 cells were grown in the presence of purified human antibodies for 48 hours. AR and PR data were compared with control subjects (n = 28 subjects each group).

**Table 3: Follow-up studies on seropositive patients**

| Patients | | | | | Antibody Testing | | |
|---|---|---|---|---|---|---|---|
| Number | Sex | Age | Specificity at onset of vision loss | Diagnosis of cancer | No. of Samples | No. of positive | After treatment |
| P409 | M | 64 | Recoverin | Before | 11 | See Fig. 3 | Negative after chemotherapy and radiation |
| P785 | M | 66 | Recoverin | 5 years after | 2 | 2 | |
| P536 | F | 72 | Recoverin | Before | 2 | 2 | |
| P718 | F | 64 | Recoverin | 6 months after | 8 | See Fig. 3 | Reduction after Rituxan |
| P626 | F | 73 | Recoverin | 2 years after | 5 | 2 | |
| P426 | F | 66 | Recoverin | After | 34 | See Fig. 3 | Negative after Tolpa [29] |
| P570 | M | 72 | Recoverin | Before | 2 | 1 | |
| P692 | M | 58 | P35 | 9 months after | 2 | 1 | Negative after systemic steroids |
| P613 | M | 68 | P35 | Before | 2 | 2 | |
| P566 | M | 59 | P35 | Before | 3 | 2 | Negative after INFa/b |
| P559 | F | 51 | Bipolar cells | After | 3 | 1 | Negative after chemotherapy [13] |
| P561 | F | 55 | Enolase | 3 months after | 5 | 5 | Reduction after IVIG |
| P474 | F | 35 | Enolase | Before | 3 | 1 | |
| P593 | F | 46 | Enolase | 17 years after | 2 | 2 | |
| P660 | F | 65 | Enolase | No cancer | 3 | 2 | |
| P436 | M | 43 | IPL | No cancer | 19 | 8 | Negative after IVIG |
| P662 | F | 47 | P35, p46 | No cancer | 2 | 2 | |
| P580 | M | 54 | P53, p46 | No cancer | 3 | 3 | No change after oral steroids |
| P630 | M | 40 | P35 | No cancer | 2 | 2 | |
| P579 | M | 30 | P35 | No cancer | 6 | 4 | No change after oral steroids |
| P513 | F | 52 | Carbonic anhydrase | No cancer | 2 | 2 | |
| P577 | M | 68 | P40 | No cancer | 2 | 2 | |
| P623 | F | 34 | Arrestin | No cancer | 2 | 2 | |
| P653 | F | 28 | P52 | No cancer | 3 | 3 | |

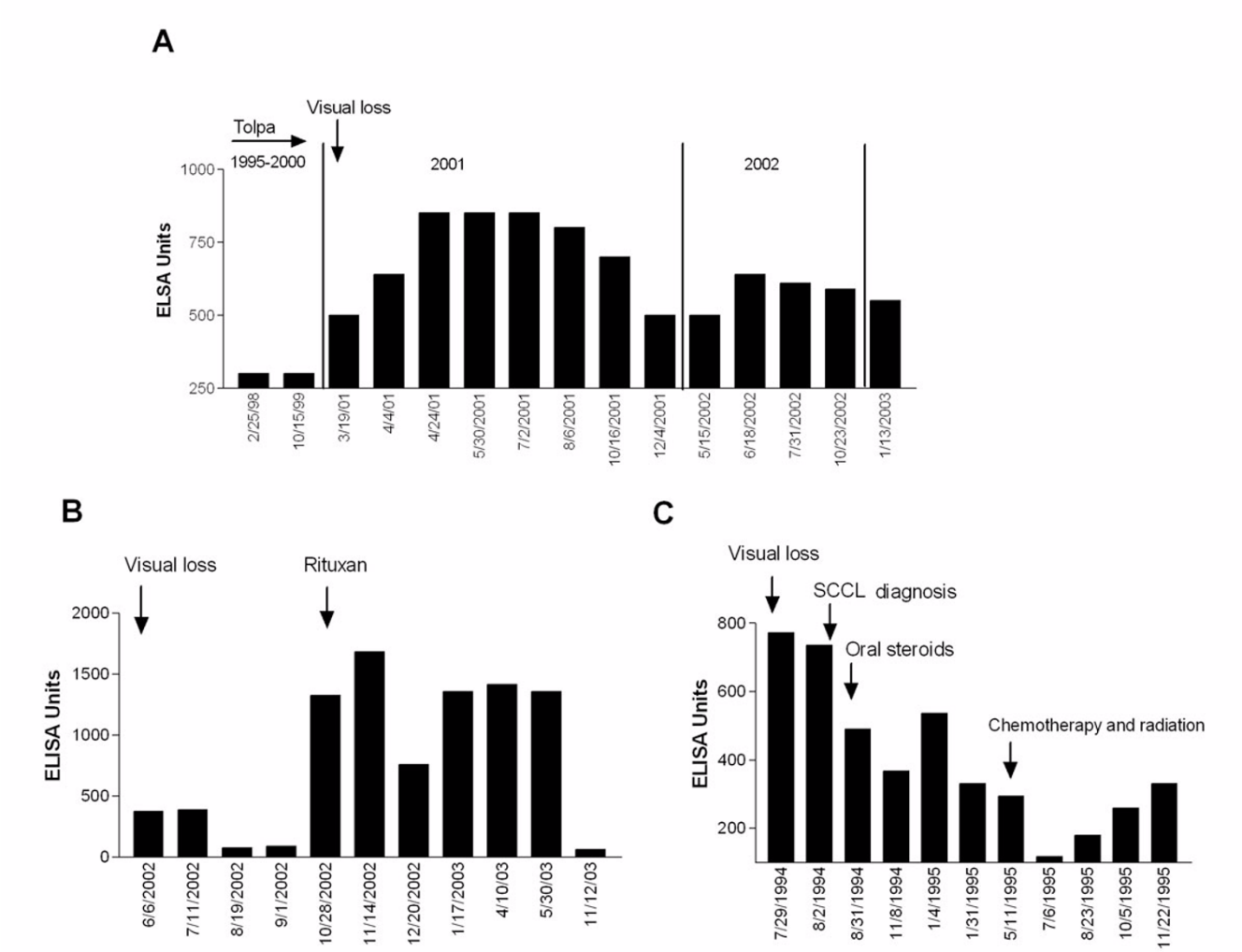


**Figure 3**
Longitudinal analysis of serum anti-recoverin antibodies in three CAR patients from serum samples obtained during follow-up clinic visits. Patients' antibody levels were measured by ELISA using recoverin-coated plates. (A) 61-year-old woman (P403) diagnosed with endometrial cancer in 1991. The graph presents tests performed after the original publication of this case report in 1998 [29]. Tolpa is a natural immunosuppressive drug. (B) 64-year-old woman (P718) who was diagnosed with breast carcinoma in 2002 who experienced an onset of visual loss 6 months later. (C) 62-year-old man (P409) who presented with an onset of visual loss and antibodies before the diagnosis of SCCL in 1995. Note that chemotherapy and radiation completely diminished the presence of antibodies due to the damage of the immune cells. The level of antibodies increased after the immune system recovered.

occurrence in relation to treatment or reoccurrence of tumor, we showed that, as in other autoimmune diseases, anti-retinal autoantibodies persisted and the levels fluctuated over a long period of time after the onset of visual symptoms. Follow-up tests of autoantibody levels can be used as a biomarker of disease activity associated with worsening of vision. (iv) Patients with PR retinopathy are older at the onset of visual symptoms. (v) Because the occurrence of autoantibodies does not necessarily indicate the presence of an autoimmune disease, findings from experimental studies are crucial to establish the role of such antibodies in retinal pathology. Therefore, testing for specificity, in particular for cytotoxicity, is important to determine the pathogenic potential of circulating anti-

retinal autoantibodies in high titers, especially light of our findings that autoantibodies against retinal proteins from retinopathy patients with and without cancer had similar cytotoxic effects on retinal cells.

Pathogenic autoantibodies may impact the retina by influencing function, leading in turn to morphological damage. Previously, we showed that anti-recoverin autoantibodies induce apoptotic death of photoreceptor cells, which leads to retinal degeneration [22,23]. This study presented the first evidence that the entry of anti-recoverin antibodies into cells resulted in apoptosis. Antibodies with other specificities have been shown to have similar effects on retinal cells, for example antibodies against hsp27 [24], which led to our hypothesis that antibody-induced cell dysfunction leads to cell death and retinal degeneration. Thus, autoantibodies can be, and we believe often are, pathogenic.

The evidence supporting the effects of antibodies on retinal cells are the following findings: a) Autoantibodies against recoverin specifically labeled retinal photoreceptor cells and were internalized by cells causing their apoptotic death [22]. b) In CAR patients, autoantibodies against α-enolase induced the apoptotic death of retinal cells, and in glaucoma patients, autoantibodies against γ-enolase labeled retinal ganglion cells and induced their death through apoptosis [21,25]. c) Intravenous injection or administered to isolated eyecup of anti-arrestin antibodies induced ERG changes [26]. d) Intravitreous injection of autoantibodies against retinal bipolar cells induced changes in bipolar cell function as shown by ERG recording [27]. e) Autoantibodies against p35 and enolase react with retinal bipolar cells, Müller cells, and ganglion cells (rather than with photoreceptor cells) [19,21]. Thus, in the pathogenesis of retinopathy, antibodies may act on cells other than photoreceptors. Our recent finding that anti-enolase autoantibodies induce the death of retinal ganglion cells supports the idea that the broad range of specificities of antibodies does not exclude their role in the pathogenicity of autoimmune retinopathy [28]. The heterogeneity in antigenic recognition may result in complex variations of clinical symptoms.

## Conclusions

In conclusion, autoantibodies against retinal proteins from retinopathy patients with and without cancer had similar cytotoxic effects on retinal cells. We hypothesize that, independent of specificity, autoantibody-induced apoptosis is a pathway to retinal death in AR. However, the pathogenic mechanisms of retinopathies are complex, and our understanding of AR is still incomplete. Further studies are necessary to identify anti-retinal autoantibodies, to test their pathogenic potentials through *in vivo* and *in vitro* methods, and to define clinical and electrophysiological indicators for seropositive patients.

## Competing interests

None declared.

## Authors' contribution

AG was involved in the experimental design, data analysis, and preparation of manuscript. GR carried out samples preparation, and antibody immunoassays, and conducted statistical analysis. RGW participated in study design and the preparation of manuscript. All authors read and approved the final manuscript.

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