Major Retinal Cell Components Recognized by Onchocerciasis Sera Are Associated With the Cell Surface and Nucleoli

You Zhou,* Ewa Dziak,* Thomas R. Unnasch,† and Michal Opas*

Purpose. Cellular localization of the components recognized by onchocerciasis autoantibodies has not been investigated in any detail in cultured retinal cells. This study sought to examine, in cultured retinal cells, the subcellular localization of major components that cross-react with onchocerciasis sera.

Methods. Immunofluorescence confocal laser scanning microscopy and Western blot analysis were carried out on adult pig retinal cells.

Results. The onchocerciasis sera contain antibodies cross-reacting strongly with components of the surface and nucleoli in both the cultured retinal pigment epithelial and neural retinal cells. These epitopes are not recognized by the control sera obtained from noninfected individuals residing in an onchocerciasis hyperendemic area, and from those with or without ocular disease who have never been in any of the onchocerciasis hyperendemic countries. Double-labeling immunofluorescence microscopy does not detect any colocalization of a putative onchocerciasis autoantigen, calreticulin, and those cellular components recognized by onchocerciasis sera in either cell type. Furthermore, none of the onchocerciasis sera tested recognized recombinant calreticulin by Western blot analysis.

Conclusions. Major epitopes for onchocerciasis anti-retinal autoantibodies are associated with the surface and nucleolus components of retinal cells. Interaction of the onchocerciasis antibodies with the retinal cell surface molecules may play an important role in the development of ocular diseases initiated by the damage of retinal cells. Furthermore, the finding that the cellular components recognized by onchocerciasis sera do not colocalize with calreticulin, taken together with the observation of lack of recognition of recombinant calreticulin by these sera on Western blots, suggests that calreticulin is not a major onchocerciasis autoantigen.

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Onchocerciasis (river blindness) is a tropical filarial disease caused by infection with the parasitic nematode *Onchocerca volvulus*. Eye diseases, along with dermatitis, sclerosing lymphadenitis, and subcutaneous nodules, are the major manifestations of human onchocerciasis, and have been well described clinically.1,2 The pathogenesis of the *Onchocerca* eye diseases is still unclear, and may involve a variety of mechanisms, including both humoral or cell-mediated immune responses and autoimmunity.3,4 It has been shown, using frozen sections or extracts of retinas, that the sera of most patients with onchocerciasis contain antibodies against retinal components.5-9 Such cross-reactivity of retinal components with the antibodies produced against parasite antigens during infection may play a role in retinal damage leading to the loss of vision.10 Biochemical and molecular studies of *Onchocerca* antigens have shed some light on the pathogenesis of onchocerciasis. A major *Onchocerca* antigen, RAL-1, shares over 60% amino acid sequence identity with calreticulin,11-14 a major Ca^{2+} storage protein of non-muscle cells.15 The similarities between calreticulin and the *Onchocerca* RAL-1 antigen are most evident in

From the *Department of Anatomy and Cell Biology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada, and the †Department of Medicine, Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, Alabama.

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Proprietary interest category: N.

Reprint requests: Dr. Michal Opas, Department of Anatomy and Cell Biology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8.
The tenets of the Declaration of Helsinki were followed, informed consent was obtained, and institutional human experimentation committee approval was granted. Sera from clinically well characterized individuals with the *O. volvulus* infection were obtained from residents of Bong County, Liberia. Collection of these sera and clinical criteria were previously described.\(^2\) Two sets of control sera were obtained from (1) people who reside in an onchocerciasis hyperendemic area but are not infected by *O. volvulus*, based on parasitologic and immunologic criteria\(^2\) (serum 148), and who have never visited any onchocerciasis hyperendemic areas and have no ocular diseases (serum NS\#1 and NS\#2); and (2) people who have never been in any of the countries known to be onchocerciasis hyperendemic, but have active keratitis, which is also one of the ocular onchocerciasis symptoms (serum B0541 and N0551). Because such sera as serum 148, from “endemic normal” individuals who comprise only about 1% of the population in the hyperendemic areas in Liberia and Ghana, are difficult to define as good “normal” sera, we used two more sera (NS\#1 and NS\#2) as normal serum controls. Polyclonal goat anti-calreticulin antibodies were kindly provided by Dr. Marek Michalak (University of Alberta, Edmonton, Alberta, Canada). Polyclonal FITC-conjugated anti-human, TRITC-conjugated anti-goat, alkaline phosphatase-conjugated anti-human or anti-goat antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (Bio/Can Scientific, Mississauga, Ontario, Canada).

### Preparation of Retinal Tissues for Frozen Sectioning

All experiment procedures involving animals conformed to the ARVO Resolution on the Use of Animals in Research. For preparation of frozen sections, fresh, whole pig eyes, after removal of the adherent muscular and connective tissues, were fixed in 3.7% formaldehyde in phosphate-buffered saline (PBS) for 10 minutes and cut in half, then immersed in the same fixative for at least 30 minutes at 22°C. After three washes in PBS, eye tissues were prepared for frozen sectioning according to standard procedures. The sections (10 to 12 \( \mu \)m in thickness) were mounted on coverslips coated with 0.2% poly-L-lysine (Sigma Chemical Co., St. Louis, MO) in PBS and processed for immunofluorescence microscopy.

### Isolation of Retinal Cells for Cell Cultures

For preparation of NR cultures, the NR was separated from the underlying RPE of fresh pig eyes in serum-free \( \alpha \)-Modified Eagle Medium (MEM). After removal of the retinal blood vessels, the NR pieces were pooled and gently agitated without trypsinization. The cells were collected after a 2-minute centrifugation at 800g, and resuspended in \( \alpha \)-MEM containing 10% fetal bovine serum, 100 IU penicillin, 0.25 \( \mu \)g fungizone, and 100 \( \mu \)g streptomycin per milliliter. They were then plated onto coverslips coated with 1 mg/ml of type IV

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**MATERIALS AND METHODS**

**Sera and Antibodies**

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Retinal Epitopes for Onchocerciasis Antibodies

collagen (Peninsula Laboratories, Belmont, CA), and cultured in the same medium at 37°C.

Preparation of RPE cells was described previously.23 In brief, eye cups after removal of the NR were treated with a dispase solution (Collaborative Research, Inc., Bedford, MA) for 30 to 40 minutes at 37°C. Pieces of RPE cell sheets were removed from the dispase solution, pooled, and centrifuged at 800g for 2 minutes. The RPE sheets were resuspended in trypsin-ethylenediamine tetraacetic acid (EDTA) and incubated for about 10 minutes with gentle agitation. They were then pooled into α-MEM containing serum and antibiotics as described above, and plated onto noncoated coverslips. Both the primary RPE and NR cells were cultured at 37°C in a 5% CO2 atmosphere in α-MEM for 5 days before the immunofluorescence staining.

SDS-PAGE and Western Blot Analysis

For Western blotting, the RPE and NR sheets were isolated without trypsinization and homogenized at 4°C in Tris-HCl buffer (50 mM Tris, 250 mM sucrose, 1 mM EDTA, pH 7.3) with protease inhibitors (1 μM each phenylmethylsulfonyl fluoride and leupeptin; Sigma) added immediately before the homogenization. Total protein concentration of the homogenates was assayed using the method of Bradford.24 If necessary, proteins were concentrated by centrifugation of the homogenates at 4°C for 30 minutes at 200,000g with a Beckman (Palo Alto, CA) XL70 Ultracentrifuge. The samples were either processed immediately for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or aliquoted and stored at −80°C.

The retinal homogenates or recombinant rabbit calreticulin (a gift of Dr. M. Michalak, University of Alberta) were mixed with reducing sample buffer, and SDS-PAGE was performed using the buffer system of Laemmli.25 Samples were run on 3% stacking and 7.5% resolving acrylamide gels in a Bio-Rad Mini-Gel system (Bio-Rad Laboratories, Ltd., Mississauga, Ontario, Canada). The amount of protein loaded per lane was 50 μg for the retinal homogenates and 2 μg for calreticulin. The SDS-PAGE–fractionated material was electrophoretically transferred to nitrocellulose filters.26 The filters containing immobilized proteins were blocked with 5% nonfat milk powder and 1% bovine serum albumin (BSA) in Tris-buffed saline (50 mM Tris, 500 Mm NaCl, pH 7.4) with 0.05% Tween-20 (TTBS) for 0.5 to 1 hour. They were then incubated for 1 hour in TTBS containing the control sera or the sera from the patients with onchocerciasis or polyclonal goat anti-calreticulin (1:50 dilution). To test the onchocerciasis sera on Western blots of recombinant calreticulin, the sera were diluted 1:1 with TTBS. After three washes (10 minutes each) in TTBS containing 1% BSA, the blots were incubated in TTBS containing alkaline phosphatase-conjugated polyclonal donkey antibodies to either human or goat IgGs (1:5000) for 1 hour. The blots were developed using a standard bromochloroindolyl-phosphatase and nitroblue-tetrazolium substrate developing system (Bio-Rad Laboratories, Ltd.). All incubations were performed at room temperature.

Immunofluorescence Confocal Laser Scanning Microscopy

Samples were prepared as described previously27; in brief, cells were fixed in 3.7% formaldehyde in PBS for 10 minutes and extracted for 5 minutes in an extraction solution (pH 6.9) containing 0.1% Triton X-100, 4% polyethylene glycol 8000, 100 mM PIPES, and 1 mM EGTA. For detection of cell surface antigens, the extraction step was omitted. After three rinses in PBS, samples were incubated for 1 hour with onchocerciasis or control sera diluted in PBS (1:20), washed in PBS, and then incubated with FITC-conjugated secondary anti-human antibodies (1:30 dilution). Frozen sections were extracted for 10 to 15 minutes in the extraction solution, and then processed as described above. For double-labeling experiments, polyclonal goat anti-calreticulin antibodies were used in addition to onchocerciasis sera, and TRITC-conjugated anti-goat IgGs were used as the secondary antibodies. The samples were washed with PBS, mounted, and examined using a Bio-Rad MRC-600 confocal laser scanning microscope equipped with an argon/krypton laser.

RESULTS

Using immunofluorescence confocal microscopy, we have examined cross-reactivities of both RPE and NR cells with nine sera from clinically well characterized individuals with onchocerciasis. The immunostaining data, including those obtained from control experiments, are summarized in Table 1. All the sera we tested (with one exception) recognize epitopes on the surface of both RPE and NR cells. Over 60% of the sera also recognize the nuclear matrix and nucleoli of both types of cells. No staining of cell surfaces or nucleoli was observed in both types of cells incubated with the control sera. Some sera also cross-react with fibers, vesicles, or patches in the cytoplasm of both cell types. Here, we show the detailed data obtained by immunofluorescence and Western blot analysis with three onchocerciasis sera, and compare them to those obtained using anti-calreticulin antibodies or the control sera.

Immunofluorescence Confocal Microscopy

Serum 4568 from a patient with onchodermatitis (Fig. 1) recognizes the surface components of both the unextracted NR (Fig. 1A) and RPE (Fig. 1B) cells. In the
TABLE 1. Immunofluorescence Staining of Retinal Cell Components and Immunoreactivity of Recombinant Calreticulin with Onchocerciasis Sera

<table>
<thead>
<tr>
<th>Symptom*</th>
<th>RPE</th>
<th>NR</th>
</tr>
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<tbody>
<tr>
<td>Serum</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>4568</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>1241</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3717</td>
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</tr>
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<td>7330</td>
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<td>No</td>
</tr>
<tr>
<td>0408</td>
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<tr>
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<tr>
<td>2924</td>
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<td>Yes</td>
</tr>
<tr>
<td>2631</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>% positiveness</td>
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<td>88.9</td>
</tr>
<tr>
<td>Controls‡</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>2 NS2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 148</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4 B0541</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5 N0551</td>
<td>-</td>
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</tbody>
</table>

* Patients with (A) skin or (B) ocular onchoceriasis symptoms (for details, see Gallin et al.22).
† Sf, surface; Cp, cytoplasmic patch; CF, cytoplasmic fiber; NM, nuclear matrix; Nu, nucleolus.
‡ rCRT, recombinant calreticulin Western blotted with the onchocerciasis sera (1:1 dilution).
§ —, +, and ++ indicate negative, positive, and strongly positive immunostaining, respectively.
‡ Number of onchocerciasis sera that positively stained the listed cell structures, expressed as a percentage of the total number of sera tested.
† Control sera were selected as described in the Materials and Methods section.
ND, not determined.

permeabilized NR cells (Fig. 1C), fluorescence is localized to the cell surface and perinuclear regions, as well as to the nucleoli. Figure 1D and E show RPE cells that were double labeled with serum 4568 and anti-calreticulin. In addition to the surface staining, strong labeling with serum 4568 is observed in the nucleoli of RPE cells (Fig. 1D). Calreticulin is detected in the endoplasmic reticulum in the same cells (Fig. 1E). In a section of the retina (Fig. 1F,G), both the RPE and the NR are labeled by serum 4568. The regions corresponding to the outer segments of the photoreceptors and to the inner layer of NR have a much stronger staining than the rest of the retina.

Immunofluorescence staining with serum 1241 from a patient with limbal haze (Fig. 2) yields strong fluorescence of the cell surface in the unextracted RPE cells (Fig. 2A), but not in unextracted NR cells (not shown). In permeabilized RPE cells (Fig. 2B), the nucleoli and nuclear matrix are strongly labeled, and fluorescence of the cell surface is discernible. In permeabilized NR cells (Fig. 2C), the nucleoli react positively with this serum, and some fibrillar structures in the cytoplasm are also stained. The distribution of calreticulin in the same cells is shown in Figure 2D. The staining patterns obtained with serum 1241 and with anti-calreticulin were found to be entirely different. Immunostaining of both the RPE and the NR in a frozen section of the retina with serum 1241 is shown in Figure 2E. Fluorescent patches are localized to RPE cells, whereas labeling of the cytoplasm and nucleoli is evident in NR cells.

Fluorescence patterns obtained with serum 3717 from a patient with sclerosing keratitis (Fig. 3) are quite similar to those obtained with serum 4568 (Fig. 1) in unextracted NR (Fig. 3A) and RPE (Fig. 3B) cells, as well as in the permeabilized NR (Fig. 3C) and RPE (Fig. 3D) cells. Although some patches in the cytoplasm are stained, the strongest fluorescence is localized to the nucleoli and, to a lesser extent, the nuclear matrix adjacent to the nuclear envelope. The staining pattern of the nucleoli with this serum, however, is different from that observed using other sera, in that with serum 3717 the fluorescence is predominantly at the periphery of the nucleolus (Fig. 3C). Staining of the nucleolar periphery with serum 3717 is evident in the X-Z (vertical section) image (insert in Fig. 3C) reconstructed from serial X-Y confocal sections of the same nucleolus (arrow). The staining patterns in RPE and NR cells after double labeling with this serum and with anti-calreticulin antibodies are essentially identical to those in RPE and NR cells shown in Figures 1 and 2, respectively. In frozen sections of the retina (Fig. 3E-G), both the RPE and the NR are strongly labeled with serum 3717 (Fig. 3E). At higher magnification, labeling of the nucleoli is clearly discernible in the inner layer of the NR (Fig. 3C).

Fluorescence staining of both the cultured cells and tissue sections with the control sera selected as...
described in Materials and Methods did not yield any of the cell surface or nucleolus labeling patterns shown above. The sera (NS#1 and NS#2), from two healthy individuals who have never been in any of the onchocerciasis hyperendemic areas, show negative staining of both the cultured cells and tissue sections, except that one of the sera (NS#2) stained very weakly the nuclear matrix of cultured NR cells (not shown). The other three control sera, however, revealed different fluorescence staining patterns in permeabilized retinal tissues and cells (Fig. 4). Serum 148, from a person living in the onchocerciasis area but without the *O. volvulus* infection, stained some perinuclear structures in RPE cells (Fig. 4A), cytoplasmic fibers, and the nuclear matrix in NR cells (Fig. 4B). This serum yielded a strong fluorescent NR and a relatively weaker fluorescent RPE (Fig. 4C,D). With the exception of only a few cells in which some cytoplasmic patches were very weakly stained, no staining was seen in RPE cells with serum B0541, obtained from a patient who has never been in any of the onchocerciasis hyperendemic countries, but who has keratitis (Fig. 4E). This serum also cross-reacted with some cytoplasmic structures at the nuclear periphery in NR cells (Fig. 4F). In the retinal frozen sections (Fig. 4G,H), serum B0541 did not stain the RPE, or stained it very weakly, but strongly labeled the segments of the photoreceptor cells, and weakly labeled the rest of the NR cells. Serum N0551, from another patient with keratitis who has never had *O. volvulus* infection, yielded nothing but very weak, if any, fluorescence of the nuclear matrix in RPE cells (Fig. 4I), and relatively stronger labeling of the nuclear matrix in NR cells (Fig. 4J). This serum appeared to contain antibodies...
FIGURE 2. Confocal images of retinal cells immunostained with serum 1241 from an onchocerciasis patient with ocular disease. (A) Unextracted and (B) permeabilized RPE cells; (C,D) the same NR cells double labeled (C) with serum 1241 and (D) with anti-calreticulin; (E) immunofluorescence and (F) phase contrast images of the same section of the retina showing labeling of the RPE (RP) and NR (NR) layers. Scale bars: (A-D) 30 μm; (E,F) 100 μm.

against retinal photoreceptor cells as seen in the retinal sections (Fig. 4K,L). Unlike serum B0541, however, serum N0551 yielded a strong fluorescence staining of the outer ends of photoreceptors adjacent to the RPE layer. Serum N0551 also weakly stained the other NR cells, but did not label the RPE layer (Fig. 4K).

Western Blot Analysis
Immunoblot data, obtained using the three different onchocerciasis sera, anti-calreticulin antibodies, and five nononchocerciasis control sera, are shown in Figure 5. As expected, anti-calreticulin antibodies clearly detected the 60-kD recombinant calreticulin (Fig. 5, lane A). The same antibodies also detected a 60-kD peptide (lane 1) in homogenates of both RPE and NR cells (Fig. 5B,C, respectively). The peptide profiles revealed by the three onchocerciasis sera (4568, lanes B2, C2; 5717, lanes B3, C3; and 1241, lanes B4, C4) that were obtained from people with various onchocerciasis symptoms are markedly different. There are some similarities, however, between the patterns of the major bands recognized by serum 4568 in the RPE (lane B2) and the NR (lane C2) cell extracts. Both the control sera (NS#1, lanes B5, C5; NS#2, lanes B6, C6), from healthy individuals living outside the onchocerciasis countries, show no cross-reactivity with RPE proteins (lanes B5, B6). One of the normal sera (NS#2), however, revealed two minor polypeptide bands on the blot of NR homogenates (lane C6). Sera (B0541, lane 7; N0551, lane 8) from patients with keratitis but without Onchocerca infection also weakly de-
Retinal Epitopes for Onchocerciasis Antibodies

FIGURE 3. Confocal images of retinal cells immunostained with serum 3717 from an onchocerciasis patient with ocular disease. (A) Unextracted NR cells; (B) unextracted RPE cells; (C) permeabilized NR cells; (C, insert) vertical section reconstructed from a stack of serial confocal sections of the nucleolus, indicated by the arrowhead in (C). The step-like appearance is an artifact of image processing. (D) Permeabilized RPE cells; (E) frozen section of the retina demonstrating the strong immunofluorescent labeling of both the RPE (RP) and NR (NR) layers; (F) phase contrast image of the same section; (G) a higher magnification of the frozen section of the inner retina showing the nucleoli labeled with serum 3717. Scale bars: (A-D) 30 μm; (insert in C) 1 μm; (E-G) 100 μm.

dected one or two minor peptides in both the RPE and the NR homogenates. The peptide profiles detected by serum 148, from a person residing in a hyperendemic area but remaining infection free, are shown in lanes B9 and C9 in Figure 5. This serum appeared to have antibodies strongly cross-reacted with NR cells (lane C9). The major peptides detected by Western blotting with the *Onchocerca* sera, but not recognized by any of the control sera, are summarized in Table 2. In agreement with our microscopy data, none of the onchocerciasis sera recognized the recombinant rabbit calreticulin (Table 1). We also tested several less well characterized onchocerciasis sera (a generous gift from Dr. T. Keystone, Toronto General Hospital, Toronto, Ontario, Canada) on the recombinant protein, and did not detect any cross-reactivity (not shown).

DISCUSSION

In the current study, we have shown the immunofluorescence labeling patterns in retinal cells produced by nine onchocerciasis sera and five nononchocerciasis control sera (Table 1), and the peptide profiles detected by three of the *Onchocerca* sera but not by any of the control sera (Table 2) on Western blots of the cell homogenates. These data indicate the presence of anti-retinal autoantibodies in onchocerciasis sera that react strongly with antigens associated with surface
FIGURE 4. Confocal images of retinal cells immunostained with control sera 148 (A–D), B0541 (E–H), and N0551 (I–L). All cells shown are permeabilized. (A,E,I) RPE cells; (B,F,J) NR cells; (C,G,K) immunofluorescence and (D,H,L) phase contrast images of the same sections, respectively, of the retinal tissues including NR (NR) and the underlying RPE (RP). Scale bars: (A,B,E,F,I,J) 50 µm; (C,D,G,H,K,L) 120 µm.
components (89% of the sera) and nucleoli (66% of the sera) of both RPE and NR cells. Two of the nucleolus-negative *Onchocerca* sera from patients with ocular disease recognize the epitopes located at the periphery of the nucleolus, yielding a ring-shaped immunofluorescence staining pattern. Such cross-reactivity of the onchocerciasis sera with retinal cell surfaces and nucleoli is not observed in retinal cells labeled with any of the control sera, including serum 148, which had a peptide profile similar, in the lower-molecular-weight range, to that detected by some of onchocerciasis sera such as serum 3717. Except for serum NS*1*, however, all other control sera appeared to have some cross-reactivity with the components associated with either nuclear matrix, cytoplasmic fibers, or some other cytoplasmic structures in the cultured NR cells. The sera from patients with keratitis also have antibodies cross-reacted strongly with retinal photoreceptors. Although the in vivo immunologic and pathogenetic mechanisms behind the interactions between such cross-reactive antibodies and the intracellular, intranuclear, or even intranucleolar components still are far from clear, our finding that epitopes at the surface of both RPE and NR cells are recognized by onchocerciasis sera provides strong evidence that these antiretinal antibodies may play a significant role in retinal damage in onchocerciasis.310

![Western blots of retinal cell homogenates with onchocerciasis sera and anti-calreticulin antibodies.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933180/)

**TABLE 2. Peptides Strongly Detected by Onchocerciasis Sera but not Recognized by any of the Control Sera on Western Blots of RPE and NR Homogenates**

<table>
<thead>
<tr>
<th>Serum</th>
<th>RPE</th>
<th>NR</th>
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<tr>
<td>4568</td>
<td>130, 58</td>
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<tr>
<td>3717</td>
<td>110, 90, 80, 70, 47, 35</td>
<td>205, 190, 70, 50</td>
</tr>
<tr>
<td>1241</td>
<td>120, 110, 80, 65, 35</td>
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Data indicate the approximate molecular weight (kDa) of the peptides.
In cultured RPE and NR cells, calreticulin and the cellular components recognized by the onchocerciasis sera do not colocalize, suggesting the absence of antibodies to calreticulin in these sera. This is supported further by our Western blot data, which show that none of the onchocerciasis sera we tested recognized recombinant rabbit calreticulin that is highly homologous to human calreticulin. Our Western blot data are in contrast to those reported by others, and no good interpretation of the difference is available at this time. It has been suggested that Onchocerca and Ro/SS-A antigens share cross-reactive epitopes, which also may be shared by calreticulin. The cross-reactivity of onchocerciasis sera with the Ro/SS-A autantigen suggests that anti-Ro/SS-A autoantibodies may be present not only in the serum of patients with systemic lupus erythematosus, but in that of patients with onchocerciasis as well. Others, however, have reported that this Ro/SS-A antigen does not immunoprecipitate with either onchocerciasis sera or anti-calreticulin antibodies.

Rokeach et al and Pruijn et al have challenged the conclusion of McCauliffe et al that calreticulin is an Ro/SS-A autantigen by suggesting that the high degree of homology between Ro/SS-A and calreticulin reported by these authors might be a result of accidental sequencing of calreticulin that was copurified with the Ro/SS-A antigen, rather than a feature of the Ro/SS-A protein itself. Although the Onchocerca RAL-1 antigen has over 60% sequence homology with calreticulin, no evidence has been shown for the presence of antibodies in the onchocerciasis sera to native calreticulin. It also is possible that calreticulin may contain certain peptide regions (or epitopes) that react nonspecifically with the sera of some patients with systemic lupus erythematosus, Sjögren's syndrome, or onchocerciasis. Our immunofluorescence data showing that calreticulin, with its conformation preserved by aldehyde cross-linking, is not recognized by the onchocerciasis sera indicate that nondenatured calreticulin also becomes from the observation of Rokeach et al that onchocerciasis sera did not immunoprecipitate in vitro-translated calreticulin. Differences between native proteins and their denatured counterparts often cause difficulties in the detection of relevant epitopes in the diagnosis of autoimmune diseases.

Taken together, the immunofluorescence and Western blot analysis data from this study strongly indicate that calreticulin is not a major Onchocerca autantigen. Confusing results concerning the cross-reactivities of onchocerciasis sera with the Ro/SS-A autantigen, RAL-1 antigen, and purified or recombinant calreticulin suggest the possible existence of non-specific binding site(s) on these molecules (or their denatured forms) recognized by several different antibodies. Furthermore, it is far from clear which particular immunogen induces the antibodies responsible for the autoimmune responses in onchocerciasis. Although there has been an emphasis on the characterization of the Onchocerca RAL-1 antigen, it has been reported that there is no correlation between the pattern of humoral recognition of this antigen and the epidemiologic status of onchocerciasis. It also is reasonable to consider the nonspecificity of certain autoantibodies, present in both onchocerciasis sera and non-Onchocerca sera, such as those cross-reacting with nuclear matrix and cytoplasmic fibers. The potential importance of the interactions between these onchocerciasis anti-retinal autoantibodies and the surface molecules of retinal cells cannot be ignored, however. Further biochemical and molecular characterization of the components associated with the retinal cell surface and recognized by onchocerciasis sera, such as those revealed by the current study, may lead to a better understanding of the pathogenesis of ocular disease in onchocerciasis.

Key Words
calreticulin, onchocerciasis serum, retinal pigment cell, neural retinal cell

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