Fuchs Heterochromic Cyclitis: Rubella Virus Antibodies and Genome in Aqueous Humor

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• PURPOSE: To characterize the polyspecific intraocular antibody synthesis in aqueous humor of patients with chronic inflammatory diseases of the eye and to detect the causative antigen in Fuchs heterochromic cyclitis (FHC).

• DESIGN: Retrospective case-control study.

• METHODS: Intraocular antibody synthesis is detected in aqueous humor with the Antibody Index [AI] (improved Goldmann-Witmer Index) and quantified as specific antibody fraction, F_s (intraocular specific antibody concentration in percent of intraocular total immunoglobulin G in aqueous humor). Virus detection is by nested polymerase chain reaction.

• RESULTS: Fifty-two eyes of 52 patients with clinically defined FHC (aged 16–73 years) had an intraocular synthesis of rubella antibodies (AI ≥1.5). The rubella genome was detected in 5 (18%) of 28 aqueous humor samples investigated, or in 5 (56%) of 9 patients aged <40 years. Oligoclonal IgG was synthesized in 34 (87%) of 39 eyes. Unaffected fellow eyes (n = 3) or cerebrospinal fluid (n = 2) were normal. In FHC the median rubella AI = 20.6 (total range 1.5–309) was seven times higher than in multiple sclerosis (MS) patients (n = 15) with uveitis intermedia or periphlebitis retinae. In MS the intraocular rubella antibody synthesis (frequency 73%) is part of a polyspecific immune response (increased measles AI in 80%, varicella zoster virus AI in 47%, herpes simplex virus AI in 23%). The median rubella-F_s = 2.6% in FHC (range = 0.14%–45.9%) was approximately 40 times higher than in MS, consistent with a virus-driven antibody response in FHC. Noninflammatory controls (50 senile cataracts) had neither an intraocular rubella antibody synthesis (normal AI ≤1.4) nor rubella antigen in aqueous humor. The rubella AI was normal in all patients with an intraocular toxoplasmosis (n = 24), anterior uveitis (n = 27), herpes simplex virus iritis (n = 25), and varicella zoster virus iritis (n = 14).

• CONCLUSIONS: Fuchs heterochromic cyclitis is a rubella virus-driven disease with persistence of the virus preferentially detected in the younger patients. The proposed laboratory supported diagnosis of FHC is based on the increased rubella Antibody Index. The virus etiology gives a rationale for omitting the ineffective corticosteroid therapy of FHC. (Am J Ophthalmol 2004;138:46–54. © 2004 by Elsevier Inc. All rights reserved.)

FUCHS HETEROCHROMIC CYCLITIS (FHC) IS AN INtraocular disease that usually strikes only one eye, causing a different iris color of the two eyes.[1] This disease, which counts for 2% to 11% of all cases of anterior uveitis,[2] shares the symptoms of a chronic, low-grade anterior uveitis with complications such as cataract, glaucoma, and vitreous opacities, but it does not show the typical symptoms of a cyclitis: pain, redness of the external eye, and miosis.[3,4]

Heterochromia may be absent[3] or may be overlooked in a dark brown iris,[5] and the various clinical signs[3,4] are not always present at the same time. This makes FHC difficult to diagnose. A specific diagnostic laboratory test for FHC has not been available so far. Incorrect diagnosis may lead to unnecessary corticosteroid therapy and to misleading expectations for the course of the disease.

During the last 100 years many different explanations for the pathomechanism of FHC have been proposed,[6] but the etiology remained obscure. Increased levels of gamma globulins (including oligoclonal immunoglobulin G) in aqueous humor[7–10] supported the hypothesis of an intraocular immune reaction. A recent publication[11] concluded from the CD8-positive T cells in aqueous humor that FHC is an antigen-driven process.

Intraocularly synthesized antibodies, detected in aqueous humor, can have two different sources: a causative persisting antigen or a polyspecific concomitant immune response. The classical view of the immune response is the clonal selection of a B-lymphocyte clone producing specific antibodies against the invading microorganism. As several clones are always found to fit the specific antigen,

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Accepted for publication Feb 23, 2003.

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0002-9394/04/$30.00
doi:10.1016/j.ajo.2004.02.055

TABLE 1. Data of Patient Groups

	Number	Sex Female/Male	Age (years) Median (range)	Inflammation*
Fuchs heterochromic cyclitis	52	25/27	43 (16–73)	Chronic
Cataract	50	31/19	68 (22–88)	No
Multiple sclerosis	15	10/5	40 (18–60)	Chronic
Anterior uveitis	27	16/11	54 (21–91)	Acute
Varicella zoster virus iritis	14	8/6	50 (5–85)	Acute
Herpes simplex virus iritis	25	11/14	58 (24–87)	Acute
Toxoplamosis retinitis	24	13/11	32 (16–72)	Acute

*Status of intraocular inflammation at the time of aqueous humor sampling.

we speak about an "oligoclonal" immune reaction. In addition to the oligoclonal antibodies against the specific causative antigen each immune reaction produces a wide spectrum of different antibody species not connected with the causative antigen. This "polyspecific immune response" does not depend on the presence of a corresponding persisting antigen, and is of lower intensities than for the causative antigen.[12,13]

The detection of oligoclonal immunoglobulin G (IgG) in cerebrospinal fluid (CSF) is a basic part of a laboratory-supported diagnosis of multiple sclerosis (MS).[14] An unexplained high frequency of combined intrathecal measles, rubella, and varicella zoster virus (VZV) antibodies in cerebrospinal fluid of patients with multiple sclerosis[13] or autoimmune diseases with involvement of the CNS[15] allows the diagnosis of a chronic inflammatory process (autoimmune type) at the time of the first clinical manifestation.[13,16] This is also important for diagnosis of cases with a monosymptomatic start of the disease like an optical neuritis or an uveitis intermedia and periphlebitis retinae. We report these intraocular immune response patterns of the virus-specific antibodies in aqueous humor of MS patients.

The detection of intraocular antibody synthesis in aqueous humor has a long tradition (in Liekfeld and associates[17] and Remky[18]). The linear Goldmann-Witmer Index[19] (GW-I) frequently used in ophthalmology (GW-I = Q_{spec}/Q_{IgG}) is improved by the corrected Antibody Index (AI),[20] established in cerebrospinal fluid CSF analysis[13,16,20–22] for cases with a strong intrathecal IgG synthesis, in which the GW-I leads to false negative interpretations.

The AI presents a relative value for the quantity of intraocularly synthesized specific antibodies. With the invention of the measurement of absolute antibody concentrations,[12] the evaluation of quantitative intraocular antibody synthesis becomes possible. With corresponding calculation of the specific fraction, F_s, in aqueous humor, a virus-driven antibody synthesis can now be discriminated from a polyspecific, network-related immune response.

These methodologic improvements are based on the evaluation of immunoglobulin quotients Q_{IgG}, Q_{IgA}, and Q_{IgM} with a nonlinear, hyperbolic discrimination function, Q_{Lim},[16,22] which allows the sensitive discrimination between blood- and eye-derived immunoglobulin fractions (i.e., intraocular synthesis of IgG, IgA, and IgM) in aqueous humor. This replaces the earlier linear approaches, like IgG index,[9,23,24] which lead to false interpretations in cases of a blood/aqueous humor barrier dysfunction as demonstrated in detail for CSF analysis.[16]

During the last 13 years we investigated the spectrum of measles, rubella, varicella zoster, herpes simplex virus, and toxoplasma antibodies in aqueous humor and serum for diagnosis of acute and chronic inflammatory processes of the eye. This led us to the discovery of the predominant rubella antibody synthesis in the eye of FHC patients and the subsequent discovery of local persistence of the virus in a fraction of these cases.

METHODS

IN THE DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY Göttingen, the aqueous humor of patients with an iridocyclitis or uveitis of unknown origin is investigated routinely as a standard protocol with informed consent of the patient. Under local anasthetic eye drops the anterior chamber is entered with a 30-gauge needle and approximately 0.1 ml of aqueous humor is aspirated. In parallel, a blood sample is collected from each patient. The demographic data of the patient groups are summarized in Table 1.

Fuchs heterochromic cyclitis is clinically diagnosed by the unilaterality of the anterior uveitis with absence of acute symptoms (severe redness, pain, miosis) but typical white keratic precipitates, few cells and flare in the aqueous humor, missing synechia, and a variable degree of depigmentation of the iris.[3,4] Fifty-two eyes of Caucasian patients with FHC have been studied in the years 1989 to

2003. From 31 eyes the aqueous humor was obtained during surgery of a secondary cataract or secondary glaucoma (n = 11).

As a noninflammatory control group, we report the data of 50 patients (Table 1) with senile cataract without any signs of an intraocular inflammation.

We collected aqueous humor from 15 neurologically diagnosed multiple sclerosis patients (Table 1) with an uveitis intermedia or a periphlebitis retinae. Typical observations were low-grade anterior segment inflammation with vitreous floaters, cellular debris, and snowballs in the periphery of the fundus. Some patients demonstrated isolated sheathing of the retinal vessels. This group is representative for a chronic inflammation with intraocular polyspecific immune response.

Twenty-seven patients had an anterior uveitis of unknown etiology (Table 1).

The diagnosis of 14 patients (Table 1) with a VZV iritis is laboratory supported, based on the increased VZV AI. In case of a concomitantly increased HSV AI the higher absolute antibody titer is used to discriminate the causative antigen. Frequent observations are brownish pigmented keratic precipitates, cells, fibrin, and sectoral iris atrophy.

The laboratory-supported diagnosis of 25 patients with a HSV iritis (Table 1) is based on the increased HSV AI. In case of a concomitantly increased VZV AI the higher absolute antibody titer is used to discriminate the causative antigen. Frequent observations are cells, fibrin, medium-sized keratic precipitates, and diffuse iris atrophy.

The increased toxoplasma AI is the base of the laboratory-supported diagnosis of toxoplasmosis retinitis[20] in 24 patients (Table 1), with a focus of retinitis surrounded by retinal edema, and pigmented atrophic retinochoroidic scars adjacent to the lesion or elsewhere in the fundus.

For protein analysis, albumin and IgG in aqueous humor and serum were analyzed with immunochemical nephelometry (Nephelometer; Dade-Behring, Marburg, Germany). To match the requested volume for routine analysis some aqueous humor samples need a manual predilution with 0.9% NaCl (1:2–1:10, depending on total protein concentration).

Data are evaluated numerically and graphically in the CSF/serum quotient diagrams,[16] which fit also the aqueous humor/serum quotients, shown in Figure 1 for noninflammatory controls. An intraocular immune reaction is detected by reference to an empirically and theoretically founded discrimination line (hyperbolic function), Q_{Lim}, between the reference range of blood-derived immunoglobulins and eye-derived immunoglobulins in aqueous humor (Figure 1). The intraocular fraction, IgG_{IF} in %, or the locally synthesized contribution to aqueous humor concentration, IgG_{Loc} in mg/l, is calculated[16,22] with $Q_{Lim} = [0.93(Q_{Alb}^2 + 6)^{0.5} - 1.7] \cdot 10^{-3}$. The albumin quotient, Q_{Alb} = Alb(aqueous humor)/Alb(ser) is calculated with the empirical albumin (Alb) concentrations in aqueous humor and serum (the Q_{Alb} value is multiplied with 10^3 for insertion in the above version of Q_{Lim}). $IgG_{Loc} = (Q_{IgG} - Q_{Lim}) \cdot IgG(ser)$ in mg/l is calculated from Q_{IgG} = IgG(aqueous humor)/IgG(ser), with the empirical IgG concentrations in aqueous humor and serum. The intraocular IgG fraction is calculated according to IgG_{IF} =

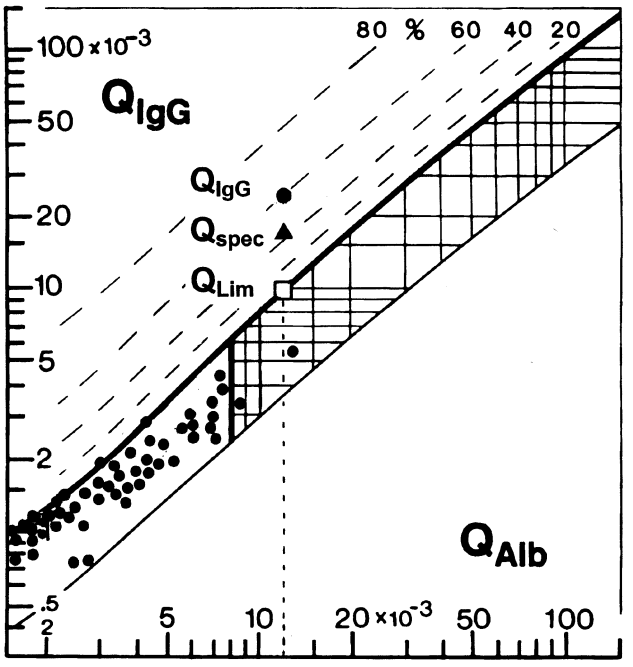

FIGURE 1. Aqueous humor/serum quotient diagram for immunoglobulin G (IgG) with a hyperbolic discrimination line according to Reiber.[22] The IgG quotient diagram was developed for cerebrospinal fluid analysis with an empirically and theoretically founded, hyperbolic reference range for blood-derived IgG. The dots in the reference range represent aqueous humor/serum quotients for IgG (Q_{IgG}) as a function of the aqueous humor/serum albumin quotient, Q_{Alb} (blood/aqueous humor barrier function). This control group of patients with senile cataract had no signs of an inflammation in the eye. They fit as well into the cerebrospinal fluid/serum quotient diagram, in particular regarding the hyperbolic discrimination line (Q_{Lim}) between the blood-derived (below) and the eye-derived IgG fraction (above, with dashed lines, which indicate the % intraocular fraction) As an example from a patient with $Q_{IgG} = 24 \cdot 10^{-3}$ and $Q_{Alb} = 12 \cdot 10^{-3}$, the corresponding upper limit of the reference range ($Q_{Lim} = 9.7$) and the intraocular fraction ($IgG_{IF} = 60\%$) are calculated (see Methods). With the rubella-specific aqueous humor/serum quotient (rubella-$Q_{spec} = 16.5 \cdot 10^{-3}$) for the same patient, we get the (corrected) Antibody Index (AI) with $AI = Q_{spec}/Q_{Lim} = 16.5/9.7 = 1.7$, a pathologic value. The Goldman-Witmer Index (GW-I = 16.5/24 = 0.7) would give a false negative interpretation. As shown earlier,[23] Q_{Alb} (and the total protein content in aqueous humor) depends on the age of the patient. The solid line at $Q_{Alb} = 8 \cdot 10^{-3}$, developed for cerebrospinal fluid data of patients with age 60 years and younger, needs further evaluation for the age-related reference values of the blood/aqueous humor barrier function (Reiber and Quentin, in preparation).

TABLE 2. Reference Range for Antibody Index (AI) Values of Measles (M), Rubella (R), Varicella zoster (VZV), Herpes Simplex (HSV), and Toxoplasma (Toxo) Antibodies in AH from Control Patients with Senile Cataract (Noninflammatory Disease)

	M AI	R AI	VZV AI	HSV AI	Toxo AI
Mean	0.91	0.94	0.88	0.91	0.94
SD	0.2	0.19	0.16	0.17	0.15
Range	0.6–1.4	0.5–1.3	0.6–1.2	0.5–1.2	0.7–1.2
Number*	42	44	45	40	20

*Number of AI values as far as detectable from n = 50 cataract patients.

(IgG_{Loc}/JgG aqueous humor) · 100 [%] or $IgG_{IF} = (1 - Q_{Lim}/Q_{IgG}) \cdot 100$ [%]. A calculation example is shown in Figure 1. For a patient with $Q_{Alb} = 12 \cdot 10^{-3}$ and $Q_{IgG} = 24 \cdot 10^{-3}$, we get $Q_{Lim} = 9.7 \cdot 10^{-3}$ and $IgG_{IF} = 60\%$.

• **ANTIBODY INDEX:** Antibody analysis (measles, rubella, herpes simplex, varicella zoster, toxoplasmosis) is performed on commercial microtiter plates (Dade-Behring, Marburg, Germany) as described for CSF.[21] The samples of aqueous humor (1:15 dilution) and serum (1:3,000 dilution) are analyzed paired in the same analytical run. The measured optical density was evaluated as arbitrary concentration units (AU), by reference to a standard curve.[21] After multiplication with the dilution factor, we calculate with the aqueous humor and serum antibody concentrations the specific antibody quotient, Q_{spec} = AB(aqueous humor)/AB(ser) = AU(aqueous humor)/AU(ser) (for example, $Q_{spec} = 16.5 \cdot 10^{-3}$ in the example in Figure 1). The AI is calculated either with $AI = Q_{spec}/Q_{IgG}$ (like the GW-I) if $Q_{IgG} < Q_{Lim}$, or $AI = Q_{spec}/Q_{Lim}$ if $Q_{IgG} > Q_{Lim}$ (for example, in Figure 1). The Q_{Lim} is calculated from the function above.[22] With the data of the example in Figure 1, we get $AI = Q_{spec}/Q_{Lim} = 16.5 \cdot 10^{-3}/9.7 \cdot 10^{-3} = 1.7$. The GW-I, GW-I $= Q_{spec}/Q_{IgG} = 16.5 \cdot 10^{-3}/24 \cdot 10^{-3} = 0.7$ would give a false-negative interpretation.

Reference range of normal AI and clinically defined cut-off for pathologically increased AI values: according to the results in Table 2 and Table 3, we get for all antibody species investigated AI ≤1.4 as normal reference range. In the group of patients with an antibody synthesis against a causative antigen (Table 3), we detected the pathologically increased AI ≥1.5 (see Results).

Fraction of specific intraocular antibodies in aqueous humor: the specific fraction, F_s, is the ratio of the intraocularly synthesized concentration of specific antibodies (AB_{Loc}), and the intraocularly synthesized concentration of total IgG (IgG_{Loc}) in %. For comparison of means in different groups, the calculation of F_s refers to Q_{mean}, the mean function[22] of the reference range instead of the upper limit Q_{Lim} used for AI (above): $F_s = AB_{Loc}(mean)/IgG_{Loc}(mean) \cdot 100$ in %. With Q_{mean} (IgG) = $(0.65 (Q_{Alb}^2 + 8)^{0.5} - 1.4) \cdot 10^{-3}$, we calculate for total IgG: IgG_{Loc} (mean) = $(Q_{IgG} - Q_{mean}) \cdot$ IgG(ser) in mg/l, and for the single antibody species AB_{Loc} (mean) = $(Q_{spec} - Q_{mean}) \cdot$ AB(ser) in mg/l.

For AB_{Loc} the absolute concentrations of $AB_{aqueous\ humor}$ and AB_{ser} are needed. These values are obtained by a modification of the method of Conrad and associates[12] from the AU concentrations and multiplication with the empirical conversion factor (for example, 0.149 μg/l = 1 AU for rubella concentration in our selfmade standard).[13]

The detection of oligoclonal bands in aqueous humor is performed by immune detection after isoelectric focusing and interpretation, as described in the international consensus for CSF.[14]

Rubella reverse transcriptase polymerase chain reaction (PCR): the rubella genom in aqueous humor samples was detected by two different laboratories: Dr Renate Seelig, Karlsruhe, Germany (results: 4/17 FHC samples positive and all 22/22 cataract patients were rubella negative) and Dr Benedikt Weissbrich, Institut für Virologie und Immunbiologie, Universität Würzburg, Würzburg, Germany (results: 1/11 FHC samples was rubella positive).

The method used for detection of rubella RNA was as follows (R. Seelig): a 105 bp portion of the glycoprotein E1 gene was amplified by nested PCR. Rubella RNA was isolated using proteinase K digestion followed by phenol chloroform extraction. Isolated RNA was reverse transcribed in a buffer containing MMLV-RT, primer 1 (ATGGCACACACACCACTGCT) and primer 2 (CAAGCGAG(CT)AAGCC(AG)GCGAG) at 37°C for 50 minutes. Nested PCR was conducted using primer 1 and primer 2 (first round), resp. primer 3 (ACCACTGCTGTGTCGGAGACCCGG) and primer 4 (TAAGCCAGAGAGT(AG)GGAGGGCGCA) in second round, with the following temperature profile: denaturating at 95°C for 5 minutes, 30 cycles of 30 seconds 94°C, 45 seconds 55°C, and 72°C for 60 seconds, 5 minutes at 72°C.

Sensitivity of the test was 10 geq/reaction using synthetic template RNA. Positive PCR results were confirmed by cloning and sequencing (ABI Prism 310 Genetic Analyzer (Perkin-Elmer, Germany) of the amplification product using standard techniques. The sequences were compared with published sequences using BLAST search (NCBI database, www.rzpd.de/db/html/blast/blast_databases.html).

RESULTS

• **REFERENCE RANGES AND CUT-OFF VALUE FOR INTRAOCULAR ANTIBODY SYNTHESIS:** The normal AI values of measles, rubella, varicella zoster, herpes simplex, and toxoplasma antibodies in aqueous humor are shown in Table 2. All AI values of this noninflammatory control (senile cataract) in Table 2 were AI ≤1.4, in particular

TABLE 3. Frequencies of Intraocular Immune Reactions in the Aqueous Humor of Acute and Chronic Inflammations

Disease	Intraocular IgG		Increased Antibody Index (AI ≥1.5)‡				
	IF*	Oligo†	M AI	R AI	VZV AI	HSV AI	Toxo AI
Fuchs heterochromic cyclitis (n = 52)	50% (25/51)	87% (34/39)	6% (3/48)	100% (52/52)	6% (3/44)	0% (0/36)	12% (2/16)§
Multiple sclerosis (n = 15)	87% (13/15)	100% (14/14)	80% (12/15)	73% (11/15)	47% (7/15)	23% (3/13)	—‖
Anterior uveitis (n = 27)	4% (1/27)	20% (2/10)	0% (0/27)	0% (0/27)	0% (0/27)	0% (0/27)	0% (0/21)
VZV iritis (n = 14)	7% (1/14)	62% (5/8)	0% (0/13)	0% (0/13)	100% (14/14)	46% (6/13)	0% (0/11)
HSV iritis (n = 25)	12% (3/25)	58% (7/12)	0% (0/23)	0% (0/23)	52% (13/25)	100% (25/25)	0% (0/17)
Toxoplasmosis retinitis (n = 24)	42% (10/24)	83% (10/12)	0% (0/20)	0% (0/20)	0% (0/22)	0% (0/21)	100% (24/24)
Senile cataract (n = 50)	0% (0/50)	0% (0/50)	0% (0/50)	0% (0/50)	0% (0/50)	0% (0/50)	0% (0/50)

*Intraocular IgG fraction, $IgG_{IF} > 0$.

†Oligoclonal IgG in aqueous humor.

‡Increased antibody index values (AI ≥1.5) for measles (M), rubella (R), varizella zoster (VZV), herpes simplex (HSV), and toxoplasma gondii (toxo).

§Increased AI in two cases with AI = 1.8 and AI = 5.1; 6 cases with AI = 0.7–1.2; 8 cases not detectable. The patient with AI = 5.1 showed scars from former toxoplasmosis infection of the retina of both eyes.

‖Not analyzed in AH but increased toxo AI detected in CSF in 10% of the multiple sclerosis patients (8/80).

rubella AI ≤1.3 (Figure 2). The average of the empirical means and SD in Table 2 was 0.91 ± 0.17. All AI values for the noncausative antigens from inflammatory controls, like the anterior uveitis of unknown cause, VZV iritis, HSV iritis, or toxoplasmosis retinitis (Table 3), were AI ≤1.4, unbiased by barrier dysfunctions, that is, high albumin quotients (up to $Q_{Alb} = 400 \cdot 10^{-3}$, median $25 \cdot 10^{-3}$).

In general, all normal AI values were between 0.5 and 1.4, that is, the reference range of normal AI with only blood-derived antibodies in aqueous humor was AI ≤1.4. But all AI values (100%) for antibodies against the causative antigen in clinically definite cases of FHC, HSV iritis, VZV uveitis, and toxoplasmosis retinitis were AI ≥1.5 (Figure 2 and Table 3). This led to the clinically defined cut-off for pathologic values with AI ≥1.5, indicating an intraocular antibody synthesis.

• **INTRAOCULAR ANTIBODY SYNTHESIS IN FHC:** We investigated aqueous humor from 52 eyes and serum of clinically definite FHC patients (Table 3). All 52 patients (100%) had an intraocular rubella antibody synthesis (rubella AI ≥1.5) with a median of rubella AI = 20.6 (total range 1.5–309; Table 4). There was no overlap of the AI values in Figure 2 between FHC and the normal control of the rubella AI values. In addition 6% of the FHC patients had an intraocular measles, 6% a varicella-zoster, and 12% an intraocular toxoplasma antibody synthesis (Table 3). The AI values for these noncausative microorganisms were small (AI = 1.5–2.0) with one exception (Table 3).

In three FHC patients, the aqueous humor of both eyes was analyzed. The AI values were normal in all three cases for the uninflamed fellow eye compared with the inflamed eye (in parantheses) with rubella AI = 1.3 (2.6); 0.9 (5.3), or 0.9 (1.5), respectively. In two patients with FHC we could analyze both aqueous humor and CSF. The rubella AI in CSF was normal (rubella AI = 0.8) or below the detection limit compared with aqueous humor with rubella AI = 18 and rubella AI = 37, respectively.

• **ANTERIOR UVEITIS OF UNKNOWN CAUSE:** A humoral immune response was detected in two (20%) of 10 patients investigated for oligoclonal IgG, but in all of the patients an intraocular antibody synthesis against measles, rubella, VZV, HSV, or toxoplasma was absent, detected either with AI values between 0.6 and 1.4 or not detectable at all (Table 3).

• **VARICELLA ZOSTER IRITIS:** All 14 patients had an increased VZV AI (total range AI = 1.5 to 44), but no increased rubella AI or measles AI (AI ≤1.4). The HSV AI was increased concomitantly in 5 cases (39%). This concomitant activation is a well-known phenomenon in VZV and HSV infections (Table 3).

• **HERPES SIMPLEX IRITIS:** All 25 patients had an increased HSV AI (total range AI = 1.5–22.6) but no raised rubella, measles, or toxoplasma AI (AI ≤1.4). A concomitant intraocular VZV antibody synthesis was observed in 13 (52%) cases (Table 3).

• **TOXOPLASMOSIS RETINITIS:** 24 patients had an increased toxoplasma AI (total range AI = 1.9 to 138) but no elevated rubella, measles, zoster, or herpes AI (AI ≤1.4).

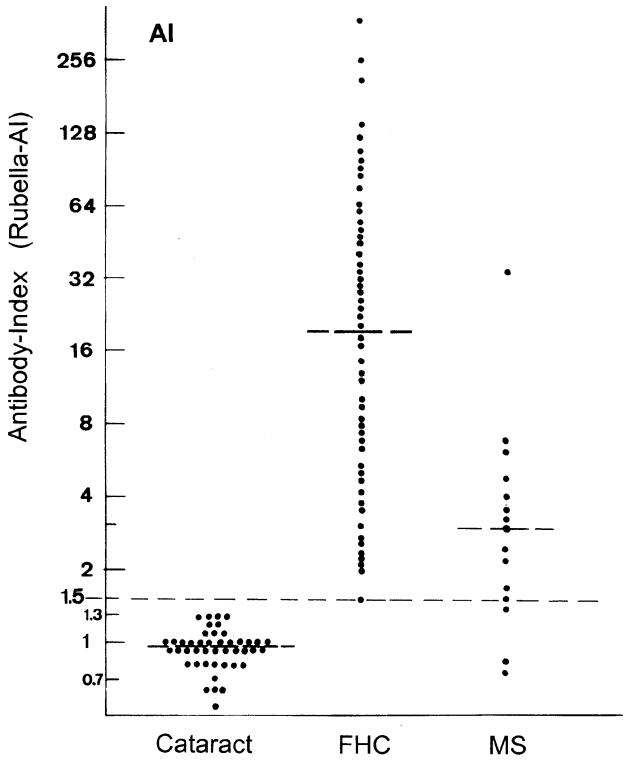

FIGURE 2. Evaluation of the antibody index (AI) for rubella antibodies in aqueous humor of patients with senile cataract (reference range AI = 0.5–1.3, Table 2), for Fuchs heterochromic cyclitis (FHC) and multiple sclerosis ([MS] Table 4). The mean of n = 44 cataract patients with a detectable rubella AI (Table 2) and the medians of n = 52 FHC patients and n = 15 MS patients are indicated by the dashed lines. The cut-off, AI ≥1.5, is also represented by a dashed line. The antibody index is presented in a binary logarithmic scale.

• **MULTIPLE SCLEROSIS:** In MS patients with an uveitis intermedia or periphlebitis retinae the frequencies of intraocular measles, rubella, zoster, and herpes simplex antibody synthesis (Table 3) are similar to the intrathecal synthesis reported from CSF analysis of MS patients.[13] Eleven (73%) of 15 MS patients had an intraocular rubella antibody synthesis (Figure 2), but the corresponding rubella AI values have been smaller, with a median of rubella AI = 3.0 (total range 0.7–35) compared with FHC (Figure 2) with a median of rubella AI = 20.6 (Table 3).

• **QUANTITY OF RUBELLA ANTIBODIES IN AQUEOUS HUMOR OF FHC AND MS:** The median of the rubella specific antibody fraction in aqueous humor of FHC patients was rubella-F_s = 2.6% (total range 0.14%–45.9%), (Table 4), that is, in average 2.6% of the intraocularly synthesized total IgG were rubella antibodies. For multiple sclerosis we calculated a median rubella-F_s = 0.06% (total range 0.01%–0.25%), that is, the mean quantity of intraocular rubella antibody synthesis in FHC is approximately 40-fold higher than in MS. The specific fraction, F_s, discriminates better between the intensities of the immune reaction in both groups, that is, 40-fold compared with sevenfold with the AI (Table 4).

• **RUBELLA GENOM IN AQUEOUS HUMOR:** With the PCR, the rubella genom has been detected in five (18%) of 28 aqueous humor samples of FHC patients investigated. The PCR-positive samples have been controlled by a sequence analysis in both laboratories (see Methods). All 22 aqueous humor samples from control eyes with a senile cataract were PCR negative. The rubella AI values in the 5 PCR-positive cases were rubella AI = 1.5, 2.2, 6.6, 11.0, and 21.6. Intraocular IgG fractions (IgG_{IF}) in aqueous humor of these PCR-positive patients were between 0% and 63%. In one sample, oligoclonal IgG was not detectable. The albumin quotients were all normal with Q_{Alb} = 2.5 to 5.8 · 10^{-3}. The age of the 5 patients was 16 to 37 years.

• **DIAGNOSTIC SENSITIVITY AND SPECIFICITY:** The rubella-specific AI with rubella AI ≥1.5 reached a clinical sensitivity of 100% compared with 87% for oligoclonal IgG and 50% for the calculated intrathecal IgG fraction (IgG_{IF}; Table 3). The detection of the rubella genom in aqueous humor had the lowest sensitivity (18% for the total group) among the parameters analyzed. Oligoclonal IgG in aqueous humor and an increased rubella AI in FHC are not specific, as shown by the comparison with multiple sclerosis (Table 3 and Figure 2). Values of rubella AI >40 (Figure 2) or rubella- F_s >0.25 (Table 4) are not observed in MS. But regarding the clinically relevant groups for differential diagnosis of FHC, it is important to note that not a single case (0%) of a total number of 83 cases with anterior uveitis, VZV iritis, HSV iritis, or toxoplasmosis retinitis (Table 3) had an increased rubella AI. This indicates a high positive predictive value approaching 100% in the clinically relevant groups for the detection of an intraocular rubella antibody synthesis.

The information from blood/aqueous humor barrier function (Q_{Alb}) did not provide any particular diagnostic help, owing to the wide range of values between normal (Q_{Alb} <10 · 10^{-3}) and extreme barrier dysfunctions (Q_{Alb} = 492 · 10^{-3}).

DISCUSSION

THE DETECTION OF RUBELLA ANTIBODIES AND THE RUbella virus in FHC has diagnostic and therapeutic consequences and can point to a new approach in the search for the pathomechanism.

• **LABORATORY-SUPPORTED FHC DIAGNOSIS:** With a sensitivity of 100%, the intraocular rubella antibody synthesis should become the most relevant criterion for a

TABLE 4. Intensity of Intraocular Rubella Antibody Synthesis and Blood/Aqueous Humor (B/AH) Barrier Function in Fuchs Heterochromic Cyclitis (FHC) and Multiple Sclerosis (MS)

	B/AH Barrier* $Q_{Alb} \cdot 10^3$			Antibody Index Rubella AI†			Antibody Fraction Rubella F_s‡		
	Median	Range	(*n*)	Median	Range	(*n*)	Median	Range	(*n*)
FHC	4.6	0.9–492	(51)	20.6	1.5–309	(52)	2.6	0.14–45.9	(37)‖
MS§	18.8	1.4–193	(15)	3.0	0.7–35	(11)	0.06	0.01–0.25	(11)

*Blood/aqueous humor barrier function, characterized as albumin AH/serum concentration quotient, Q_{Alb}.

†Rubella antibody index (AI): rubella AI = $Q_{rubella}/Q_{IgG}$ or rubella-AI = $Q_{rubella}/Q_{Lim}$ in case $Q_{IgG} > Q_{Lim}$.

‡Intraocularly synthesized rubella antibody fraction, rubella F_s = rubella-$AB_{Loc}/IgG_{Loc} \cdot 100$ in [%].

§Multiple sclerosis patients with a periphlebitis retinae or uveitis intermedia of the eye.

‖Rubella F_s could be calculated only for 37 patients, for 12 FHC patients with $Q_{IgG} < Q_{mean}$ the IgG_{Loc} (mean) and subsequently rubella F_s could not be calculated.

laboratory-supported diagnosis of FHC in connection with clinical signs.[3,4] The absence of a rubella antibody synthesis in the eye would make the diagnosis of FHC very implausible. By comparison with MS, it is clear that an increased rubella AI is not specific for FHC (Tables 3 and 4, Figure 2). But for the clinical differential diagnosis of FHC it is not a problem to discriminate an MS-related periphlebitis retinae or uveitis intermedia (MS is diagnosed in neurology with CSF analysis and MRT together with eventual polyspecific immune response). In the clinically relevant differential diagnostic groups (Table 3), the detection of intraocular rubella antibody synthesis has a very high positive predictive value approaching 100% for FHC (0% of 83 patients with anterior uveitis, VZV iritis, HSV iritis, or toxoplasmosis retinitis showed an increased rubella AI, Table 3).

In particular cases with rubella AI >40, the rubella AI becomes absolutely specific for a rubella-driven antibody synthesis unlike a concomitant polyspecific immune reaction in MS with AI <40 (Figure 2 and Table 4). The low sensitivity of the direct detection of the rubella virus makes PCR the diagnostically least sensitive parameter, particularly in elderly patients (see below).

Oligoclonal IgG with 87% sensitivity for FHC remains the second choice for FHC diagnosis but is the most sensitive parameter for detection of any unspecified humoral immune response in the eye[8,10,20,26] and can therefore be recommended for general screening.

• **IMPROVEMENT OF AQUEOUS HUMOR DATA INTERPRETATION:** The application of improved interpretation concepts[13,14,16,21,25] that refer to the nonlinear, hyperbolic discrimination line[22] (Figure 1) for the detection of an intraocular IgG-synthesis helped to avoid the flaws of earlier reports[8,17] with false high numbers for intraocular IgG synthesis[8] and in particular false positive interpretations of noninflammatory controls.[8,24]

• **SENSITIVE DETECTION OF INTRAOCULAR ANTIBODY SYNTHESIS:** Owing to the correction for the polyspecific immune response (example in Figure 1 and Methods), the AI is the diagnostically relevant, most sensitive parameter to detect an intraocular antibody synthesis. Owing to the possible false negative interpretations (example in Figure 1), with the frequently used Goldmann-Witmer Index (GW-I = Q_{spec}/Q_{IgG}), an intraocular rubella synthesis would have been missed in 42% of the MS cases (and in 2% of FHC), compared with the interpretation with the corrected AI = Q_{spec}/Q_{Lim} (if $Q_{IgG} > Q_{Lim}$). The reference range of normal AI values in CSF is confirmed for aqueous humor by the data reported in Table 3 and Figure 2. All normal values from the patients with senile cataract (Table 3) were between 0.5 and 1.4 in this study. Together with a clinically defined cut-off for pathologic values with AI ≥1.5 we avoid false positive[8] and false negative[17] interpretations of intraocular antibody synthesis. In the case of antibodies against the causative antigen there was no overlap between pathologic and normal values (Table 3).

• **VIRUS-DRIVEN VS POLYSPECIFIC IMMUNE RESPONSE:** With the calculation of the absolute amount of antibodies synthesized in the eye[12] we could demonstrate that the mean intraocular fraction of rubella- F_s in FHC is 40-fold higher than the mean rubella-F_s in MS (Table 4). Similar results were found in other cases of immune response against a causative antigen, for example, the quantitation of the intrathecal measles antibody synthesis in subacute sclerosing panencephalitis (caused by measles)[12,13] or HSV antibody synthesis in HSV encephalitis[13] also showed a mean 60- or 20-fold (correspondingly) higher intrathecal antibody synthesis than measles or HSV antibody synthesis observed in CSF of MS patients.[13] This quantitative analysis of a strong immune response represents a very sophisticated argument to support the hypothesis of a virus-driven antibody synthesis in the eye or CNS. In the cases of an accompaning weak, polyspecific immune re-

sponse there was no virus genome persistent; for example, the rubella genom[28,29] as well as the measles genome[28] could not be detected postmortem in the brain of MS patients.

The similar frequencies of increased measles, rubella, VZV, and HSV AI in aqueous humor (Table 3) and CSF[13] fit to earlier reports about the similarities of intraocular and intrathecal immune reactions in MS patients.[27]

In FHC the frequencies of intraocular synthesis of antimeasles antibodies (6%) or antivaricella zoster antibodies (6%) are much lower than the corresponding frequencies in multiple sclerosis, with approximately 80% (for measles) or 47% for varicella zoster (Table 3). In FHC the values of measles AI and zoster AI between 1.5 and 2.0 also are low compared with the intensity of the rubella antibody synthesis in FHC with rubella AI values up to 309. The presence of many different antibody species (polyspecific reaction) in FHC is not unexpected, as only 0.14% to 45.6% (median 2.6%) of the intraocular IgG synthesis are rubella antibodies (Table 4). It is clear from these data that the rubella antibody synthesis in FHC and MS is the result of different causes: FHC presents a virus driven antibody response[32] of highest frequency and in particular of high intensity for the causative, persisting rubella antigen; MS presents an antibody response against the rubella virus of high frequency but of low intensity, due to the network properties of the immune system.[33]

• **PATHOMECHANISM OF FHC:** Many causes for FHC have been proposed.[6] The increasing consensus that an immunologic reaction is involved in the pathologic process[1,8,9,11,24] is consistent with our discovery of a rubella virus-driven antibody synthesis in the eye. Occasionally, *Toxoplasma gondii* has been discussed as a possible cause of FHC.[3] This is contradicted by our results (Table 3), where only two of 16 FHC cases had increased toxoplasma AI values (AI = 1.8 and 5.1), including 1 case (AI = 5.1) with an unilateral FHC but with scars from a former, clinically diagnosed toxoplasmosis of both eyes. Our investigations of the healthy uninflamed fellow eyes and the comparison of aqueous humor and CSF from the same patient confirm that FHC is a local process, as suggested in earlier reports.[30] In our study only 3 of 52 FHC patients (6%) had the disease in both eyes, which is consistent with other reports.[3,4] All these observations support the statement that FHC is connected with a rubella virus-driven local immune response with a slowly progredient pathology. The detection of the rubella virus in only 5 (18%) of the investigated 28 aqueous humor samples needs some particular comments. The virus was detected in aqueous humor samples of FHC patients with an age between 21 and 37 years (median = 28 years). In the PCR subgroup of 28 FHC patients (aged 21–73 years) only 9 patients were younger than 40 years. This would mean that the frequency of detection would be 5 of 9 or 56%, which is above that for toxoplasma gondii (31%) in acute toxoplasmosis of the eye[31] but below that of cytomegalovirus (91%) or VZV and HSV (81%) in the corresponding acute diseases.[31] So it seems reasonable to speak about the persistence of the virus in a chronic disease, but the duration of the persistence of the rubella virus in the eye of FHC patients remains an open question and needs further investigation. The pathology of FHC is similar to the persistence of the measles virus in subacute sclerosing panencephalitis. A congenital rubella infection seems implausible[29] because of the late onset of symptoms in the group of FHC patients with a median age of 44 years (16–73 years). The discovery that FHC could be caused by the persistent rubella virus also offers a rationale for the empirical experience of many clinicians that the corticosteroid therapy used in FHC treatment is ineffective.

CONCLUSION

WITH THE DETECTION OF RUBELLA VIRUS-DRIVEN INtraocular antibody synthesis in FHC, for the first time a highly sensitive laboratory test is established to confirm a clinically suspected FHC. With the aqueous humor analysis FHC can be differentiated from Posner-Schlossmann syndrome, pars planitis, sarcoidosis, and panuveitis. This is important, because FHC has a good prognosis, and the therapeutic strategy is different. The risk of developing a secondary glaucoma was 20% of our 52 patients, and surgery on the secondary cataract had a good prognosis. The laboratory-supported diagnosis together with the virus etiology justifies the clinician in omitting the long-lasting corticosteroid treatments that reduce inflammation only minimally but increase the chance of developing cataract and glaucoma. With detection of the local persistence of the virus, we get new perspectives for investigation of the pathomechanism and development of a treatment of the cause of FHC.

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